# SCIENTIFIC REPORTS

Received: 07 October 2016 Accepted: 10 February 2017 Published: 17 March 2017

## **OPEN** Sex differences in DNA methylation of the cord blood are related to sexbias psychiatric diseases

Mariana Maschietto<sup>1</sup>, Laura Caroline Bastos<sup>2</sup>, Ana Carolina Tahira<sup>2</sup>, Elen Pereira Bastos<sup>2</sup>, Veronica Luiza Vale Euclydes<sup>2</sup>, Alexandra Brentani<sup>3</sup>, Günther Fink<sup>4</sup>, Angelica de Baumont<sup>2</sup>, Aloísio Felipe-Silva<sup>5</sup>, Rossana Pulcineli Vieira Francisco<sup>6</sup>, Gisele Gouveia<sup>2</sup>, Sandra Josefina Ferraz Ellero Grisi<sup>3</sup>, Ana Maria Ulhoa Escobar<sup>3</sup>, Carlos Alberto Moreira-Filho<sup>3</sup>, Guilherme Vanoni Polanczyk<sup>2</sup>, Euripedes Constantino Miguel<sup>2</sup> & Helena Brentani<sup>2</sup>

Sex differences in the prevalence of psychiatric disorders are well documented, with exposure to stress during gestation differentially impacting females and males. We explored sex-specific DNA methylation in the cord blood of 39 females and 32 males born at term and with appropriate weight at birth regarding their potential connection to psychiatric outcomes. Mothers were interviewed to gather information about environmental factors (gestational exposure) that could interfere with the methylation profiles in the newborns. Bisulphite converted DNA was hybridized to Illumina HumanMethylation450 BeadChips. Excluding XYS probes, there were 2,332 differentially methylated CpG sites (DMSs) between sexes, which were enriched within brain modules of co-methylated CpGs during brain development and also differentially methylated in the brains of boys and girls. Genes associated with the DMSs were enriched for neurodevelopmental disorders, particularly for CpG sites found differentially methylated in brain tissue between patients with schizophrenia and controls. Moreover, the DMS had an overlap of 890 (38%) CpG sites with a cohort submitted to toxic exposition during gestation. This study supports the evidences that sex differences in DNA methylation of autosomes act as a primary driver of sex differences that are found in psychiatric outcomes.

Epigenetics is a dynamic biological mechanism involved in the modulation of an individual's genome to environmental stimuli. Much of the epigenome is established during embryogenesis and early gestation and is transmitted in a relatively stable fashion<sup>1</sup>. Among the epigenetic factors, DNA methylation is thought to contribute to stable long-term regulation of gene expression. A large study in families with twins reported methylation differences dependent on genetic factors and environment<sup>2</sup>. Due to the stability, DNA methylation might be related to the development of psychiatric diseases later in life<sup>3</sup>. Several studies pointed out to a correspondence at methylation levels between blood and brain within individuals, suggesting that, although with some restrictions, blood can be used as a source to study psychiatric diseases<sup>4,5</sup>.

During perinatal sensitive periods, the environment exerts a critical impact on the maturation of brain structure and function<sup>6-10</sup>. It has been well characterized in humans that exposure to external damaging agents, such as smoking, mercury and arsenic during gestation induced epigenetic alterations in newborns<sup>11-13</sup>. Likewise, early life adversity, including pre- and post-natal stress, increased the risk of psycopathologies in animal models<sup>14</sup>. In humans, the Dutch Hunger Winter studies demonstrated the relationship between famine exposure at different times of prenatal development and phenotypic outcomes. Famine exposure around the period of conception was associated with increased risk of adult schizophrenia<sup>15</sup>, and with persistent changes in DNA methylation for INSIGF, GNASAS, LEP, MEG3, IL10 and ABCA116. Furthermore, epidemiological studies indicated that psychological stress such as

<sup>1</sup>Brazilian Biosciences National Laboratory (LNBio), Brazilian Center for Research in Energy and Materials (CNPEM), Campinas, Brazil. <sup>2</sup>Institute of Psychiatry, University of São Paulo Medical School, SP, Brazil. <sup>3</sup>Department of Pediatrics, University of São Paulo Medical School, SP, Brazil. <sup>4</sup>Department of Global Health and Population, Harvard School of Public Health, USA. <sup>5</sup>Department of Pathology, University Hospital of Sao Paulo, SP, Brazil. <sup>6</sup>Department of Obstetrics and Gynecology, University of São Paulo Medical School, SP, Brazil. Correspondence and requests for materials should be addressed to H.B. (email: helena.brentani@gmail.com)

bereavement, unwanted pregnancies, military invasion and natural disasters increased the risk for the offspring to develop neurodevelopmental diseases later in life, such as schizophrenia, depression and anxiety<sup>17-20</sup>.

The relation between psychiatric diseases and adverse experience in early life were included in the Developmental Origins of Health and Disease (DOHaD) hypothesis. Most DOHaD diseases have a sex-biased susceptibility<sup>21,22</sup>, suggesting that the response to stress is different in males and females. Accordingly, when exposed to stress, males suffer from behavioral problems earlier in development - at one year of age<sup>23</sup>, whilst females show the effects at later life periods<sup>24,25</sup>. In addition, there is a clear temporal specificity and sexually dichotomous influence of the effects of prenatal stress<sup>26–28</sup>. Males seem to be more vulnerable to develop schizophrenic, autism and attention-deficit/hyperactivity disorder (ADHD) symptoms in response to perinatal stress, all these were associated with intra uterus growth restriction (IUGR)<sup>29,30</sup>.

Animal models also contributed to association between methylation alterations and different response to stress exposition in females and males. For instance, intrauterine exposure to Bisphenol A (BPA), an estrogenic endocrine disruptor, seems to induce DNA methylation changes in male cortex and in female hypothalamus as result of expression changes in *ESR1* and *DNMT1*<sup>31</sup>. In mice, treatment with valproic acid during gestation induced sex-specific morphological alterations at a discreet region of the midbrain, including a higher cell counts in males compared to females<sup>14</sup>. In mice, exposure to stress in the prenatal period caused elevation of *DNMT1* expression levels in females' placenta, but not males. Contrariwise, males showed response to maladaptive behavioral stress together with change in methylation and expression of the glucocorticoid receptor and corticotropin-releasing factor in the fetal brain<sup>28</sup>.

A correlation between changes in methylation and gene expression were suggested to be involved with the differential brain development<sup>32</sup>. Thus, differences in the regulation of gene expression between females and males could explain the variances in specific brain structure<sup>8,27,28,32,33</sup>, cognitive abilities and stress-coping strate-gies<sup>28</sup> Some studies already evaluated sex differences at methylation levels between females and males in the cord blood<sup>34</sup>, prefrontal cortex<sup>32,35</sup> and pancreatic isolates<sup>35</sup>, nevertheless, none considered exposition to stress. Whilst it is known that the developing brain has enhanced sex-biased sensitivity to environmental stimuli at specific periods of gestation that contributes to vulnerability to psychiatric diseases, the possibility that DNA methylation differences between females and males could be involved with psychiatric diseases, independently of exposition to stress, remains to be addressed.

To unveil the contribution of stress exposition and the sexual antagonism effects in DNA methylation as well as their association with psychiatric vulnerabilities, we conducted a genome-wide methylation analysis of cord-blood in a well-characterized cohort of children born at term and of adequate weight/size at birth. Adjustments for cell heterogeneity and stress exposure co-variables were performed followed by the identification of differentially methylated CpG sites (DMSs) between females and males. These methylation differences were explored in other cohorts, including brain development, neurodevelopmental disorders and a cohort where children were exposed to stress during gestation.

#### Results

**General characteristics of the study population.** We used a natural cohort of newborns collected at the University Hospital of São Paulo Medical School, where, typically, only mothers with a not at risk pregnancy do have labor. We randomly collected cord blood samples from 96 individuals. Then, we excluded six pre-term and two post-term newborns, as well as 11 small and seven large for gestational age newborns, since those presentations have already been associated with psychiatric outcomes and stress exposition<sup>36</sup>. The final cohort included 71 cases, comprised of 39 females and 32 males, all with adequate weigh/size for gestational age and born at term. Moreover, to maximize differences in methylation probably related to sexes differences, we corrected for other exposure variables that could interfere with the analysis (Table 1, Supplemental Table 1) explored by this study (females versus males). Although smoking were different between females and males (p=0.034), only psychiatric disease in father's family presented a significant value as given by the sva package analysis (Supplemental Figure 1) and was corrected accordingly. In addition, data were adjusted for batch effects and cell type composition. Only as a proof of concept, the sex of each sample was confirmed by methylation levels of sexual chromosomes. The study design was included as a chart showing the analyses flow (Supplemental Figure 2).

**Methylation differences between sexes correlate with gene expression.** To characterize methylation changes in the cord blood of females and males, methylation levels were derived for 464,964 CpG sites, excluding XYS probes. Supervised comparison between sexes identified 2,332 differentially methylated CpG sites (DMSs) with females presenting twice the number of hypermethylated sites compared to males (adjP < 0.05, 1,546 and 786 CpG sites more methylated in females and males, respectively), with 1,499 CpG sites associated to 1,113 genes (Fig. 1; Supplemental Table S2). Upon clustering of the 2,332 CpG sites (Euclidian distance with average linkage), two clusters formed, completely separating both sexes (Supplemental Figure 3).

To evaluate if DMSs were associated with differentially expressed genes between females and males, we used an expression dataset of 2,500 samples from 13 tissues<sup>37</sup>. Excluding genes located in the sexual chromosomes, the 1,113 genes were enrichment among differentially expressed genes between females and males in five tissues, four being brain regions (anterior cingulate cortex, frontal cortex, cerebellum and hippocampus), and the fifth being heart (Supplemental Table 3).

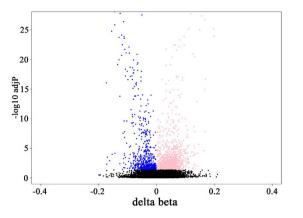
Considering that the 1,113 genes have a sex-biased expression, we used MiSig/WebGestalt to verify if there was an enrichment of binding sites for estrogen and androgen receptors and binding sites located at 2 Kb up or downstream from transcriptional start site (TSS) of each of gene. This analysis resulted in 105 genes regulated by estrogen receptor alpha or beta (five motifs, adjP < 0.05), 24 genes regulated by androgen receptor (three motifs, adjP < 0.05), and ten genes regulated by both transcriptional factors. Then, to explore more

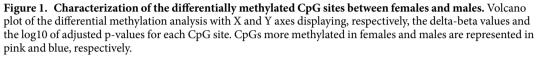
		Male	e (n = 32)		Female (n = 39)							
	n	%	Mean (±SD)	n	%	Mean (±SD)	P value					
Mothers												
-Age (years)	1		26.9 (5.9)			29.4 (7.0)	0.133					
≤18	0			1	2.56							
19–35	29	90,7		31	79.5							
≥36	3	9.3		7	17.9							
-Educational level							0.840					
Unlettered	2	6.5		1	2.6							
Elementary school	11	35.5		14	35.9							
High school	17	54.8		20	51.3							
College	0			1	2.6							
Unknown	1	3.2		3	7.7							
Income (Minimal wages)			4.0 (1.0)			4.5 (0.8)	0.399					
$\leq 2$	9	29.0		18	46.1							
3-4	11	35.5		14	35.9							
≥5	7	22.6		3	7.7							
Unknown	4	12.9		4	10.3							
-Type of delivery							0.815					
Normal	17	57.7		21	53.9							
Cesarean section	13	43.3		18	46.1							
-Planned gestation	26	83.9		34	87.2		0.727					
-Self-reported environmental fact	ors											
-Stress during gestation	15	48.4		20	51.3		0.525					
-Feelings during gestation							0.546					
Most time, positive	18	58.1		18	46.2							
Soft stress	4	12.9		10	25.6							
Moderate stress	6	19.4		5	12.8							
Abortion was considered	2	6.5		5	12.8							
Severe stress	1	3.1		1	2.6							
-Psyquiatric drugs during pregnancy	0			2	5.1		0.201					
-Smoking during gestation	2	6.3		10	25.6		0.034					
-Drugs/alcool during pregnancy	12	38.7		9	23.1		0.156					
		N	Veonates									
-Gestational age (weeks)	30		39.5 (1.4)	39		39.4 (1.2)	0.876					
-Birth Weight (g)	30		3359.2 (396)	39		3298.2 (321)	0.482					
-Length	30		49.3 (1.7)	39		48.1 (1.8)	0.009					
-Cephalic circumference	30		34.7 (1.3)	38		34.2 (1.0)	0.123					
-Thoracic circumference	30		33.2 (1.4)	38		33.2 (1.4)	0.963					
-Abdominal circumference	30		31.9 (1.6)	38		32.3 (1.8)	0.361					

 Table 1.
 Characteristics of the cohort and factors evaluated as covariables of the analyses.

distant motifs, we used Opossum<sup>38</sup> that searched for transcriptional factors target sites located at 5 Kb or 10 Kb up or downstream from TSS of 838 (out of 1,113) genes, revealing no enrichment for *ESR1* or *AR* binding sites (Supplemental Table 4).

**Differentially methylated CpG sites are not randomly distributed within the genome.** Females have twice the number of methylated CpGs sites than males, with a non-random distribution in the genome. CpG sites more frequently methylated in females were more often located near transcriptional start sites (TSS1500) and within CpG islands, S\_shores and S\_shelves. Conversely, CpG sites more frequently methylated in males were enriched within gene bodies and 3'UTR, and were associated with open sea areas (Supplemental Table 5, chi-square test, p < 0.05). Based on the predefined regions, the differentially methylated regions (DMRs) between females and males were distributed as follow: 171 CpG islands (98 and 73 hyper- and hypomethylated in females), 131 promoters (80 and 51 hyper- and hypomethylated in females), 127 genes (64 and 63 hyper- and hypomethylated in females) (Supplemental Figure 4, Supplemental Table 6, adjP < 0.05). The top DMRs (adjP < 0.05 and at least 10 CpG sites) are shown in Table 2.





Differentiated methylated sites between males and females are related to genes associated with normal brain development and neurodevelopment disorders. To gather a global view of the role of these genes, the 1,113 genes were annotated into gene ontology, revealing over-representation of genes related to system development, neurogenesis and neuron differentiation, striated muscle cell differentiation, and single organism behavioral and synaptic transmission. In addition, we found enrichment of genes belonging to the metabolic pathway (60 genes), the MAPK signaling pathway (23 genes), and focal adhesion (19 genes), among others (adjP < 0.01), including the insulin signaling pathway (12 genes). Finally, we observed an enrichment of genes related to neuropsychiatric disorders, stress, cancer, congenital disorders, musculoskeletal including cardiovascular diseases, endocrinological and female and male genitourinary diseases (Supplemental Table 7).

To test if the 1,113 genes were associated with brain development, we searched in the Allen Brain Atlas and found that 930 genes were expressed in at least one of the 26 brain regions (8<sup>th</sup> post-conception week to 40 years of age). Next, the comparison between the 2,332 DMS and the 7% of CpGs that changes the methylation during brain development<sup>39</sup> showed an enrichment, represented by 238 CpGs (p < 0.00001, Supplemental Figure 5). Further, we compared to the 521 CpG sites that, in addition to being modulated during brain development, were also differentially methylated between brains of females and males, resulting in 195 common CpG sites (p < 0.00001, Supplemental Figure 5). Finally, extracting data from the only study in schizophrenia that had used the 450 K Beadchip arrays with publicly available data<sup>40</sup> we searched for enrichment between 2,332 DMS between females and males and the 4,641 differentially methylated CpG sites in brain tissue from schizophrenic cases and controls. The analysis reported an overlap of 116 CpG sites between both studies, indicating an enrichment for CpG sites related to schizophrenia among CpG sites that differ between females and males in cord blood (p = 0.0139, Supplemental Figure 5).

**Differentiated methylated sites between females and males are enriched for psychiatric disorders in a stress exposition model.** To further explore the enrichment of DMSs between sexes after correction for stress exposition among genes associated with psychiatric disorders, we compared our DMSs to the differentially methylated CpG sites in the cord blood between females and males exposed to stress (toxic agents) during gestation from an independent cohort. A study from CHAMACOS (a longitudinal birth cohort study of the effects of exposure to pesticides and environmental chemicals on the health and development of Mexican-American children living in the agricultural region of Salinas Valley, CA) assessed the methylation changes related to sex differences in the cord blood of females and males, taking into account batch effects and cell composition. Excluding XYS CpG sites, they found 3,031 DMS (25.7% of the total) located in the autosomes, with most of them (82%) more methylated in females<sup>34</sup>. We compared these to our 2,332 DMS and found 890 (38%) common DMSs, all presenting the same pattern regarding hypo- and hypermethylation.

To assess whether there was an enrichment of genes associated with psychiatric diseases in both data sets, CHAMACOS' and ours, we used only those DMSs related to genes. Considering that one gene may have more than one CpG site, and different CpG sites may have opposing methylation differences (hypo- or hypermethylated), depending on their location, we excluded genes represented by more than one CpG site. This analysis resulted in 472 common genes between CHAMACOS' and ours, 1,309 and 500 genes belonging exclusively to CHAMACOS' or our datasets, respectively. Next, an enrichment analysis for diseases were performed with full datasets followed by the enrichment analysis with common and exclusive genes of the two cohorts that showed that, considering all diseases, most of the psychiatric diseases were enriched in all datasets, although there were differences in the ranking as exemplified by the top ten diseases (Supplemental Table 8, diseases and schizophrenia).

These gene sets were tested for enrichment in modules of co-expressed genes along human neocortical development<sup>41</sup> (Supplemental Table 9). M8, M13 and M17 were enriched within the full datasets and among common genes. M8 is comprised of genes more expressed in the beginning of gestation; genes from M13 present a decreasing expression until postconception week (PCW) 16, from which point present an increasing pattern;

Cybin         Cybin <th< th=""><th>ID</th><th>Chromosome</th><th>Start</th><th>End</th><th>Gene symbol</th><th>entrezID</th><th>Mean difference</th><th>Combined adjP</th><th>CpG sites</th></th<>	ID	Chromosome	Start	End	Gene symbol	entrezID	Mean difference	Combined adjP	CpG sites
2minchar120799992080012Cond-0.04637.87-041123002char196405136406225C-0.0071.946-63102307char227.0681527.06815C0.0071.116-02126417char237.98700228.977060.0071.018-02104444char349.99892C0.00171.088-021013864char1510.0942110.9999900.00241.228-021316894char162569560257.071C0.0263.768-02121888char162569560257.071NNN-0.0421.028-02121894char32587.620NANA-0.0421.028-0212121805Sch17.568NANA-0.0421.028-0212121805char35591.559561.7568NANA-0.0421.028-02121805char32587.162StrNANA-0.0421.028-02121805char32587.163StrNANA-0.0421.028-02121805char32587.163NANA-0.0421.028-02121805char32587.163NANA-0.0421.028-02121805char32587.163NANA0.0674.458-02131805char32587.162PILANA </th <th></th> <th>Chromosome</th> <th>oturt</th> <th>Liiu</th> <th>Gene symbol</th> <th>Children</th> <th>incun unterence</th> <th>Combined adjr</th> <th>opusites</th>		Chromosome	oturt	Liiu	Gene symbol	Children	incun unterence	Combined adjr	opusites
2202         ch.19         640713         640714         27106815         27106213         1         -0.003         2.13E-03         1.01           25607         ch.21         27106815         27106211         -0.003         2.13E-03         1.01           3768         ch.2         28857002         0.016         1.48E-03         1.01           3768         ch.23         2885700         0.017         1.08E-02         1.01           1864         ch.15         101049521         101094921         0.020         7.85E-03         1.3           1889         ch.16         258950         2571071         0.022         0.026         3.76E-02         1.2           7284         ch.5         19546028         16914608         NA         NA         -0.021         1.22E-02         1.2           ENSCO0000132708         ch.7         565159         557868         NA         NA         -0.012         1.92E-02         1.1           ENSCO00013060         ch.7         579462         579567         CNN1         15105         0.017         1.91E-03         1.0           ENSCO00013060         ch.16         258858         2570857         ADD112         5105772014         0.027	-	chr17	20798958	20800112			-0.046	3 79F-03	11
25607ch/c127106112710611271061127106-0.0032.13.E-03106417ch/c357993395799470C0.0071.11E-0212758ch/c220877100C0.01611.348E-03104494ch/3499985649995942C0.01241.028E-021318864ch/r15101095211019993C0.02047.58E-03131889ch/r16259799525876200C0.0283.76E-02127284ch/r5194402819440988C0.03683.76E-0212FSC00002752ch/r75894598587758NANA-0.0479.82E-0313ENSC000003528ch/r759792675974667FL/R113146-0.0421.92E-0212ENSC000003529ch/r12988746128877663C/N11151464-0.0471.92E-0212ENSC000013540ch/r12988746128875763C/N11151490.0471.91E-0213ENSC000013591ch/r12547422587645C/N11151490.0464.45E-0213ENSC000013791ch/r12547422587657C/N1115140.0464.45E-0213ENSC000013791ch/r11574397579657C/N1115140.0464.45E-0213ENSC000013794ch/r11574397579669C/N1115140.0464.45E-0213EN							-		
4617         chr.3         57994704         cm         0.007         1,11E-02         12           3768         chr.2         20857706         0.016         1.488-02         10           1854         chr.15         10109421         101099431         0.017         1.488-02         10           1854         chr.15         10109421         101099431         0.026         3.768-02         12           1849         chr.16         2587500         0.026         0.026         3.768-02         12           7248         chr.1         1954028         16954088         0.026         3.768-02         11           ENSCO000032726         chr.17         25798954         2080955         CCDC144NL         31814         -0.042         1.02E-02         12           ENSCO00013508         chr.6         5799462         7579462         FLNB         2317         0.007         1.39E-02         11           ENSCO00013060         chr.16         258876         CNX1         11819         0.017         1.39E-02         12           ENSCO00013072         chr.10         2587462         2877676         CNX1         1918         0.027         4.45E-02         12           ENSCO000016206									
3768         ch.2         29857706         29857706         0         0.016         1.485-03         10           4494         ch.3         43934856         4393942         0.017         1.085-03         10           18864         chrif 5         10104521         10109405         0.020         7.587-03         13           18880         chrif 5         14954028         2571071         0.024         3.761-02         12           7248         chr5         14954028         14954098         0.058         2.201-03         10           Promoter          5615569         5617568         NA         NA         -0.047         9.62E-03         13           ENSCO000020512         chr17         26914669         5034486         RATTL         13914         -0.042         1.02E-02         11           ENSCO000015304         chr3         5799267         5793462         CNN1         15195         0.017         1.41F-03         13           ENSCO0000136304         chr12         2857472         2857473         CNN1         15195         0.017         1.41F-03         13           ENSCO0000136304         chr6         14954458         14954657         CDN1         1981									
4444         chr3         4939485         69395942         cm         0.017         1.085-02         10           1854         chr15         101094511         10109493         cm         0.020         7.65E 03         13           1889         chr16         2587095         257701         0.024         1.22E-02         13           16491         chr13         2587095         2887200         0.024         0.024         1.22E-02         12           784         chr5         14954028         4834698         CM         NA         -0.042         1.02E-02         12           RNSC0000013212         chr17         5651568         S617568         NA         NA         -0.042         1.02E-02         12           RNSC000013206         chr64         1598462         FLNB         2317         0.007         1.39E-02         12           RNSC000013206         chr14         2587663         CNTH         151195         0.017         1.14E-02         13           RNSC000013721         chr1         248777         236766         PEND         5192         0.040         & 4.24E-02         13           RNSC000013721         chr1         2544162         2576160         NPL1 </td <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
18564         chr.16         269660         2571071         c         0.0,20         7,65E.03         13           18889         chr.16         2567995         257797         c         0.0,26         1,765.2.0         12           728.8         chr.3         19546028         149546028         149546028         10         10           Pmmoter          5651569         56517568         NA         NA         -0,047         9,62E.03         13           ENSCO0000205212         chr.17         2079894         2080953         CCDC144NI, 339184         -0,042         1,02E-02         12           ENSCO000013998         chr.13         2587673         CCNTL1         151195         0,007         1,39E.02         13           ENSCO000013998         chr.13         2587763         CCNTL1         151195         0,007         1,45E.02         13           ENSCO000013994         chr.13         2587763         CCNTL1         19119         0,016         1,21E.02         13           ENSCO000013994         chr.13         2587661         NVEL1         9818         0,027         4,45E.02         13           ENSCO000013991         chr.14         17378989         ETF1         2107 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
18889         chr16         2569500         2571071         0         0.024         1.22E.02         13           16401         chr13         2587300         0.026         3.76E.02         12           2788         chr3         19545028         19254028         19254028         102         10           Prometer         5515595         5617588         NA         NA         -0.047         9.62E-03         13           ENSCO000025212         chr17         20798944         20800953         CCDC144NL         339184         -0.042         1.02E.02         12           ENSCO0000156806         chr3         150345169         150345763         CCNT14         151195         0.017         1.91E-03         10           ENSCO000015206         chr13         2587162         2587616         NUPL1         9818         0.027         4.45E-02         13           ENSCO000015791         chr13         2547162         2587616         NUPL1         9818         0.027         4.45E-02         13           ENSCO000015791         chr14         2547162         2587616         NUPL1         98165         -0.022         9,38E-05         10           ENSCO000015791         chr11         2344737 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
16491         chr.13         25876200         n         0.0,26         3,76E 02         12           7248         chr.5         14956028         14956028         0.0,58         2,20E.03         10           Promotes         ENSCO0000237268         chr.7         25078954         2080955         CCDC144NL         339184         -0.047         9,62E.03         13           ENSCO0000135068         chr.7         25034669         15034168         RAETTL         154664         -0.022         1,21E.02         11           ENSCO000013608         chr.3         57994626         FINB         2317         0.007         1,3956-02         12           ENSCO0000136949         chr.3         25874762         2687763         CCNTL         15119         0.017         4,45E 02         13           ENSCO000013791         chr.13         2587162         2578757         CDNT         1044         0,466         4,24E-02         10           ENSCO000013791         chr.5         13784789         14787636         PENCH         1912         0.040         8,816-0         12           ENSCO000013702         chr.1         13784789         1797456         RAD21         5885         -0.003         4,848-02         12 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
7248         dn/s         14954028         14954028         0.058         2.20E 03         10           Promoters         ENSC00000205212         chr/7         2675559         S6517568         NA         NA         -0.047         9.02E-03         13           ENSC00000205212         chr/17         2078944         20809953         CCDC144NL         339184         -0.042         1.02E-02         12           ENSC0000015606         chr/1         20879763         CCNYLI         151195         0.007         1.91E-03         10           ENSC0000015606         chr/16         2568858         2570877         AMDHD2         51005,752014         0.040         4.61E-03         13           ENSC00000157911         chr/1         2346736         PEX10         5192         0.040         4.61E-03         13           ENSC00000157911         chr/1         2346736         PEX10         5192         0.040         4.61E-03         10           ENSC0000015791         chr/1         2347787         2346736         PEX10         5192         0.040         4.61E-03         10           ENSC0000015791         chr/3         17987181         17387198         ETF1         2070         -0.004         2.32E-02									
Promoters         Free         Free         Procession									
ENSC000023728         chr7         5651569         56517568         NA         NA         -0.047         9.62E-03         13           ENSC000023212         chr17         2079894         20800953         CCDC144NL         39184         -0.042         10.2E-02         12           ENSC000015918         chr6         150546161         10544161         RACT         39184         -0.042         1.02E-02         12           ENSC000015918         chr1         5595627         CCNYL1         151195         0.007         1.39E-02         12           ENSC00001566         chr16         258585         257887         AUDHD         51057,752014         0.026         1.21E-02         13           ENSC00000157911         chr1         2344737         2346736         PEX10         5192         0.040         8.61E-03         13           ENSC0000015721         chr1         134945857         CDX1         1044         0.046         4.24E-02         10           ENSC000015721         chr3         8507012         8781859         ETF1         2107         -0.004         3.24E-02         12           ENSC000016725         chr3         13784784         17887969         PSMA6         5687         -0.004		chr5	149546028	149546988			0,058	2,20E-03	10
ENSCO00020212         chri         20798954         20800953         CCDC144NL         393184        0.02         3.75E-02         11           ENSCO00001568         chri         15046169         15034168         RAETIL         154064         -0.020         3.75E-02         11           ENSCO0001568         chri         208574764         208576753         CCNVL1         151195         0.017         1.91E-03         10           ENSCO0001569         chri         2587462         208576753         CCNVL1         151195         0.040         8.61E-03         13           ENSCO000139946         chri         2587462         25874161         CNUL1         19848         0.027         4.45E-02         10           ENSCO00013979         chri         13744774         13787898         CDX1         1044         0.666         2.22E-02         12           ENSCO00001474         chri         13784783         3578669         PSMA6         5687         -0.004         3.62E-02         12           ENSCO000014724         chri         13784783         3578669         PSMA6         5687         -0.003         4.88E-02         15           ENSCO000015727         chri1         1378178         RASP <td< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>									
ENSCO000155918         chr         150346169         150348168         RAET1L         154064        0.020         3.75E-02         111           ENSCO000015068         chr3         57998426         FLNB         2317         0.007         1.91E-03         100           ENSCO00016206         chr16         256858         2570577         CNTL         151195         0.017         1.91E-03         13           ENSCO00015791         chr1         2544737         2347676         PCX10         19818         0.027         4.85E-02         13           ENSCO00015791         chr1         2344737         2346767         PCX10         1044         0.046         4.2E-02         10           ENSCO00015791         chr1         13787898         FTF1         2107         -0.004         4.2E-02         10           ENSCO00016795         chr3         13787898         FTF1         2107         -0.004         3.62E-02         12           ENSCO00016791         chr8         11788714         17387498         ETF1         2107         -0.004         3.62E-02         12           ENSCO000167927         chr21         2706881         2714771         GABP         2551         -0.004         4.35E-02									
ENSG0000116068         chr3         57992627         57994626         FLNB         2317         0.007         1.39E-02         12           ENSG000010249         chr2         20857763         CCNYL1         151195         0.017         1.91E-03         10           ENSG0000102606         chr16         2588783         ZST0857         AMDHD2         51005,752014         0.026         1.21E-02         13           ENSG0000151372         chr1         248746         2587453         PEX10         5192         0.040         8.61E-03         13           ENSG000011372         chr1         249548851         19545857         CDX1         1044         0.040         4.24E-02         10           ENSG000011372         chr15         85070012         8511447         UBE2Q2P1         388165         -0.022         9.38E-05         10           ENSG000010692         chr14         3774739         3578699         PSMA6         5687         -0.004         3.212-02         12           ENSG0000154723         chr14         3774739         3578699         PSMA6         5687         -0.004         3.218-02         112           ENSG0000154723         chr14         1784894         A1755         522         -0.									
ENSG0000163249         chr2         208574764         208576763         CCNYL1         151195         0.017         1.91E-03         10           ENSG00000162066         chr16         256888         2570857         AMDHD2         51005,752014         0.026         1.21E-02         13           ENSG00000157911         chr13         25874162         NUPL1         9818         0.027         4.45E-02         10           ENSG00000157911         chr13         2346737         2346736         PEX10         5192         0.040         8.61E-03         13           ENSG0000101372         chr5         14954858         H9546857         CDX1         1044         0.046         8.421E-02         10           ENSG0000104754         chr5         13781781         13786798         FTF1         2107         -0.009         1.24E-02         12           ENSG0000164727         chr21         27106881         2714771         GABPA         2551         -0.004         2.32E-02         12           ENSG000015727         chr21         27106881         2714794         ATP51         522         -0.016         7.33E-03         18           ENSG0000015771         chr21         2788159         CDC1211         7088									
ENSG0000162066         chrl6         2568858         2570857         AMDHD2         51005,752014         0.026         1,21E-02         13           ENSG0000139496         chrl3         23874162         25876161         NUPL1         9818         0.027         4,455.02         13           ENSG000013722         chrl3         149544858         149546857         CDX1         1044         0.046         4,24E-02         10           Gene          S         557017         8511447         UBE2Q2P1         388165         -0.022         9,38E-05         110           ENSG0000189136         chrl5         8570712         8511447         UBE2Q2P1         388165         -0.004         2,32E-02         12           ENSG000010705         chrl4         3578738         3578699         PSMA6         5687         -0.004         2,32E-02         12           ENSG000017714         chrl2         2784850         2781879         CCDC11         79635         0.016         6,56E-05         12           ENSG000017718         chrl1         1308488         1202         774810         CCDC18         33985         0.016         7,33E-02         12           ENSG00000176714         chrl1         1308488 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
ENSG0000139496         chrl 1         25874162         25876161         NUPL1         9818         0.027         4.45E-02         13           ENSG00000157911         chrl 1         234737         2346736         PEX10         5192         0.040         8,61E-03         13           ENSG0000113722         chr5         149544857         CDX1         1044         0.046         4,24E-02         10           ENSG000018705         chr15         8570012         85114447         UBE2Q2P1         388165        0.022         9,38E-05         10           ENSG0000104775         chr4         17881784         11788717         RAD21         5885        0.004         2,32E-02         12           ENSG000015727         chr11         2708879         2710784         ATP51         522         -0.001         4,83E-02         15           ENSG000016774         dhr2         2784850         2701784         ATP51         522         -0.016         6,66E-05         12           ENSG0000116774         chr2         2784850         2701784         ATP51         502         0.016         6,56E-05         16           ENSG0000116774         chr2         2784850         170681         0.034         1,58E-02<	ENSG00000163249	chr2	208574764	208576763			0,017		10
ENSG0000157911         chr1         2344737         2346736         PEX10         5192         0,040         8,61E-03         13           ENSG000013722         chr5         149544858         149546857         CDX1         1044         0,040         4,24E-02         10           Genes         ENSG0000189136         chr15         85070012         8511447         UBEQ2P1         388165         -0.022         9,38E-05         10           ENSG0000169136         chr15         137841784         13787899         ETF1         2107         -0.009         1,24E-02         12           ENSG00000102000         chr14         35787899         PSMA6         5687         -0.004         2,32E-02         12           ENSG0000014777         chr21         2706881         2714771         GABPA         2551         -0.001         6,56E-05         12           ENSG0000176714         chr21         2798845         ATP51         522         -0.016         6,56E-05         18           ENSG0000117418         chr21         2788453         TDCF1         6997         0.016         7,326         16           ENSG0000116781         chr11         11660083         1166136         APOA5         116519         0.034 <td></td> <td>chr16</td> <td>2568858</td> <td>2570857</td> <td>AMDHD2</td> <td>51005; 752014</td> <td>0,026</td> <td>1,21E-02</td> <td>13</td>		chr16	2568858	2570857	AMDHD2	51005; 752014	0,026	1,21E-02	13
ENSG0000113722         chr5         149544858         149544857         CDX1         1044         0,046         4,24E-02         10           Gene                  ENSG000018913         chr15         85070012         8511447         UBE2Q2P1         388165        0,022         9,38E-05         10           ENSG0000120705         chr14         17378174         17378799         ETF1         2107        0,003         4,32E-02         12           ENSG000016774         chr84         17787893         5786699         PSMA6         5687        0,003         4,38E-02         15           ENSG0000157272         chr14         27088815         2714771         GABPA         2551        0,001         3,91E-02         14           ENSG000015714         chr2         278850         2714773         CDC121         79655         0,016         6,56E-05         12           ENSG000016774         chr2         278850         2710784         ATP51         S22         -0.001         3,5E-02         16           ENSG0000175714         chr1         11660083         116E1         70835         0.034         4,5E-02         16 </td <td>ENSG00000139496</td> <td>chr13</td> <td>25874162</td> <td>25876161</td> <td>NUPL1</td> <td>9818</td> <td>0,027</td> <td>4,45E-02</td> <td>13</td>	ENSG00000139496	chr13	25874162	25876161	NUPL1	9818	0,027	4,45E-02	13
Genes	ENSG00000157911	chr1	2344737	2346736	PEX10	5192	0,040	8,61E-03	13
ENSG0000189136         chr15         85070012         8511447         UBE2Q2P1         388165        0.022         9,38E-05         10           ENSG0000012075         chr5         137841784         137879898         FTF1         2107        0.009         1,24E-02         10           ENSG00000002         chr14         35736699         FSMA6         5687        0.004         2,32E-02         12           ENSG0000002         chr14         35736699         FSMA6         5687        0.003         4,83E-02         12           ENSG0000002         chr14         27106881         27144771         GABPA         2551        0.003         4,83E-02         14           ENSG0000015773         chr11         46616045         4666803         TDCF1         6997         0.016         6,56E-05         12           ENSG0000156781         chr9         84198598         8430420         TLE1         7088         0.034         3,55E-02         10           ENSG00000156781         chr1         12466083         IDCF18         339865         0.034         3,55E-02         12           ENSG000015737         chr11         13018488         13026368         NA         NA         0.039         2,72F-0	ENSG00000113722	chr5	149544858	149546857	CDX1	1044	0,046	4,24E-02	10
ENSG0000120705chrs13781784137878989ETT121070.0991.24E-02110ENSG000016747chr8117858174117887105RAD2158850.0083.62E-0212ENSG000016727chr1435787833578699PSMA656870.0042.32E-0212ENSG000015727chr12271058827147784ATP515220.0013.91E-0214ENSG000017714chr227888152716784ATP515220.0163.91E-0214ENSG000017574chr2278885027851879CCDC121796350.0166.56E-0512ENSG0000175761chr9841985988430422TLE170880.0305.00E-0516ENSG000017578chr1111666008311665136APOA51165190.0341.68E-0212ENSG000017579chr1111848881302688NANA0.0397.27E-0514ENSG000017579chr115885645818G10.0501.42E-0212ENSG000017579chr1158856145588465A1BG10.0501.42E-0212ENSG000017579chr15731000552000C0.0462.44E-0212ENSG000017579chr15745000115741000C0.0462.44E-0212ENSG000017579chr15745000115741000C0.0462.44E-0212ENSG000017579chr1157	Genes								
ENSG0000164754         chr.8         11785174         117887105         RAD21         5885        0.008         3.62E-02         12           ENSG0000019020         chr.14         35747839         35786699         PSMA6         5687        0.004         2.32E-02         12           ENSG00000154727         chr.21         27108881         27114771         GABPA         2551        0.001         3.91E-02         14           ENSG0000176714         chr.21         2788815         2707984         ATP51         522         -0.001         6.56E-05         12           ENSG0000176714         chr.2         2784856         2781879         CCDC11         79683         0.016         6.56E-05         12           ENSG0000176714         chr.9         84198598         8430420         TLE1         7968         0.030         5.00E-05         16           ENSG000015479         chr.4         77334154         77343021         CCDC18         339955         0.034         3.55E-02         12           ENSG000012410         chr.19         5885645         A1BG         1         0.050         1.42E-02         12           ENSG000012410         chr.19         5885645         A1BG         1         0.05	ENSG00000189136	chr15	85070012	85114447	UBE2Q2P1	388165	-0,022	9,38E-05	10
ENSG0000109002         chr14         35747839         35786699         PSMA6         5687        0.004         2.32E-02         12           ENSG00000154727         chr21         27106881         27114771         GABPA         2551        0.003         4,83E-02         15           ENSG00000154723         chr21         27888505         2705784         ATP51         522        0.001         3.91E-02         14           ENSG0000176714         chr2         27848506         27851879         CCDC121         79635         0.016         6,56E-05         12           ENSG000011243         chr1         11666045         46668033         TDCF1         6997         0.016         7,33E-02         12           ENSG0000163749         chr4         77234154         77343021         CCDC188         339965         0.034         3,58E-02         12           ENSG0000175773         chr1         3018488         13026368         NA         NA         0.036         1,42E-02         12           ENSG0000121410         chr19         5885917         5886549         A1BG-AS1         503538         0.054         2,79E-03         12           ENSG000021410         chr19         5885917         5885910	ENSG00000120705	chr5	137841784	137878989	ETF1	2107	-0,009	1,24E-02	10
ENSG0000154727         chr21         27106881         27144771         GABPA         2551        0.003         4,83E-02         15           ENSG00000154723         chr21         2708815         27107984         ATP5J         522        0.001         3,91E-02         14           ENSG00000176714         chr2         27841850         27851879         CCDC121         79635         0.016         6,56E-05         12           ENSG0000016714         chr3         46616045         46668033         TDGF1         6997         0.016         7,33E-03         18           ENSG000016281         chr9         8419858         84304220         TEE1         708         0.034         1,68E-02         10           ENSG0000163749         chr4         77243154         77343021         CCDC158         339965         0.034         3,55E-02         12           ENSG000012840         chr19         5885644         S866549         A1BG         1         0.050         1,42E-02         12           ENSG000012840         chr19         5885645         A1BG         1         0.054         2,44E-02         14           ENSG000002885         chr19         5885649         A1BG-AS1         50338         0.054	ENSG00000164754	chr8	117858174	117887105	RAD21	5885	-0,008	3,62E-02	12
ENSG0000154723         chr21         2708815         27107984         ATP5J         522        0.011         3.91E-02         14           ENSG00000176714         chr2         27848506         27851879         CCDC121         79635         0.016         6.56E-05         12           ENSG00000167714         chr3         46616045         46666033         TDGF1         6997         0.016         7,33E-03         18           ENSG000016374         chr4         77234154         7743021         CCDC188         339955         0.034         3,5E-02         12           ENSG0000163749         chr44         77234154         7734021         CCDC188         339955         0.034         3,5E-02         12           ENSG000015773         chr11         13018488         13026368         NA         NA         0.039         7,27E-05         14           ENSG0000121410         chr19         588517         586659         AIBG-AS         1503538         0.054         2,79E-03         12           ENSG0000026895         chr19         585170         56515001         5652000         14         2,44E-02         14           528037         chr17         157405001         15741000         16        0,046<	ENSG00000100902	chr14	35747839	35786699	PSMA6	5687	-0,004	2,32E-02	12
ENSG0000176714         chr2         27848506         27851879         CCDC121         79635         0.016         6,56E-05         12           ENSG000016781         chr3         46616045         46668033         TDGF1         6997         0.016         7,33E-03         18           ENSG000016781         chr9         84198598         8430420         TLE1         7088         0.030         5,00E-05         16           ENSG000016373         chr11         116660083         11663136         APOA5         116519         0.034         1,68E-02         10           ENSG0000175773         chr11         13018488         13026368         NA         NA         0.039         7,27E-05         14           ENSG0000025895         chr19         5885644         5886456         A1BG         1         0.050         1,42E-02         12           ENSG0000268895         chr19         5885644         586549         A1BG         1         0.050         1,42E-02         12           ENSG00000268895         chr19         56515001         5652000         -0.046         2,44E-02         15           27817         chr7         157405001         15741000         16741000         -0.046         -0.044	ENSG00000154727	chr21	27106881	27144771	GABPA	2551	-0,003	4,83E-02	15
ENSG0000241188         chr3         46616045         46668033         TDGF1         6997         0.016         7,33E-03         18           ENSG0000196781         chr9         84198598         84304220         TLE1         7088         0.030         5,00E-05         16           ENSG000010243         chr11         116660083         11665136         APOA5         116519         0.034         1,68E-02         10           ENSG00001275773         chr11         13018488         13026368         NA         NA         0.039         7,27E-05         14           ENSG000012410         chr19         5885644         5846545         AIBG         1         0.050         1.42E-02         12           ENSG000012410         chr19         5885544         5846545         AIBG         1         0.054         2,79E-03         12           ENSG000012400         chr19         5885611         5866549         AIBG-AS1         503538         0.054         2,79E-03         12           ENSG00000226895         chr17         15740500         15741000         15741000         160         -0.044         4,02E-02         14           503210         chr17         20795001         2080000         -0.014	ENSG00000154723	chr21	27088815	27107984	ATP5J	522	-0,001	3,91E-02	14
ENSG0000196781         chr9         84198598         84304220         TLE1         7088         0,030         5,00E-05         16           ENSG00001010243         chr11         116660033         116663136         APOA5         116519         0,034         1,68E-02         10           ENSG00000163749         chr4         77234154         77343021         CCDC158         339965         0,034         3,55E-02         12           ENSG0000175773         chr11         1308488         130263688         NA         NA         0,039         7,27E-05         14           ENSG0000126805         chr19         5885645         886645         A1BG         1         0,050         1,42E-02         12           ENSG000026805         chr19         5885617         588654         A1BG         1         0,050         1,42E-02         12           ENSG000026805         chr19         5885017         5886509         A1BG         1         0,050         1,42E-02         12           ENSG000026805         chr7         56515001         15740000         1         -0.046         2,44E-02         14           528027         chr1         20795001         2081000         -0.014         1,94E-02         12<	ENSG00000176714	chr2	27848506	27851879	CCDC121	79635	0,016	6,56E-05	12
ENSG0000110243         chr11         116660133         116663136         APOA5         116519         0,034         1,68E-02         10           ENSG0000163749         chr4         77234154         77343021         CCDC158         339965         0,034         3,55E-02         12           ENSG000015773         chr11         130184888         130263688         NA         NA         0,039         7,27E-05         14           ENSG00000268895         chr19         5885654         5886549         A1BG         1         0,050         1,42E-02         12           ENSG00000268895         chr19         5885017         5886549         A1BG         1         0,050         1,42E-02         12           Genome-wide tiling regions          -0,046         2,44E-02         14           528039         chr7         157405001         15741000         -0,046         -0,044         4,02E-02         14           54201         chr17         20795001         2080000         -0         -0,044         4,02E-02         12           478486         chr19         56915001         650000         -0,014         1,94E-02         12           41882         chr19         20945001         209	ENSG00000241186	chr3	46616045	46668033	TDGF1	6997	0,016	7,33E-03	18
ENSG0000163749chr47723415477343021CCDC1583399650.0343,55E-02112ENSG0000175773chr111301848813026368NANA0.0397,27E-0514ENSG0000121410chr1958856544586649A1BG10,0501,42E-0212ENSG00000268895chr1958850175866549A1BG10,0501,42E-0212Genome-wide tilingerses591175866549A1BG5035380,0542,79E-0312Genome-wide tilingerses591175651500156520001-0,0462,44E-0215278217chr17207950120800001-0,0404,02E-0214504201chr17207950120800001-0,0404,02E-021353197chr19649500185150001-0,0141,94E-021241882chr12940500129410001-0,0141,94E-021241882chr12278500127850001-0,0141,96E-02125422chr22785001278500010,0112,56E-022091567chr22085750120857500208575000,0212,99E-0311340633chr1510109501101000010,0212,99E-0311340633chr162325000227700010,0232,47E-0213367640chr1738020013825000 <t< td=""><td>ENSG00000196781</td><td>chr9</td><td>84198598</td><td>84304220</td><td>TLE1</td><td>7088</td><td>0,030</td><td>5,00E-05</td><td>16</td></t<>	ENSG00000196781	chr9	84198598	84304220	TLE1	7088	0,030	5,00E-05	16
ENSG0000163749chr47723415477343021CCDC1583399650.0343,55E-02112ENSG0000175773chr111301848813026368NANA0.0397,27E-0514ENSG0000121410chr1958856544586649A1BG10,0501,42E-0212ENSG00000268895chr1958850175866549A1BG10,0501,42E-0212Genome-wide tilingerses591175866549A1BG5035380,0542,79E-0312Genome-wide tilingerses591175651500156520001-0,0462,44E-0215278217chr17207950120800001-0,0404,02E-0214504201chr17207950120800001-0,0404,02E-021353197chr19649500185150001-0,0141,94E-021241882chr12940500129410001-0,0141,94E-021241882chr12278500127850001-0,0141,96E-02125422chr22785001278500010,0112,56E-022091567chr22085750120857500208575000,0212,99E-0311340633chr1510109501101000010,0212,99E-0311340633chr162325000227700010,0232,47E-0213367640chr1738020013825000 <t< td=""><td>ENSG00000110243</td><td>chr11</td><td>116660083</td><td>116663136</td><td>APOA5</td><td>116519</td><td></td><td></td><td>10</td></t<>	ENSG00000110243	chr11	116660083	116663136	APOA5	116519			10
ENSG0000175773chr11130184888130263688NANA0,0397,27E-0514ENSG0000121410chr19588554458864865A1BG10,0501,42E-0212ENSG00000268895chr19588591175886549A1BG-AS15035380,0542,79E-0312Genome-wide tilling regions258039chr7565150015652000-0.0462,44E-0215278217chr7157405001157410000-0.0444,02E-0214504201chr17207950012080000-0.014-0.0392,28E-0213478486chr196495001650000-0.014-0.0141,94E-021241882chr120940500120941000-0.0101,88E-0210447146chr14357600035765000-0.0101,88E-021055422chr227850012785000-0.0141,06E-021241883chr151010950110110000-0.0140,0112,56E-022091567chr22785001277000-0.020,0141,66E-0211340633chr102765001277000-0.010,0212,99E-0311340633chr173802000138025000-0.0332,59E-0211195502chr3466150014662000-0.0140,0332,59E-0211196502chr3195945000-0.0332,59E-021111 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>12</td>									12
ENSG0000121410chr19588554458864865A1BG10,0501,42E-0212ENSG0000268895chr19588591175886549A1BG-AS15035380,0542,79E-0312Genome-wide tiling regions258039chr7565150015652000-0.0462,44E-0215278217chr7157405001157410000-0.0444,02E-0214504201chr17207950012080000-0.0392,28E-0213478486chr15851100185115000-0.014-0.0206,65E-0411533197chr19649501650000-0.014-0.0141,94E-021241882chr12094050120941000-0.014-0.0101,88E-0210447146chr1435760013576500-0.0054,10E-021291567chr2278500128850000.0212,99E-0311340633chr15101095001101100000.0241,52E-0211107815chr34661500123250000.0274,67E-0211107815chr34661500138025000.0274,67E-0211107816chr17380200138025000.0332,59E-0213507646chr17380200138025000.0332,59E-0213517646chr17380200138025000.0332,43E-031121529chr6508501509000									
ENSG0000268895chr19588591175886549A1BG-AS15035380.0542.79E-0312Genome-wide tills regionschr775651500156520000 </td <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
Genome-wide tiling regions258039chr7565150015652000-0,0462,44E-0215278217chr7157405001157410000-0,0444,02E-0214504201chr17207950012080000-0,0392,28E-0213478486chr158511000185115000-0,0141,94E-021241882chr1209405001209410000-0,0101,88E-0210447146chr143576000135765000-0,00141,94E-022091567chr22785001278550000,0112,56E-022091567chr2208575001208800000,0141,66E-0211340633chr1022765001227700000,0212,99E-031196502chr2233250002332550000,0325,47E-0310137680chr3195940001159450000,0332,59E-0213507646chr173802001380250000,0332,59E-021313529chr65085015090000,0332,59E-0213206237chr5149545001149550000,0452,93E-0214206237chr630700130075000,0473,07E-021266673chr284110000,0502,35E-0811									
258039chr756515001565200000,0462,44E-0215278217chr71574050011574100000,0444,02E-0214504201chr1720795001208000000,0392,28E-0213478486chr1585110001851150000,0141,94E-021241882chr12094050012094100000,0101,88E-0210447146chr1435760001357650000,0054,10E-021055422chr22785001278550000,0112,56E-022091567chr22085750012085800000,0141,66E-0211340633chr1022765001227700000,0241,52E-021196502chr22332500012332550000,0332,59E-0213107815chr346615001466200000,0332,59E-0213507646chr1738020001380250000,0332,59E-021313529chr650850015090000,0382,43E-0311213529chr650850015090000,0452,93E-0213549738chr2030070001300750000,0473,07E-021266673chr28410500184110000,0502,35E-0811							-,		
Z8217chr71574050011574100000,0444,02E-0214504201chr172079500120800000-0,0392,28E-0213478486chr158511000185115000-0,0206,65E-0411533197chr1964950016500000-0,0141,94E-021241882chr1209405001209410000-0,0101,88E-0210447146chr14357600013765000-0,0054,10E-022055422chr2278500127855000-0,0112,56E-022091567chr2208575001208580000,0112,56E-0211340633chr102276500122770000,0212,99E-0311340633chr1022325000233255000,00274,67E-021196502chr34661500146620000,0332,59E-0213177680chr319594001195945000,0332,59E-0213137680chr17380200138025000,0332,59E-0213137680chr65085015090000,0332,59E-0213137680chr17380200138025000,0332,59E-0213137680chr65085015090000,0332,59E-0213137680chr17380200138075000,0452,93E-0214206237chr6508501199000119590000,0452,93E-0213 <td></td> <td></td> <td>56515001</td> <td>56520000</td> <td></td> <td></td> <td>-0.046</td> <td>2.44E-02</td> <td>15</td>			56515001	56520000			-0.046	2.44E-02	15
504201chr172079500120800000,0392,28E-0213478486chr1585110001851150000,0206,65E-0411533197chr1964950016500000,0141,94E-021241882chr1209405001209410000,0101,88E-0210447146chr1435760001357650000,0054,10E-021055422chr2278500127855000,0112,56E-022091567chr220857501208580000,0141,66E-0212481683chr102276500122770000,0212,99E-0311340633chr102275001233250000,0224,67E-0211107815chr3466150146620000,0332,59E-0213507646chr17380200138025000,0332,59E-021313529chr65085005090000,0332,59E-0214206237chr514954500149550000,0452,93E-021354778chr20307000130750000,0473,07E-021266673chr20841100000,0502,35E-0811							-		
478486chr1585110001851150000,0206,65E-0411533197chr19649500165000000,0141,94E-021241882chr12094050012094100000,0101,88E-0210447146chr1435760001357650000,0054,10E-021055422chr22785000127855000-0,0141,66E-022091567chr22085750012085800000,0112,56E-022091567chr151010950011011000000,0212,99E-0311340633chr1022765001227700000,0211,52E-021196502chr223325000233255000,0214,67E-0211107815chr346615001466200000,0332,59E-0213507646chr173802001380250000,0332,59E-0213213529chr650850015090000,0332,93E-0311213529chr650850015090000,0452,93E-0213549738chr20300700130075000,0473,07E-021266673chr284105001841100000,0502,35E-0811									
533197chr19649500165000000,0141,94E-021241882chr12094050012094100000,0101,88E-0210447146chr1435760001357650000,0054,10E-021055422chr227850001278550000,0112,56E-022091567chr22085750012085800000,0141,66E-0212481683chr151010950011011000000,0212,99E-0311340633chr1022765001227700000,0241,52E-021196502chr223325000233255000,0274,67E-0211107815chr346615001466200000,0332,59E-0213507646chr173802001380250000,0382,43E-0311213529chr650850015090000,0452,93E-0213549738chr20300700130075000,0473,07E-021266673chr284105001841100000,0502,35E-0811									
41882chr12094050012094100000,0101,88E-0210447146chr1435760001357650000,0054,10E-021055422chr227850001278550000,0112,56E-022091567chr22085750012085800000,0141,66E-0212481683chr151010950011011000000,0212,99E-0311340633chr1022765001227700000,0241,52E-021196502chr22332500012332550000,0274,67E-0211107815chr346615001466200000,0332,59E-0213507646chr173802001380250000,0382,43E-0311213529chr650850015090000,0452,93E-0213549738chr203007001300750000,0473,07E-021266673chr284105001841100000,0502,35E-0811									
447146chr1435760001357650000,0054,10E-021055422chr2278500012785500000,0112,56E-022091567chr220857500120858000000,0141,66E-0212481683chr1510109500110110000000,0212,99E-0311340633chr10227650012277000000,0241,52E-021196502chr223325000123325500000,0325,47E-0310107815chr346615001466200000,0332,59E-0213507646chr1738020013802500000,0382,43E-0311213529chr65085001509000000,0394,10E-0214206237chr51495450011495500000,0473,07E-021266673chr284105001841100000,0502,35E-0811									
55422         chr2         27850001         27855000         208580000         0,011         2,56E-02         20           91567         chr2         208575001         208580000         0,014         1,66E-02         12           481683         chr15         101095001         101100000         0,021         2,99E-03         11           340633         chr10         22765001         22770000         0,024         1,52E-02         11           96502         chr2         23325001         233255000         0,027         4,67E-02         11           107815         chr3         46615001         46620000         0,033         2,59E-02         13           507646         chr17         3802001         38025000         0,038         2,43E-03         11           213529         chr6         5085001         509000         0,039         4,10E-02         14           206237         chr5         149545001         14955000         0,047         3,07E-02         13           549738         chr20         30070001         30075000         0,047         3,07E-02         12           66673         chr2         84105001         84110000         0,0500         2,35E-08 <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>									
91567chr22085750012085800000,0141,66E-0212481683chr151010950011011000000,0212,99E-0311340633chr1022765001227700000,0241,52E-021196502chr2233250002332550000,0325,47E-0310107815chr346615001466200000,0332,59E-0213357640chr173802001380250000,0382,43E-0311213529chr650850150900000,0394,10E-0214206237chr5149545011495500000,0473,07E-021266673chr2841050084110000,0502,35E-0811							-		
481683chr151010950011011000000,0212,99E-0311340633chr10227650012277000000,0241,52E-021196502chr22332500023325500000,0274,67E-0211107815chr3466150014662000000,0325,47E-0310137680chr319594000119594500000,0332,59E-0213507646chr17380200013802500000,0382,43E-0311213529chr65085001509000000,0452,93E-0213549738chr2030070013007500000,0502,35E-0811									
340633chr10227650012277000000,0241,52E-021196502chr2233250002332550000,0274,67E-0211107815chr34661500146620000,0325,47E-0310137680chr31959400011959450000,0332,59E-0213507646chr1738020001380250000,0382,43E-0311213529chr6508500150900000,0394,10E-0214206237chr51495450011495500000,0452,93E-0213549738chr2030070001300750000,0473,07E-021266673chr28410500184110000,0502,35E-0811									
96502         chr2         233250001         233255000         233255000         0,027         4,67E-02         11           107815         chr3         46615001         46620000         0,032         5,47E-03         10           137680         chr3         195940001         195945000         0,033         2,59E-02         13           507646         chr17         3802001         38025000         0,038         2,43E-03         11           213529         chr6         5085001         5090000         0,045         2,93E-02         13           206237         chr5         149545001         14955000         0,045         2,93E-02         13           549738         chr20         3007001         30075000         0,047         3,07E-02         12           66673         chr2         84105001         84110000         0,0500         2,35E-08         11									
107815         chr3         46615001         46620000         0.032         5,47E-03         10           137680         chr3         195940001         195945000         0.033         2,59E-02         13           507646         chr17         38020001         38025000         0.033         2,43E-03         11           213529         chr6         5085001         509000         0.03         0,039         4,10E-02         14           206237         chr5         149545001         14955000         0.045         2,93E-02         13           549738         chr20         3007001         30075000         0.045         0,0457         3,07E-02         12           66673         chr2         84105001         84110000         0.045         0,0500         2,35E-08         11									
137680chr319594000119594500000,0332,59E-0213507646chr17380200013802500000,0382,43E-0311213529chr65085001509000000,0394,10E-0214206237chr51495450011495500000,0452,93E-0213549738chr203007001300750000,0502,35E-081166673chr284105001841100000,0502,35E-0811									
507646         chr17         38020001         38025000         0,038         2,43E-03         11           213529         chr6         5085001         5090000         0         0,039         4,10E-02         14           206237         chr5         149545001         14955000         0,045         2,93E-02         13           549738         chr20         3007001         30075000         0         0,0500         2,35E-08         11									
213529         chr6         5085001         5090000         0,039         4,10E-02         14           206237         chr5         149545001         149550000         0,045         2,93E-02         13           549738         chr20         3007001         30075000         0         0,045         3,07E-02         12           66673         chr2         84105001         8411000         0         0,050         2,35E-08         11									
206237         chr5         149545001         14955000         0,045         2,93E-02         13           549738         chr20         3007001         30075000         0,047         3,07E-02         12           66673         chr2         84105001         8411000         0         0,050         2,35E-08         11									
549738         chr20         30070001         30075000         0,047         3,07E-02         12           66673         chr2         84105001         8411000         0         0,050         2,35E-08         11									14
66673 chr2 84105001 84110000 0,050 2,35E-08 11	206237	chr5	149545001	149550000			0,045	2,93E-02	13
	549738	chr20	30070001	30075000			0,047	3,07E-02	12
543670 chr19 58860001 58865000 0,065 7,02E-04 10	66673	chr2	84105001	84110000			0,050	2,35E-08	11
	543670	chr19	58860001	58865000			0,065	7,02E-04	10

Table 2. Differentially methylated regions in predefined genomic regions with adjusted p-value < 0.05 and containing at least 10 CpG sites.

and genes from M17 increase after PCW 16 until birth, from which point it seems to reach a plateau. Genes exclusively from this study (n = 500) were enriched for M9 (p = 0.0009), which contains genes highly expressed in the beginning of gestation that decrease expression levels at around 12<sup>th</sup> week whereas genes exclusively from CHAMACOS' (n = 1,309) were enriched for M15 (p = 0.0246), that presents an increasing level of expression during the whole neocortical development. Genes found only by CHAMACOS study (total or exclusive genes) were enriched for M1, that present a decreasing expression until week 12<sup>th</sup>, from which point present a constant increasing expression levels (Fig. 2).

#### Discussion

Differences underlying sexes have been associated with distinct incidence and severity of a group of psychiatry diseases, although the mechanisms are not well understood, moreover, differential methylation has been involved with this sex bias prevalence of psychiatry disorders. The immune system response during certain periods of development has sex-specific effects on regulation of various neurotransmitters important for the stress response throughout life, with consequences for physical and psychiatric disorders in later life<sup>36</sup>. Besides, there is a clear sex dimorphism in structures during brain development that were implicated to a sex-bias vulnerability to psychiatric disorders independent of stress exposition<sup>42</sup>. The potential underlying mechanisms driving these sex differences regarding stress responses and their relevance to disease were deeply discussed. The authors concluded that the continued focus and appreciation for how sex differences in stress responses may predict disease risk and resiliency is critical for developing preventive strategies and treatments<sup>43</sup>. In this study, we conducted a genome-wide DNA methylation profile of cord blood of a well-characterized cohort composed of babies born at term and with adequate weight, after correcting for stress exposition factors that could interfere with DNA methylation. We showed that differential methylation between females and males found in the cord blood can represent gene expression differences in tissues with sex dimorphism.

The DMS were equally distributed across all 22 autosomes, as previously described<sup>34</sup>, but their locations were not random. Whilst females had more methylated CpGs associated with repressed gene expression, males had more methylated CpGs in the open sea, which is more often positively correlated with expression<sup>44,45</sup>.

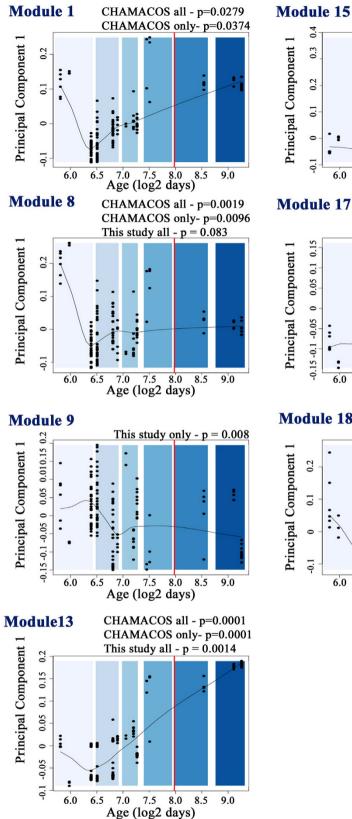
Pathways enriched for the 1,113 genes related to the DMS between sexes included metabolic and MAPK pathways. Changes in the metabolism of 1-carbon and methyl donor availability in the fetal liver, a major metabolic organ, were suggested to have a sex influence in a subset of genes<sup>46</sup>. Several pathways related to muscle were over-represented, such as the hypertrophic cardiomyopathy and vascular smooth muscle contraction pathways. The development and establishment of musculature is thought to be a major difference between adult males and females. At molecular level, these differences seem to be present already at birth. Accordingly, cardiovascular and musculoskeletal disorders were enriched in the diseases analyses.

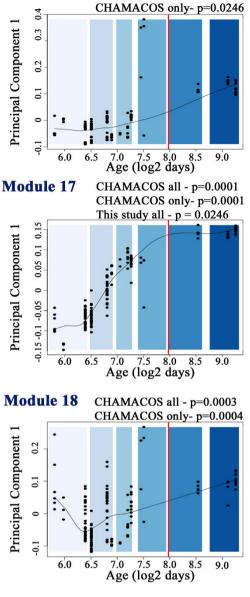
The enrichment analysis revealed a possible role of our gene set to the brain as CpG sites and genes are modulated during brain development on top of being differently methylated between sexes<sup>39</sup>. Furthermore, these genes have been associated to psychiatry diseases as demonstrated by the enrichment of the 2,332 DMSs between females and males among the 4,641 differentially methylated CpG sites in brain tissue between patients with schizophrenia and controls<sup>40</sup>. Among the common genes, *SHANK* genes participate in synaptic formation, including in *SHANK2* and *SHANK3*, in which mutations were described in a wide spectrum of neurodevelopmental and neuropsychiatric diseases<sup>47</sup>.

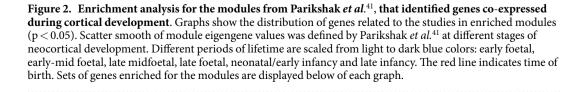
When analyzing the near and distant motifs for sex hormones, we suggested that: (1) the differential methylation found in CpG sites could interfere with the binding in motifs close to TSS of transcriptional factors classically related to females and males and; (2) other factors that are not exclusively associated with the regulation performed by sexual hormones are contributing to differentially expressed and methylated genes between females and males. Also, most of the DMS (66%) showed higher DNA methylation in females compared to males. It has been reported that females have higher methylation levels than males, although most studies used the X and Y chromosomes in their analyses, where a substantial portion of the DMSs are located<sup>34</sup>. Importantly some of these studies were performed in tissues other than cord blood, such as brain or pancreas, and with older subjects, that present changes in methylation because of the aging process<sup>35,48,49</sup>.

X dosage and the presence of Y chromosome exerts a role for the methylation patterns on the autosomes. One X was associated to hypomethylation on the autosomes but the presence of Y changes this pattern, suggesting the existence of an interaction between X and Y dosages<sup>50</sup>, meaning that, in addition to the organization effects of gonadal hormones, the sex chromosomes contribute to the differential methylation between females and males and these differences might contribute the sex bias psychiatric vulnerabilities, agreeing with a previous published discussion<sup>43</sup>.

The importance of considering the sex differences relies on the different response from females and males to stress to maintain homeostasis, which may have different consequences for the long-term responses to perturbations from the environment<sup>33</sup>. Prenatal stress can induce long-term neurodevelopmental diseases, particular those related to the hypothalamic-pituitary-adrenal (HPA) response to stress<sup>51</sup>. This exposure to maternal stress may alter the stress-vulnerability of the embryo leading to an increased risk of psychopathology<sup>36</sup>. The cohort from CHAMACOS was subjected to toxic stress (e.g. exposure to pesticides)<sup>34</sup> whilst our study excluded factors already associated with psychiatric vulnerabilities (not born at term and not adequate size/weight for gestational age); and corrected for stress factors covariables that potentially interfere with DNA methylation. Psychiatric disorders were associated to genes from both studies. Nevertheless, comparing both data, we found 890 overlapping CpG sites, with our methylation differences (delta-beta) being smaller than theirs were, suggesting that exposition to stress might increase these differences. Comparing both datasets to the co-expressed gene modules during neocortical development revealed that some modules were similarly enriched. In addition, genes from these modules (M8: SOX1, CDKN2C, TRIP10, SALL1, EFNA4, PARD3B, CDH23, PRDM16, NEK9, Clorf61, HSPB1,







*CCDC8*, *FLNB* and *TEAD3*; M13: *RTKN*, *SYN2*, *SYNGR1*, *NRXN3*, *PRSS16*, *CPLX1*, *ZNF365* and *SLC17A7*; *M17*: *CYP1A1*, *GRIN1*, *GRIN2D*, *PRPH*, *CSMD1*, *BTBD9*, *UPP2*, and *USP12*) were found among genes enriched for the overrepresented psychiatric diseases. For instance, *SYN2*, more methylated in females (cg10245988, island), encodes a neuron-specific phosphoprotein that selectively binds to small synaptic vesicles in the presynaptic nerve terminal. Polymorphisms in this gene were associated with abnormal presynaptic function and increased risk for related neuronal disorders, including autism, bipolar disorder and schizophrenia<sup>52–54</sup>. SNPs in *UPP2* were associated with a sex-bias factor in children diagnosed with autism spectrum disorder<sup>55</sup>. Nonetheless, the enrichment analysis of specific datasets reported different groups of genes regarding their expression levels during neurodevelopment, suggesting specific contributions.

We are aware that this study has limitations. First, we controlled for a limited number of stress factors and cannot guarantee that the methylation analyses were not influenced by unexplored stress exposition factors. Second, we could not measure contamination of maternal cells and thus, did not correct for it. Third, comparisons between data from cord blood and brain were not performed with samples collect from the same individuals. Fourth, we used only exposure to pesticides and environmental chemicals as a model toxic stress. Finally, we found only one study with publicly available data that used the H450K to evaluate DNA methylation in the brain of patients with schizophrenia representing psychiatric diseases found enriched in our analyses.

This study corroborated previous findings from the literature that showed that methylation differences between females and males in the cord blood methylation are associated with brain developing tissues. We describe there that genes related to this differential methylation are enriched for genes previously associated with psychiatric diseases, even after correcting the methylation analysis for stress exposition covariables, in additional to the technical effects. These differences are not solely explained by gonadal hormone effects, but also by sex chromosomes effects on the autosomes. Moreover, they present specific pattern of gene co-expression organized in modules of neocortical development, which implies temporal specific effects.

#### **Material and Methods**

**Sample collection.** Samples came from the Western region of Sao Paulo city, where 6,200 women were enrolled between 26<sup>th</sup> and 34<sup>th</sup> week gestation between 2012–2014 (Regiao Oeste Cohort, ROC). For this study, a questionnaire was administered to mothers to evaluate levels of stress and toxic exposures during gestation (smoke, use of drugs). These children are being followed up to gather additional information related to neurode-velopmental diseases and behavioral phenotypes as a continuum of this study; this will not be explored in here due to the relatively small time-lapsed. We collected blood from the umbilical cord of 96 women that delivered at the University Hospital of Sao Paulo. All women gave written informed consent to participate in the study. The study itself, as well as the use of samples, were conducted with ethical approval granted by the Hospital's Internal review board – CEPHU under protocol number 1076/10, with all experiments performed in compliance with the Helsinki Declaration.

**Sample preparation and quality control.** DNA was isolated from umbilical cord blood samples using QIAamp DNA Blood Midi Kit (Qiagen). NanoDrop (Thermo Fisher Scientific) and Qubit (Thermo Fisher Scientific) was used to assess DNA purity and quantity. DNA quality was checked by electrophoresis in 0.8% agarose gels followed by bisulfite conversion whereby 1 ug DNA was treated using the EZ DNA Mehylation kit (Zymo Research Corp), following manufacture recommendations.

High quality bisulfite-converted DNA from 96 samples were hybridized in the Human Methylation 450 BeadChip microarrays (HM450K, Illumina), following the Illumina Infinium HD methylation protocol. Both experiments were processed by Deoxi Biotecnologia (www.deoxi.com) according to manufacturer's instructions. Raw data were extracted by the iScan SQ scanner (Illumina) using GenomeStudio software (v.2011.1), with the methylation module v.1.9.0 (Illumina), into IDAT files, which were used for further analyses. The HM450K platform interrogates DNA methylation levels of 485,577 *loci* distributed across the genome at single-nucleotide resolution. Probes were annotated according regarding their nearest genes using FDb.InfiniumMethylation.hg19 package with an additional annotation for those probes that were located at more than one gene, Hg19 coordinates from UCSC were selected. This resulted in 20,461 unique entrezID genes. The methylation levels for each CpG probe were provided in beta-values (0 indicating unmethylated CpGs, and 1, fully methylated CpGs). All analyses were performed in R environment using Bioconductor packages (http://www.bioconductor.org). All samples had high quality data passing QC using standard parameters.

**Genome-wide methylation analysis.** The RnBeads package<sup>56</sup> was applied to the dataset. Unreliable measurements were identified using Greedycut (p > 0.05) that removed 1,478 probes. Probes located in SNPs (n = 4,776) as well those located in specific contexts (n = 5,926 - non-CpG sites as well as additional SNPs) were also excluded. Samples were retained only if >99% of sites assayed had detection p > 0.05. The background was corrected using noob method, which is based on a normal-exponential convolution using out-of-band probes<sup>57</sup>. Signal intensities values from probes type I and II were normalized using SWAN method that adjusts the intensities based on a quantile approach<sup>58</sup>. Probes located in the sexual chromosomes were filtered out remaining 464,964 probes for analyses.

To quantify methylation differences resultant from cellular composition of the blood, a purified dataset containing six blood cell types was used as ref. 59 to estimate the blood cell type contribution for each cord blood sample. Based on the estimated percentages of each cell type, a projection of the methylation levels for each cord blood sample was used in the limma based analysis of differential DNA methylation, as implemented by RnBeads<sup>56</sup>. Variables from the questionnaire were tested for association with the main group comparison (female versus male) indicating that only psychiatric disease in the father family was acting as co-variables. Moreover, adjustment for technical effects (e.g. batch effects) and cell type composition were performed before the differential methylation analyses. To identify differentially methylated CpG sites (DMSs), the M-values (loggit of B-values) were used employing an empirical Bayesian framework linear model from limma<sup>60</sup>. CpG sites with FDR adjusted p-value (adjP) < 0.05 were selected as differentially methylated. RnBeads also identified differentially methylated regions (DMRs) in pre-defined CpG islands, promoters, genes and genome-wide tiling regions, that exhibit DNA methylation changes between females and males (FDR adjP < 0.05).

*In silico* functionally exploration of data. Functional and disease enrichment analysis were performed in WebGestalt<sup>61</sup> using the whole genome as background. Significant values were considered to be those features comprised by at least 10 genes and adjusted p-value (adjP) <0.01 adjusted by the multiple test as given by Benjamini–Hochberg procedure.

CpGs were compared to differentially methylated CpG sites found by three studies with publicly available data<sup>34,40,62</sup> using all probes from 450 K BeadChip as background. In addition, genes associated to DMSs were compared to 17 modules comprised of co-expressed genes during human cortical development<sup>41</sup>. For both cases, we applied the Modular Single-set Enrichment Test (MSET)<sup>62</sup>, which assess enrichment for a gene or CpG site (here named as feature) list of interest within a set of features using a randomization testing. First, the probability of our list of features being significantly represented in each module were compared to 10,000 simulated sets of features generated randomly from the 450 K Beadchip array (for DMS) or RefSeq (for genes) as background. Then, a p-value was calculated based on the number of simulated randomized sets that contains equal or higher number of features belonging to our list of features in the list generated by each of the three studies. We considered as significant, those comparisons with a p-value < 0.05. For the comparison with modules of genes co-expressed during neocortex development, the module eigengene values from the original study<sup>41</sup> were plotted in a scatter smooth plot for the significant modules.

**Data access.** The HumanMethylation450 BeadChip data set from this study is available in NCBI Gene Expression Omnibus (GEO) under accession number GSE85042.

#### References

- 1. Reik, W. Stability and flexibility of epigenetic gene regulation in mammalian development. Nature 447, 425-32 (2007).
- 2. van Dongen, J. *et al.* Genetic and environmental influences interact with age and sex in shaping the human methylome. *Nat. Commun.* **7**, 11115 (2016).
- Babenko, O., Kovalchuk, I. & Metz, G. A. S. Stress-induced perinatal and transgenerational epigenetic programming of brain development and mental health. *Neurosci. Biobehav. Rev.* 48, 70–91 (2015).
- 4. Walton, E. et al. Correspondence of DNA Methylation Between Blood and Brain Tissue and Its Application to Schizophrenia Research. Schizophr. Bull. 42, 406-414 (2016).
- 5. Kundakovic, M. et al. DNA methylation of BDNF as a biomarker of early-life adversity. Proc. Natl. Acad. Sci. USA 112, 6807–13 (2015).
- 6. Weinstock, M. The long-term behavioural consequences of prenatal stress. Neurosci. Biobehav. Rev. 32, 1073-1086 (2008).
- 7. Korosi, A. & Baram, T. Z. The pathways from mother's love to baby's future. Front. Behav. Neurosci. 3, 27 (2009).
- 8. Fox, S. E., Levitt, P. & Nelson, C. A. III. How the timing and quality of early experiences influence the development of brain architecture. *Child Dev.* 81, 28-40 (2010).
- Loman, M. M. & Gunnar, M. R. Early Experience, Stress, and Neurobehavioral Development Center. Early experience and the development of stress reactivity and regulation in children. *Neurosci. Biobehav. Rev.* 34, 867–76 (2010).
- 10. Lucassen, P. J. *et al.* Perinatal programming of adult hippocampal structure and function; emerging roles of stress, nutrition and epigenetics. *Trends Neurosci.* **36**, 621–631 (2013).
- 11. Ivorra, C. et al. DNA methylation patterns in newborns exposed to tobacco in utero. J. Transl. Med. 13, 25 (2015).
- 12. Bouwland-Both, M. I. et al. Prenatal parental tobacco smoking, gene specific DNA methylation, and newborns size: the Generation R study. Clin. Epigenetics 7, 83 (2015).
- 13. Cardenas, A. *et al.* Differential DNA methylation in umbilical cord blood of infants exposed to mercury and arsenic in utero. *Epigenetics* **10**, 508–15 (2015).
- Mowery, T. M. et al. Embryological exposure to valproic acid disrupts morphology of the deep cerebellar nuclei in a sexually dimorphic way. Int. J. Dev. Neurosci. 40, 15–23 (2015).
- 15. Susser, E. et al. Schizophrenia after prenatal famine. Further evidence. Arch. Gen. Psychiatry 53, 25-31 (1996).
- Tobi, E. W. *et al.* DNA methylation differences after exposure to prenatal famine are common and timing- and sex-specific. *Hum. Mol. Genet.* 18, 4046–53 (2009).
- 17. Mueller, B. R. & Bale, T. L. Early prenatal stress impact on coping strategies and learning performance is sex dependent. *Physiol. Behav.* **91**, 55–65 (2007).
- Varese, F. et al. Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective- and crosssectional cohort studies. Schizophr. Bull. 38, 661–71 (2012).
- Koenig, J. I., Kirkpatrick, B. & Lee, P. Glucocorticoid hormones and early brain development in schizophrenia. Neuropsychopharmacology 27, 309–18 (2002).
- 20. Scott, K. M. et al. Association of childhood adversities and early-onset mental disorders with adult-onset chronic physical conditions. Arch. Gen. Psychiatry 68, 838–44 (2011).
- Intapad, S., Ojeda, N. B., Dasinger, J. H. & Alexander, B. T. Sex differences in the developmental origins of cardiovascular disease. *Physiology (Bethesda)*. 29, 122–32 (2014).
- Gilbert, J. S. & Nijland, M. J. Sex differences in the developmental origins of hypertension and cardiorenal disease. Am. J. Physiol. Regul. Integr. Comp. Physiol. 295, R1941–52 (2008).
- 23. Gerardin, P. et al. Depression During Pregnancy. J. Clin. Psychiatry 72, 378-387 (2011).
- 24. Buss, C., Davis, E. P., Hobel, C. J. & Sandman, C. A. Maternal pregnancy-specific anxiety is associated with child executive function at 6-9 years age. *Stress* 14, 665–76 (2011).
- Buss, C., Entringer, S., Swanson, J. M. & Wadhwa, P. D. The Role of Stress in Brain Development: The Gestational Environment's Long-Term Effects on the Brain. Cerebrum 2012, 4 (2012).
- Bock, J., Murmu, M. S., Biala, Y., Weinstock, M. & Braun, K. Prenatal stress and neonatal handling induce sex-specific changes in dendritic complexity and dendritic spine density in hippocampal subregions of prepubertal rats. *Neuroscience* 193, 34–43 (2011).
- 27. Mychasiuk, R., Gibb, R. & Kolb, B. Prenatal stress produces sexually dimorphic and regionally specific changes in gene expression in hippocampus and frontal cortex of developing rat offspring. *Dev. Neurosci.* 33, 531-8 (2011).

- Mueller, B. R. & Bale, T. L. Sex-specific programming of offspring emotionality after stress early in pregnancy. J. Neurosci. 28, 9055–65 (2008).
- 29. van Os, J. & Selten, J. P. Prenatal exposure to maternal stress and subsequent schizophrenia. The May 1940 invasion of The Netherlands. Br. J. Psychiatry 172, 324–6 (1998).
- 30. Leff, S. S. *et al.* Social cognitions, distress, and leadership self-efficacy: Associations with aggression for high-risk minority youth. *Dev. Psychopathol.* **26**, 759–772 (2014).
- Kundaković, M. et al. Sex-specific epigenetic disruption and behavioral changes following low-dose in utero bisphenol A exposure. Proc. Natl. Acad. Sci. 110, 9956–9961 (2013).
- 32. Xu, H. et al. Sex-biased methylome and transcriptome in human prefrontal cortex. Hum. Mol. Genet. 23, 1260–70 (2014).
- 33. Bale, T. L. Sex differences in prenatal epigenetic programming of stress pathways. Stress 14, 348–56 (2011).
- 34. Yousefi, P. et al. Sex differences in DNA methylation assessed by 450 K BeadChip in newborns. BMC Genomics 16, 911 (2015).
- Hall, E. *et al.* Sex differences in the genome-wide DNA methylation pattern and impact on gene expression, microRNA levels and insulin secretion in human pancreatic islets. *Genome Biol.* 15, 522 (2014).
- Monk, C., Spicer, J. & Champagne, F. A. Linking prenatal maternal adversity to developmental outcomes in infants: the role of epigenetic pathways. *Dev. Psychopathol.* 24, 1361–76 (2012).
- 37. Mayne, B. T. et al. Large Scale Gene Expression Meta-Analysis Reveals Tissue-Specific, Sex-Biased Gene Expression in Humans. Front. Genet. 7, 183 (2016).
- Ho Sui, S. J. et al. oPOSSUM: identification of over-represented transcription factor binding sites in co-expressed genes. Nucleic Acids Res. 33, 3154–64 (2005).
- 39. Spiers, H. et al. Methylomic trajectories across human fetal brain development. Genome Res. 25, 338-52 (2015).
- 40. Wockner, L. F. *et al.* Genome-wide DNA methylation analysis of human brain tissue from schizophrenia patients. *Transl. Psychiatry* **4**, e339 (2014).
- 41. Parikshak, N. N. *et al.* Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. *Cell* **155**, 1008–21 (2013).
- 42. Shi, L. et al. Sex Biased Gene Expression Profiling of Human Brains at Major Developmental Stages. Sci. Rep. 6, 21181 (2016).
- 43. Bale, T. L. & Epperson, C. N. Sex differences and stress across the lifespan. Nat. Neurosci. 18, 1413-20 (2015).
- Ball, M. P. et al. Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells. Nat. Biotechnol. 27, 361–8 (2009).
- Maunakea, A. K., Chepelev, I., Cui, K. & Zhao, K. Intragenic DNA methylation modulates alternative splicing by recruiting MeCP2 to promote exon recognition. *Cell Res.* 23, 1256–69 (2013).
- Drake, A. J. et al. In utero exposure to cigarette chemicals induces sex-specific disruption of one-carbon metabolism and DNA methylation in the human fetal liver. BMC Med. 13, 18 (2015).
- 47. Sala, C., Vicidomini, C., Bigi, I., Mossa, A. & Verpelli, C. Shank synaptic scaffold proteins: keys to understanding the pathogenesis of autism and other synaptic disorders. J. Neurochem. 135, 849–58 (2015).
- 48. Liu, J., Morgan, M., Hutchison, K. & Calhoun, V. D. A Study of the Influence of Sex on Genome Wide Methylation. *PLoS One* 5, e10028 (2010).
- 49. Bacalini, M. G. et al. A meta-analysis on age-associated changes in blood DNA methylation: results from an original analysis pipeline for Infinium 450k data. Aging (Albany. NY). 7, 97–109 (2015).
- Sharma, A. et al. DNA methylation signature in peripheral blood reveals distinct characteristics of human X chromosome numerical aberrations. Clin. Epigenetics 7, 76 (2015).
- 51. Weinstock, M. in 3-25, doi: 10.1007/978-1-4939-1372-5\_1 (2015).
- 52. Le-Niculescu, H. et al. Towards understanding the schizophrenia code: an expanded convergent functional genomics approach. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 144B, 129–58 (2007).
- Saviouk, V., Moreau, M. P., Tereshchenko, I. V. & Brzustowicz, L. M. Association of synapsin 2 with schizophrenia in families of Northern European ancestry. Schizophr. Res. 96, 100–11 (2007).
- Steeb, H. et al. Serum proteomic analysis identifies sex-specific differences in lipid metabolism and inflammation profiles in adults diagnosed with Asperger syndrome, doi: 10.1186/2040-2392-5-4.
- Chen, C., Van Horn, J. D. & GENDAAR Research Consortium. Developmental neurogenetics and multimodal neuroimaging of sex differences in autism. *Brain Imaging Behav.*, doi: 10.1007/s11682-015-9504-3 (2016).
- 56. Assenov, Y. et al. Comprehensive analysis of DNA methylation data with RnBeads. Nat. Methods 11, 1138-1140 (2014).
- Triche, T. J., Weisenberger, D. J., Van Den Berg, D., Laird, P. W. & Siegmund, K. D. Low-level processing of Illumina Infinium DNA Methylation BeadArrays. Nucleic Acids Res. 41, e90 (2013).
- Maksimovic, J., Gordon, L. & Oshlack, A. SWAN: Subset-quantile Within Array Normalization for Illumina Infinium HumanMethylation450 BeadChips. *Genome Biol.* 13, R44 (2012).
- 59. Houseman, E. et al. DNA methylation arrays as surrogate measures of cell mixture distribution. BMC Bioinformatics 13, 86 (2012).
- Ritchie, M. E. *et al.* limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Res.* 43, e47 (2015).
- Zhang, B., Kirov, S. & Snoddy, J. WebGestalt: an integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res. 33, W741–8 (2005).
- 62. Zhao, C., Eisinger, B. E., Driessen, T. M. & Gammie, S. C. Addiction and reward-related genes show altered expression in the postpartum nucleus accumbens. *Front. Behav. Neurosci.* **8**, 388 (2014).

#### Acknowledgements

We thank our colleagues Alda Valéria Neves Soares Gomes and Chang Yi Wei from Obstetric Center of University Hospital of São Paulo, Brasil, for allowing the umbilical cord blood collection. We also thank Kyle Wiley from Yale University of USA for contributing to the writing of the manuscript. We thank funding agencies FAPESP (2015/06281-7, 2012/19472-7 (ROC), 2008/57896-8), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (573974/2008-0), Instituto de Psiquiatria do Desenvolvimento da Infância e da Adolescência (INPD), Saving Brains. Finally, we extend our gratitude to the mothers and their families who volunteered their time and efforts during this research.

#### **Author Contributions**

Wrote the manuscript: M.M., H.B. Collected information, material and performed experiments: L.B., A.B., G.G., R.F., A.E. and S.G., A.S. Analyzed data: M.M., A.T., V.E., H.B. Conceived and designed the study: H.B., A.B., G.F., C.M., G.P., E.M. Prepared figures, tables and supplemental material: M.M., E. B., V.E. All authors revised the manuscript.

### **Additional Information**

Supplementary information accompanies this paper at http://www.nature.com/srep

Competing Interests: The authors declare no competing financial interests.

How to cite this article: Maschietto, M. *et al.* Sex differences in DNA methylation of the cord blood are related to sex-bias psychiatric diseases. *Sci. Rep.* **7**, 44547; doi: 10.1038/srep44547 (2017).

**Publisher's note:** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article's Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/

© The Author(s) 2017