Journal of Neuroimmunology 328 (2019) 38–49

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Cumulative influence of parity-related genomic changes in multiple sclerosis

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ARTICLE INFO

Keywords:
Multiple sclerosis
Pregnancy
Gene expression
DNA methylation

ABSTRACT

Pregnancy reduces the frequency of relapses in Multiple Sclerosis (MS) and parity also has a beneficial long term effect on disease outcome. We aimed to uncover the biological mechanisms underlying the beneficial long-term effects of parity in MS.

Genome-wide gene expression revealed 574 genes associated with parity; 38.3% showed significant DNA methylation changes (enrichment p = 0.029). These genes overlapped with previous MS genes in humans and a rat MS model and were overrepresented within axon guidance (P = 1.6e-05), developmental biology (P = 0.0094) and cell-cell communication (P = 0.019) pathways.

This gene regulation could provide a basis for a protective effect of parity on the long-term outcome of MS.

1. Introduction

Multiple Sclerosis (MS) is a common neurological disorder characterized by inflammation, demyelination and axonal damage in the central nervous system and is considered to be an autoimmune disease. The risk of developing MS can be attributed to genetic and environmental factors, and large genome-wide studies have identified 103 discrete genomic loci associated with susceptibility to MS (International Multiple Sclerosis Genetics et al., 2013; International Multiple Sclerosis Genetics et al., 2011).

MS generally is diagnosed at the age of 20–40 years, which overlaps with the period of reproduction and family planning and is more common in women (Trojano et al., 2012). Recently it has been shown that there is a prodromal period of non-specifc symptoms for some years before diagnosis (Disanto et al., 2018; Hogg et al., 2018; Wijnands et al., 2017). Pregnancy is a state that induces changes in the maternal physiology, due to effects of circulating hormones including estrogen, progesterone, prolactin, leptin and placental lactogen that act through receptors to regulate gene transcription in many cell types. Disease activity and level of disability influence the decision to become pregnant among women with MS (Confavreux et al., 1998). The majority of studies showed that risk of complications during pregnancy and birth outcome is similar to that of healthy women (Finkelsztejn et al., 2011; van der Kop et al., 2011).

Pregnancy is associated with fewer relapses in women with MS (McCombe and Greer, 2013), with effects most pronounced in the third trimester, but with increased relapses during the first three months postpartum which then returned to the pre-pregnancy rate (Confavreux et al., 1998). The mechanism by which pregnancy protects against MS relapses is thought to be related to changes in the maternal immune system, with changes in cytokines and T cells in pregnant compared to non-pregnant MS patients (Gilmore et al., 2004). It is thought that changes in immune response are necessary to prevent the maternal immune system from rejecting the fetus (Patas et al., 2013).

The long-term effects of parity on MS is less certain. There have been some studies suggesting that parity is associated with better long-term outcome, but this could arise because women with more severe MS might decide not to have children (Runmarker and Andersen, 1995; D'Hooghe, 2013; D'Hooghe et al., 2010; Verdru et al., 1994). However, there is accumulating evidence that parity is beneficial. Ponsonby and colleagues examined the association between past pregnancy, offspring number, and risk of a first episode of demyelination (Ponsonby et al., 2012). The study demonstrated that higher parity was associated with reduced risk of a first episode of clinical demyelination and the results were consistent with a cumulative beneficial effect of pregnancy.

In another study using the MSBase registry, where patients were matched according to clinical characteristics, pregnancy was 4.5 times more potent than first-line disease modifying therapies (interferon-beta,

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https://doi.org/10.1016/j.jneuroim.2018.12.004
Received 28 July 2018; Received in revised form 6 December 2018; Accepted 12 December 2018
0165-5728/ © 2018 Published by Elsevier B.V.

glatiramer acetate) in preventing long-term disability accrual, and any time spent pregnant (including induced and spontaneous abortions) was beneficial (Jokubaitis et al., 2016).

For parity to have a long-term effect on MS, it would be expected that there would be permanent changes, that persist after the woman is no longer pregnant and hormone levels have returned to normal. This could lead to accumulation of changes with successive pregnancies or with the experience of parenthood. One possible mechanism for such an effect could be epigenetics, with DNA methylation being one of the most common and widely studied epigenetic mechanisms. DNA methylation is the addition of a methyl group at cytosine–phosphate-guanine dinucleotide (CpG) sites in the genome, leading to changes in gene expression (Robertson, 2005). DNA methylation can also be altered by endogenous hormones such as estrogen (Jost and Saluz, 1993). Therefore, changes in DNA methylation are one mechanism by which the environment, social circumstances and changes in physiology, including hormone levels, can later gene expression (Greer and McCombe, 2012). Such changes in DNA methylation across the genome can be measured via microarray technology.

The aim of this study was to gain biological insight of the cumulative influence of pregnancy on MS. To achieve this, we a) investigated genome-wide gene expression and DNA methylation changes in women with MS associated with the number of pregnancies b) assessed biological pathways that were associated with pregnancy-related genomic changes and c) compared our results to previous studies of MS.

2. Methods

2.1. Subjects

Women with clinically definite MS were recruited to this study. The study was approved by the Human Research Ethics Committees of the Royal Brisbane and Women's Hospital and the University of Queensland. All subjects gave written informed consent to participation. The Extended Disability Status Scale (EDSS) at the time of blood collection was recorded. The numbers and dates of pregnancies were recorded. The details of smoking and use of medications were recorded. The details of use of diseasse modifyng therapies were recorded. All subjects were Caucasian, and all were patients of one neurologist. They all lived in the same region and were of similar socio-economic status. All had blood collected with the same protocol using the same tubes.

3. RNA sequencing

Total RNA was extracted from whole blood in PAXgene tubes using the PAXgene Blood miRNA Kit from Qiagen. RNA was quantified on the Nanodrop1000 and on a Qubit™ fluorimeter using the RNA HS Assay Kit followed by quality evaluation using the Agilent Bioanalyzer RNA 6000 Nano kit. mRNA libraries were prepared using the TruSeq® Stranded mRNA Library Prep Kit for NeoPrep™ following manufacturer's instructions. The Qubit quantitation was used to determine the 100 ng input for each sample. The libraries were to be sequenced as pools of up to 24 libraries. Post library construction each library was quantified using the Qubit™ dsDNA HS Assay Kit, followed by size determination using the Agilent High Sensitivity DNA Kit. Up to 24 unique indexed libraries were equimolar pooled and run on the NextSeq500/550 using NextSeq 500/550 High Output v2 kit (75 cycles) following manufacturer's instructions at a final concentration of 1.5pM. Sequencing runs were monitored in real time through Illumina BaseSpace. RNASeq read filtering, alignment and normalization was carried out using the RNA Aligner application hosted on BaseSpace. An average of 22 Million single 75 base pair reads were generated across the 47 samples and 97.8% reads were aligned to the human reference genome.

4. DNA methylation

DNA samples were extracted from whole blood in EDTA tubes collected at the same time and from the same subjects as the RNA samples using a salting out method followed by ethanol precipitation. Quality assessment was performed using Picogreen assay (Molecular Probes) and the Fluoroskan Ascent Microplate Fluorometer (Thermo Scientific). DNA samples were also assessed on the Epoch Microplate Reader (Biotek) to obtain DNA concentration. 500-1000 ng of DNA bisulphite converted with Zymo EZ DNA Methylation kit(Mehta et al., 2013). Methylation arrays and processed as per the manufacturers protocols for the Infinium II workflow. Arrays were scanned on the Illumina iScan.

5. Analysis of gene expression

Data analysis was performed in R (http://www.R-project.org/). The edgeR Bioconductor package in R was used for assessing differential expression across parity as a quantitative trait using RNA-seq data using non-normalised reads after quality control and normalization (Ritchie et al., 2015; Robinson et al., 2010). The gene-level counts were imported into edgeR and pre-processing involved a filter threshold of > 0.7 counts per million (CPM) to remove low expressed genes in at least 50% of the samples followed by trimmed mean of M-values (TMM) normalization to scale for library size, allowing a total of 13,884 genes for further analysis. The edgeR package was then used to test for differential expression by fitting a model to the negative binomial distribution with the model including the CPM data against the phenotype of interest (number of pregnancies) and adjusting for age and smoking status.

Using raw gene expression data on the Illumina HT12-V3 microarray (GEO GSE17048), we compared our study to genes previously found to be differentially expressed between 45 healthy controls (16 males and 29 females, parity unknown) and 99 untreated MS patients (33 males and 66 females, parity unknown) belonging to three principal clinical subtypes of MS, including relapsing remitting (RRMS), primary progressive (PPMS) and secondary progressive (SPMS)(Gandhi et al., 2010; Riveros et al., 2010). Data were analysed using an established analysis pipeline (Barfield et al., 2014; Mehta et al., 2011; Mehta et al., 2013) via generalised linear regression models where variance stabilizing normalised gene expression was regressed against the clinical subtype in R to identify genes differentially associated between controls and the three MS clinical subtypes. Comparison of variables across the study groups was performed using Pearson's chi-squared tests and regression models. Enrichment testing was performed in R across different gene sets using a hypergeometric test to indicate an overlap greater than expected by chance alone.

6. Analysis of DNA methylation

Raw beta values from 450 k Illumina arrays were exported into R for statistical analysis. Intensity read outs, normalization and methylation beta values calculation were performed using the minfi Bioconductor R package version 1.10.2 (Aryee et al., 2014). Probes with $> 50\%$ of the samples with a detection P-value $> .05$, probes with single-nucleotide polymorphisms present within 10–50 bp from query site, and within < 10 bp from query site were removed. These resulted in a total of $n = 452{,}885$ CpG probes that were used for all subsequent analysis. Whole blood cell counts were estimated using the minfi package in R. Data were analysed using an established analysis pipeline (Barfield et al., 2014; Mehta et al., 2011; Mehta et al., 2013) via generalised linear regression models by regressing the DNA methylation beta values against the number of pregnancies and adjusting for age, cell counts, medication use and smoking status.

7. Functional annotation

To investigate the underlying biology, we compared the list of MS associated genes that we had identified as regulated across pregnancy to all genes that were regulated across pregnancy using the Pathway Commons tool and disease and drug-related gene analysis via the Webgestalt tool (Wang et al., 2013). Pathway Commons is a collection of publicly available pathway data from multiple organisms (http://www.pathwaycommons.org) that allows users to disseminate results via biological pathways. The disease and drug analysis allows to identify an overrepresentation of genes from the query list with genes involved in diseases and drug action respectively. Results for the functional annotation were corrected for multiple testing using the Benjamini Hochberg correction for the number of genes tested in each pathway. The Drug Gene Interaction database (DGIdb) was used to query for drug-gene interactions (http://dgidb.genome.wustl.edu/api) for clinically proven drug targets as well as potentially druggable genes that give important information and highlights additional likely drug targets among the differentially expressed gene list.

8. Results

A total of 47 women with Multiple sclerosis (MS) were analysed in this study. Of these, 12 women had never been pregnant, 7 women had been pregnant only once, 17 women had been pregnant twice, 8 women had been pregnant thrice and 3 women had been pregnant 4 times. Detailed demographics and clinical characteristics of the women are shown in Table 1. There were no differences in age (p = 0.62), disease duration (p = 0.20), duration since last pregnancy (p = 0.193) and Expanded Disability Status Scale (p = 0.82) between the parous and non-parous women. There were also no differences in the number of women on current medication between the parous and non-parous groups (p = 0.14) nor in the type of medication (p = 0.56).

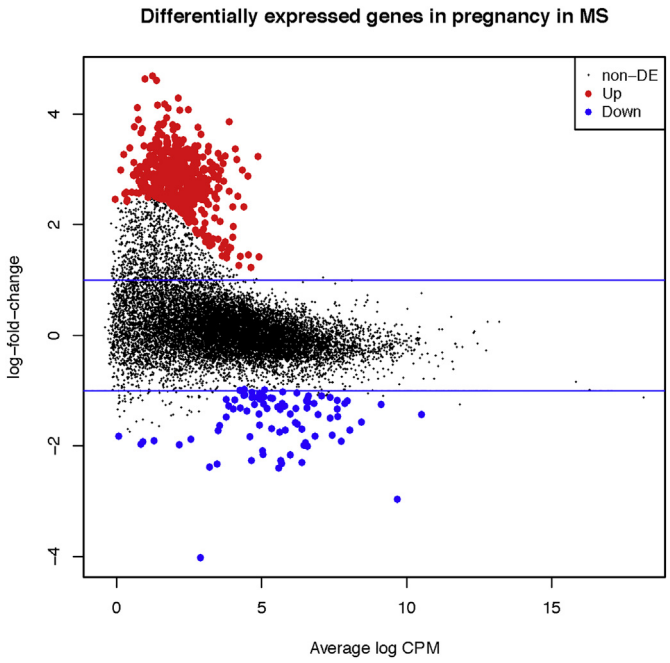

Fig. 1. Volcano plot showing up and down-regulated genes differentially expressed with parity in MS.

9. Gene expression and DNA methylation changes associated with parity

To test for gene expression changes related to parity, we used genome-wide RNA sequencing data and identified gene expression changes associated with number of pregnancies, after adjusting for age and smoking status.

A total of 574 genes showed significant changes in expression changes with number of pregnancies even after multiple testing correction of 5% false discovery rate (Table 2). Post-hoc analysis revealed

Table 1
Demographics of the 47 women included in the study.

Characteristic	Mean[SD]/N[%]
Ethnicity: Caucasian	47 [100%]
Age (in years)	47.1[12.1]
Smoking Status	
Smoker	44 [93.6%]
Non-smoker	3 [6.4%]
Number of pregnancies	
None	12 [25.5%]
One	7 [14.9%]
Two	17 [36.2%]
Three	8 [17%]
Four	3 [6.4%]
Expanded Disability Status Scale (EDSS)	2.0 [2.2]
Duration since last pregnancy (in years)	19.35 [2.19]
Disease duration (in years)	14.67 [1.36]

Mean[SD] Or N [%]	Zero Pregnancy (n = 12)	One Pregnancy (n = 7)	Two Pregnancies (n = 17)	Three Pregnancies (n = 8)	Four Pregnancies (n = 3)
Age	45.58 [4.4]	39.43 [2.3]	48.65 [2.9]	51.25 [1.9]	51 [11.2]
EDSS	2.08 [0.8]	1.14[0.3]	2.0 [0.6]	1.88 [0.7]	3.33[1.8]
Smoker	0 [0%]	2 [28.6%]	1 [5.9%]	0 [0%]	0 [0%]
Disease Duration	17.7 [2.1]	5 [1.3]	14.19 [2.1]	19 [3.5]	17 [8.7]
Current medication	5 [100%]	6 [86%]	15 [88%]	7 [88%]	1 [33.3%]
Medication type	4 [80%]	3 [50%]	6 [40%]	4 [57%]	0 [0%]
Immunosuppresive	0 [0%]	3 [50%]	7 [47%]	2 [29%]	0 [0%]
Non-immunosuppressive	1 [20%]	0 [0%]	2 [13%]	1 [14%]	1 [100%]
Monoclonal antibody					

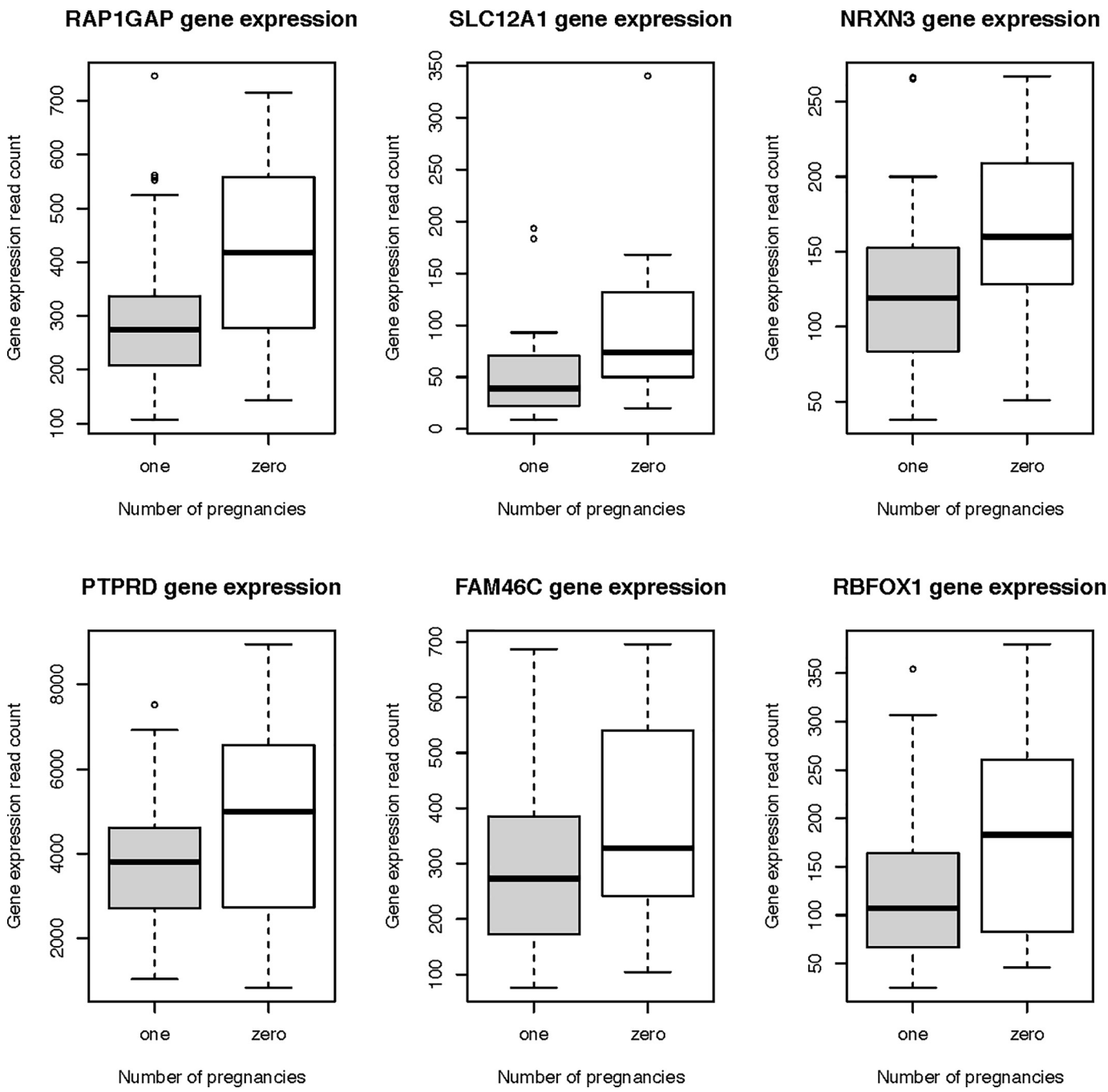

Fig. 2. Differentially expressed genes. a) Figure showing differentially expressed genes associated with parity in MS, b) Figure showing differentially expressed genes associated with parity in MS across different numbers of pregnancies.

that the genes were significant even after correcting for disease duration, disability burden (EDSS), duration since last pregnancy and medication use. Of the differentially expressed genes, 488 were upregulated and 86 genes were downregulated (Fig. 1). Post-hoc analysis revealed that all the genes were parity- associated and none of these were significantly differentially expressed between the parous and non-parous group (Supplementary Table 1). Examples of the differentially expressed genes are shown in Fig. 2a and b. The top genes included *RAP1GAP*, implicated in diverse processes such as cell proliferation, adhesion, differentiation and embryogenesis and implicated in inhibition of tumor progression in endometrial cancer (Tamate et al., 2017).

Among the 574 differentially expressed genes, a total of 355 genes also had at least one probe present on the genome-wide DNA methylation array. From these 355 genes, a total of 136 genes (38.3% overlap, enrichment $p = 0.029$) also showed significant DNA methylation changes with parity in the same women at the nominal level, after adjusting for age, cell types and smoking status ($p < 0.05$, Supplementary Table 2a and b and Fig. 3). There was no association of cell types (CD8, CD4, NK, B cells, monocytes and granulocytes) with parity nor were there any differences in these cell types between the parous and non-parous women ($p > 0.05$).

10. Parity-associated genes overlap with genes differentially expressed in clinical subtypes of MS

We compared our list of genes associated with changes in expression with parity with genes previously found to be differentially expressed in 99 untreated MS patients belonging to three principal clinical subtypes

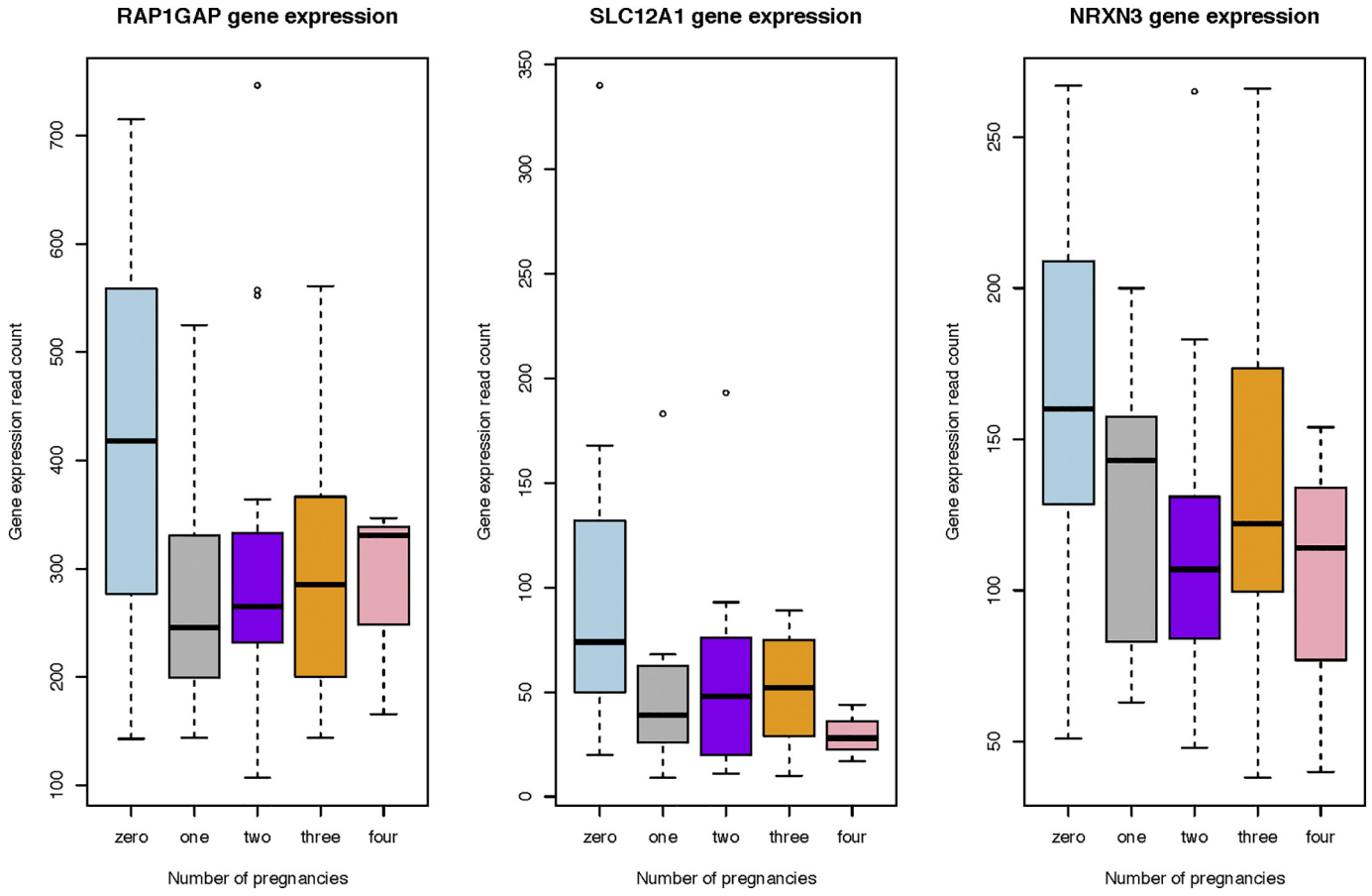

Fig. 2. (*continued*)

of MS and 45 healthy controls (n = 16 males and 29 females), including relapsing remitting (RRMS), primary progressive (PPMS) and secondary progressive (SPMS)(Gandhi et al., 2010; Riveros et al., 2010) (33 males, 66 females, parity status unknown). Using the raw gene expression data on the Illumina HT12-V3 microarray available from the original study we performed analysis to identify genes differentially associated between controls and the three MS clinical subtypes. Systematic testing was performed for the 574 parity-associated genes from which 436 genes were also present on the microarray from the clinical gene expression study. Of the 574 genes associated with parity in the current study, we observed that 85 genes (20% of those tested, enrichment p = 0.0012) showed significant gene expression changes between the controls and either of the 3 MS clinical subtypes (Supplementary Table 3).

11. Overlap of differentially expressed genes with other MS studies

We tested whether the genes associated with risk of MS in previous studies were also associated with parity in the current study.

Comparison with the large MS GWAS revealed that 10 out of 81 MS-associated GWAS genes (International Multiple Sclerosis Genetics et al., 2013; International Multiple Sclerosis Genetics et al., 2011) present in our data also showed significant gene expression changes with parity (enrichment p = 0.003, Supplementary Table 4a). Analysis of a more recent unpublished set of MS-associated genes (https://www.biorxiv.org/content/early/2017/07/13/143933), showed that 19 of 155 MS genes showed significant gene expression changes with parity (enrichment p = 0.018, Supplementary Table 4b).

Comparison to genes differentially expressed between MS and healthy controls at baseline (MS signature) in humans (Gilli et al., 2010), indicated that 28 of 401 of these genes were also associated with significant gene expression changes (enrichment p = 0.046, Supplementary Table 5) with parity.

Similarly, when compared to genes differentially expressed between rats with MBP-EAE and control rats (Inglis et al., 2012), an overlap of 149 of 1257 genes ($p < 0.05$) was found among those regulated with parity in this study (Supplementary Table 6); this was significantly higher than expected by chance (enrichment p = 0.022).

12. Biological insights of genes associated with the number of pregnancies in MS

We functionally annotated the differentially expressed genes associated with parity using publicly available databases including Pathway commons and disease and drug enrichment via the Webgestalt interface and the Drug Gene Interaction Database (Table 3).

Pathway analysis of the differentially expressed genes revealed that they were overrepresented in pathways associated with axon guidance (p = 1.6e-05), developmental biology (p = 0.0094) and cell-cell communication (p = 0.019). The genes were enriched for those associated with Mental disorders, Neurobehavioural manifestations and Neurologic manifestations. The top-enriched drug category was bupropion (p = 3.96e-38), an anti-depressant and smoking cessation drug.

From the 574 genes, a total of 176 genes were shown to have a potentially druggable genome and 19 of the genes had been reported to be clinically actionable genes as per the Drug Gene Interaction Database (DGIdb, Supplementary Table 7).

Genome-wide analysis of DNA methylation did not reveal any genes that survived multiple testing corrections however pathway analysis of nominally significant parity-associated differentially methylated genes

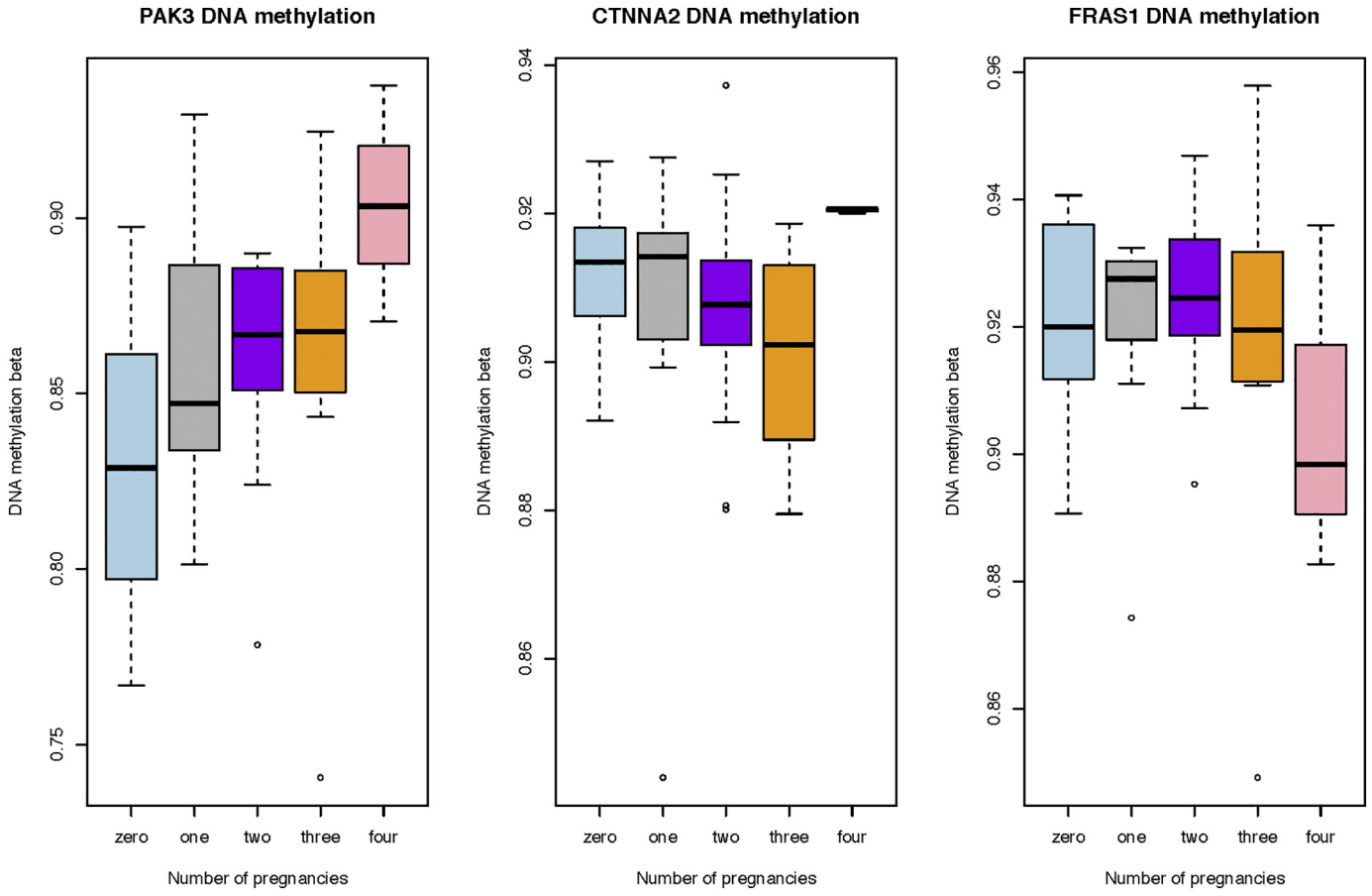

Fig. 3. Figure showing differentially expressed genes also showing significant DNA methylation changes associated with parity in MS.

revealed similar pathways as the differentially expressed genes including axon guidance, developmental biology and cell-cell communication (Supplementary Table 8).

13. Discussion

To explore a possible biological basis for a long-term beneficial effect of pregnancy, in this study we looked for changes in gene expression and DNA methylation that were related to parity in a cohort of women with MS. We studied peripheral blood cells of patients with MS. These cells are relevant to MS, as it is thought that peripheral blood lymphocytes enter the CNS to cause inflammation in MS.

Among women with MS, our analysis revealed a total of 574 genes associated with parity after multiple testing corrections. The majority of the differentially expressed genes (85%) were upregulated among parous MS women. For the genes showing an association with parity we would expect to find a difference between parous and no-parous subjects. No significant differences were found, but this likely reflects the low power for a test comparing 12 non-parous vs 35 parous subjects (we have power to detect 1 SD difference at the nominal significance threshold of $p = 0.05$). However, 81% of the non-parous vs parous differences are in the expected direction given the direction of association with parity, and this rate of agree is highly significant compared to chance ($p < 2e\text{-}16$). In peripheral blood cells of women with MS, there has been a prior study showing changes in the expression of genes related to inflammation; these returned to normal after pregnancy (Gilli et al., 2010). We are not aware of any prior studies of the effects of long term effects of parity in blood cells from MS patients or controls.

Functional analysis revealed that the differentially expressed genes in peripheral blood in MS patients were overrepresented in axon guidance, developmental biology and cell-cell communication pathways. The axon guidance pathway is important in repair and might underlie perturbed neuronal connectivity that is a characteristic of neurological disorders such as MS (Van Battum et al., 2015). Moreover, the genes were enriched for those associated with Mental disorders, Neurobehavioural manifestations and neurologic manifestations. A total of 16 of the 574 genes had been reported to be clinically actionable genes. This included *PTPRD*, encoding a transmembrane protein involved in neuronal differentiation, neurite out-growth and excitatory synapse formation. *PTPRD* was associated with MS in the International Multiple Sclerosis Genetics Consortium study (International Multiple Sclerosis Genetics et al., 2011). In murine models, *PTPRD* has been shown to regulate motoneuron axon guidance (Uetani et al., 2006). It is of note that all many of these genes were associated with neuronal function rather than immune function. Immune abnormalities are thought to underlie relapses of MS and the formation of new lesions. However, long term disability in MS results from neurodegeneration (Mahad et al., 2015), and beneficial regulation of genes involved in neuronal growth and repair could possibly underlie a reduction in progression of disability. It must be noted that these changes in "neuronal" genes were observed in peripheral blood cells and we have not shown these changes in the brain. Nevertheless, there have been attempts to explore the degree of correlation between DNA methylation in brain and peripheral blood cells and these studies have shown a substantial overlap between the two (Qi et al., 2018; Walton et al., 2016).

Next, we examined DNA methylation in the same subjects as a likely molecular mechanism that might underlie the observed gene expression changes. Among genes present on the DNA methylation array, 38.3% showed significant DNA methylation changes associated with parity. This is a novel observation and shows a possible mechanism by which parity could lead to changes in gene expression. DNA methylation could arise as an effect of the hormonal changes in pregnancy. For example,

Table 2
List of the 574 differentially expressed genes associated with the number of pregnancies.

SYMBOL	ENTREZID	logFC	FDR Pvalue
RAP1GAP	5909	−4.0218123	9.87E-018
SLC12A1	6557	3.85879555	1.50E-011
FAM46C	54855	−2.4037702	1.50E-011
TMEM176A	55365	−2.2587646	8.33E-011
ALAS2	212	−2.9685476	8.33E-011
RBFOX1	54715	3.23026022	9.18E-011
TMEM176B	28959	−2.3191977	9.18E-011
NRXN3	9369	3.37142474	9.18E-011
TRIM58	25893	−2.2635609	2.86E-010
CSMD1	64478	3.17925281	5.47E-010
SLC6A8	6535	−2.1501104	9.29E-010
SELENBP1	8991	−2.2976818	9.87E-010
DMD	1756	2.98875442	1.73E-009
KRT1	3848	−2.0890746	3.72E-009
PTPRD	5789	2.87422595	4.52E-009
GMPR	2766	−2.1664966	5.06E-009
CALN1	83698	3.24249513	5.86E-009
OR2W3	343171	−2.3268352	2.66E-008
DPP10	57628	3.1027739	4.94E-008
LRP1B	53353	3.20699672	4.94E-008
ERC2	26059	3.28918093	7.66E-008
ANKRD9	122416	−2.3816701	3.11E-007
PTCHD1-AS	100873065	2.97751798	4.14E-007
FECH	2235	−1.8346761	5.57E-007
AHSP	51327	−2.0054823	5.57E-007
MXI1	4601	−1.9893099	5.57E-007
MACROD2	140733	2.50914915	7.50E-007
DAB1	1600	2.78237453	9.03E-007
KIAA1217	56243	3.0415018	9.03E-007
CDH13	1012	2.60483471	1.05E-006
HYDIN	54768	3.41222801	1.30E-006
MIR6087	102464835	−1.9420027	1.32E-006
IL1RAPL1	11141	2.88269991	1.78E-006
SOX5	6660	3.08703718	2.85E-006
MAGI2	9863	2.81325004	2.87E-006
PCDH15	65217	3.32629266	2.87E-006
DLG2	1740	2.31611993	3.06E-006
CTNNA2	1496	3.12908058	5.90E-006
ASIC2	40	2.91353081	6.25E-006
DPP6	1804	3.63103258	6.45E-006
GLRX5	51218	−1.7538347	7.42E-006
SUGCT	79783	2.95752711	7.49E-006
NPAS3	64067	3.75971475	7.49E-006
PTPRT	11122	2.85859692	7.89E-006
DOCK3	1795	2.66756521	8.57E-006
CNTNAP2	26047	2.320812	8.72E-006
PRKG1	5592	2.67821181	8.81E-006
GPC6	10082	2.99100156	1.07E-005
SDK1	221935	2.64726072	1.07E-005
CA1	759	−1.6901648	1.41E-005
HBD	3045	−1.8287948	1.48E-005
EPB42	2038	−1.7198241	1.62E-005
SLC4A1	6521	−1.9142017	1.90E-005
BPGM	669	−1.6228268	2.59E-005
TRPM3	80036	4.08026499	2.81E-005
SHANK2	22941	3.22346297	2.84E-005
CADM2	253559	3.42108535	4.83E-005
BCL2L1	598	−1.8040106	6.01E-005
SNCA	6622	−1.6954836	6.01E-005
OPCML	4978	2.81354014	6.84E-005
LRRC4C	57689	2.54459702	7.25E-005
ANKS1B	56899	3.01011428	7.89E-005
PARD3	56288	2.46295786	8.01E-005
CTNNA3	29119	2.50484659	8.59E-005
WBSCR17	64409	2.98000523	8.59E-005
CNTN4	152330	3.4255113	0.00010003
GRID2	2895	2.99067135	0.00010206
DGKB	1607	4.28888055	0.00013765
LOC730100	730100	3.22621823	0.00014211
NRG3	10718	3.20786546	0.00014756
NLGN1	22871	3.32060759	0.00014895
AKAP6	9472	3.36661425	0.00016592
SIAH2	6478	−1.6079215	0.00016674
SYN3	8224	2.84109745	0.00016897
PARD3B	117583	2.75253028	0.00016897
TTC28	23331	2.61498106	0.00016897
RIMS2	9699	3.16436027	0.00019058
TENM2	57451	2.69504322	0.00019949
TSTA3	7264	−1.5823414	0.00020956
TMEM132D	121256	2.76034207	0.00020956
CADPS2	93664	4.06548881	0.00020956
PRKN	5071	1.96115937	0.00022398
IFIT1B	439996	−1.7232914	0.0002321
ESRRG	2104	3.24684759	0.00024401
ERBB4	2066	3.10224803	0.00025656
GRIN2A	2903	3.34195396	0.00028611
CASC15	401237	2.29801482	0.00028911
PHEX	5251	4.68520493	0.00029256
LINC01924	284294	4.60274041	0.00031758
PPARGC1A	10891	3.34367167	0.00037844
DLC1	10395	3.1301994	0.00037844
CNTN5	53942	2.90096473	0.00037844
DMTN	2039	−1.7202136	0.00037844
NELL1	4745	3.16680541	0.00040535
CDH12	1010	2.80978015	0.0004062
DCC	1630	2.63864167	0.00051534
CSMD2	114784	3.24707253	0.00051534
XK	7504	−1.6389761	0.00051593
CHPT1	56994	−1.4249886	0.00052253
DSCAM	1826	2.84130029	0.00052456
RARB	5915	2.99815233	0.00057067
RALYL	138046	3.36822656	0.00060802
CNTNAP5	129684	2.79683686	0.00063135
ZFPM2-AS1	102723356	4.63165383	0.00067697
ADCY2	108	4.18259361	0.00068269
LUZP2	338645	3.53564045	0.00068269
NKAIN3	286183	3.7683525	0.00068269
LARGE1	9215	2.05576745	0.00068269
SHROOM3	57619	4.10655671	0.00068269
NCKAP5	344148	3.067499	0.00070943
GRM7	2917	2.93879733	0.00073542
IL1RAPL2	26280	2.71221556	0.00079939
PDE1C	5137	3.27331925	0.0008051
ANOS1	3730	4.16401717	0.00084914
RASAL2	9462	2.54501427	0.0009133
GPC5	2262	2.36485521	0.00092756
CDH18	1016	2.94421048	0.00095039
LINC02147	102467224	3.80839491	0.00095039
GREB1L	80000	3.2068302	0.00096982
PPP2R5B	5526	−1.37196	0.00097966
KCNIP4	80333	2.42185425	0.00098627
NRG1	3084	1.77667188	0.00101991
LOC101929710	101929710	3.31048191	0.00102256
NTM	50863	2.76241813	0.00104666
AGBL1	123624	2.75946822	0.00104935
EPHA6	285220	3.46703845	0.00107389
YOD1	55432	−1.47506	0.00108408
PTPRM	5797	2.36292356	0.00108408
SHISA9	729993	3.52588547	0.00109876
UNC13C	440279	3.52256785	0.00114124
MYO18B	84700	3.92886997	0.00116982
CADPS	8618	3.34426363	0.00116982
THSD4	79875	2.12184024	0.00116982
TACC2	10579	3.71846422	0.00124131
NAALADL2	254827	2.96947756	0.00127684
RORA	6095	2.48356208	0.00127684
RUNDC3A	10900	−1.4220645	0.00127684
RGS7	6000	2.75667083	0.0015344
FMN2	56776	3.22894226	0.0015344
CTNND2	1501	2.79209196	0.00154467
GPR158	57512	3.58605583	0.00154467
SGCG	6445	4.11175566	0.00154467
ZNF423	23090	3.55736799	0.00156269
FGF12	2257	3.53698284	0.00156574
MYO3B	140469	3.5268278	0.00160517
KIF26B	55083	2.71894924	0.00160517
GALNTL6	442117	2.54205596	0.00160517
RBMS3	27303	2.88703143	0.00165458

(*continued on next page*)

Table 2 (*continued*)

SYMBOL	ENTREZID	logFC	FDR Pvalue
SGCZ	137868	2.4862359	0.00171391
ZBTB7C	201501	3.808667	0.00171391
LINC01483	101928122	3.82849305	0.0017141
SNTG1	54212	3.27461971	0.00178484
SCML2	10389	3.81043684	0.00190594
SERPING1	710	−1.3269581	0.00196308
TYW1B	441250	2.45337649	0.00196779
ROBO1	6091	2.47446455	0.00198593
ROR1	4919	3.34341764	0.00198593
DLGAP2	9228	2.65577487	0.00198593
LOC101928516	101928516	3.02434185	0.00198593
FAM210B	116151	−1.4941159	0.00204565
MALRD1	340895	2.61429541	0.00204565
ALK	238	2.8921911	0.00204565
ZNF804B	219578	3.36785128	0.00214683
UNC5D	137970	2.73507057	0.00216417
NKAIN2	154215	2.66382942	0.00220712
COL6A4P2	646300	3.89323354	0.00221848
PTGDS	5730	−1.316872	0.00225964
FSTL5	56884	3.21748095	0.00228048
SLC25A21	89874	3.42126968	0.00233807
ST6GALNAC4	27090	−1.3389156	0.00236782
FAM155A	728215	3.21749452	0.00236782
FBN1	2200	3.7499638	0.00236782
RANBP17	64901	3.07114721	0.00236782
FMN1	342184	2.45909196	0.00236782
FRMPD4	9758	3.13533996	0.00236782
C9orf78	51759	−1.4346225	0.00248349
TENM3	55714	3.15746741	0.00248349
MIR548F3	100302159	3.10977986	0.00248349
YBX3	8531	−1.5712044	0.00252979
CADM1	23705	3.33627994	0.00252979
IGF2BP2	10644	−1.3356924	0.00262984
PHACTR1	221692	2.4812356	0.00263449
BMPR1B	658	3.66536237	0.00263449
LOC339975	339975	3.76507064	0.00265442
MAGI1	9223	2.52496634	0.0026828
SYT14	255928	3.62144121	0.0027145
NLGN4X	57502	3.56638907	0.0027145
RNU5F-1	26828	3.73633853	0.00275776
OPHN1	4983	2.52798486	0.00276987
ADGRB3	577	3.13669656	0.00276987
GRIN2B	2904	3.08555365	0.00276987
WNK3	65267	3.32971913	0.0028642
EGFEM1P	93556	3.17363135	0.0028642
DNER	92737	3.62021147	0.00300716
GRIK2	2898	3.20721448	0.0030217
PPM1E	22843	2.84680867	0.00306103
LINC01122	400955	2.56389339	0.00311215
CRTC3-AS1	101926895	3.6638633	0.00311551
ZNF536	9745	3.05944416	0.00311551
KIF6	221458	3.22943932	0.00312802
RBM38	55544	−1.4682061	0.00313976
ERG	2078	3.49149126	0.00322858
DISC1FP1	101929222	2.80237389	0.00325773
LINC00907	284260	2.68974642	0.00331439
FOXP2	93986	3.49715958	0.00331721
ENOX1	55068	2.92340363	0.00331721
SLC38A5	92745	−1.2965141	0.00336877
SLIT3	6586	2.68333263	0.00340006
GPHN	10243	1.6953826	0.00340396
CLIC3	9022	−1.2491443	0.00349709
MIPOL1	145282	3.47173269	0.00353286
GRM5	2915	3.44329274	0.00354213
AGBL4	84871	2.58778793	0.00356855
GABRG3	2567	3.03040326	0.00367598
ADAMTSL3	57188	3.24089206	0.00387854
RNF187	149603	−1.323721	0.00399574
C8orf34	116328	3.24384441	0.00399574
UNC5C	8633	3.52957174	0.00399574
LINC01572	101927957	2.18854716	0.00427451
CFAP61	26074	3.26828731	0.00446491
SLC1A5	6510	−1.24223	0.00452579
FAM135B	51059	3.37526098	0.00452579
XKR4	114786	3.32835429	0.00452579

Table 2 (*continued*)

SYMBOL	ENTREZID	logFC	FDR Pvalue
TEAD1	7003	3.00335103	0.00452579
LAMA1	284217	3.4669973	0.00452579
KCNMA1	3778	2.46004471	0.00467263
DLGAP1	9229	1.57792972	0.00467263
TEX11	56159	3.17286413	0.00467263
RIOK3	8780	−1.2800503	0.00467263
GPC3	2719	2.67971368	0.00467263
MEIS2	4212	3.54349246	0.00467263
ANK2	287	2.26795769	0.00467263
PCDH7	5099	3.3251062	0.00477099
PLEKHA5	54477	2.26125292	0.00477379
C4orf22	255119	3.30514469	0.00479521
CACNA2D3	55799	1.58859066	0.00494617
CEP112	201134	2.56204956	0.00502616
LINC01317	104355287	2.78209577	0.00507133
DEC1	50514	3.4375646	0.00510505
SORCS1	114815	2.51034568	0.00510505
LINC00578	100505566	3.42872744	0.00513454
KCNH1	3756	3.05153147	0.005205
WWTR1	25937	2.77808447	0.005205
CNTN1	1272	3.42682482	0.005205
PPP1R14C	81706	3.38207289	0.00535968
OSBP2	23762	3.18786581	0.00539483
CNPPD1	27013	−1.319913	0.00539483
HMCN1	83872	3.34815835	0.00544922
DIAPH2	1730	1.45045085	0.00545085
GHR	2690	3.39297223	0.00545085
SNAP25-AS1	100131208	3.42054465	0.0054561
NCAM2	4685	2.82429454	0.0054561
LOC102724084	102724084	3.30944759	0.00554022
EDIL3	10085	3.38089074	0.00560656
ANK3	288	1.64202088	0.00560656
DCDC1	341019	3.29982945	0.00589419
NEGR1	257194	1.73358105	0.00593571
ROR2	4920	3.29101671	0.00594596
ADGRL3	23284	2.51979272	0.00600262
SLC24A2	25769	3.08051737	0.00601325
FGF14	2259	3.26922777	0.00618547
DOK5	55816	3.38106683	0.0061955
SYT1	6857	2.6911991	0.0061955
GABBR2	9568	2.78671412	0.00633564
UNC45B	146862	−1.2800713	0.00633564
PRAMEF18	391003	2.90890146	0.00635425
ROBO2	6092	3.1671528	0.0064121
PIEZO2	63895	3.09108444	0.00653543
MDGA2	161357	2.56341998	0.00653543
CCBE1	147372	3.28994304	0.00653543
NFIB	4781	2.96825459	0.00653543
HERC5	51191	−1.2343939	0.00654483
KIR2DS4	3809	−1.8778527	0.00654483
SHISA6	388336	2.82151274	0.00656851
CSMD3	114788	2.58357678	0.0066899
FAM189A1	23359	2.91845808	0.0066899
RFLNA	100533183	2.94841724	0.0066899
ZNF521	25925	2.83639958	0.00669919
PLD5	200150	3.06424112	0.00669919
WWOX	51741	1.42246353	0.00679438
LIMCH1	22998	3.12291285	0.00699079
ULK4	54986	1.53712711	0.00699079
MIR100HG	399959	3.11713461	0.00699079
LOC101927815	101927815	3.33415855	0.00702928
EBF2	64641	3.22216042	0.00706816
MIR924HG	647946	3.00918248	0.00735053
STXBP5L	9515	3.04027558	0.00739546
BRIP1	83990	3.20346094	0.00739546
UNC79	57578	2.62878806	0.00741302
TARID	100507308	3.16822733	0.00741316
RNF219-AS1	100874222	3.12439519	0.00742513
AUTS2	26053	1.42308922	0.00768292
STK32B	55351	3.03887889	0.00770769
KCNIP1	30820	3.25860164	0.007751
RYR2	6262	2.02448126	0.00778983
SOX2-OT	347689	2.36912621	0.00802549
ADAMTSL1	92949	3.08372713	0.00813148
HPSE2	60495	2.48758857	0.00824249

(*continued on next page*)

Table 2 (*continued*)

SYMBOL	ENTREZID	logFC	FDR Pvalue
PCDH11X	27328	2.60352791	0.00864873
DOCK1	1793	2.87298547	0.00895601
PDE11A	50940	3.00375865	0.00895846
GRM8	2918	3.039845	0.00895846
GLIS3	169792	2.39247426	0.00896702
FRMD5	84978	2.52696278	0.00898219
WDR93	56964	3.12408028	0.00914095
RAPGEF4	11069	2.85945038	0.00934051
BICC1	80114	3.13504716	0.00934051
RIMBP2	23504	3.20796003	0.00934051
FAM186A	121006	3.1592952	0.00959275
SORCS3	22986	2.49100979	0.00959275
MGAT4C	25834	3.06330097	0.00989028
RIT2	6014	3.19049268	0.00989028
EFNA5	1946	3.08539456	0.00989028
PADI6	353238	3.26647377	0.00989028
COL25A1	84570	3.04432203	0.00990474
ADAMTS6	11174	3.06041308	0.01002973
LAMA2	3908	2.74401724	0.01022101
TSHZ2	128553	1.57308478	0.01025207
ADGRG5	221188	−1.1489045	0.01030038
DCAF12	25853	−1.3304172	0.01030208
CACNA1B	774	3.07327792	0.01033395
CECR2	27443	2.85309356	0.01041915
LRRTM4	80059	2.89299423	0.01045703
MTUS2	23281	2.73824493	0.01050701
NUP210L	91181	2.36989328	0.01051666
REEP1	65055	3.25068796	0.01054538
LOC284581	284581	3.24362783	0.01054618
RELN	5649	2.94378739	0.01063391
NTN4	59277	3.14111365	0.01091092
RGS6	9628	1.74709738	0.01111973
COL27A1	85301	3.12943148	0.01124918
MIR4300HG	101928989	3.06848623	0.01125258
TEX41	401014	3.09390288	0.01128576
GYPB	2994	−1.9809801	0.01129346
SEMA3A	10371	3.08452303	0.01129346
RIMS1	22999	3.07153136	0.01163252
CHST9	83539	3.0323384	0.01169511
LINC01170	103724389	3.08284746	0.01204637
PRR16	51334	3.05206967	0.01211971
PKHD1	5314	2.85504463	0.01211971
STOX2	56977	2.93205321	0.01211971
PDZRN3	23024	3.0497591	0.01211971
LINC00534	100874052	3.06900161	0.01213703
ABCA13	154664	2.59894119	0.01215312
NPDC1	56654	−1.1217298	0.01215312
MEIKIN	728637	3.05449797	0.01221356
DNAH8	1769	2.71978632	0.01234199
FBXL7	23194	2.8988468	0.01236296
LINC01002	399844	−1.1218402	0.01241106
FRAS1	80144	2.777216	0.01261237
PAK3	5063	2.88758129	0.01273219
LSAMP	4045	2.62401894	0.01275952
LINC02267	105377338	3.10143308	0.01303745
PPP1R9A	55607	2.71427422	0.01305192
TSPAN5	10098	−1.1447888	0.01324011
IL23R	149233	3.03717224	0.01368389
KCTD16	57528	2.93972349	0.01369344
MARCH8	220972	−1.2334934	0.01373073
KCNN3	3782	2.98964281	0.01373073
OAS3	4940	−1.2160643	0.01373234
TMEM178B	100507421	3.01588444	0.01402483
TNS1	7145	−1.1341683	0.01411861
DNAH3	55567	2.35437654	0.01415046
CLSTN2	64084	3.00038546	0.0142041
GTF2IRD1	9569	2.99905741	0.01422291
SPTB	6710	−1.1188858	0.01442906
SV2B	9899	2.97511376	0.01450827
SPOCK3	50859	2.89704443	0.01454555
DDR2	4921	3.10290276	0.01454763
VAT1L	57687	2.98946902	0.01454763
BRINP3	339479	2.96790781	0.01454763
DIAPH3	81624	2.34587502	0.01454763
NTNG1	22854	2.74335238	0.0146713

Table 2 (*continued*)

SYMBOL	ENTREZID	logFC	FDR Pvalue
ATRNL1	26033	2.44473152	0.01469118
NRXN1	9378	1.79157689	0.01488806
SMIM1	388588	−1.1679071	0.01507316
SEC14L6	730005	2.98229255	0.01520825
WDR17	116966	2.90147505	0.01523279
HECW2	57520	1.92200086	0.01534565
PRELID2	153768	2.90385101	0.01534565
LOC105370802	105370802	2.95456103	0.01563257
CACNA2D1	781	2.54461876	0.01624451
GNG12	55970	2.89306622	0.01624451
MIR99AHG	388815	2.34287701	0.01644128
ATP8A2	51761	2.3025645	0.01647064
MYO3A	53904	2.91453887	0.01647064
SEL1L2	80343	2.83601748	0.0165065
SRGAP1	57522	2.54225495	0.0165065
MYO10	4651	2.38182122	0.01664269
RSAD2	91543	−1.1902442	0.01666928
ANO3	63982	2.85560172	0.01677131
CYP7B1	9420	2.92629313	0.01763903
PTPN20	26095	2.71197189	0.01763903
SCN8A	6334	2.85024798	0.01763903
KCNT2	343450	2.9169965	0.01763903
CDON	50937	2.89538839	0.01777101
LOC643339	643339	2.19566923	0.01777101
GRHL2	79977	2.91383445	0.01803108
EFHD1	80303	2.9205491	0.01808072
NIM1K	167359	2.89970117	0.01808072
EYA2	2139	2.75341147	0.01839645
TENM4	26011	2.23670954	0.01839645
PITPNM3	83394	2.88570014	0.01840282
DEPTOR	64798	2.48501534	0.01850311
CACNB2	783	1.39629938	0.01850311
MYO9A	4649	1.6672351	0.01850311
TAL1	6886	−1.0858084	0.01890576
ANK1	286	−1.0838048	0.01890576
PINK1	65018	−1.0831827	0.01900842
CENPP	401541	2.3556018	0.01922481
TMC1	117531	2.73525733	0.01929564
PKIB	5570	2.7125454	0.0194237
LOC284930	284930	2.85350802	0.0194237
PLCH1	23007	2.63676282	0.02003509
DCLK1	9201	2.78221161	0.02005241
CFAP77	389799	2.84645891	0.02005241
TMC2	117532	2.88222476	0.02026109
AGAP1	116987	1.64375328	0.02042475
LOC101928437	101928437	2.64519801	0.02044546
NTRK2	4915	2.64787617	0.02054075
ARHGEF28	64283	2.85324836	0.02062306
LINC00536	100859921	2.85200361	0.02062306
AKR1C3	8644	−1.1591934	0.02067998
SLC35F4	341880	2.87473106	0.02084034
TMEM132C	92293	2.72804714	0.02097965
ZNRF3	84133	2.75068639	0.02180189
DNAH7	56171	2.71908351	0.02180189
DNAH5	1767	2.79268078	0.02180189
OLFM4	10562	2.82254611	0.02200501
CD177	57126	1.47582598	0.02202918
XACT	105463123	2.79067946	0.02236798
CUX2	23316	2.45417581	0.02265167
ZFPM2	23414	2.72660662	0.02274938
CELF4	56853	2.77353907	0.02290677
AOC1	26	−1.9693855	0.02290677
ARHGAP44	9912	2.36988885	0.02318599
ITGA8	8516	2.67617253	0.02341314
DNAH9	1770	2.15316906	0.02390959
NAV2	89797	2.065501	0.02402937
RPH3A	22895	1.43395877	0.02416397
HAGH	3029	−1.1505944	0.02416397
HS6ST3	266722	2.66248559	0.02416397
BNC2	54796	1.80494118	0.02416397
CDKAL1	54901	1.26064238	0.0242273
TANC1	85461	2.64768736	0.02427805
ADAMTS2	9509	2.77219477	0.02439429
FOXO3	2309	−1.0545568	0.02439429
LYPD6B	130576	2.78658275	0.02439429

(*continued on next page*)

Table 2 (*continued*)

SYMBOL	ENTREZID	logFC	FDR Pvalue
CIT	11113	2.54496196	0.02439429
CEP128	145508	2.04997475	0.02439429
KNL1	57082	2.77179892	0.02458248
LINC02346	100128714	2.7669875	0.02490947
WWC1	23286	2.62251704	0.02592827
B4GALNT3	283358	2.75591717	0.02632826
NAV3	89795	2.7358429	0.02673933
LGALS2	3957	1.2287913	0.0269841
PPFIA2	8499	2.69474193	0.0269841
RNF10	9921	−1.2311401	0.02702731
GNG12-AS1	100289178	2.73634565	0.02730122
KIF14	9928	2.7445241	0.02730849
NRCAM	4897	2.71555438	0.02730849
PDE6A	5145	2.71472323	0.02739376
CNBD1	168975	2.55991084	0.02739376
LOC401478	401478	2.44180061	0.02739801
PDE1A	5136	2.64230754	0.02809386
APBB2	323	2.25646628	0.02825678
SLC25A39	51629	−1.4306285	0.02879629
CA10	56934	2.56152343	0.02903683
CPEB3	22849	1.70000573	0.02903683
PRKD1	5587	2.61501449	0.02966374
CLNK	116449	2.68620975	0.02986698
PTMS	5763	−1.0308857	0.0301041
TSPO2	222642	−1.8291999	0.03074921
MYO1D	4642	1.61470213	0.03086006
CDH1	999	2.67289944	0.03100687
SPAG16	79582	1.85588648	0.03100687
TMEM121	80757	−1.924579	0.03104366
ALPK2	115701	2.67469782	0.03113099
HTR2C	3358	2.58253876	0.03113099
LINC02008	105377180	2.64679864	0.03116966
SEMA5A	9037	2.18001735	0.03203209
SCARA5	286133	2.66970312	0.03203623
TTC23	64927	2.66297408	0.03220676
DAB2IP	153090	2.65119261	0.03402921
SLC14A2	8170	2.35086711	0.03414497
PAPPA	5069	2.65526668	0.03414497
CPNE4	131034	2.48215727	0.03417291
WNT3	7473	2.58261406	0.03417291
USH2A	7399	2.27803586	0.03433533
FAM160A1	729830	2.57323376	0.03452578
DPH6-AS1	100507466	2.65394494	0.03461824
HHAT	55733	2.26314432	0.0347966
CDH4	1002	1.69028834	0.03495799
NA	110175910	2.53821495	0.03528998
CDKL4	344387	2.61792306	0.03570367
LINC02006	105374165	2.53601608	0.03595085
MAP2K3	5606	−1.1743433	0.03631631
ZNF711	7552	2.5533031	0.03631631
STK31	56164	2.64481627	0.03639862
NUDT4	11163	−1.9039016	0.03671002
PTPN14	5784	2.62020165	0.03735752
EFCAB6	64800	2.24116343	0.03793506
TMEM67	91147	2.62332653	0.03793506
ANO2	57101	2.51557751	0.03793506
CDC42BPA	8476	2.14076506	0.03799511
SLC30A8	169026	2.60552628	0.03842174
SLC44A5	204962	2.48350216	0.0385174
MX1	4599	−1.1903098	0.03881084
MYRIP	25924	2.53512408	0.03932354
KCNB1	3745	2.57732458	0.03951005
DPY19L1P1	100129460	2.63132374	0.04021626
NPRL3	8131	−1.0930346	0.04022775
ENAH	55740	2.44319559	0.04058568
SNTG2	54221	2.60859525	0.04062622
GNA14	9630	2.5767033	0.04062622
LINC01091	285419	2.56552207	0.04062622
STARD13	90627	1.89826281	0.04068121
DNAH14	127602	2.37255686	0.04068121
CHODL	140578	2.32912509	0.04068121
KALRN	8997	1.83356475	0.04114319
MIR3681HG	100506457	1.86532682	0.04129726
LOC440084	440084	2.58593834	0.04195192
MID1	4281	2.35149464	0.04195192

Table 2 (*continued*)

SYMBOL	ENTREZID	logFC	FDR Pvalue
KSR2	283455	2.11708233	0.04195192
LOC105376360	105376360	2.20093756	0.04195192
FAT3	120114	2.50751039	0.04201238
IQGAP3	128239	2.45515763	0.0425702
DGKI	9162	2.09418059	0.04281863
KCND3	3752	2.60313477	0.04329305
TEX14	56155	2.50183571	0.04329305
GSG1L	146395	2.33606432	0.0432938
THSD7B	80731	1.84656211	0.04367269
ALDH7A1	501	2.55179243	0.04393667
KIAA1549	57670	2.55671791	0.04443463
CRB1	23418	2.38815525	0.04454282
DYNC1I1	1780	2.50845142	0.04454282
ZNF890P	645700	2.44851754	0.04455788
AK4	205	2.56189847	0.04511996
HOMER1	9456	2.46496922	0.04519707
COBL	23242	2.49407406	0.04541562
TET1	80312	2.21826314	0.04541562
LINC00999	399744	−0.9962417	0.04567608
LINC01476	101927728	2.52088016	0.04567608
OCA2	4948	2.56051575	0.04572101
BEND3	57673	2.59073602	0.04581295
TLN2	83660	2.50778299	0.04581295
PLCB4	5332	2.28127475	0.04601789
C7orf73	647087	−1.0213255	0.04607736
ADIPOR1	51094	−1.252617	0.04620559
MYO5B	4645	2.43362467	0.04662833
NLRP6	171389	−0.9867137	0.04708896
CACNA1D	776	2.34034906	0.04722698
HDAC2-AS2	101927768	2.42198886	0.04725405
HELLS	3070	2.53248427	0.04725405
GBA3	57733	2.04192516	0.04725405
PNPLA2	57104	−1.119976	0.0473186
PLPP3	8613	2.41863804	0.04746373
LOC100287944	100287944	2.49172557	0.04751111
TP63	8626	2.432457	0.04760674
GRID1	2894	2.39595254	0.04785942
PQLC1	80148	−1.0425829	0.04800528
FANK1	92565	2.46103611	0.04842995
GPC4	2239	2.50875495	0.04919306
MPP1	4354	−1.0939679	0.04949817
SORBS1	10580	2.47822236	0.04949817
SPOCK1	6695	2.40999057	0.04949817
NALCN-AS1	100885778	2.48612732	0.04949859
PACRG	135138	2.38156648	0.04949859
NFIX	4784	−0.9779112	0.04949859
LINC01088	100505875	2.46957799	0.04949859
DENND2C	163259	2.49293865	0.04949859
LOC100506207	100506207	2.47000662	0.04973983
MYT1L	23040	2.32705564	0.04973983
GLI3	2737	2.4956466	0.04976421

estrogen exposure can lead to DNA methylation (Yang et al., 2018). It is also possible that the changes could arise from the long term emotional and physical experience of parenthood. For example, it has been shown that parental stress can alter DNA methylation (Wright et al., 2017); however, this was regarded by the authors as harmful whereas we are considering the possibility that parity could be beneficial.

Among the differentially methylated genes was the *ESRRG* gene, a transcriptional activator of *DNMT1* found to be differentially methylated in in relapsing-remitting MS patients (Kulakova et al., 2016). Another gene was the *RELN* (reelin), a gene reported to show significant association with age of onset in MS (Baranzini et al., 2009). Another differentially expressed and differentially methylated gene *PAK3* is involved in certain types of intellectual disability and regulated myelin sheath development (Maglorius Renkilaraj et al., 2017). Again, we have shown these changes in peripheral blood rather than in brain cells. It is possible that the effects of hormones such as estrogen would affect all cells equally and the effects that we observe are the cumulative effects of repeated exposure to pregancy hormones. However it is also known that methylation changes can be specific for cells and tissues, so further

Table 3
Functional annotation of the differentially expressed genes using the Webgestalt interface and the drug gene interaction database.

Druggable Gene Category	# Genes	
Druggable genome	176	
Clinically actionable	19	
Webgestalt functional annotation		
Pathway common		
PathwayName	#Gene	Statistics
Axon guidance	21	R = 3.81;rawP = 1.74e-07;adjP = 1.64e-05
Developmental Biology	25	R = 2.30;rawP = 0.0001;adjP = 0.0094
Cell-Cell communication	11	R = 3.74;rawP = 0.0002;adjP = 0.0188
Disease	#Gene	Statistics
Mental Disorders	56	R = 4.32;rawP = 2.04e-20;adjP = 5.63e-18
Autistic Disorder	25	R = 7.89;rawP = 8.83e-16;adjP = 2.44e-13
Autism Spectrum Disorder	25	R = 7.05;rawP = 1.38e-14;adjP = 3.81e-12
Schizophrenia	36	R = 4.47;rawP = 5.66e-14;adjP = 1.56e-11
Bipolar Disorder	35	R = 4.55;rawP = 7.80e-14;adjP = 2.15e-11
Adhesion	51	R = 3.20;rawP = 2.27e-13;adjP = 6.27e-11
Anxiety Disorders	21	R = 6.01;rawP = 4.39e-11;adjP = 1.21e-08
Mood Disorders	25	R = 4.11;rawP = 2.56e-09;adjP = 7.07e-07
Depression	20	R = 4.57;rawP = 1.68e-08;adjP = 4.64e-06
Neurobehavioral Manifestations	24	R = 3.38;rawP = 2.20e-07;adjP = 6.07e-05
Chromosome Disorders	25	R = 3.16;rawP = 4.62e-07;adjP = 0.0001
Mental Retardation	25	R = 3.00;rawP = 1.15e-06;adjP = 0.0003
Neurologic Manifestations	25	R = 2.63;rawP = 1.20e-05;adjP = 0.0033
Drug	#Gene	Statistics
bupropion	42	R = 15.76;rawP = 1.80e-39;adjP = 3.96e-38
ziprasidone	8	R = 8.84;rawP = 2.46e-06;adjP = 5.41e-05
nifedipine	5	R = 8.65;rawP = 0.0002;adjP = 0.0044
azacitidine	9	R = 3.73;rawP = 0.0007;adjP = 0.0154
melatonin	5	R = 5.85;rawP = 0.0015;adjP = 0.0330
decitabine	7	R = 3.92;rawP = 0.0020;adjP = 0.0440

R = enrichment ratio.
rawR = nominal p-value.
adjR = Benjamini Hochnerg adjusted *p*-value.

information about brain methylation is needed.

The genes associated with parity also overlapped with the large MS GWAS ($n = 10$), genes differentially expressed between MS and healthy controls at baseline in humans (Gilli et al., 2010) ($n = 28$). There was also overlap with genes differentially expressed between rats with MBP-EAE and control rats (Inglis et al., 2012) ($n = 149$). This study was used as it is the only published list of genes regulated in the CNS in EAE. Also, comparison to gene expression profiles from untreated MS patients indicated an overlap of our findings with differentially expressed genes between controls and three clinical MS subtypes ($n = 85$). This implies that the shaping of the transcriptome by prior pregnancy is particularly detectable in the MS genes, consistent with the clinical effects of pregnancy on MS. The modest overlap of genes with some of the previous studies is expected, given that our study only assessed women with MS and investigated gene expression changes associated with the number of pregnancies in MS, in contrast to other studies. Nevertheless, these results demonstrate that parity-related gene expression changes in MS candidate genes might be one mechanism explaining the apparent protective effect of parity on long-term outcome of MS.

This study has several limitations. This was a small study and replication in larger samples is needed. Given the cross-sectional nature of this study, it might be worthwhile to perform a larger longitudinal analysis of parous and non-parous women with MS to assess different gene expression trajectories. While we have accounted for all known environmental factors, we acknowledge that disease modifying therapies and other unknown environmental factors might also affect the epigenome. Pregnancy itself has a vast impact on gene expression, so it is hard to discern between a true effect and simple confounding despite accounting for all known variables. Nevertheless, to the best of our knowledge this is the first and only study where we have examining the beneficial effects of parity in MS by assaying genome-wide gene expression patterns and DNA methylation to uncover the biological underpinnings of the apparent (Alwan and Sadovnick, 2012) long-term protective effect of parity on MS outcome.

Our findings suggest that prior pregnancy alters the transcriptomic landscape among women with MS with alteration of key genes and pathways, particularly those related to neuronal growth, this could possibly provide long term protection against the progressive pathology that contributes to long-term disability in MS.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jneuroim.2018.12.004.

Conflict of interest/financial disclosures

There were no commercial sponsors or commercial relationships related to the current work.

Authors' contributions

Divya Mehta analysed and interpreted the data and wrote the first draft of the manuscript. Shivangi Wangi, Leanne Wallace and Anjali Henders performed the experiments in the lab of Naomi Wray. Pamela A McCombe conceived the study and supervised the project. All authors provided critical comments on the manuscript.

Declaration of interest

None.

Acknowledgements

The authors would like to thank all the participants involved in this study. This project was funded by the University of Queensland via an Early Career Research grant to Divya Mehta. Divya Mehta received salary support from the Queensland University of Technology Vice Chancellor Fellowship. NRW acknowledge NHMRC grants (1078901, 1113400).

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