Variable DNA methylation in genes involved in the immune response is a potential predictor of chronic widespread pain after motor vehicle collision

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Background

DNA methylation at CpG dinucleotides is an important epigenetic mechanism regulating gene expression. Loci of variable DNA methylation often correlate with regulatory DNA regions controlling gene transcription. Identifying regulatory regions differentially methylated between individuals who do and do not develop chronic widespread pain (CWP) after motor vehicle collision (MVC) may provide insights into biologic mechanisms mediating CWP development.

Methods

Study design: Nested case-control study using data from a prospective cohort study of 948 European Americans who presented to the emergency department (ED) after MVC and received follow-up evaluation at 6 wks and 6 & 12 months.

Subjects: 11 females with CWP (≥7 body regions with pain at 6 weeks, 6 months and/or 1 year) were selected and age-matched to 11 female controls with no pain or only mild regional pain (Table 1).

DNA was procured from patient blood samples obtained in the ED. Genome-wide CpG methylation analyses were performed using the Illumina HumanMethylation 450k platform.

Data analyses: Two approaches were utilized:

a) Identification of loci with differentially variable CpG methylation
   1) Interquartile range of CpG methylation levels at each CpG locus was calculated for both groups (Fig.1);
   2) Loci with $[IQR_{CWP} - IQR_{control}] > 0.10$ were identified;
   3) Ingenuity Pathway Analysis software was used to identify overrepresented pathways (Table 2, Fig.2).

b) Identification of genomic regions with CpG methylation levels associated with CWP development:
   1) *bumphunter* function in *minfi* package (Bioconductor, R 3.0.2) was used to identify genomic regions of significant association with CWP (Fig.3-5);
   2) *GREAT:* Genomic Regions Enrichment of Annotations Tool (great.stanford.edu/) was used to identify overrepresented pathways among the DNA regions associated with CWP.

Table 1. Characteristics of study participants

	Cases	Controls
N	11 females	11 females
Mean age (range)	41 (26-50)	41 (25-60)
N of hit body sites*	1.3 (0-5)	0.9 (0-2)
Rear-ended	3/10	1/11
Overall pain , 0-10 NRS		
week 6	8.6 (6-10)	0.1 (0-1)
month 6	8.3 (7-10)	0 (0-0)
year 1	8.7 (8-10)	0.2 (0-2)
N of body pain regions		
week 6	10.7 (3-19)	0.5 (0-1)
month 6	11.1 (5-19)	0 (0-0)
year 1	11.0 (4-19)	0.1 (0-1)

*Number of body regions striking an object during collision

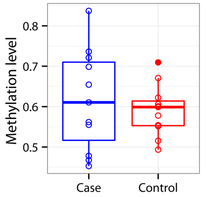

Fig.1. Example of differentially variable methylation (cg01035815 locus in ITPR3)

Table 2. Top overrepresented pathways among genes with differentially variable methylation (Ingenuity Pathway Analysis)

Name of pathway	Genes	p-value
Antigen Presentation Pathway	CALR, HLA-DQB2, HLA-DRA, HLA-DRB1, HLA-DMB, HLA-F, TAP2	6.80E-05
Role of NFAT in Regulation of the Immune Response	GNA15, GNA12, HLA-DRA, ITPR3, GNA11, HLA-DRB1, HLA-DMB, ITPR1, GNG7, NFATC1	4.80E-04
CXCR4 Signaling	DOCK1, GNA15, ADCY5, GNA12, ITPR3, GNA11, RHOJ, ITPR1, GNG7	2.90E-03

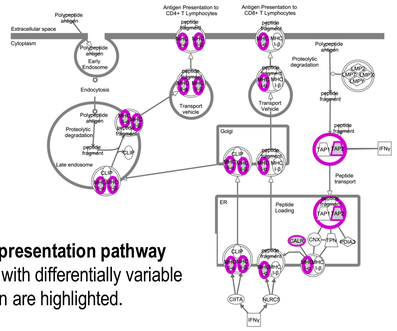

Fig.2. Antigen presentation pathway
Genes/proteins with differentially variable CpG methylation are highlighted.

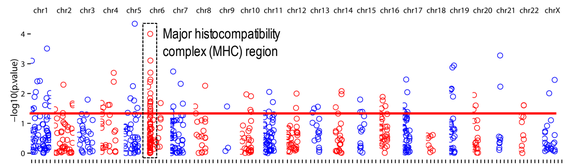

Fig.3. Manhattan plot of association p-values (Bioconductor package *minfi*)

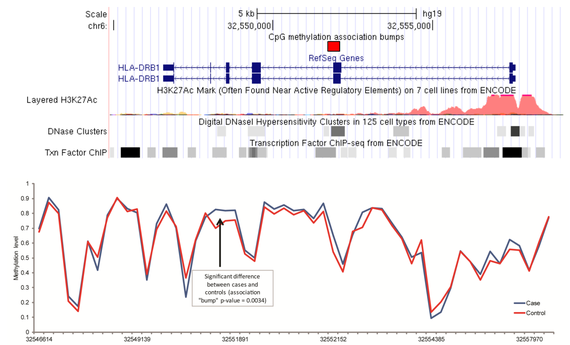

Fig.4. Region of association in HLA-DRB1 gene

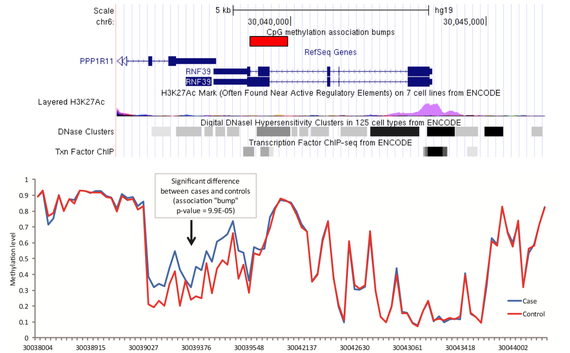

Fig.5. Region of association in RNF39 gene

Table 3. Top overrepresented gene ontology (GO) categories among all genes significantly associated with CWP (*GREAT* analysis)

GO category	Term name	FDR Q-Value	Fold Enrichm	Gene Hits
GO Molecular Function	MHC class II receptor activity	1.40E-03	56.2	4
GO Biological Process	Antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	2.01E-08	47.3	8
Cellular component	MHC protein complex	1.01E-09	29.6	10

Results

a) Differential variability of DNA methylation between individuals who did and did not subsequently develop CWP: A total of 398 genes with differentially variable methylation at CpG loci were identified. Top statistically significant overrepresented biologic pathways were the antigen presentation pathway, the nuclear factor of activated T-cells (NFAT) pathway, and the chemokine CXCR4 signaling pathway (Table 2, Figure 2).

b) Genomic regions with CpG methylation levels associated with CWP development: 112 genomic regions were identified with methylation levels associated with CWP ($p<0.05$). Of those regions, 14/112 (13%) were located within the major histocompatibility complex (MHC) region on chromosome 6 (Fig.3). These regions generally localized to potentially important regulatory regions of MHC genes (Fig.4-5). *GREAT* analysis confirmed statistically significant overrepresentation of multiple pathways involved with antigen presentation (Table 3).

Conclusion

These preliminary results suggest that variable methylation of MHC gene regions may influence vulnerability to chronic widespread pain after motor vehicle collision. Further studies are needed to confirm these findings and to evaluate the association between methylation and the expression of specific MHC genes.

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