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Functional characterization of the *MECP2/IRAK1* lupus risk haplotype in human T cells and a human *MECP2* transgenic mouse

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Abstract

Genetic polymorphism in *MECP2/IRAK1* on chromosome Xq28 is a confirmed and replicated susceptibility locus for lupus. High linkage disequilibrium in this locus suggests that both *MECP2* and *IRAK1* are candidate genes for the disease. DNA methylation changes in lupus T cells play a central role in the pathogenesis of lupus, and MeCp-2 (encoded by *MECP2*) is a master regulator of gene expression and is also known to recruit DNA methyltransferase 1 (DNMT1) during DNA synthesis. Using human T cells from normal individuals with either the lupus risk or the lupus protective haplotype in *MECP2/IRAK1*, we demonstrate that polymorphism in this locus increases *MECP2* isoform 2 mRNA expression in stimulated but not unstimulated T cells. By assessing DNA methylation levels across over 485,000 methylation sites across the entire genome, we further demonstrate that the lupus risk variant in this locus is associated with significant DNA methylation changes, including in the HLA-DR and HLA-DQ loci, as well as interferon-related genes such as *IFI6*, *IRF6*, and *BST2*. Further, using a human *MECP2* transgenic mouse, we show that overexpression of *MECP2* alters gene expression in stimulated T cells. This includes overexpression of *Eif2c2* that regulates the expression of multiple microRNAs (such as miR-21), and the histone demethylase *Jhdm1d*. In addition, we show that *MECP2* transgenic mice develop antinuclear antibodies. Our data suggest that the lupus associated variant in the *MECP2/IRAK1* locus has the potential to affect all 3 epigenetic mechanisms: DNA methylation, microRNA expression, and histone modification. Importantly, these data support the notion that variants

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within the *MECP2* gene can alter DNA methylation in other genetic loci including the HLA and interferon-regulated genes, thereby providing evidence for genetic-epigenetic interaction in lupus.

Keywords

MECP2, *IRAK1*; lupus; epigenetics; polymorphism; DNA methylation; T cells; transgenic mouse

1. Introduction

The etiology of lupus is incompletely understood. However, both genetic and environmental factors are implicated in disease pathogenesis. Indeed, the current paradigm suggests that lupus develops in genetically susceptible individuals upon the exposure to disease-triggering environmental factors. While the genetic etiology of lupus has been supported by a number of confirmed genetic susceptibility loci for the disease, identification of environmental triggers for lupus has been more challenging [1]. Nonetheless, a number of environmental triggers implicated in disease pathogenesis, such as UV light exposure, procainamide, and hydralazine, have been shown to induce autoimmunity by altering T cell DNA methylation [2]. Indeed, abnormal DNA methylation in CD4⁺ T cells plays an important role in the pathogenesis of lupus [2–7], and DNA demethylation in CD4⁺ T cells is sufficient to cause lupus in mice [8]. Epigenetic regulation plays an important role in CD4⁺ T cell responses including differentiation into Th17 cells and regulatory T cells that are implicated in autoimmunity [9]. Importantly, a recent report in identical twins discordant for lupus identified significant DNA methylation changes between the lupus-affected and unaffected twin pairs, emphasizing the role for environmental exposures on inducing pathogenic epigenetic changes [6].

MeCp-2 (methyl-CpG-binding protein 2) is a transcriptional regulator intimately involved in regulating the expression of methylation sensitive genes. The binding of MeCp-2 to methylated CpG sites in gene promoter sequences recruits histone deacetylase complexes, leading to histone tail deacetylation, transcriptionally-repressive chromatin conformation, and silencing of gene expression [10]. MeCp-2 is also known to recruit DNA methyltransferase 1 (DNMT1) during DNA synthesis [11]. Furthermore, recent evidence suggests that MeCp-2 can act as a transcriptional activator via recruiting the transcription factor CREB1 [12], and that MeCp-2 also has a role in regulating RNA splicing [13].

We have previously established and confirmed the genetic association between SNPs located within the gene encoding for MeCp-2 (*MECP2*) and lupus [14, 15]. Genetic association between lupus and a neighboring gene, *IRAK1*, has been also established [16, 17]. The two genes, *MECP2* and *IRAK1* are in high linkage equilibrium, suggesting that lupus-associated variants in this locus might have functional consequences on either of the two genes or possibly both *MECP2* and *IRAK1* [14, 18].

Herein, we examine the functional effects of the *MECP2/IRAK1* lupus-associated polymorphism and demonstrate increased expression of an *MECP2* transcript variant in stimulated T cells from normal healthy individuals carrying the lupus risk haplotype in this locus. We further determine the effects of this lupus-associated haplotype on genome-wide DNA methylation in stimulated human T cells, and study the effect of increased *MECP2* expression in T cells using a human *MECP2* transgenic mouse.

2. Methods

2.1 Human T cell culture and activation

Primary T cells from healthy female European-American donors homozygous for the lupus risk or protective *MECP2/IRAK1* haplotypes were studied. These haplotypes were tagged by the SNP rs17435 in this locus. For each sample, T cells were isolated from 1×10^7 fresh frozen peripheral blood mononuclear cells (PBMCs) obtained from the Oklahoma Immune Cohort collection through the Oklahoma Rheumatic Disease Resources Cores Center biorepository, by negative selection using the Human Pan T Cell Isolation Kit II (Miltenyi Biotec, Cambridge, MA). Briefly, PBMCs were thawed and rested overnight in complete T cell media (RPMI, 10% FBS, 1% penicillin-streptomycin solution and 50uM β -mercaptoethanol). The primary T cells were then isolated and were immediately re-plated with and without stimulation. For T cell stimulation, T cells were re-plated onto anti-CD3-coated (10ug/ml) 12-well plates with complete T cell media containing anti-CD28 (2.5ug/ml) and IL-2 (25U/ml) to begin activation. Cells were incubated at 37°C for time points 6, 12, and 24 hours, after which cells were collected and RNA or DNA isolated. RNA extraction was performed using a combination of TRIzol (Invitrogen, Carlsbad, CA) and RNeasy kits (Qiagen, Valencia, CA), in a hybrid protocol as previously described [15]. DNA was isolated by the DNeasy Kit (Qiagen, Valencia, CA) for subsequent use in the DNA methylation studies.

2.2 Real-Time PCR

Transcript levels of *MECP2A* (isoform 1), *MECP2B* (isoform 2), and *DNMT1* were measured by real-time PCR using the iScript One Step RT-PCR Kit with SYBR green (Bio-Rad, Hercules, CA) and the Rotor-Gene 3000 Thermocycler (Corbett Research, Australia). Samples were quantified with a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE) after removing any contaminating genomic DNA using the Turbo DNA-Free Kit (Ambion, Austin, TX) according to manufacturer's directions. All samples were normalized to the internal control housekeeping gene, *ACTB* (β -actin), in 20ul reaction volumes with 25ng RNA in each reaction. The PCR protocol was as follows: The following primers were used at 300nM concentrations: *MECP2A* (isoform 1) forward: 5'-CTGGGATGTTAGGGCTCAGGGA-3', reverse: 5'-AGAGTGGTGGGCTGATGGCT-3'; *MECP2B* (isoform 2) forward: 5'-AGGCGAGGAGGAGAGACTGGAA-3', reverse: 5'-AGAGTGGTGGGCTGATGGCT-3'; *DNMT1* forward: 5'-CGACTACATCAAAGGCAGCAACCTG-3', reverse: 5'-TGGAGTGGACTTGTGGGTGTTCTC-3'; and *ACTB* forward: 5'-GCACCACACCTTCTACAATGAGC-3', reverse: 5'-GGATAGCACAGCCTGGATAGCAAC-3'. All of the primers were purchased from Integrated DNA Technologies (Coralville, IA). The PCR conditions used were as follows: 10 minutes at 50°C, 5 minutes at 95°C, and 45 cycles of 95°C for 10 seconds followed by 55°C for 30 seconds. Relative transcript levels were quantified using the $2^{-(\Delta\Delta CT)}$ method [19].

2.3 DNA methylation analysis

Genome-wide DNA methylation was quantified in T cells from healthy individuals with the *MECP2/IRAK1* risk and protective haplotypes 24 hours post stimulation using the Illumina Infinium HumanMethylation450 BeadChip platform (San Diego, CA), which allows for the assessment of the methylation status of over 485,000 methylation sites across the entire genome. This platform covers 99% of RefSeq genes, with an average of 17 CpG sites per gene distributed across the promoter, 5'-UTR, first exon, gene body, and 3'-UTR. Validation of the methylation array data was performed using bisulfite sequencing in known hypermethylated and hypomethylated loci as previously described [20].

2.4 MECP2 transgenic mice

Female mice transgenic for human *MECP2* (FVB-Tg(*MECP2*)1Hzo/J), hereafter referred to as *MECP2*-Tg, and age- and sex-matched wild-type mice (FVB/NJ) were obtained from The Jackson Laboratories (Bar Harbor, Maine). At 8 weeks of age the mice were sacrificed and splenic CD4+ T cells were isolated using indirect labeling and magnetic bead separation (Miltenyi Biotec, Cambridge, MA). Serum samples were also collected at the same time. RNA was isolated from 5×10^6 purified CD4+ T cells immediately after cell separation. For stimulated CD4+ T cells, another 5×10^6 cells were stimulated over night with PMA (50ng/ml) and ionomycin (750ng/ml) and then RNA extracted. RNA extraction was performed using a combination of TRIzol (Invitrogen, Carlsbad, CA) and RNeasy kits (Qiagen, Valencia, CA) as previously described [15]. RNA concentration was determined with a NanoDrop 1000 Spectrophotometer (Thermo Scientific, Wilmington, DE) and then qualitatively assessed for degradation with 28:18S ribosomal RNA, using a capillary gel electrophoresis system (Agilent 2100 Bioanalyzer, Agilent, Wilmington, DE). Gene expression profiling was performed in stimulated and unstimulated CD4+ T cells from *MECP2*-Tg mice and wild-type controls using the MouseWG-6 v2 BeadChip arrays (Illumina).

Sera obtained from *MECP2*-Tg and wild-type mice at 8 weeks of age were tested for the presence of antinuclear antibody (ANA) and anti-dsDNA antibody using quantitative ELISA assays (Alpha Diagnostic, San Antonio, TX).

2.5 Statistical analysis

Microarray expression analysis was performed and corrected for multiple testing using Significance Analysis of Microarrays (SAM) [21]. Gene ontology, network, and pathway analysis was performed using Ingenuity Pathway Analysis (Redwood City, CA). A Student's *t*-test was used in comparisons of continuous variables. DNA methylation analysis was performed using GenomeStudio (Illumina). Differentially methylated loci were defined as CpG loci with an average difference in DNA methylation level of at least 1.2-fold, and differential methylation score of $|\beta| \geq 0.3$ after adjusting for multiple testing using the Benjamini and Hochberg false discovery rate. Differential methylation score is defined as $10 * \text{sgn}(\beta_{\text{Risk}} - \beta_{\text{Protective}}) * \log_{10} p$. β is the average level of DNA methylation on each CpG site and ranges from 0 to 1 (0, completely demethylated; 1, completely methylated).

3. Results

3.1 Effect of the MECP2/IRAK1 lupus-associated haplotype on MECP2 and IRAK1 expression in human T cells

Since a DNA methylation defect in T cells has been previously shown to play an important role in the pathogenesis of lupus, we tested the effect of the lupus-associated *MECP2/IRAK1* haplotype on *MECP2* mRNA expression in primary T cells isolated from healthy donors. The expression levels of the two known *MECP2* transcript variants (*MECP2A* and *MECP2B*) were measured in unstimulated and stimulated T cells from individuals homozygous for the risk haplotype ($n = 14$) and the non-risk haplotype ($n = 20$). Twenty four hours after stimulation with anti-CD3/anti-CD28 antibodies, the expression of *MECP2B* was significantly higher in T cells from individuals carrying the lupus-associated *MECP2/IRAK1* risk haplotype ($P = 0.04$). There was no difference in *MECP2A* expression in stimulated T cells, and no difference in the expression of either *MECP2* transcript variants in unstimulated T cells (Figure 1).

Next, we examined if the *MECP2/IRAK1* haplotype also affects *IRAK1* expression in T cells similar to *MECP2*. We did not observe any difference in *IRAK1* mRNA expression as

influenced by the presence of the *MECP2/IRAK1* lupus-associated haplotype in T cells (Unstimulated, risk= 1.56 ± 0.23 , non-risk= 1.36 ± 0.16 , $P= 0.46$; stimulated, risk= 0.87 ± 0.14 , non-risk= 0.60 ± 0.093 , $P= 0.11$).

3.2 Effect of the *MECP2/IRAK1* lupus-associated haplotype on DNMT1 expression in human T cells

MeCp-2 (encoded by *MECP2*) represses gene expression primarily by recruiting the histone deacetylase complex to methylated DNA sequences. In addition, MeCp-2 recruits DNMT1 during DNA replication to ensure efficient replication of DNA methylation patterns during DNA synthesis. Further, the expression of *Mecp2* has been previously shown to be increased in *Dnmt1* knockout mice [22]. Therefore, we tested the expression of *DNMT1* in the same normal T cells from individuals with the lupus risk and non-risk *MECP2/IRAK1* haplotype but observed no differences in the mRNA levels between the two groups with or without T cell stimulation ($P= 0.48$ and 0.55 , respectively).

3.3 Effect of the *MECP2/IRAK1* lupus-associated haplotype on Genome-wide DNA methylation in human T cells

Since the expression of at least one *MECP2* transcript variant was increased in stimulated T cells (24 hrs post stimulation) from individuals with the lupus-associated *MECP2/IRAK1* haplotype, and MeCp-2 recruits DNMT1 outside of the replication fork, we tested the hypothesis that the lupus-risk haplotype could influence DNA methylation patterns. To do this, we examined global DNA methylation by quantifying the methylation status of over 485,000 methylation sites simultaneously in T cells 24 hours post stimulation from normal individuals with the risk and the non-risk *MECP2/IRAK1* haplotype ($n=6$) using the Infinium HumanMethylation450 BeadChip arrays. Array validation was performed using the same samples by bisulfite sequencing in known hypermethylated and hypomethylated loci as described previously [20], and showed a correlation r^2 value of 0.83 between the array data and bisulfite sequencing data.

We did not observe a difference between the average global DNA methylation between the risk and protective haplotypes (risk= 0.5562 ± 0.0021 , protective= 0.5576 ± 0.0022 [mean $\pm$ SEM], $P= 0.64$). However, we identified 133 hypermethylated and 112 hypomethylated CpG sites in individuals with the risk compared to the protective *MECP2/IRAK1* haplotype (fold difference > 1.20). Interestingly, the most significant canonical pathways identified in these differentially methylated loci was the protein ubiquitination pathway ($P= 9.16 \times 10^{-3}$) and the antigen presentation pathway ($P= 1.44 \times 10^{-2}$). The *MECP2/IRAK1* risk haplotype is associated with significant methylation changes in the HLA, and in particular HLA class II (Table 1). The *BST2* gene, an interferon-inducible membrane-bound protein that helps restrict the release of retroviral particles, is hypomethylated in individuals with the risk *MECP2/IRAK1* haplotype. Other interferon-related genes that are hypomethylated include *IFI6* and *IRF6*. Indeed, network analysis of differentially methylated genes highlight interferon-alpha and NFkB (Figure 2). The complete list of differentially methylated CpG sites between stimulated T cells with and without the *MECP2/IRAK1* lupus-associated haplotype are shown in Supplementary Table 1.

3.4 The effect of *MECP2* overexpression in stimulated and unstimulated T cells and autoantibody production in a human *MECP2* transgenic mouse

Since MeCp-2 is a transcriptional regulator, and the expression of *MECP2* is higher in stimulated T cells from individuals with the lupus-associated risk variants, we studied the effect of *MECP2* overexpression using a mouse transgenic for human *MECP2* (*MECP2*-Tg). This mouse includes the entire human *MECP2* gene and expresses MeCp-2 at approximately 2-fold wild-type levels [23]. Stimulated and unstimulated CD4⁺ T cells from the spleen

were isolated and gene expression profiles determined using expression microarrays. In unstimulated T cells, we detected 4 overexpressed and 18 underexpressed transcripts in *MECP2*-Tg mice compared to wild-type controls. However, upon stimulation, we detected 100 significantly overexpressed and 9 underexpressed genes (Figure 3). Network analysis indicated the differentially expressed genes in these stimulated T cells were statistically overrepresented in several networks including “Gene Expression, Post-Translational Modification, Cellular Assembly and Organization”, “Protein Synthesis, Antigen Presentation, Cell-To-Cell Signaling and Interaction”, “Cell Cycle, Cellular Movement, Cell Death”, “Cell Death, Cell Morphology, Cellular Development”, and “Connective Tissue Disorders, Inflammatory Disease, Inflammatory Response”. Interestingly, *Eif2c2* (eukaryotic translation initiation factor 2C,2) is among the upregulated genes in *MECP2*-Tg mice. This gene regulates the expression of a number of microRNAs, including *miR-21* which is known to be overexpressed in lupus CD4⁺ T cells [24]. Several other translation associated factor genes were overexpressed in the human *MECP2*-Tg mouse including *Eif4ebp2* (1.76-fold). Other overexpressed genes in stimulated T cells from the *MECP2* transgenic include the histone demethylase *Jhdm1d* (also known as *A630082K20Rik*) (1.69-fold), and *Cd44* (1.18-fold), a recently reported susceptibility gene for lupus [25]. The expression of *Faf2* (Fas associated factor family member 2) is also increased in *MECP2*-Tg mice (1.38-fold). *FAF2* is upregulated in T cells from patients with atopic dermatitis and is suspected to play a role in modulating T cell apoptosis [26]. Canonical pathway analysis highlighted several overlapping pathways including IL-6 signaling ($P=1.62\times10^{-2}$), IL-17 signaling ($P=4.3\times10^{-2}$), PPAR α /RXR α activation ($P=4.63\times10^{-2}$), and CD40 signaling ($P=3.38\times10^{-2}$).

Because the expression of *MECP2* is increased in T cells with the risk *MECP2/IRAK1* polymorphism, and *MECP2*-Tg mice differentially express genes involved in inflammatory pathways, we hypothesized that overexpression of *MECP2* might result in autoantibody production. Indeed, we detected elevated antinuclear antibody production in the sera obtained from the *MECP2*-Tg mouse compared to wild-type controls (Figure 4). However, no anti-dsDNA production was detected by ELISA (data not shown).

4. Discussion

The genetic locus containing *MECP2* is a confirmed susceptibility locus for systemic lupus erythematosus, though the causal variant(s) in this locus has not yet been identified. Because T cell DNA methylation defects play an important role in the pathogenesis of lupus, and MeCP-2 is a key regulator for the expression of methylation sensitive genes, we performed studies to characterize the effect of the lupus-associated *MECP2/IRAK1* haplotype using human T cells homozygous for the risk and protective haplotypes in this locus. We demonstrated increased expression of one *MECP2* transcript variant in stimulated but not unstimulated T cells from normal healthy individuals carrying the lupus-associated haplotype.

Next we performed a genome-wide DNA methylation study in a subset of the same stimulated human T cells to determine if the lupus-associated *MECP2/IRAK1* haplotype and the observed difference in *MECP2B* mRNA expression were associated with DNA methylation changes. Indeed, these data suggest significant wide-spread locus-specific DNA methylation changes, though global DNA methylation levels were not different. We detected significant DNA methylation differences in the HLA region and in particular involving CpG sites in the HLA-class II loci including HLA-DR, and HLA-DQ (Table 1). These data suggest that the lupus-associated *MECP2/IRAK1* haplotype is associated with DNA methylation changes which, in turn, might influence other disease-relevant pathways and other susceptibility loci outside of the *MECP2/IRAK1* locus. Indeed, HLA-DR contains one

of the most robustly associated genetic susceptibility loci for lupus, and both HLA-DR and HLA-DQ are known susceptibility loci for primary Sjögren's syndrome [27]. We have previously reported the genetic association between *MECP2* and primary Sjögren's syndrome [28]. Therefore, the genetic-epigenetic interaction suggested by our findings can extend beyond lupus and might also play a role in the pathogenesis or genetic susceptibility of other autoimmune diseases. Whether there is also a genetic epistasis effect between the lupus susceptibility loci in the HLA region and *MECP2* remains to be determined.

Our network analysis of differentially methylated genes associated with the *MECP2/IRAK1* haplotype highlights NFkB and interferon alpha (Figure 2). Several interferon-regulated genes such as *BST2*, *IFI6*, and *IRF6* were hypomethylated in the presence of the lupus-associated *MECP2* variants. We have previously shown that *BST2* is hypomethylated in CD4+ T cells from lupus patients compared to controls [20]. This interferon-regulated gene (also known as Tetherin) restricts the release of viral particles, such as HIV-1, by tethering them to the cell surface [29]. We previously suggested that hypomethylation and overexpression of *BST2* in lupus CD4+ T cells might provide a plausible mechanistic explanation for the reported lower HIV-1 infection in lupus patients [30]. Indeed, our data suggest that the *MECP2* variants associated with increased risk for lupus might be at the same time protective against retroviral infections such as HIV-1, as indicated by hypomethylation of *BST2*. This phenomenon has been previously reported with other genetic variants, with the classical example being the sickle cell mutation that is protective against malaria [31].

To further examine the extent to which *MECP2* upregulation affects T cell function, we used a transgenic mouse that expresses human *MECP2*. Gene expression profiles in stimulated CD4+ T cells identified significant gene expression differences in the *MECP2*-Tg mouse compared to wild-type controls. Importantly, these studies found overexpression of *Eif2c2* in *MECP2*-Tg mice. *Eif2c2* regulates the expression of *miR-21*, which has been reported to be overexpressed in lupus CD4+ T cells, and resulting in reduced DNMT1 expression, as well as hypomethylation and overexpression of methylation sensitive genes in CD4+ T cells [24]. Further, the histone demethylase *Jhdm1d* is ~ 2 times overexpressed in stimulated T cells from *MECP2*-Tg mice compared to controls, suggesting a possible direct effect of increased *MECP2* expression on histone tail modification in T cells.

Our studies suggest that the disease-associated variants in *MECP2* could influence all three mechanisms of epigenetic regulation; DNA methylation, histone modification, and microRNA expression, which adds to the functional pathogenic complexity of the lupus-associated variants in this locus.

5. Conclusions

sMECP2 was previously implicated as a susceptibility gene for lupus, yet how it might affect disease etiology was not known. In this study, we identified specific functional consequences of the *MECP2* polymorphism that extend beyond the genetic locus containing *MECP2* and *IRAK1*. We found that the risk variant of *MECP2* correlates with higher relative expression of isoform 2 (*MECP2B*) in stimulated T cells. We show evidence for genetic-epigenetic interaction associated with the lupus-associated *MECP2* variants, suggested by differential DNA methylation in multiple genetic loci including class II HLA genes. Future studies of disease-associated polymorphism in *MECP2* are warranted, both to determine the functional effects in other cell types and to identify the causal variants.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Research Highlights

- The lupus variants in *MECP2/IRAK1* increase *MECP2* expression in stimulated T cells
- These variants alter DNA methylation in the HLA and interferon-related genes
- MeCP2 overexpression in mice alters gene expression in stimulated T cells.
- MeCP2 overexpression is associated with antinuclear antibody production in mice

Figure 1. Relative mRNA expression of *MECP2A* (A) and *MECP2B* (B) in stimulated T cells (A and B, respectively), and unstimulated T cells (C and D, respectively) from healthy individuals with the lupus risk and the non-risk haplotype in the *MECP2/IRAK1* locus. Expression is depicted relative to β -actin. NS, not significant.

Figure 2.

Network analysis of differentially methylated genes in stimulated T cells from normal healthy individuals with the lupus-risk compared to the protective *MECP2/IRAK1* haplotype. Green and red colors represent hypomethylated and hypermethylated genes in the risk haplotype, respectively. Colorless genes and molecules are not differentially methylated but brought into the network by the analysis software to denote relationships with differentially methylated genes.

Figure 3. A heat map depicting differentially expressed genes in stimulated CD4+ T cells from *MECP2*-Tg mice compared to controls (n=6). Red and green colors represent upregulated and downregulated transcripts, respectively.

Figure 4. Anti-nuclear antibody levels in the serum of mice transgenic for human MECP2 compared to wild-type age- and sex-matched controls (174.7 ± 23.92 versus 49.59 ± 10.87 ug/ml (mean $\pm$ SD), n=6, P= 0.0008)

CpG sites in the HLA class II loci with differential DNA methylation in T cells between individuals with the lupus-risk and the protective haplotype in MECP2/IRAK

Table 1

Target CG ID	Location on Chr. 6	Gene	Methylation (β)				Differential methylation score
			Risk	Protective	Ratio		
cg04418355	32729174	HLA-DQB2	0.73	0.60	1.20		31.86
cg00103771	32525805	HLA-DRB6	0.80	0.42	1.93		340.34
cg06559318	32526260	HLA-DRB6	0.70	0.36	1.93		340.34
cg11888470	32526021	HLA-DRB6	0.83	0.53	1.55		340.34
cg24638099	32526027	HLA-DRB6	0.70	0.43	1.62		340.34

Differential methylation score = $10^{\ast} \text{sgn}(\beta_{\text{Risk}} - \beta_{\text{Protective}}) \ast \log_{10} p$, where β is the average level of DNA methylation on each CpG site and ranges from 0 to 1 (0, completely demethylated; 1, completely methylated).