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Variants within *MECP2*, a key transcriptional regulator, are associated with increased susceptibility to lupus and differential gene expression in lupus patients

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Abstract

Objective—Both genetic and epigenetic factors play an important role in the pathogenesis of lupus. Herein, we study methyl-CpG-binding protein 2 (*MECP2*) polymorphism in a large cohort of lupus patients and controls, and determine functional consequences of the lupus-associated *MECP2* haplotype.

Methods—We genotyped 18 SNPs within *MECP2*, located on chromosome Xq28, in a large cohort of European-derived lupus patients and controls. We studied the functional effects of the lupus-associated *MECP2* haplotype by determining gene expression profiles in B cell lines from female lupus patients with and without the lupus-associated *MECP2* risk haplotype.

Results—We confirm, replicate, and extend the genetic association between lupus and genetic markers within *MECP2* in a large independent cohort of European-derived lupus patients and controls (OR= 1.35, p= 6.65×10^{-11}). MECP2 is a dichotomous transcriptional regulator that either activates or represses gene expression. We identified 128 genes that are differentially expressed in

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lupus patients with the disease-associated *MECP2* haplotype; most (~81%) are upregulated. Genes that were upregulated have significantly more CpG islands in their promoter regions compared to downregulated genes. Gene ontology analysis using the differentially expressed genes revealed significant association with epigenetic regulatory mechanisms suggesting that these genes are targets for MECP2 regulation in B cells. Further, at least 13 of the 104 upregulated genes are interferon-regulated genes. The disease-risk *MECP2* haplotype is associated with increased expression of the MECP2 transcriptional co-activator CREB1, and decreased expression of the co-repressor HDAC1.

Conclusion—Polymorphism in the *MECP2* locus is associated with lupus and, at least in part, contributes to the interferon signature observed in lupus patients.

Introduction

Systemic lupus erythematosus (SLE or lupus) is a chronic debilitating autoimmune disease associated with significant morbidity and mortality. The disease can affect multiple organs including the brain, kidney, lung, heart, and joints. Lupus is characterized by the production of autoantibodies to a variety of nuclear antigens and by the presence of an autoreactive T cell phenotype in the peripheral blood (1,2). The pathogenesis of both drug-induced and idiopathic lupus involves a defect in T cell DNA methylation resulting in overexpression of a number of methylation sensitive genes such as *ITGAL* (CD11a), *TNFSF7* (CD70), *PRF1* (perforin), and *CD40LG* (CD40L) (3,4). Normal CD4+ T cells treated with DNA methylation inhibitors such as 5-azaC overexpresses the same methylation sensitive genes similar to T cells from lupus patients. T cells treated with DNA methylation inhibitors become autoreactive *in vitro*; capable of spontaneously lysing syngeneic macrophages, and inducing autologous B cell activation and immunoglobulin production (5). Further, T cells treated with DNA methylation inhibitors induce a lupus-like disease with glomerulonephritis and autoantibody production upon adoptive transfer into mice (6). Interestingly, a CD4+ T cell methylation defect has also been reported in at least one murine model of the disease (7).

We have previously reported the genetic association between lupus and common variants within the methyl-CpG-binding protein 2 (*MECP2*) gene in two independent cohorts of lupus patient and controls and identified both risk and protective haplotypes (8). *MECP2*, located on chromosome Xq28, encodes for a 486 amino acid protein that binds methylated DNA and is intimately involved in the transcriptional regulation of methylation-sensitive genes.

MECP2 had been largely thought of as a transcriptional repressor exerting its effects, at least in part, by recruiting histone deacetylases to promoter sequences of target genes, thereby inducing a transcriptionally inaccessible chromatin configuration (9). Recent evidence, however, indicates that MECP2 is also a transcriptional activator capable of recruiting the transcription factor CREB1 (10). Indeed, MECP2 acts as a transcriptional activator in the majority (~85%) of genes regulated by MECP2 in the murine hypothalamus (10).

In this report, we first confirm the association of lupus with variants within *MECP2* in a large independent cohort of European-derived lupus patients and controls. We next determined the expression of the two known mRNA isoforms of *MECP2* in B cell lines from lupus patients with the risk and the protective haplotypes. Furthermore, we demonstrate that the *MECP2* risk haplotype dictates global changes in B cell gene expression relative to the protective non-risk haplotype and thereby provides multiple paths toward realization of the phenotype.

Methods

Patients and controls

A cohort of 1,418 European-derived unrelated lupus patients and 1,876 race-matched controls were recruited at the Oklahoma Medical Research Foundation as well as at collaborating institutes in the United States, the United Kingdom, and Sweden. This cohort is independent of the previously studied European-derived cohort reported in Sawalha et al (8). All patients met the 1997 American College of Rheumatology classification criteria for lupus. All protocols were approved by the institutional review boards at the University of Oklahoma Health Sciences Center and the Oklahoma Medical Research Foundation.

Genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells (PBMCs). Genotyping of 18 SNPs within the *MECP2* gene was performed using an Illumina BeadStation 500GX instrument using Illumina Infinum II genotyping assays following manufacturer's recommendations. These 18 SNPs were selected from the published SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/) to cover the entire length of *MECP2*, and were previously genotyped by our group in two independent cohorts of lupus patients and controls.

Statistical analysis

Analysis of genotyping data—SNPs with minor allele frequencies of ≥ 5% and Hardy-Weinberg equilibrium p value of >0.01 were used for further analysis. All SNPs analyzed had a genotyping success rate of ≥97.4%. Allele frequencies were determined in both cases and control groups, and a Pearson's Chi square and p value were calculated to assess differences between the two groups. Permutation p values were calculated using Haploview 4.1 to correct for multiple testing (11). Haploview 4.1 was also used to generate a linkage disequilibrium (LD) plot for the analyzed SNPs and to calculate correlation coefficient (r^2) values between SNPs. Common haplotypes (with a frequency of >1%) produced by the disease-associated SNPs were determined and haplotype frequencies calculated using Haploview 4.1. Principal component analyses (PCA) were computed to identify populations substratification in our cohort (12). A total of 64 samples that violated the assumption of sample homogeneity based on the PCA (41 cases and 23 controls) were removed prior to data analysis. We then performed genomic control analysis to calculate the inflation factor λ (Lambda) using 2218 null SNPs, which produced a λ =1.04, further indicating no evidence for population substratification. The inflation factor is a measure that quantifies the degree to which population stratification increases the chi^2 test statistics.

Bioinformatics and Statistical Analysis of Microarray Data—Statistical analysis of microarray data was performed using associative analysis of expression as previously described (13). CpG islands in the 5kb upstream and 5kb downstream regions of the transcription start site of differentially expressed genes were identified algorithmically using Build 36.3 (released Mar 24, 2008) of the Human genome (ftp://ftp.ncbi.nih.gov/genomes/H_sapiens/). CpG islands were defined as a stretch of DNA of at least 200bp with a C+G content of at least 50% and an observed/expected CG frequency of at least 0.6. The IRIDESCENT algorithm (14) was used to identify and score the relevance of "objects" (i.e., genes, diseases, phenotypes, small molecules and ontology categories) that co-occurred in MEDLINE abstracts with the differentially expressed genes. Names and synonyms for these objects are obtained from publicly available databases including, but not limited to, OMIM (diseases), Disease Ontology Database (phenotypes), Entrez Gene (genes), CHEMID (chemicals) and the Gene Ontology database (GO

categories). A shared relationship between a subset of differentially expressed genes and another object in the IRIDESCENT database identifies common processes and associations.

Cell culture and RNA extraction

Epstein-Barr virus (EBV) transformed B cell lines from lupus patients were used to study the effect of *MECP2* risk and protective haplotypes. B cell lines were prepared from PBMCs isolated from lupus patients by density gradient centrifugation and then suspended in RPMI 1640 with 10% bovine serum, supplemental glutamine, streptomycin, and penicillin. A small concentration of cyclosporine is added (1 μg/ml) to inhibit T cell suppression of transformed B cell growth. Finally, an aliquot of a fresh culture supernatant from a B95-8 marmoset cell line culture producing infectious Epstein-Barr virus is added as the transforming agent. Cell lines grow in a few weeks, are expanded, and frozen in 90% fetal calf serum and 10% DMSO in aliquots of 20 million cells at −70°C. After having equilibrated at this temperature the cells are transferred to liquid nitrogen for long-term storage. EBV transformed B cell lines from 10 lupus patients homozygous for the *MECP2* risk haplotype and 10 lupus patients homozygous for the protective haplotypes were thawed into medium, washed and grown in RPMI 1640 supplemented with 10% fetal calf serum, glutamine, streptomycin and penicillin. Twenty-four hours prior to isolating RNA all cell lines were washed and grown into fresh media. RNA was isolated using a combination of Trizol (Invitrogen, Carlsbad, CA) and RNeasy kits (Qiagen, Valencia, CA). Briefly, 15×10^6 cells were lysed in 1 ml of Trizol reagent, 200μl of chloroform added, then mixed by inversion for 15 seconds and incubated at room temperature for 3 minutes. The lysate was then centrifuged for 15 minutes at 4°C and 14,000 RPM. Ethanol (100%) was added to the supernatant at 0.53 volume and the mixture loaded into the RNeasy column and RNA isolation was completed following the RNeasy protocol.

Real time RT PCR

To measure the levels of *MECP2* transcripts (isoform 1 and isoform 2), real time RT PCR was performed using iScript One-Step RT-PCR Kit With SYBR Green (Bio-Rad, Hercules, CA) and the Rotor-Gene 3000 real-time thermocycler (Corbett Research, Australia). RNA was first treated with Turbo DNA-free (Ambion, Austin, TX) to digest any contaminating DNA. A total of 62.5ng RNA was used per reaction. The following PCR protocol was used: 10 min at 50 °C, 5 min at 95 °C, 45 cycles of 10 s at 95 °C and 30 s at 55 °C. Internal standards prepared by serial dilutions were used to quantify expression levels of both *MECP2* isoforms, *CREB1*, and *HDAC1*, followed by normalization to a housekeeping gene (*GAPDH* or *ACTB* (β actin)). The following primers were used: *MECP2A* (isoform 1) forward: 5′-CTGGGATGTTAGGGCTCAGGGA-3′, reverse: 5′-AGAGTGGTGGGCTGATGGCT-3′; *MECP2B* (isoform 2) forward: 5′-AGGCGAGGAGGAGAGACTGGAA-3′, reverse: 5′-AGAGTGGTGGGCTGATGGCT-3′; *CREB1* forward: 5′-CCAGCAGAGTGGAGATGCAG-3′, reverse: 5′-GTTACGGTGGGAGCAGATGAT-3′; *HDAC1* forward: 5′-ACCCGGAGGAAAGTCTGTTAC-3′, reverse: 5′-GGTAGAGACCATAGTTGAGCAGC-3′; *GAPDH* forward: 5′-TGTTGCCATCAATGACCCCTTC-3′, reverse: 5′-CTCCACGACGTACTCAGCGC-3′; *ACTB* forward: 5′-GCACCACACCTTCTACAATGAGC-3′; reverse: 5′-GGATAGCACAGCCTGGATAGCAAC -3′. Real time RT PCR as described above was also used to validate the expression microarray data. Genes examined include *CLIC2, IFITM3, IGJ, ITM2B,* and *TEX15*. Primer sequences are available upon request. All primers were purchased from Integrated DNA Technologies, Inc. (Coralville, IA).

Expression microarray

After purification, RNA concentration was determined with a Nanodrop scanning spectrophotometer, and then qualitatively assessed for degradation using the ratio of 28:18s rRNA using a capillary gel electrophoresis system (Agilent 2100 Bionalalyzer, Agilent Technologies). Biotinylated amplied RNA was produced from 250ng total RNA per sample using a modification of the Eberwine protocol (15) as described in the Illumina® TotalPrep RNA Amplification Kit from Ambion, Inc (Austin, TX). Briefly, RNA was reverse-transcribed with oligo(dT) primer containing a T7 promoter. RNA containing biotin-UTP ribonucleotides was amplified by in vitro transcription to generate anti-sense RNA. This RNA was hybridized overnight at 58C to human WG-6 version 3 Expression BeadChip™ microarrays (Illumina Corp, San Diego, CA). These arrays contain 48,804 50-mer oligonucleotide probes coupled to beads that are mounted on glass slides. Each bead has approximately a 20–30-fold redundancy per microarray. Microarrays are washed under high stringency, labeled with streptavidin-Cy3, and scanned with an Illumina BeadStation 500 scanner.

Results

Lupus is associated with polymorphisms within the *MECP2* gene

We confirm the association between SNPs (single nucleotide polymorphisms) within *MECP2* and lupus in a large independent cohort of European-derived lupus patients and controls. We genotyped 18 SNPs within *MECP2* in a cohort of 1,418 European-derived lupus patients and 1,876 controls. Principle component analysis was used to detect population substratification and identified ‘outlier’ samples (41 cases and 23 controls) that were excluded from further analysis. A total of 1,377 lupus patients (1,293 females, and 84 males) and 1,853 controls (1,097 females, and 756 males) were analyzed.

SNPs with minor allele frequencies of ≥ 5%, and a Hardy-Weinberg equilibrium (HWE) p value of >0.01 were included in subsequent analysis. HWE p value measures the difference between the observed genotype frequency and the expected genotype frequency based on the observed allele frequency. A high HWE p value indicates random mating in a study population. We confirm the association between lupus and all 8 SNPs within *MECP2* previously reported in European-derived and Korean lupus patients and controls (8). Indeed, the SNPs with the strongest association rs3027933, rs1734791, rs1734792, rs1734787, and rs2075596, have odds ratios of 1.38, 1.37, 1.37, 1.35, and 1.35, respectively, and p values of 1.50×10^{-5}, 1.92×10^{-5}, 2.80×10^{-5}, 5.22×10^{-5}, and 5.66×10^{-5}, respectively in the new independent cohort (Table 1). All the 8 lupus-associated SNPs identified are in linkage disequilibrium (LD) with pair-wise r^2 values of ≥0.64. The SNPs with the strongest association, mentioned above, are in strong LD with pair-wise r^2 values of ≥0.95 suggesting that they are surrogates for the same genetic effect.

Using the 8 SNPs in *MECP2* that are associated with lupus in our cohort, we identified 3 haplotypes with a frequency of >1%. Haplotype 1 “ACTGCAAA” is a disease-risk haplotype (OR= 1.38, 95%CI= 1.19–1.60, p= 2.36×10^{-5}) while Haplotype 2 “GGAAATCG” is a protective haplotype (OR= 0.82, 95%CI= 0.72–0.93, p= 0.0022). These data are consistent with and confirm our previously reported findings (8).

Table 2 summarizes the odds ratios and the Fisher’s combined p values for the *MECP2* SNPs associated with lupus in three independent cohorts of lupus patients and controls that have been studied to date. *MECP2* SNPs with the strongest association are rs1734787, rs1734792, and rs1734791, with Fisher’s combined p values of 6.65×10^{-11}, 9.67×10^{-11}, and 1.52×10^{-10}, respectively.

Expression of *MECP2* in lupus patients with and without the lupus-associated haplotype

To determine if the disease-associated polymorphism within the *MECP2* locus alters the expression of *MECP2*, we determined the expression of the two known *MECP2* transcript isoforms (*MECP2A and MECP2B*) in female lupus patients who are homozygous for the disease risk haplotype and in female lupus patients homozygous for the protective haplotype. *MECP2A* (isoform 1) includes exon 2 where translation is reported to start. The more recently identified transcript variant, *MECP2B* (isoform 2), lacks exon 2, and has a translation start site in the first exon (16,17). There was no detectable difference in the level of either transcript variant in lupus patients with the risk haplotype compared to lupus patients with the protective haplotype as measured by real time RT PCR and primers specific for the two transcript isoforms (Fig. 1A). However, statistical power to find differences in this experiment is limited by the number of B cell lines available with the risk and protective homozygous *MECP2* haplotypes.

Identification of functional consequences of the disease-associated *MECP2* haplotype

MECP2 binds methylated DNA, recruits histone deacetylase or CREB1, and functions as a transcriptional repressor or activator for genes with CG-rich promoter sequences. Therefore, if the lupus-risk *MECP2* haplotype we identified alters the function of MECP2, it is likely to affect the expression of a number of target genes that are regulated by MECP2. To test this hypothesis, we examined the expression patterns of genes in B cell lines from five European-derived female lupus patients homozygous for the disease-risk haplotype compared to six European-derived female lupus patients homozygous for the protective haplotype using expression microarrays. We identified 128 genes that are differentially expressed as a result of the *MECP2* haplotype **(**Table 3 and Table 4). The majority of differentially expressed genes (104 genes, ~81%) are upregulated (≥1.5 fold) in patients with the risk haplotype compared to patients with the protective haplotype, while 24 genes (~19%) were downregulated. Interestingly, the number of CpG islands in the 5kb region upstream and 5kb region downstream of the transcription start site was significantly higher in the upregulated genes compared to genes that are downregulated ($t=2.07$, $df=120$, $p=0.04$) (Fig. 1B). A number of genes that are upregulated in patients with the *MECP2* risk haplotype are interferon-regulated genes. These include *BTN3A2*, *CEBPD*, *CECR1*, *IFI6 (G1P3)*, *IFI35*, *IFITM1*, *IFITM3*, *IRF7*, *ISG20*, *LY6E*, *PHGDH*, *S100A10*, and *ZBP1*. An interferon signature is well documented in peripheral blood mononuclear cells of lupus patients (18,19). We conducted a literature-based analysis of shared commonalities for these genes as previously described (14), and found that several of these genes are associated with epigenetic mechanisms (Table 5). We confirmed the microarray data by examining the expression of 5 genes (3 upregulated and 2 down regulated) using real time RT PCR. The genes examined are *CLIC2*, *IFITM3*, *IGJ*, *ITM2B*, and *TEX15* (Fig. 1C). We next determined mRNA expression levels of histone deacetylase 1 (*HDAC1*) and *CREB1* in patients homozygous for the disease-risk compared to patients homozygous for the protective haplotype. HDAC1 and CREB1 are recruited by MECP2 and function as a transcriptional co-repressor and a transcriptional co-activator, respectively. We found that the presence of the lupus-risk *MECP2* haplotype is associated higher expression levels of *CREB1* (0.04) and lower expression levels of *HDAC1* ($p=0.018$) and (Fig. 1D, 1E).

Discussion

We first replicate the association between SNPs within the *MECP2* gene and systemic lupus erythematosus in an independent large cohort of European-derived lupus patients and controls (Table 2). Similarly, using this independent European-derived cohort, we further confirm the previously identified *MECP2* lupus risk haplotype “ACTGCAAA” and the protective haplotype “GGAAATCG”.

To study the functional consequences of *MECP2* polymorphism upon lupus susceptibility, we used transformed B cell lines from lupus patients that are homozygous for the *MECP2* risk haplotype and lupus patients that are homozygous for the *MECP2* protective haplotype. This approach has the advantage of removing any potential confounding effects of environmental factors or medication experiences among lupus patients. We observe no difference in the steady state mRNA levels of the two known *MECP2* transcript variants between lupus patients with the risk and protective haplotypes. MECP2 binds to methylated CG dinucleotides in promoter sequences of methylation sensitive genes and functions as a key transcriptional repressor, in part by recruiting histone deacetylases thereby altering chromatin configuration to a transcriptionally inaccessible form (9,20).

Surprisingly, recent evidence suggests that MECP2 is a key transcriptional activator that associates with the transcription factor CREB1 in promoter sequences of MECP2-activated genes (10). Moreover, MECP2 is directly involved in the activation of the transcription factor CREB1. Indeed, MECP2 functions as a transcriptional activator in the majority of genes dysregulated in the hypothalamus of *MECP2*-transgenic and *Mecp2*-null mice (10). MECP2 target genes are tissue specific, perhaps related to the relative abundance of the various co-repressors or co-activators that facilitate the effects of MECP2. Given the dichotomous effects of MECP2 on gene expression, we determined the functional consequences of the lupus-risk *MECP2* haplotype compared to the lupus-protective *MECP2* haplotype in B cell lines from lupus patients using expression microarrays. We identified 128 genes that were differentially expressed as a result of the *MECP2* haplotype carried. Interestingly, the majority of the differentially expressed genes (~81%) are upregulated in lupus patients homozygous for the risk haplotype. Approximately 85% of genes regulated by *MECP2* in the hypothalamus are overexpressed in *MECP2*-transgenic mice and underexpressed in *Mecp2*-null mice (10). If this relationship remains true in human B cells, then the lupus-associated *MECP2* polymorphism is surrogate for a gain of MECP2 function. This hypothesis will more readily explain the predominance of lupus in females, who have a copy of *MECP2* on each of the 2 X chromosomes coupled with reactivation of the normally inactive X chromosome due to defective DNA methylation that has been described in lupus patients (21). Genes that are upregulated in patients homozygous for the risk haplotype contain significantly more CpG islands in their promoter regions compared to downregulated genes (p=0.04) **(**Fig. 1B). This is consistent with a gain of MECP2 function as a result of the *MECP2* risk haplotype, as genes that are activated by MECP2 were reported to contain more CpG islands compared to genes repressed by MECP2 (10). The expression of *CREB1* is increased in patients with the *MECP2* risk haplotype as compared to patients with the protective haplotype. On the contrary, the expression of *HDAC1*, which is an important MECP2 transcriptional co-repressor, is decreased. This predicts that the *MECP2* disease-risk haplotype induces an overall overexpression of MECP2 regulated genes, consistent with the results of our expression microarray experiment.

Gene ontology analysis reveals several interesting features in the group of genes that are differentially expressed as a result of *MECP2* haplotypes. A number of genes upregulated in B cell lines carrying the risk haplotype are interferon-regulated genes. This is particularly interesting since upregulation of interferon regulated genes in PBMCs (peripheral blood mononuclear cells) of lupus patients is well established and is linked to the disease activity and the production of anti-dsDNA antibodies (18,22,23). Of note, both IFN-γ and IFN-β are known to be regulated by epigenetic mechanisms (24,25), suggesting that epigenetic dysregulation of interferon genes is a plausible functional consequence of *MECP2* polymorphism in lupus patients.

In a mouse model with an inducible ERK signaling defect resulting in reduced DNA methyltransferase 1 expression and abnormal expression of methylation sensitive genes,

differential expression of interferon-regulated genes has also been reported (26). Further, stimulated T cells from female mice with a truncated form of MECP2 (*Mecp2*$^{308/308}$) demonstrate significant overexpression of IFN-γ compared to wild-type mice (Sawalha et al. Unpublished observation).

We used an algorithm called IRIDESCENT (27,28) to search the Medline database for relationships in the literature with the list of the differentially expressed genes as a result of *MECP2* haplotypes. Several interesting significant relationships were identified with epigenetic-related mechanisms (Table 5). For example, among the upregulated genes, *TMS1* (Target of Methylation-induced Silencing 1, (*PYCARD, ASC*) is a pro-apoptotic gene that is methylation sensitive and is epigenetically silenced in some cancers (29) and was recently found to affect the innate inflammatory response (30). Our data here suggests it is also sensitive to MECP2, either directly or indirectly. *Vimentin* and *p18* (*CDKN2C*), genes found to be hypermethylated in some cancers (31,32) were also upregulated. The expression of *ITGAL* (CD11a), an integrin molecule, is known to be regulated by DNA methylation (33). *ITGAL* is hypomethylated and overexpressed in lupus T cells, and its overexpression is associated with T cell autoreactivity in lupus patients (2,34).

Among the down-regulated genes, there was the proto-oncogene *MYC* (*c-Myc*), which is known to affect DNA methylation and histone modifications (35,36) and has been implicated in autoimmunity and SLE before (37,38). *SMARCA2* is a member of the chromatin remodeling family (SWI/SNF) of genes that regulate transcription by altering chromatin structure, and was recently reported as upregulated in the immunodeficiency syndrome ICF that is known to result from a mutation in the DNA methylating enzyme DNMT3B (39). PEG10 (Paternally Expressed Gene 10), an imprinted gene (40), was also downregulated.

We find a strong relationship in the literature between the differentially expressed genes, as the consequence of *MECP2* haplotype carried, and epigenetic mechanisms including DNA methylation and histone modification. This further argues for a role of the identified *MECP2* haplotypes in epigenetic dysregulation and supports the fact that the differentially expressed genes reflect target genes for MECP2 that are altered as a result of the lupus-associated *MECP2* polymorphism. Of interest, this literature search identified a set of our differentially expressed genes and CREB1. CREB1 is a known transcription factor that has recently been identified as a key player in MECP2-induced transcriptional activation (10). Further, we identify a relationship in the literature with brain-derived neurotrophic factor (BDNF), which is the first mammalian neuronal target gene for MECP2 identified and is thought to play a pathogenic role in patients with Rett Syndrome-associated *MECP2* mutations (41).

In conclusion, our data replicate and further confirm the genetic association of polymorphism within the *MECP2* gene and lupus. We identify a number of target genes that are dysregulated in B cells from lupus patients with the *MECP2* lupus-risk haplotype. Importantly, *MECP2* risk haplotype is associated with increased expression of a number of interferon-regulated genes and may play a role in the interferon signature observed in lupus patients. Further, the list of *MECP2* target genes identified in lupus patients' B cells can potentially uncover various aspects in the pathogenesis of the disease and help provide new therapeutic targets for lupus.

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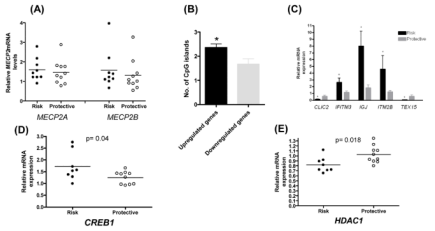

Fig. 1.

(A) mRNA expression levels of *MECP2* transcript variants (*MECP2A* and *MECP2B*) in B cells from lupus patients homozygous for the *MECP2* risk haplotype (*Risk*) compared to patients homozygous for the protective haplotype (*Protective*). (B) Genes that are upregulated (104 genes) in lupus patients with the disease-associated *MECP2* haplotype have significantly more CpG islands in their promoter region compared to downregulated genes (24 genes) (t= 2.07, p=0.04). (C) Confirmation of expression micraoarray data by real time RT PCR. The expression of 5 genes differentially expressed in B cells from 5 patients with the *MECP2* risk haplotype (*Risk*) compared to 6 patients with the *MECP2* protective haplotype (*Protective*) ($p<0.05$). (D, E) mRNA expression levels of *CREB1* and *HDAC1* in B cells from lupus patients homozygous for the *MECP2* risk haplotype (*Risk*) compared to patients homozygous for the protective haplotype (*Protective*).

Table 1

Genetic association between SNPs within MECP2 and lupus in an independent European-derived lupus patients and controls. Only SNPs with minor allele frequencies of ≥5% were analyzed.

SNP	Risk allele	Risk allele frequency		Chi2	OR (95% CI)	p value	Permutation p value	HWE p value
		Cases n (%)	Controls n (%)					
rs2075596	A	446 (16.8)	383 (13.0)	16.212	1.35 (1.17–1.57)	5.66×10^{-5}	0.0003	0.25
rs3027933	C	464 (17.4)	390 (13.2)	18.734	1.38 (1.19–1.60)	1.50×10^{-5}	1.00E-04	0.28
rs3027935	G	2476 (93.3)	2721 (92.4)	1.581	1.14 (0.93–1.40)	0.2086	0.5385	0.76
rs3027939	A	2447 (94.8)	2721 (93.9)	1.828	1.17 (0.93–1.48)	0.1763	0.4747	0.99
rs17435	T	608 (22.8)	575 (19.5)	9.005	1.22 (1.07–1.38)	0.0027	0.0136	0.79
rs7050901	G	2542 (95.2)	2786 (94.5)	1.534	1.16 (0.92–1.47)	0.2155	0.5508	0.98
rs1624766	G	604 (22.7)	576 (19.5)	8.4	1.21 (1.06–1.37)	0.0038	0.0176	0.96
rs7884370	A	2523 (94.7)	2773 (94.0)	1.198	1.14 (0.90–1.43)	0.2737	0.6497	0.75
rs1734787	C	459 (17.2)	393 (13.3)	16.366	1.35 (1.17–1.56)	5.22×10^{-5}	0.0003	0.40
rs5987201	G	2529 (94.8)	2772 (94.0)	1.655	1.16 (0.92–1.46)	0.1982	0.5189	1.00
rs1734791	A	464 (17.5)	395 (13.4)	18.269	1.37 (1.18–1.59)	1.92×10^{-5}	0.0002	0.24
rs1734792	A	462 (17.3)	392 (13.3)	17.546	1.37 (1.18–1.58)	2.80×10^{-5}	0.0003	0.31
rs11156611	G	2530 (94.8)	2773 (94.0)	1.387	1.15 (0.91–1.44)	0.239	0.5891	1.00
rs2239464	A	583 (21.9)	541 (18.4)	10.811	1.25 (1.09–1.42)	0.001	0.0053	0.94

OR, odds ratio; CI, confidence interval; HWE, Hardy-Weinberg equilibrium.

Table 2

Fisher's combined p values for risk alleles in lupus-associated *MECP2* SNPs in all three lupus cohorts reported.

SNP	Risk allele	Odds ratio (95% CI)			Fisher combined p value
		Korean*	European-derived 1*	European-derived 2**	
rs2075596	A	1.49 (1.24–1.80)	1.28 (1.09–1.52)	1.35 (1.17–1.57)	1.57×10^{-9}
rs3027933	C	1.48 (1.23–1.79)	1.30 (1.10–1.53)	1.38 (1.19–1.60)	2.90×10^{-10}
rs17435	T	1.58 (1.31–1.90)	1.29 (1.11–1.49)	1.22 (1.07–1.38)	1.45×10^{-9}
rs1624766	G	1.50 (1.24–1.82)	1.28 (1.10–1.48)	1.21 (1.06–1.37)	3.76×10^{-8}
rs1734787	C	1.55 (1.29–1.87)	1.32 (1.12–1.56)	1.35 (1.17–1.56)	6.65×10^{-11}
rs1734791	A	1.51 (1.25–1.82)	1.31 (1.11–1.54)	1.37 (1.18–1.59)	1.52×10^{-10}
rs1734792	A	1.53 (1.27–1.83)	1.31 (1.11–1.54)	1.37 (1.18–1.58)	9.67×10^{-11}
rs2239464	A	1.51 (1.25–1.82)	1.24 (1.07–1.45)	1.25 (1.09–1.42)	2.92×10^{-8}

* Cohorts published in Sawalha AH et al. 2008 (8). The Korean cohort included 628 lupus patients and 736 controls; European-derived 1 cohort included 1,080 patients and 1,080 controls.

** Current study. The European-derived 2 cohort included 1,377 lupus patients and 1,853 controls.

Table 3

Genes upregulated (≥1.5 fold) in lupus patients homozygous for the lupus-associated *MECP2* risk haplotype as compared to lupus patients homozygous for the *MECP2* protective haplotype

Gene	Definition/Description	Ratio Risk/Protective	Associative P value
AES	Amino-terminal enhancer of split, transcript variant 3	1.55	5.63×10^{-09}
AIM2	Absent in melanoma 2	1.62	9.06×10^{-06}
ANG	Angiogenin, ribonuclease, RNase A family, 5	1.71	3.85×10^{-05}
ARSD	Arylsulfatase D, transcript variant 1	1.54	2.47×10^{-18}
B3GALT4	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 4	1.58	1.69×10^{-21}
BTG2	BTG family, member 2	1.77	2.07×10^{-34}
BTN3A2	Butyrophilin, subfamily 3, member A2	1.78	8.00×10^{-07}
BTN3A3	Butyrophilin, subfamily 3, member A3, transcript variant 1	1.59	1.76×10^{-19}
C19ORF10	Chromosome 19 open reading frame 10	1.50	1.28×10^{-13}
CCDC53	Coiled-coil domain containing 53	1.77	1.31×10^{-05}
CCR6	Chemokine (C-C motif) receptor 6, transcript variant 2	2.53	1.76×10^{-19}
CD1C	CD1C antigen, c polypeptide	1.52	1.47×10^{-08}
CD79A	CD79A antigen (immunoglobulin-associated alpha), transcript variant 1	2.16	4.05×10^{-20}
CD96	CD96 molecule, transcript variant 1	1.89	1.69×10^{-40}
CD96	CD96 molecule, transcript variant 2	1.62	3.59×10^{-37}
CDKN2C	Cyclin-dependent kinase inhibitor 2C, transcript variant 2	1.70	7.19×10^{-05}
CEBPD	CCAAT/enhancer binding protein (C/EBP), delta	1.91	6.19×10^{-38}
CECR1	Cat eye syndrome chromosome region, candidate 1, transcript variant 1	3.01	1.35×10^{-33}
CHST12	Carbohydrate (chondroitin 4) sulfotransferase 12	1.56	4.86×10^{-05}
CLEC2D	C-type lectin domain family 2, member D, transcript variant 3	1.86	1.38×10^{-06}
CRIM1	Cysteine rich transmembrane BMP regulator 1 (chordin-like)	1.51	2.74×10^{-09}
CRKRS	Cdc2-related kinase, arginine/serine-rich	2.23	7.28×10^{-10}
DHRS8	Hydroxysteroid (17-beta) dehydrogenase 11	1.58	3.14×10^{-28}
EAF2	ELL associated factor 2	1.70	2.97×10^{-07}
EDG6	Endothelial differentiation, G-protein-coupled receptor 6	2.04	5.91×10^{-09}
EVI2B	Ecotropic viral integration site 2B	1.75	6.98×10^{-18}
FAM46C	Family with sequence similarity 46, member C	2.92	9.17×10^{-20}
FAM55C	Family with sequence similarity 55, member C	1.62	1.42×10^{-08}
FLJ11000	Hypothetical protein FLJ11000	2.26	4.20×10^{-28}
FLJ20021	PREDICTED: Hypothetical LOC90024	1.52	8.68×10^{-09}
FLJ43692	ARHGEF5-like	1.83	4.81×10^{-05}
G1P3	Interferon, alpha-inducible protein (clone IFI-6-16), transcript variant 1	1.53	3.20×10^{-05}
GPR18	G protein-coupled receptor 18	2.02	1.92×10^{-10}
GSDML	Gasdermin-like	1.76	6.37×10^{-114}
H2AFJ	H2A histone family, member J, transcript variant 2	1.66	2.26×10^{-04}
HCST	Hematopoietic cell signal transducer, transcript variant 1	1.64	4.50×10^{-04}

Gene	Definition/Description	Ratio Risk/Protective	Associative P value
HIST1H1C	Histone cluster 1, H1c	2.03	2.82×10^{-07}
HIST1H2BH	Histone cluster 1, H2bh	1.52	1.41×10^{-12}
HIST1H4K	Histone cluster 1, H4k	1.69	2.71×10^{-06}
HLA-E	Histocompatibility complex, class I, E	1.77	1.01×10^{-17}
HLA-F	Major histocompatibility complex, class I, F	1.75	2.64×10^{-45}
HLA-H	Major histocompatibility complex, class I, H	2.33	1.21×10^{-09}
HS.276808	CDNA FLJ43371 fis, clone NTONG2005969	1.63	1.30×10^{-06}
HS.487766	CDNA clone L17N670205n1-10-D09 5	1.78	3.46×10^{-09}
IFI35	Interferon-induced protein 35 (IFI35), mRNA.	1.53	1.73×10^{-04}
IFI6	Interferon, alpha-inducible protein 6 (IFI6), transcript variant 2	2.13	4.86×10^{-05}
IFITM1	Interferon induced transmembrane protein 1	2.03	1.03×10^{-05}
IFITM3	Interferon induced transmembrane protein 3	3.41	0.00
IGJ	Immunoglobulin J polypeptide	2.89	8.82×10^{-21}
IRF7	Interferon regulatory factor 7, transcript variant b	1.87	2.13×10^{-14}
IRF7	Interferon regulatory factor 7, transcript variant c	1.77	2.86×10^{-18}
ISG20	Interferon stimulated exonuclease gene 20kDa	2.00	5.27×10^{-08}
ITGAL	Integrin, Lymphocyte function-associated antigen 1; alpha polypeptide	1.87	2.43×10^{-15}
ITM2B	Integral membrane protein 2B	3.15	2.78×10^{-46}
ITM2C	Integral membrane protein 2C, transcript variant 2	1.66	2.92×10^{-04}
ITM2C	Integral membrane protein 2C, transcript variant 1	1.58	1.09×10^{-03}
KIAA0125	KIAA0125	2.47	6.86×10^{-16}
LBA1	PREDICTED: Lupus brain antigen 1	1.72	1.59×10^{-08}
LDLR	Low density lipoprotein receptor (familial hypercholesterolemia)	1.71	2.35×10^{-10}
LIME1	Lck interacting transmembrane adaptor 1	1.76	2.69×10^{-21}
LMBRD1	LMBR1 domain containing 1	1.57	1.62×10^{-05}
LMO4	LIM domain only 4	1.80	1.67×10^{-05}
LOC387882	Hypothetical protein	1.60	4.73×10^{-08}
LOC541471	PREDICTED: Hypothetical LOC541471	1.71	3.16×10^{-17}
LOC554223	PREDICTED: Hypothetical LOC554223, transcript variant 3	1.57	3.18×10^{-24}
LY6E	Lymphocyte antigen 6 complex, locus E	1.82	1.44×10^{-04}
MGC13057	Hypothetical protein MGC13057	2.56	3.06×10^{-07}
MGC24039	Hypothetical protein MGC24039	1.68	4.01×10^{-13}
MGC29506	Hypothetical protein MGC29506	1.74	1.33×10^{-15}
MGST3	Microsomal glutathione S-transferase 3	1.60	4.75×10^{-06}
MID1IP1	MID1 interacting protein 1 (gastrulation specific G12-like (zebrafish))	1.50	3.35×10^{-07}
NCF4	Neutrophil cytosolic factor 4, 40kDa, transcript variant 1	1.85	6.11×10^{-61}
NPC2	Niemann-Pick disease, type C2	1.54	6.04×10^{-07}
P2RY5	Purinergic receptor P2Y, G-protein coupled, 5	2.59	5.91×10^{-07}
PHGDH	Phosphoglycerate dehydrogenase	1.54	6.46×10^{-06}

Gene	Definition/Description	Ratio Risk/Protective	Associative P value
PIM2	Pim-2 oncogene	1.61	4.07×10^{-17}
PIP3-E	Phosphoinositide-binding protein PIP3-E	2.30	4.37×10^{-06}
PNOC	Prepronociceptin	1.75	7.40×10^{-16}
PRDM1	PR domain containing 1, with ZNF domain, transcript variant 1	1.67	1.42×10^{-06}
PRDX4	Peroxiredoxin 4	1.52	1.01×10^{-12}
PTPRCAP	Protein tyrosine phosphatase, receptor type, C-associated protein	1.68	1.55×10^{-07}
PYCARD	PYD and CARD domain containing, transcript variant 3	1.54	4.76×10^{-04}
RABAC1	Rab acceptor 1 (prenylated)	1.54	1.09×10^{-09}
RNASET2	Ribonuclease T2	1.94	2.42×10^{-06}
RNF36	Ring finger protein 36, transcript variant b	2.00	8.84×10^{-12}
RNF36	Ring finger protein 36, transcript variant a	1.72	2.74×10^{-10}
S100A10	S100 calcium binding protein A10	2.42	1.76×10^{-15}
SCOTIN	Scotin	1.61	3.53×10^{-13}
SGK	Serum/glucocorticoid regulated kinase	2.08	3.28×10^{-04}
SLC27A3	Solute carrier family 27 (fatty acid transporter), member 3	1.62	9.09×10^{-09}
SORT1	Sortilin 1	1.62	1.81×10^{-10}
SPRY2	Sprouty homolog 2 (Drosophila)	1.63	3.95×10^{-07}
SSR4	Signal sequence receptor, delta (translocon-associated protein delta)	1.67	3.93×10^{-10}
ST3GAL1	ST3 beta-galactoside alpha-2,3-sialyltransferase 1, transcript variant 2	1.85	4.02×10^{-36}
TBX15	T-box 15	1.66	1.33×10^{-12}
TCL6	T-cell leukemia/lymphoma 6, transcript variant TCL6d1	1.54	2.56×10^{-29}
TMEM156	Transmembrane protein 156	1.50	3.11×10^{-07}
TNFRSF17	Tumor necrosis factor receptor superfamily, member 17	1.50	8.82×10^{-08}
TNFRSF7	Tumor necrosis factor receptor superfamily, member 7	2.43	2.91×10^{-07}
TRIB1	Tribbles homolog 1 (Drosophila)	1.57	5.01×10^{-05}
TRIM69	Tripartite motif-containing 69, transcript variant b	1.90	5.28×10^{-11}
TXNDC11	Thioredoxin domain containing 11	1.66	1.90×10^{-05}
TXNDC5	Thioredoxin domain containing 5, transcript variant 1	1.82	4.20×10^{-08}
VIM	Vimentin	2.03	3.97×10^{-06}
XBP1	X-box binding protein 1, transcript variant 1	1.90	2.44×10^{-10}
XBP1	X-box binding protein 1, transcript variant 2	1.87	2.73×10^{-08}
ZBP1	Z-DNA binding protein 1	2.09	1.96×10^{-25}
ZFP36	Zinc finger protein 36, C3H type, homolog (mouse)	1.77	1.04×10^{-31}
ZSWIM6	PREDICTED: Zinc finger, SWIM-type containing 6	1.52	2.05×10^{-05}

Table 4

Genes downregulated (≥1.5 fold) in lupus patients homozygous for the lupus-associated *MECP2* risk haplotype as compared to lupus patients homozygous for the *MECP2* protective haplotype

Gene	Definition/Description	Ratio Risk/Protective	Associative P value
AP3M2	Adaptor-related protein complex 3, mu 2 subunit	0.64	2.81×10^{-06}
CD80	CD80 antigen (CD28 antigen ligand 1, B7-1 antigen)	0.62	3.12×10^{-08}
CLIC2	Chloride intracellular channel 2	0.53	1.96×10^{-09}
CRY1	Cryptochrome 1 (photolyase-like)	0.53	3.97×10^{-51}
FUCA1	Fucosidase, alpha-L- 1, tissue	0.63	1.77×10^{-04}
GCET2	Germinal center expressed transcript 2, transcript variant 2	0.57	1.86×10^{-05}
HDGFRP3	Hepatoma-derived growth factor, related protein 3	0.58	3.68×10^{-27}
HRASLS3	HRAS-like suppressor 3	0.61	5.99×10^{-08}
HSPA4L	Heat shock 70kDa protein 4-like	0.65	1.34×10^{-10}
KDELC2	KDEL (Lys-Asp-Glu-Leu) containing 2	0.63	7.11×10^{-34}
LOC134147	Similar to mouse 2310016A09Rik gene	0.52	1.29×10^{-07}
LRPPRC	Leucine-rich PPR-motif containing	0.66	3.97×10^{-25}
MYC	V-myc myelocytomatosis viral oncogene homolog (avian)	0.63	1.87×10^{-07}
PEG10	Paternally expressed 10, transcript variant 1	0.66	1.95×10^{-10}
PRRT3	Proline-rich transmembrane protein 3	0.64	1.17×10^{-05}
RASGRP1	RAS guanyl releasing protein 1 (calcium and DAG-regulated)	0.58	6.84×10^{-11}
RPS7	Ribosomal protein S7	0.66	7.04×10^{-04}
SACS	Spastic ataxia of Charlevoix-Saguenay (sacsin)	0.64	7.06×10^{-42}
SMARCA2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 2, transcript variant 2	0.59	4.75×10^{-07}
SORD	PREDICTED: Sorbitol dehydrogenase	0.64	2.77×10^{-05}
STEAP3	STEAP family member 3, transcript variant 2	0.64	1.25×10^{-06}
TEX15	Testis expressed sequence 15	0.50	5.83×10^{-62}
TGFBR3	Transforming growth factor, beta receptor III	0.48	7.92×10^{-13}
TMOD1	Tropomodulin 1	0.50	6.25×10^{-06}

Table 5

IRIDESCENT algorithm analysis showing shared relationships identified in MEDLINE with genes that are differentially expressed as a result of the *MECP2* haplotype present. The ratio of observed to expected relationships (Obs/Exp) is given below, which reflects a statistical enrichment score for the association. The empirically determined average Obs/Exp ratio for a list of 128 genes is 1.42 ± 0.07. Only associations greater than 3 standard deviations were reported.

Shared relationship	Genes shared	Obs/Exp
Histone deacetylase	14	2.48
BDNF	13	2.12
Interferon-inducible	13	4.53
Chromatin structure	12	2.15
CREB1	12	2.03
Hypermethylation	11	2.36
Trichostatin A	11	2.83
CpG methylation	9	3.61
Promoter methylation	8	2.69
Aberrant methylation	7	3.61