

## REVIEW ARTICLE

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# Epigenetics and Behçet's Disease: DNA Methylation Specially Highlighted

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## ABSTRACT

Behçet's disease (BD) is a multisystem inflammatory disease with unknown etiology. Although evidence about the pathogenesis of BD is growing, the actual cause of this disease is unclear. Both genetic and epigenetic factors are claimed to play significant roles in BD. Epigenetic factors such as age, gender, smoking as well as exogenous factors like diet, infection, stress are related to the onset and clinical manifestations of BD. DNA methylation refers to a major epigenetic element which influences gene activities with catalyzing DNA using a set of DNA methyltransferases (Dnmts). DNA methylation status of many genes in patients with BD is different from that of healthy people. For example, cytoskeletal gene, *Human Leukocyte Antigen (HLA)* loci, Long interspersed nuclear element (LINE-1), and *Arthrobacter luteus (Alu)* repetitive sequences are different in the DNA methylation status in patients with BD and healthy controls. In this paper we reviewed, according to previous studies, the mechanisms of epigenetic, the epigenetic factors involved in the BD, and especially the effect of DNA methylation in the Behçet's disease. Future studies are needed to identify the capability of specific DNA methylation alterations in BD in order to predict disease manifestations, medical course, and response to treatment.

**Keywords:** Behçet's disease; DNA methylation; Epigenetics

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## INTRODUCTION

Behçet's disease (BD) is a multigenic and systemic autoimmune-mediated inflammatory disease first time

described by Hulusi Behcet in 1937, categorized by uveitis, genital ulcers, recurrent aphthous stomatitis, and skin lesions as an inflammatory process of unrevealed etiology. BD has an extensive and worldwide prevalence in different parts of the world, however, the peak of incidences in the Middle East, the Mediterranean, and the Far East. The highest rate of incidence in Turkey has been reported, including 421 people per  $10^5$ .<sup>1</sup> BD is a multisystem inflammatory disease with unknown etiology. Although evidence about the pathogenesis of BD is growing, the actual cause of the disease is unclear, both genetic and epigenetic factors are claimed to play significant roles in BD.<sup>2-4</sup>

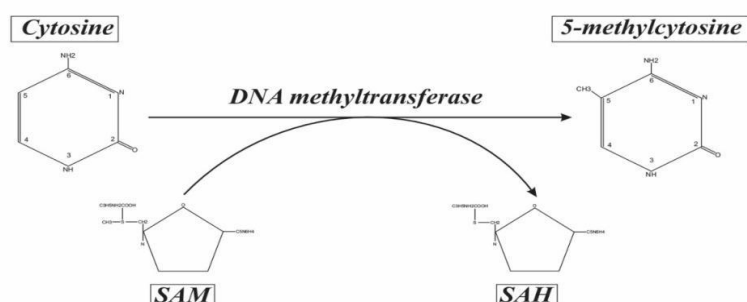
Epigenetics refers to the study of genome-wide inherited alterations in gene function and expression in the offspring of cells or individuals, without any alteration in the DNA sequence, as well as their contribution to cellular development and differentiation disorder and aging.<sup>5</sup> It is believed that epigenome is a crossing point between the environment and the hereditary fixed genome; it also organizes during growth to form a variation of gene-expression courses for diverse cell types via a greatly systematized procedure. Physical, biological and chemical compounds along with social aspects alter the epigenome at critical stages. Epigenetic regulation of gene expression leads to typical growth through dynamic transcriptional actions from gametogenesis at embryonic and newborn stages and endures throughout life. Temporary and permanent epigenetic alterations occur throughout the lifetime in response to endogenous stimuli and exogenous elements in the environment.<sup>5</sup> Epigenetic alterations take place through

DNA methylation, histone adjustment, micro-RNA, and chromatin remodeling which are extremely interrelated.<sup>6</sup>

### Overview of DNA Methylation

DNA methylation refers to a major epigenetic element which influences gene activities with catalyzing DNA using a set of DNA methyltransferases (Dnmts). Such enzymes are categorized into three types: writers, erasers, and readers. Addition of methyl groups onto cytosine residues catalyzed by the writer's enzymes (e.g. Dnmt1, Dnmt3a, and Dnmt3b) (Figure 1). De novo methylation requires DNMT3A and DNMT3B. DNMT1 is necessary for the maintenance of methylation in mammalian cells. DNA demethylation is carried out by eraser enzymes which transform and remove the methyl group. Reader enzymes contribute to the Reading DNA Methylation by three distinct families of proteins (e.g. the Methyl-CpG-binding domain protein (MBD), ubiquitin-like, containing PHD and RING finger domains (UHRF), and the zinc-finger proteins). These proteins identify and are bound to methyl groups to finally affect gene expression.<sup>7,8</sup>

While most DNA methylation arises on CpG sites, some studies indicate that non-CpG methylation occurs in human embryonic stem cells. However, these methylations are missing in adults. DNA methylation is necessary for X chromosome inactivation, regulating tissue-specific gene expression, genomic imprinting, and silencing of retroviral elements.<sup>7,10</sup> In different genomic areas, based on the inherent genetic sequence, DNA methylation might inflict different impacts on activities of the genes.<sup>7</sup>



**Figure1.** Formation of 5-methylcytosine by Dnmt using S-adenosylmethionine (SAM). Methylated to 5-methylcytosine (5mC) by DNA methyltransferases (DNMT). -Methyl cytosine has been characterized as the “fifth base” of the human genome. This indicates that this modification is relatively high in the genome, as about 4% of the cytosine residues in the human genome have been found to be methylated. The important element of cytosine methylation is its development or even specificity for “symmetric” CpG dinucleotides.<sup>7,9</sup>

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**Table 1. The effect of epigenetics on the risk of different autoimmune diseases based on its impact on the gene methylation rate**

<i>Diseases</i>	<i>Genes/pathway</i>	<i>Effect</i>	<i>Methylated alterations</i>	<i>samples type</i>	<i>References</i>
Behçet disease	IL-10	risk of BD ↑	Hypermethylation	PBMCs	(11)
	IL-6	risk of BD ↑	Hypomethylation	PBMCs	(12)
Type 1 diabetes	INS	risk of T1D ↑	Hypermethylation or hypomethylation	Whole blood cell	(13)
	IL2RA	risk of T1D ↑	Hypermethylation or hypomethylation	Whole blood cell	(14)
	IGFBP-1	circulating IGFBP-1 levels ↑	Hypermethylation	PBMCs	(15)
Systemic Lupus Erythematosus	MAPK	activity of DNMT ↓		T cell	(16)
	ERK	expression of Dnmt1 and Dnmt3a ↓		T cell	(17)
	PP2Ac	MEK/ERK phosphorylation ↓	Hypomethylation	CD3+T cell	(18)
	RFX1	activity of DNMT ↓	Hypomethylation	CD4+Tcell	(19)
	PRF1	Involved in autoreactive killing	Hypomethylation	CD4+Tcell	(20)
	GADD45A	expression of CD11a and CD70 ↑	Demethylation	CD4+Tcell	(21)
	HMGB1	expression of CD11a and CD70 ↑	Hypomethylation	CD4+Tcell	(22)
	ITGAL	expression of and CD11a	Hypomethylation	T cell	(23)
	TNFSF7	expression of and CD70	Demethylation	CD4+Tcell	(20)
	CD40LG	expression of and TNFSF7	Demethylation	CD4+Tcell	(24)
	IL-6	expression of Foxp3 ↓	Hypomethylation	CD4+Tcell	(25)
	IL-10	expression of IL10 ↑	Hypomethylation	CD4+Tcell	(26)
Rheumatoid Arthritis	IL-13	expression of IL13 ↑	Hypomethylation	CD4+Tcell	(26)
	IL-6	expression of IL6 ↓	Hypermethylation	PBMCs	(27)
	IL-10	expression of IL10 ↑	Hypermethylation	PBMCs	(28)
	DR3	resistance to apoptosis	Hypermethylation	Synovial cells	(29)
	CXCL12	expression of MMPs ↑	Hypermethylation	Synovial cells	(30)
	TBX5	Inflammatory processes ↑	Hypermethylation	Synovial cells	(31)
	MST1	expression of MST1 ↓	Hypermethylation	T cell	(32)
	FoxP3	expression of FoxP3 ↓	Hypermethylation	Treg cell	(33, 34)
Multiple Sclerosis	TNF- $\alpha$	local inflammatory ↑	Hypermethylation	Blood cell	(35)
	SHP-1	leukocyte-mediated inflammation ↑	Hypermethylation	Peripheral blood leukocytes	(36)
	PAD2	synthesis of PAD2 protein ↑	Hypermethylation	Brain and thymus tissue	(37)
	HERV-W	activity of proinflammatory ↑	Hypermethylation or hypomethylation	PBMCs	(38)
	TET2	expression of TET2 ↓	Hypermethylation	PBMCs	(39)
	HLA-DRB1	risk of MS ↑	Hypermethylation or hypomethylation	CD4+Tcell	(40)

IL-10: interleukin-10, IL-6: interleukin-6, INS: insulin, IL2RA: interleukin 2 receptor subunit alpha, IGFBP-1: insulin like growth factor binding protein 1, MAPK: mitogen-activated protein kinase, ERK: extracellular regulated MAP kinase, PP2Ac: protein phosphatase 2 catalytic subunit alpha, RFX1: regulatory factor X1, PRF1: perforin 1, GADD45A: growth arrest and DNA damage inducible alpha, HMGB1: high mobility group box 1, ITGAL: integrin subunit alpha L, TNFSF7: TNF receptor superfamily7, CD: cluster of differentiation, CD40LG: CD40 ligand, DR3: hypothetical protein, CXCL12: C-X-C motif chemokine ligand 12, TBX5: T-box transcription factor 5, MST1: macrophage stimulating 1, FoxP3: forkhead box P3, TNF- $\alpha$ : tumour necrosis factor alpha-like, SHP-1: protein phosphatase, regulator SHP1, PAD2: proteasome alpha subunit D2, HERV-W: endogenous retrovirus group W, TET2: tet methylcytosine dioxygenase 2, HLA-DRB1: major histocompatibility complex, class II, DR beta 1 PBMC: *peripheral blood mononuclear cell*

## Epigenetic Factors

### *DNA Methylation in Autoimmune Diseases*

Few studies have been done about the role of epigenetics in the Behcet disease as summarized in Table 1.

### Age

Aging is the gentle decline of multiple biological functions and is dependent on time. Epigenetic patterns are biologically programmed and necessary to change dramatically during development (Figure 2). However, such alterations in adult somatic tissue may be an indication of aging-dependent deleterious events.<sup>10</sup> Aging has been shown to be associated with significant changes in the innate immune system such as neutrophil and macrophage phagocytic activity impairment, a rise in the production of pro-inflammatory cytokines and a reduction in antibacterial defense. Previous studies have shown that the higher expression of such cytokines in older individuals caused by age-related changes in the DNA methylation profile of the TNF and IL-10 promoter.<sup>11,41,42</sup> Inflammation is often attributed to the DNA hypermethylation of specific genes as was reported in ulcerative colitis for the first time.<sup>43</sup> It has been demonstrated that methylation alterations in Mesenchymal Stromal Cells (MSC), especially total DNA methylation levels decrease during later cultures in these cells. Moreover, DNA methylation alterations upon aging may depend on personal differences.<sup>44</sup> It has been reported that methylation levels of LINE-1 (long interspersed element), Alu, and HERV-K (human endogenous retrovirus K) are different at different age intervals.<sup>45</sup> Previous studies have provided evidence determining the mean age at the onset of the BD at 29 years.<sup>46</sup> In recent studies, it has been shown that cutaneous involvement is more frequently observed in patients younger than 20 years old while articular involvement is more frequent in patients older than 40.<sup>47</sup> BD is typically diagnosed between the ages of 20 to 40, and is less common in other age groups. Many studies have addressed the relationship between the onset of the disease in the childhood period and clinical features of BD.<sup>48</sup> To the best of our knowledge, few or no studies have dealt with the onset in older ages.

### Gender

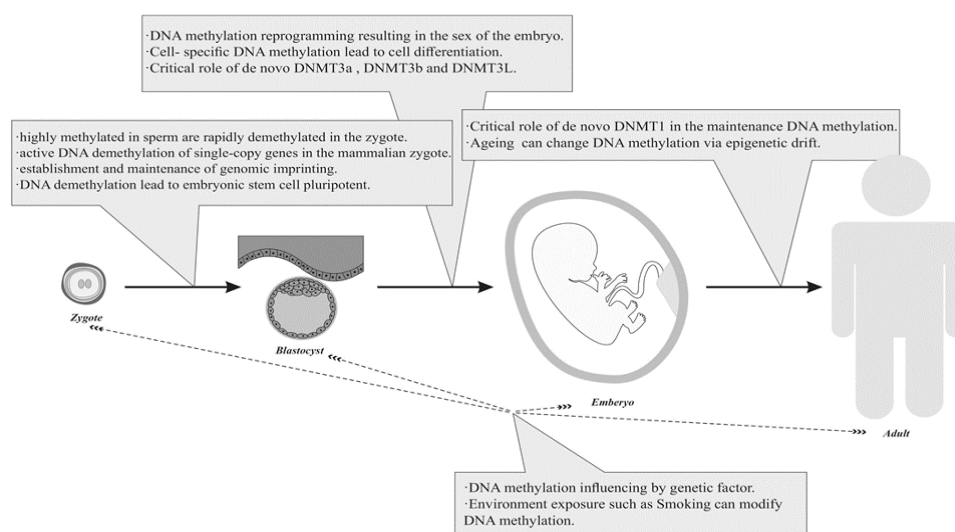
Autoimmunity has been shown to be more predominant in females.<sup>50</sup> However, BD has been

shown to follow a geographically different trend. Studies have reported female predominance in countries like the United States, Korea, and Brazil while male predominance has been reported in some other countries (e.g., Asia, Turkey, Middle East, and the Mediterranean).<sup>51</sup> There are no clear-cut mechanisms for gender bias in autoimmunity and studies which delve more deeply into sex differences seem imperative. Such studies may need to address the contribution of sex chromosomes particularly the X chromosome in the modification of the immune system.<sup>19</sup> Furthermore, differences in clinical presentation, onset, progression, and outcome of certain autoimmune diseases are deemed to be associated with gender. There is a possibility that sex hormones are to some extent responsible for the dominance of autoimmune disorders in females. Experimental studies have shown that the estrogen and prolactin affect the autoimmune and rheumatic diseases through immune modulation in animal models.<sup>52</sup> Meanwhile, although the influence of gender on BD is not fully known, several studies show that BD has more severe courses among young males.<sup>53</sup> In addition, it has been suggested that gender is an important factor for oral health-associated disease activation and poor prognosis in BD.<sup>54</sup>

### Smoking

Smoking is the most common addiction in the human being. Approximately 4000 toxic components exist in the cigarette, which influences the vascular and immune systems. Smoking is also related to many ocular diseases.<sup>55</sup> Moreover, smoking is a risk factor for vascular diseases.<sup>56</sup> Many studies have reported that smoking is associated with uveitis.<sup>57,58</sup> The access of organisms to intraocular tissue facilitated by the proinflammatory components of cigarette resulting in a rise in the response of inflammatory cells to the microorganism.<sup>55</sup> Previous studies have indicated that cytokines such as IL-1, IL-2, TNF, and nuclear factor- $\kappa$ B activation are upregulated via smoking.<sup>58,59</sup> It has also been reported that cigarette smoking relieves oral aphthous ulcers in BD.<sup>60</sup> Also, studies have reported that the HLA-B51 gene is a risk factor in the progression of central nervous system inflammation in smokers with BD.<sup>61</sup> However, it has been reported that smoking may have a positive effect on symptoms of certain diseases such as ulcerative

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**Figure2.** DNA methylation during development, from gametogenesis at embryonic and newborn stages, enduring throughout life. As a fertilized egg grows into an infant, the received signals cause steady changes in gene expression patterns. Epigenetic codes substantially record the cell's skills on the DNA and steady gene expression. Each signal activates some genes and inactivates others, as the cell progresses into its final destination. Primary in development, most signals come from within cells or from adjacent cells. Different involvements cause the epigenetic shapes of each cell type to grow gradually different over time. Ultimately, many cell types form, each with different characteristics and particular function. Particular genes are turned on and off at a confident stage interims. The fetal epigenome is most susceptible during this developmental period to epigenetic modifiers in the maternal environment.<sup>49</sup>

colitis and BD. Such a positive effect of smoking on BD may be attributed to the fact that nicotine inhibits the polymorphonuclear cells, the creation of some cytokines, and the alteration of nitric oxide synthesis.<sup>55</sup> However, the influence of smoking on BD has remained unknown.

### X chromosome

The X chromosome is about 155 million base pairs long, which includes around 1000 genes. Several previous studies have addressed the function of the gene dosage of X chromosome via inactivation or repetition in autoimmunity. Furthermore, sex chromosome alterations are likely to organize the common attributes of the predisposition toward autoimmune disorders.<sup>62</sup> Some studies suggest that nuclear factor  $\kappa$ B (NF- $\kappa$ B) indispensable modulator (NEMO) is essential for the activation of the transcription factor NF- $\kappa$ B. This gene charted in the chromosome position Xq28. In this regard, the observed heterozygous *NEMO* mutation is a reason of familial incidence of BD in female patients.<sup>63</sup>

### B Cell Abnormalities in BD

It is claimed that irregularities in B cell activation are involved in the pathogenesis of autoimmune disorders. These abnormalities in BD patients include the existence of immune complexes, incapability to produce Ig, raised levels of serum Ig, an increase in autoreactive antibodies, proliferation in quantity of cells secreting Ig and inability to react to mitogen. These irregularities are more frequent in patients with active BD and are less frequent in inactive cases. Such findings suggest that patients with active BD have declined quantities of resting B cells and amplified numbers of activated B cells.<sup>64</sup>

### Exogenous Factors

Exogenous factors can participate in oral. It should be mentioned that diet, infection, stress or anxiety, menstrual phases or trauma function as triggers of oral ulcer recurrences. But, these conclusions rely on limited evidence and occasionally disagree in results. The percentage of patients with BD With a recognizable trigger of oral ulcer recurrences and the accurate nature of these triggers are not known. Oral

ulcer recurrences seem to be triggered by an outside event in 62% to 70% of patients, with stress/fatigue and diet being the most common particular reason. Volleet al suggest that emotional features are a major reason for oral ulcer recurrences in BD. Foods are another main trigger of oral ulcer recurrence in BD. Tree nuts, cheese, citrus fruits, pineapple, and strawberries are, especially, reported as impulsive oral ulcer triggers.<sup>65</sup>

#### **DNA Methylation Condition in BD-CD4+T Cells and Monocytes**

It is now widely accepted that CD4+ T-cells are the central mediators of the immune response in humans. CD4 + T cells are critical for immune responses during host defense against harmful microorganisms.<sup>66</sup> Travis Hughes et al. demonstrated that 125 CpG sites in 62 genes in CD4+T cells of patients with BD have different methylations compared to the healthy control group. Also, they reported that 383 CpG sites in 228 genes in monocytes of patients with BD differentially methylated across patients and controls.<sup>67</sup> Moreover, the same study also evaluated the hypomethylation of, a long-established genetic susceptibility locus in BD patient monocytes, proposing a role for this gene product in ubiquitination pathways.<sup>67</sup>

#### **HLA Loci Are Hypomethylated in BD**

The genetic relationship between the human leukocyte antigen (HLA) and some autoimmune and inflammatory diseases such as BD has been demonstrated. Numerous previous reviews have focused on the genetic association between HLA-B51 and BD, discovering that extra predisposition loci in the HLA regions are complicated by the durable linkage disequilibrium in this region.<sup>68</sup> Travis Hughes et al. revealed that differential methylation in some HLA class II loci was primarily not able in CD4+T cells from BD patients. Moreover, they also specifically evaluated along with several CpG sites in *HLA-DRB5* and *HLA-DRB6*.<sup>67</sup>

#### **DNA Methylation- Status Cytoskeletal Gene in BD**

Dysregulated cytoskeletal remodeling is a determining factor in BD Pathogenesis, which is necessary for the connection and penetration of leukocytes into irritated tissues.<sup>69</sup> Also, epigenetic remodeling in cytoskeleton-related genes has been

reported to have a significant role in the pathogenesis and the therapeutic response in BD.<sup>69</sup> The cytoskeletal reorganization is a procedure involved in the movement, adhesion and cellular proliferation in leukocytes.<sup>67</sup> Various levels of the cytoskeletal organization including actin- binding motor proteins, microtubule construction, and Rho GTPase enzymes have been reported to be implicated in epigenetic dysregulation in BD.<sup>67</sup> The authors claim that the immune complex is probably convoluted in BD pathogenesis. They argue that cytoskeletal components are typical targets for autoantibody formation in BD. In addition, antibodies were observed to detect intermediary filaments of the cytoskeleton. Furthermore, in a subgroup of patients, it has been confirmed that autoantibodies target cofilin 1, tubulin-like, and actin-like self-antigens.<sup>67</sup> In line with this idea is the observation that many of the recognized sites were in genes associated with the cytoskeletal function. Remarkably, treatment for BD changed the methylation patterns in CD4+cells.<sup>70</sup> In what follows, some of the genes implicated in cytoskeletal remodeling (e.g., Fascin, IRTKS, RAC1, RIP3, SYNJ2, Obscurin), as well as their methylation condition, are discussed.

#### ***Fascin***

Fascin is the main actin filament (F-actin) bundling protein in prominent cellular constructions such as filopodia, dendrites, and invadopodia, which play significant roles in cell motility, leadership, cell relocation and attack of the extracellular medium. Fascin comprised of two actin-binding locations at either trimmings of the protein.<sup>71,72</sup> It should be noted that fascin actin-bundling protein 2 (*FSCN2*) in monocytes and CD4+ T cells from patients with BD hypomethylated.<sup>67</sup>

#### ***BAIAP2L1 (IRTKS)***

IRTKS (insulin receptor tyrosine kinase substrate; also recognized as BAIAP2L1 or BAI1-associated protein 2-like 1) which has extensive tissue spreading, is a substrate for the insulin receptor and binds Rac. It should be mentioned that Expression of IRTKS prompts groups of short actin bundles rather than filopodia-like protuberances.<sup>73,74</sup> It has been claimed that IRTKS insufficiency causes improved innate immune responses in contradiction of RNA viruses and functions as a negative modulator of

extreme irritation.<sup>75</sup> It has been shown that hypermethylation of IRTKS in monocytes and CD4+T cells occurs in BD patients.<sup>67</sup>

### **RAC1**

Rac1 GTPase is known to control cell motility through cortical actin re-organization. It also controls reactive oxygen species generation through regulation of NADPH oxidase action. Many cellular and molecular studies have dealt with Rac1 in several cardiovascular disorders.<sup>76</sup> It has recently been shown that in CD4+ T cells RAC1 demonstrated the uppermost level of hypomethylation in BD patients compared to controls. In addition, incomplete renovation of reduced RAC1 methylation has been detected in patients after attainment of syndrome retardation.<sup>67</sup>

### **MPRIIP (RIP3)**

Receptor interacting protein 3 (RIP3) indispensably implicated in TNF-induced necroses.<sup>77</sup> RIP3 is a RIP family protein kinase that has recently been referred to as an essential regulator of planned necrosis. RIP3 is implicated in downstream of expiry receptors, Toll-like receptors, or other sensors to intermediate necrotic cell death.<sup>78</sup> Similarly, RIP3 involved in the development of actin constructions that implicated in T cell immigration through the stimulation of Rac1.<sup>79</sup> Travis Hughes et al reported that RIP3 was hypermethylated in BD patient monocytes.<sup>67</sup>

### **Synaptojanin 2 (SYNJ2)**

SYNJ2 is essential for the aggressive conduct of a numeral of diverse tumor cell kinds. The role of SYNJ2 in tumor cell invasion is self-determining from its role in clathrin-mediated endocytosis and is likely to be facilitated by its function in the creation of invadopodia.<sup>80</sup> It has also been reported that synaptojanin 2 (*SYNJ2*) is hypermethylated in CD4+ T cells and monocytes from BD patients following treatment.<sup>67</sup>

### **Obscurin**

This is a giant sarcomeric protein made up of connected components and signaling domains. It has been shown that Obscurin surrounds myofibrils at the surface of the Z disk and M line. It also performs a significant role in the development and preservation of intermittent A bands in developing muscle cells.<sup>81</sup>

Studies also show that Obscurin in BD monocytes is hypermethylated.<sup>67</sup>

### **DNA Methylation in LINE-1 and Alu Repetitive Sequences in BD**

Most parts of genomic DNA are made up of a series of repeated sequences that result in portable and transposable elements. There are four sets of transposable elements in the human genome including long interspersed nuclear elements (LINEs), which are characterized by LINE-1, short interspersed nuclear elements (SINEs) which are principally characterized by Alu sequences, long-terminal repeat (LTR) transposons, and DNA transposons. LINE1 retrotransposons are susceptible to principal initiators of the autoimmunity that consequently result in inflammation. Environmental aspects like medications, demethylating CpG DNA motifs, ultraviolet light, anxiety, or hormones may play a stimulating role in this process. However, the individual's genetic complement of complete LINE1 elements that contributes to directing those elements provides a threshold way to reinforce a pathogenic self-directed immune reply.<sup>67</sup> These sequences are tightly methylated in normal somatic cells. In this condition, they are generally dormant and stay dormant. However, hypomethylation and recrudescence of these sequences are believed to have numerous functional roles, like monitoring the activity of genes by regulating enhancers and repressors or functioning as an alternate promoter upon utilization, which could lead to insertional mutations and chromosomal instability.<sup>82</sup> Yüksel et al demonstrated that *LINE-1* Methylation Analysis differences in the frequency of methylation in genomic DNA acquired from PBMCs and neutrophils of patients with active and dormant BD. In addition, the controls were not significantly different in the overall methylation across BD patients and healthy controls or across the active and dormant groups in PBMC and neutrophil subgroups. Yüksel et al found that *Alu* Methylation Analysis in the PBMC and neutrophil cell types are not significantly different in general methylation levels of PBMCs of dynamic patients compared with controls and dormant patients, and between controls and quiet patients. Finally, the occurrence of uCuC in PBMCs is meaningfully higher in BD patients compared with controls.<sup>82</sup>

### Effect of Treatment on DNA Methylation Changes in Patients with BD

It has been reported that the effect of colchicine in decreasing the severity of some of the clinical manifestations of BD is due to its capability to decrease leukocyte chemotaxis. A recent report showed that hypermethylation of the kinesin gene *KIF2A* and hypomethylation of *TPPP* is detectable in monocytes and CD4+T cells from treated patients. In addition, hypomethylation of tubulin folding cofactor D (*TBCD*) observed in monocytes of patients with BD was inverted following the treatment.<sup>35</sup> The same study also concluded that treatment contributes to the inversion of the hypomethylation of tripartite motifs containing 39 (*TRIM39*).<sup>67</sup>

In other diseases, there is a relationship between methylation and drug therapies. For example, it has been shown that Methotrexate treatment modifies DNA methylation in patients being treated for r arthritis. In addition, it has been proposed that vitamin B 12 levels can influence the effect of MTX treatment on DNA methylation patterns. Also, MTX treatment alone significantly increased LINE-1 and FKBP5 methylation in cell line.<sup>83</sup> Significant DNA demethylation following Aza treatment Furthermore Aza treatment decrease DNA methylation as demonstrated by several studies in vivo and in vitro, although the degree of demethylation seems to be limited. In other words, it takes place significant DNA demethylation following Aza treatment.<sup>84</sup> Treatment induce DNA Methylation Modifications in Animal Models and human.<sup>85</sup>

### Future Perspective

Prospect studies should provide an extensive and more in-depth analysis assay. Recent methylation studies furthermore fail to differentiate cytosine methylation from hydroxymethylation. The development of more analyses is predicted to afford an even greater sensitivity in discovering appropriate epigenetic modifications. Epigenetics may also be helpful in treating such diseases. Epigenome editing will permit to better understand epigenetic expression control and to translate such knowledge into therapeutic tools. DNA methylation is the best-characterized epigenetic mechanism to date, therefore other aspects that should be addressed in future research include methodological challenges, such as tissue specificity, and the influence of genetic variation on differential methylation patterns.

### CONCLUSION

One of the most controversial features of epigenetics is the reversibility of its alterations. Although there remains a need for novel experimental techniques including animal models and specific cell-lines to search for appropriate mediators, some medicines such as colchicine have already been used to treat BD, leading to DNA methylation changes (67). However, longitudinal training will be required to completely discover the usefulness of such modified DNA methylation as biomarkers. Future studies may be needed to identify the capability of specific DNA methylation alterations in BD and other autoimmune diseases in order to predict disease manifestations, medical course, and response to treatment.

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