Chapter

# Epigenetic Studies of Atopic Dermatitis

*Vladimir Sobolev, Elizaveta Bystritskaya and Oxana Svitich*



## Abstract

Since the pathogenesis of atopic dermatitis could not be explained only by a population genetic and phenotypic profiles, epigenetic regulator factors have been considered. Epigenetics is the study of inherited changes in gene expression that are not related to changes in its nucleotide sequence. One of the main classical regulatory mechanisms in human cells is DNA methylation. It is not clear how permanent modifications caused by this process are and whether it is possible to affect them by changing the activity of enzymes that trigger remodeling reactions. In this chapter we analyze all recent studies in this field. We focus more on methylation of innate and adaptive immune factors, with an emphasis on T-lymphocyte genes such as CD3, CD4, and CD8.

**Keywords:** atopic dermatitis, epigenetics, DNA methylation, genome-wide methylation analysis, immune system

## 1. Introduction

Atopic dermatitis (AD) is a chronic recurrent inflammation of the skin, characterized by impairment of the epidermal barrier that entailing its further dysfunction. The predisposition to IgE-mediated hypertension contributes to such a malfunction, realized in sensitization to surrounding allergens [1]. This pathology is also characterized by infiltration and accumulation of type 2 T helper cells (Th2) and eosinophils [2]. Atopic dermatitis is a multifactorial disease. The main triggers are various genetically predetermined defects of the epidermal barrier and the immune system influenced by environment [1].

Thus, the study of tissues and cells transcriptome involved in the pathogenesis of the disease is one of the best options for detecting molecular signs of complex diseases such as AD [3]. In one of these studies, it was found that the expression of a large number of genes which were responsible for terminal differentiation of keratinocytes was reduced in case of AD compared with healthy controls. These genes include filaggrin (*FLG*), loricrin (*LOR*), involucrin, late cornified envelope protein *LCE2B*, and genes encoding the S100 family of proteins [4]. This study showed that AD is associated with impaired keratinization processes in the epidermis, and confirmed another profile study by Sugiura et al., where suppression of *LOR* and *FLG* expression was determined in the lesional skin [5]. With the help of RNA sequencing technology by which transcriptomes of intact and damaged skin of patients with moderate and severe AD were compared, an increased expression of

the *TREM-1* signaling pathway, as well as *IL-36*, was revealed [6]. The laser capture microdissection method once again confirmed that the expression of genes encoding skin barrier proteins, including *FLG*, *LOR*, *CLDN4* and *CLDN8*, is reduced in affected atopic skin; and, on the contrary, the expression of cytokines Th2 and Th17 genes, such as *CCL22*, *CCL26*, *TSLP*, and *IL-22* etc., is increased [7].

Loss of function mutations in the gene encoding *FLG* are one of the most significant genetic risk factors for AD. A transcriptome profiling study realized by RNA sequencing revealed differentially expressed genes involved in the extracellular reactions, lipid metabolism, and stress response. In FLG-deficient skin, the stress response mediated by type I interferon (IFN) was expressed [8].

However, genetic changes solely do not fully shed light on the molecular mechanisms involved in the pathogenesis of AD. Therefore, epigenetic mechanisms involved in the genomic adaptation according to environmental conditions may possibly explain how environmental exposure affects the risk of allergy development.

Epigenetic mechanisms, in particular methylation, play a key role in immune regulation and are influenced by a variety of environmental factors leading to persistent molecular changes in genes. The methylation process involves the addition of a methyl group to the cytosine (C5 position; 5-methylcytosine, 5mC). DNA methylation occurs primarily in the context of CpG dinucleotides and is the main epigenetic modification involved in the regulation of chromatin structure and gene expression [9].

## 2. Targeted methylation studies

### 2.1 DNA methyltransferase studies

The main enzymes responsible for the methylation process in humans are DNA methyltransferases 1, 3a, and 3b (DNMT1, DNMT3a, and DNMT3b). It is generally accepted that DNMT3a and DNMT3b are *de novo* methyltransferases that form a model of DNA methylation at early stages of development, as well as its changes during cell differentiation [10]. DNA methyltransferase 1 (DNMT1) maintains the methylated state of DNA by attaching methyl groups to one of the DNA strands at the sites where the other complementary strand is methylated [9].

In the context of the AD study, only DNA methyltransferase-1 (DNMT-1), an enzyme that catalyzes the methylation of cytosine bases in CpG islands, has so far been considered. Nakamura et al. for the first time carried out an indirect assessment of methylation status in patients with atopic dermatitis by measuring the expression of messenger RNA (mRNA) of DNA methyltransferase-1 in peripheral blood mononuclear cells (PBMC) by quantitative RT-PCR. Although the expression level of *DNMT-1* mRNA had a tendency to decrease in patients with atopic dermatitis compared with healthy controls, there were no significant differences between these groups [11]. However, in the group of patients with AD, the IgE level was also taken into account. It was found that the level of *DNMT-1* mRNA was significantly lower in the high IgE group compared to the control group.

It is common knowledge that many local factors, such as skin impairment, play an important role in the development of AD [12, 13]. However, Th2-infiltration in response to penetration of allergens and production of cytokines by infiltrated cells (for example, IL-4 and IL-5) plays the key role in the development of IgE-mediated response and chronic inflammation involving eosinophils [14–16]. It is assumed that in these processes DNA hypomethylation contributes to the hyperreactivity of Th2 cells in response to allergens and, as a consequence, cytokine-mediated

IgE production. It has also been suggested that IL-4-mediated IgE production in patients with high serum IgE levels is associated with DNA hypomethylation in B cells [16, 17]. In this study the decrease in *DNMT-1* expression in PBMCs in patients with high IgE levels also confirmed the concept that AD is promoted by lower *DNMT-1* levels.

On the other hand, the lack of significant differences while comparing *DNMT-1* expression between groups with high and low IgE levels, along with no correlation between *DNMT-1* expression and serum IgE levels in the respective patients, indicates that *DNMT-1* cannot be a factor that solely affects serum IgE. To clarify this issue, more studies are needed in which *DMNT-1* levels would be assessed in patients without AD but with high serum IgE levels.

### 2.2 *FCER1G* methylation studies

Based on the opinion that overexpression of the high-affinity IgE receptor on monocytes and dendritic cells contributes to the pathogenesis of AD, a group of scientists studied the epigenetic mechanism of deregulation of high-affinity IgE receptors – FcεRI [18].

Liang et al. measured the methylation level of total DNA of monocytes from 10 patients with AD and 10 healthy people from the control group. Bisulfite sequencing was used as the main method to determine the methylation status of the *FCER1G* promoter region. To determine the functional significance of methylation changes in FcεRI expression, targeted methylation of the sequence and a demethylating agent, 5-azacytidine (5-aza), were used. The levels of FcεRIγ mRNA and FcεRI protein were determined using RT-PCR RT, Western blotting, and flow cytometry, respectively.

Thus, total hypomethylation in $CD14^+$ monocytes in patients with AD was revealed, as well as locus-specific hypomethylation of the *FCER1G* promoter region in comparison with healthy controls. In addition, hypomethylation of *FCER1G* contributed to its increased expression. Targeted methylation in combination with a reporter luciferase assay confirmed this association between methylation and expression. Moreover, treatment of monocytes of healthy people with 5-azacytidine caused a decrease in methylation levels and induction of FcεRIγ transcription and expression of surface FcεRI. The authors showed that demethylation of specific regulatory elements at the *FCER1G* locus promotes an increase in FcεRI expression in monocytes in patients with AD, which, in turn, leads to an enhanced allergic response.

Atopic monocytes with high FcεRI levels are thought to play an important role in the pathogenesis of AD. This is due to the fact that monocytes carrying FcεRI can differentiate into inflammatory dendritic epidermal cells (IDECs), which intensify allergic inflammatory reactions in the skin by stimulating T cells, and are also involved in the transition to the chronic course of AD with a predominance of Th2 [19, 20]. In this study, it was shown for the first time that changes in the epigenetic regulation of the *FCER1G* gene can explain the pathological activation of FcεRI on patient monocytes.

### 2.3 *TSLP methylation studies*

Thymic stromal lymphopoietin (TSLP) plays an important role in maintaining T-cell homeostasis and, apparently, is of great importance in the development of allergic symptoms, especially in atopic dermatitis and asthma [21, 22]. Human TSLP is overexpressed in keratinocytes of patients with acute and chronic AD. However, the mechanism of such TSLP expression remains unclear. The question is whether

TSLP expression is regulated by modification of aberrant DNA methylation of *TSLP* promoter in keratinocytes of AD patients [23].

It is known that the TSLP protein cannot be found in healthy skin, in skin lesions in patients with nickel contact dermatitis or in patients with disseminated lupus erythematosus, as well as in intact skin in patients with AD; however, increased levels of TSLP expression are observed in both acute and chronic atopic skin lesions [21, 24]. TSLP overexpression in keratinocytes can activate myeloid dendritic cells by enhancing the surface expression of CD54, CD80, CD83, CD86 molecules and MHC class II molecules on myeloid dendritic cells [25], which lead to Th2-inflammatory reactions [24].

Luo et al. measured the levels of mRNA and TSLP protein in samples of affected skin from 10 atopic children and 10 healthy people from the control group, using quantitative RT-PCR and immunohistochemistry [26]. Bisulfite sequencing was performed to determine the methylation status of the *TSLP* promoter; 5-aza, a DNA methyltransferase inhibitor, was used to determine the effect of DNA methylation on TSLP expression.

As a result, the levels of expression of mRNA and TSLP protein relative to β-actin were significantly higher in affected skin regions of patients with AD compared with healthy controls. In addition, hypomethylation of the promoter region of the *TSLP* gene containing 16 CG pairs was found in the affected skin regions. Upon treatment of HaCaT cell line keratinocytes with 5-aza, the methylation level of the *TSLP* promoter decreased significantly, while its transcription increased.

It can be concluded that DNA demethylation of the specific regulatory region of the *TSLP* gene may contribute to the overexpression of TSLP in the affected skin regions in atopic patients. This suggests that aberrant epigenetic modifications play an important role in the pathogenesis of this disease.

In another study, the authors tried to reveal the effect of prenatal smoking on DNA methylation in case of atopic disorders [27]. Methylation differences associated with exposure to tobacco smoke were initially identified with the use of Illumina Infinium 27 K methylation kits in 14 children in a Taiwanese study cohort. Information on the course of the disease and possible risk factors was collected. Cord blood levels of cotinine were measured in order to represent prenatal smoking. CpG loci, in which statistically significant differences in methylation were found, were validated by methylation-dependent fragment separation (MDFS). Differential methylation in three genes (*TSLP*, *GSTT1*, and *CYB5R3*) was detected during the experiment. Among these, only the *TSLP* gene showed a significant difference in the percentage of promoter methylation after testing with MDFS. The *TSLP* gene was further investigated in a larger sample group (150 children) which completed a follow-up study. The *TSLP* 5'-CpG island (5'CGI) methylation status has been found to be significantly associated with prenatal exposure to smoke and atopic dermatitis. The degree of the *TSLP* 5'CGI methylation was inversely correlated with the expression levels of the TSLP protein.

Thus, it can be assumed that changes in *TSLP* 5'CGI methylation decrease the regulatory function of the immune system and cause the development of Th2-type allergic inflammation in case of atopic dermatitis. The methylation status of *TSLP* 5'CGI was also found to differ depending on cotinine levels, and hypomethylated *TSLP* 5'CGI was positively associated with atopic dermatitis. Moreover, the degree of *TSLP* 5'CGI methylation and the level of TSLP protein showed an inverse correlation. This means that severe exposure to smoke can lead to *TSLP* 5'CGI hypomethylation. Therefore, hypomethylated *TSLP* 5'CGI is associated with increased gene expression and increased TSLP protein concentration. An increased level of TSLP protein may also activate Langerhans epidermal cells, contributing to the AD development. *TSLP* was also highly expressed in the lesional skin of atopic patients [24].

The results of Wang et al. study suggest that prenatal exposure to tobacco smoke is associated with a risk of atopic dermatitis, possibly through DNA methylation.

### 2.4 *MICAL3* methylation studies

Cho et al. conducted a research to assess the role of 25-hydroxyvitamin D (25[OH]D) deficiency in cord blood in comparison with postnatal 25[OH]D levels in AD development during the first 3 years of life and found out how 25[OH]D deficiency affects the DNA methylation profile of cord blood leukocytes [28].

Severe 25[OH]D deficiency in cord blood was associated with a higher risk of atopic dermatitis diagnosing precisely at the age from 2 to 3. Comparison of differentially methylated CpG sites in accordance with moderate and insufficient 25[OH]D levels in cord blood revealed the common *MICAL3* gene for groups with and without pathology. *MICAL3* was hypomethylated in the case of low 25[OH]D levels.

Since *MICAL3* is a member of the MICAL family of flavoprotein monooxygenases involved in axon control and actin remodeling through oxidation of its molecules or production of reactive oxygen species (ROS) [29], ROS, induced by increased expression of *MICAL3*, can then suppress the antioxidant defense of the fetus, leading to subsequent AD development during the first 3 years of life. This process probably also affects the severity of the disease, since a correlation has been established between the expression of *MICAL3* mRNA and the severity index of atopic dermatitis. In addition, *MICAL3* expression levels were associated with 25[OH]D levels in cord blood regardless of the presence of AD.

To reproduce the mechanism of atopic dermatitis associated with ROS, using the example of *MICAL3*, another gene was chosen, 8-oxoguanine-DNA glycosylase (*OGG1*), which, as is known from data on mRNA expression, contributes to the development of allergic diseases in combination with oxidative stress reactions [30]. Accordingly, in atopic children with 25[OH]D deficiency in cord blood, the expression of *OGG1* mRNA was 5.22 times higher than in healthy children with a sufficient level of 25[OH]D. *OGG1* expression levels were found to be inversely related to 25[OH]D levels and atopic dermatitis severity index. In addition, there is a significant correlation between the expression levels of *MICAL3* and *OGG1*. However, studies showing that *MICAL3* and *OGG1* are directly related have not yet been conducted.

### 2.5 *HBD-1* methylation studies

Noh et al. described patterns of DNA methylation of human β-defensin-1 (HBD-1), a unique antimicrobial peptide expressed in various tissues, including the skin [31]. HBD-1 may be associated with a variety of innate immune system defects in the AD pathogenesis. A possible mechanism for the decrease in *HBD-1* gene expression in atopic dermatitis was investigated, and the *HBD-1* transcription restoration in undifferentiated normal epidermal keratinocytes after treatment with a DNA methyltransferase inhibitor was shown.

Suppression of *HBD-1* in undifferentiated NHEK cells has been shown to be regulated by an epigenetic inactivation mechanism involving methylation of DNA 14 CpG dinucleotide in the 5′-region of *HBD-1*. In dermatitis-affected skin, the frequency of methylation at the CpG 3 and CpG 4 sites within the *HBD-1* promoter was significantly higher than in healthy skin.

To identify specific CpG sites that play a significant role in HBD-1 expression in NHEK cells, bisulfite genomic sequencing of the region upstream of the proximal site of the *HBD-1* promoter was performed and methylation profiles of 6 CpG dinucleotides (from CpG 3 to CpG 8) were determined. Since the single nucleotide

polymorphism (rs2978863) is located at the CpG 8 locus within the *HBD-1* promoter region (GenBank accession no. NC_000008.11) in the NHEK cell line, the other five CpG dinucleotides (CpG 3–7) were subjected to bisulfite sequencing analysis. Studying the methylation profile of the *HBD-1* promoter revealed detectable demethylation at the CpG 3 and CpG 4 loci in 2-deoxy-5-azacytidine-treated NHEK cells compared with untreated control cells. Such differentially methylated single CpG units in the *HBD-1* promoter may play a special role in the regulation of *HBD-1* transcription of the NHEK cell line.

Thus, epigenetic modulation of the *HBD-1* promoter, that is, DNA methylation in two separate CpG units, can affect *HBD-1* expression *in vitro*. In the affected skin, both CpG sites were hypermethylated. The failure of skin innate immunity leading to increased colonization of *S. aureus* in atopic patients may be due to an epigenetic predisposition of constitutively expressed *HBD-1*.

## 3. Genome-wide DNA methylation

### 3.1 In naive CD4$^+$

Both atopic dermatitis and psoriasis are characterized by a targeted immune response via polarized CD4$^+$ T cells. During the polarization of naive CD4$^+$ T cells, DNA methylation plays an important role in the regulation of gene transcription. Taken into consideration the similarity of immune response of atopic dermatitis and psoriasis, Han et al. conducted a study of the global DNA methylation profile in naive CD4$^+$ T cells in patients with AD and psoriasis, as well as in healthy people using the ChIP-seq method. DNA hypomethylation (more than 4 times) was found in T-cell samples isolated from patients with psoriasis and healthy people in 26 genome sites ranging in size from 10 to 70 kb. These regions were mostly pericentromeric on 10 different chromosomes and randomly overlaid with various defining epigenome signals, such as histone modifications and binding sites for transcription factors (according to the ENCODE project), which implied the potential influence of epigenetic regulation in the development of psoriasis [32].

To determine whether naive CD4$^+$ T cells from patients with AD or psoriasis have DNA methylation patterns different from those of healthy people, complex genome-wide CpG methylation profiling was performed. The uniquely mapped regions coincided with strong histone modification signals such as H3K4Me1, H3K27Ac, and H3K4Me3, as well as with transcription factor binding sites in various cell lines.

It appears that hypomethylation in some pericentromeric regions of naive CD4$^+$ T cells may be a sign of psoriasis, but not atopic dermatitis. It is not yet clear what exact role epigenetic changes of these regions play in the development of T cells. However, these data show for the first time the importance of such changes in the development of immune-mediated skin diseases [33].

The X chromosome encodes many of immune genes, which show a higher hypermethylation pattern than other genes. It is known that abnormalities, such as inactivation of the X chromosome, can contribute to the impairment of self-structures recognition and, ultimately, lead to autoimmunity [34]. In addition, DNA methylation is involved in the initiation of the X chromosome inactivation, as well as in the stable maintenance of the gene silencing state [35]. These studies suggest that DNA methylation may affect gene expression on the X chromosome or the development of T cells in psoriasis. It was found that DNA methylation is dramatically increased in the promoter region of genes on the X chromosome in patients with psoriasis. The binding sites for CDPCR3, GATA3, BRN2, and other

transcription factors were identified as slightly enriched. The data obtained on epigenome changes in T cells show that naive $CD4^+$ T cells may be involved in the development of atopic dermatitis or psoriasis even before antigenic stimulation. This may be due to the effects of various environmental factors.

### 3.2 Tissue-specific patterns

To determine the tissue-specific differences in DNA methylation associated with AD, the research group of Rodriguez et al. examined the DNA of whole blood, T cells, B cells, as well as the affected and unaffected epidermis of atopic patients and healthy people from the control group [36]. To identify functional associations, they studied the expression profiles of epidermal mRNA.

Whole-genome methylation analysis was performed using Human Methylation27 BeadCheap. The results for epidermal tissue were different from those for blood cells. To determine the intraindividual and interindividual differences in DNA methylation, the researchers identified a pairwise correlation of methylated regions in the same tissue in samples from patients of similar sex and age, as well as between different tissues in the same person. In whole blood, T cells, and B cells, there were no significant differences in genome DNA methylation in the pathology group as compared with and the control group, and in general, intraindividual differences in DNA methylation were greater than those between individuals. A clear link was shown in case of comparing similar tissue in different individuals for different CpG sites, which partially correlated with altered levels of gene transcripts, mainly related to the processes of epidermal differentiation (*S100A* genes) and reactions of the innate immune response - thus, this study confirms the high the level of tissue specificity for DNA methylation patterns.

Regarding differentially methylated CpG islands in the epidermal tissue, 9 regions were identified as reliably associated with atopic dermatitis: in the *CFLAR*, *GPR55*, *MMP7*, *LOC283487*, *SH2D2A*, and *ERP27* genes, these regions were hypomethylated, and in the *LRRC8C*, *S100A5*, and *EBP49* genes, these regions were hypermethylated.

Based on analysis of whole genome mRNA expression (using HumanHT-12v3 Expression BeadChip), significant differences were revealed in seven transcripts when comparing samples of the affected skin of patients with AD and the skin of healthy people.

From nine selected pairs of differentially methylated regions / differentially expressed transcripts using the EpiTYPER system and quantitative PCR, the following combinations associated with the development of AD were successfully validated: *KRT6A*/*KRT6A* and *KRT6A*/*KRT6B* (encode keratin); *IFI27*/*IFI27*, *OAS2*/*OAS2* (belong to the family of proteins regulated by IFN), *GDPD3*/*GDPD3* and *S100A5*/*S100A2*. In most of these pairs, an inverse correlation was observed, that is, higher levels of methylation were associated with lower expression of the relevant gene, and vice versa. Such dependence is usually observed in CpG islands near the sites of transcription initiation, where DNA methylation is associated with prolonged silencing of the relevant gene.

Olisova et al. carried out a genome-wide study of DNA methylation using the Illumina Infinium Human Methylation450 BeadChip technology [37]. When comparing the affected and unaffected skin areas in atopic patients, no difference in the methylation profile was found. This suggests that epigenetic changes affected the entire skin as a whole, although they have not yet appeared in clinically intact skin areas. However, when comparing the affected skin with the skin of healthy volunteers, differentially methylated genes of the TSS200 and TSS1500 regions were isolated, whose protein products were involved in the pathogenesis of atopic

dermatitis and related processes: steroid hormone biosynthesis and cell metabolism (*HSD17B14*, *HSD17B*), epithelial differentiation (*KRT31*, *LCE3D*), regulation of DNA-dependent transcription and RNA processing (*DMBX1*, *MTO1*, *SNORD93*, *WDR36*), immune response and activation of lymphocytes (*AIM2*, *CD300E*, *CLEC1A*, *DEFB135*, *IL23A*), activation of transforming growth factor β1 (*LTBP1*), cellular proliferation and apoptosis (*SERPINB3*, *EPR1*).

### 3.3 Replicated methylation

Another genome-wide epigenetic study examined differences in DNA methylation in atopic dermatitis together with herpetic eczema (HE), and revealed how methylation changes in patients with atopic dermatitis, complicated or uncomplicated HE [38].

490 significantly differentially methylated CpG sites were identified. Many of these were associated with indicators of disease severity, especially with the level of eosinophils (431/490 sites). One CpG region was replicated and was significantly differentially methylated based severity and phenotype.

The authors found replication for one CpG region associated with total serum IgE in the *IL4* gene, as well as possible replication for four CpG regions associated with HE in the *IL13* and *IL4* genes. It has also been shown that eosinophil levels play an important role in methylation patterns in people with AD, which via molecular mechanisms can lead to phenotypic changes.

## 4. Epigenetic regulation of immune system factors

It is known that abnormal epigenetic regulation of immune factors and skin barriers contribute to the pathogenesis of AD. During the development of immune system cells, epigenetic mechanisms are involved in specific changes in the variants of immune response [39]. Here are some examples.

Regulatory T cells (Tregs) play an important role in early immune programming and the formation of an adequate immune response in relation to pro-allergic or tolerant conditions. Tregs are best characterized by the expression of transcription factor 3 (Foxp3), which is important for the induction and stability of Tregs [40]. Foxp3 is controlled by DNA methylation of its transcriptional regulatory regions. Naturally induced by TGF-β Foxp3$^+$ Tregs indicate stable expression of Foxp3, which is associated with selective demethylation of an evolutionarily conserved element at the Foxp3 locus - a Treg-specific demethylated region. Inhibition of DNA methylation by azacytidine, even in the absence of exogenous TGF-β, not only promotes induction of Foxp3 expression *de novo* during priming, but also ensures stability of Foxp3 expression upon restimulation. Importantly, stable Foxp3 expression was detected only in cells with an increased level of TSDR demethylation [41]. Research suggests that prenatal environmental factors can alter DNA methylation at the *FOXP3* locus in cord blood. Babies with low Tregs identified by TSDR demethylation at birth may have a higher risk of AD developing or sensitization to food allergens in the first 3 years of life [42].

In the neonatal immune system, epigenetic regulation can be shifted away from Th1-mediated immunity in order to prevent dangerous cellular immune responses to the developing fetus. The IFN-γ gene (*IFNG*), a prototype Th1 cytokine gene whose activity is regulated during fetal development, is hypermethylated in the promoter regions of resting neonatal CD4$^+$ cells compared to adult ones [43]. Similarly, the availability of chromatin at the *TBX21* locus, a major regulator of Th1 clone committing, is attenuated in neonatal CD4$^+$ cells compared to mature cells,

and a decrease of transcription factor level in peripheral T cells suppresses IFN-γ production [44]. After birth, exposure to a variety of microorganisms and the formation of microbiota contributes to the essential activation of Th1 immune responses through epigenetic modifications. In a mouse model it was shown that prenatal administration of gram-negative bacteria leads to histone H4 acetylation at the *IFNG* gene and the associated increase in IFN-γ production in the offspring [45].

MicroRNAs (miRNAs) are short, single-stranded RNA molecules that function with their associated proteins and cause the degradation of targeted mRNAs, inhibiting their translation. miRNAs play an important role in a wide range of biological processes, including proliferation, differentiation, determination of cell development, apoptosis, signal transduction, and organ development. Some miRNAs are expressed specifically for each type of cells and tissues and contribute to the maintenance of cell identity. Tissue-specific miRNAs function at various levels of gene regulation, ranging from control of targeted effector genes, incompatible with the differentiated state, to control over the levels of transcriptional regulators and alternative pre-mRNA splicing. This multilevel regulation of miRNAs influences the gene expression program of differentiated cells [46]. miRNAs, including miR-21, miR-146, and miR-223, activated in patients with allergic disorders, are also activated in the skin of patients with AD [47]. A study by Herberth et al. showed that maternal exposure to tobacco during pregnancy correlated with high levels of miRNA-223 and low Treg cell levels, which predisposed children to atopic dermatitis during the first 3 years of life [48]. Sonkoly et al. found that miR-155 was one of the most activated miRNAs in lesional skin samples from atopic patients in comparison with skin samples from healthy people. It has been found that local exposure of relevant allergens to intact skin of patients with AD induces miR-155 expression. miR-155 suppresses cytotoxic T lymphocyte – associated protein 4 - CTLA-4, which negatively regulates the function of T cells. This suppression of CTLA-4, in turn, enhances the T cell proliferative response, which can then lead to a long-term chronic inflammatory state [47].

## 5. Conclusion

There is not much evidence on the role of epigenetic mechanisms of innate and adaptive immunity regulation in the pathogenesis of atopic diseases, as these mechanisms have been studied recently. The described candidate genes involved in pathological processes such as dysfunction of the epidermal barrier, enhanced transmission of Th2 immunity signals, weakened innate immune responses, etc. play an important role in the pathogenesis of AD. Epigenetic studies also indicate modifications in genes involved in these mechanisms. Dysfunction of the epithelial barrier and immune response reactions together trigger the development of atopic dermatitis.

New insights on epigenetic and immunological markers associated with the risk of development of atopic dermatitis will help to create new prognostic approaches in the management of patients with atopic pathology. In this regard, it is important to have a complete understanding of the pathogenic mechanisms of an allergic disease.

## Conflict of interest

The authors declare no conflict of interest.

## Author details

Vladimir Sobolev[1,2*], Elizaveta Bystritskaya[1] and Oxana Svitich[1,3]

1 I. Mechnikov Research Institute for Vaccines and Sera, Moscow, Russian Federation

2 Center for Theoretical Problems of Physico-Chemical Pharmacology, RAS Moscow, Russian Federation

3 Sechenov University, Moscow, Russian Federation

*Address all correspondence to: vlsobolew@gmail.com

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