Review Article

Alterations in Immune-Related Defensin Alpha 4 (DEFA4) Gene Expression in Health and Disease

Fatemah Basingab,1,2 Abeer Alsaiary,1,3 Shahad Almontashri,1 Aisha Alrofaidi,1 Mona Alharbi,1 Sheren Azhari,1 Khloud Algothmi,1 and Safiah Alhazmi1

1Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, Jeddah, Saudi Arabia
2Immunology Unit, King Fahad for Medical Research, King Abdulaziz University, Jeddah, Saudi Arabia
3Biology Department, College of Sciences, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

Correspondence should be addressed to Fatemah Basingab; fbaseqab@kau.edu.sa

Received 9 January 2022; Revised 31 March 2022; Accepted 5 May 2022; Published 28 May 2022

1. Introduction

The innate immune response is the first mechanism to protect the host from invading pathogens. This response occurs through the collaboration between many constituent elements of the innate immune system. The innate immune system is composed of anatomical and physical barriers, a variety of cells, cell receptors, soluble mediators, and host defense peptides (HDPs) (e.g., histatins, cathelicidin, defensins, etc.) [1].

Defensins are small (3–5 kDa) cysteine-rich, cationic peptides. Mature defensins contain six cysteine residues (Cys), forming three intramolecular disulfide bonds. Based on the configuration of the disulfide bonds, human defensins are classified into alpha (α) and beta (β) subfamilies [2]. Human α-defensin genes co-locate with β-defensin genes on adjacent loci on chromosome 8 (8p22-p23) [3–6].

In α-defensins, three disulfide bridges are formed (between Cys1-Cys6, Cys2-Cys4, and Cys3-Cys5 pairings) [7]. In humans, the α-defensin family consists of six members (DEFA1–6). Members 1–4 were initially identified in neutrophils. Thus, DEFA1–4 were called Human Neutrophil Peptides (HNP) (HNP-1, HNP-2, HNP-3, and HNP-4) [8, 9]. The other two members were found in the Paneth cells of the intestinal tract. These jejunal defensins are known as Human Defensin (HD5 and HD6) [5]. In this review, the DEFA abbreviation will be used instead of (HNP or HD) to indicate these peptides.

DEFA1–4 are abundantly expressed and stored in azurophil granules of neutrophils, where they comprise up to 30% of the protein content in these granules. Furthermore, DEFA1–4 can be expressed by various cell types such as lymphocytes, monocytes, natural killer (NK) cells, and mucosal surface epithelium [10–12].
Human α-defensins exert broad-spectrum activities against enveloped viruses and different types of cellular pathogens [13–16]. Additionally, α-defensins can act as immunomodulatory agents exhibiting both anti-inflammatory activity and pro-inflammatory activity [17–19]. Human α-defensins are chemoattractant for multiple different immune cell types such as immature dendritic cells (DCs), macrophages, mast cells. In addition, α-defensins can induce cytokine and chemokine production [11, 17, 20]. The α-defensins are released from dead neutrophils by apoptosis or necrosis leading to repressed inflammatory cytokines and nitric oxide release from macrophages which ultimately lead to limiting the pro-inflammatory response [18]. Moreover, α-defensins roles are not limited to fighting against pathogens or modulating the immune response. The α-defensins have therein been reported to be involved in various non-immune physiological processes such as wound healing and promoting collagen expression [21–23], activation of the Nuclear Factor Kappa B (NF-κB) pathway [20], anti-angiogenic, anti-tumor as well as activation of cancerous cell proliferation [24–28], anti-Adrenocorticotropic hormone (ACTH) activity and inhibiting glucocorticoid hormone secretion [9]. The ability of α-defensins to perform various functions has been attributed to the amphiphilic–cationic organization of these peptides. The cationic nature enables these peptides to selectively bind and disrupt the negatively charged microbial membranes. Furthermore, the cationic nature of α-defensins may facilitate interactions with different host receptors via electrostatic forces [5].

At the peptide sequence level, DEFA1, 2, and 3 are almost identical; DEFA1 and DEFA3 differ only at the N-terminal residue (alanine in DEFA1 and aspartic acid in DEFA3). Interestingly, DEFA2 is not an individual gene product and it can be encoded by both DEFA1 and DEFA3 genes redundantly. DEFA2 can therein be generated through proteolytic removal of the first N-terminal amino residue of DEFA1 or DEFA3 [29]. On the other hand, DEFA4 is different from DEFA1–3 at the amino acids sequence level. DEFA4 shares only 11 identical residues with DEFA1, 2, 3. Nine of these eleven shared residues are therein structurally conserved in mammalian α-defensins (six Cys residues) (Arginine5 (Arg)—Glutamic acid13 (Glu) ion pair) and (Glycine 17 (Gly)) (Figure 1(a)) [13, 30]. Despite these differences in sequence identity between DEFA1, 2, 3 and DEFA4, the tertiary structures of DEFA4 and DEFA1, 2, 3 are similar [16, 30].

Interestingly, the proteolytic digestion process of α-defensins may naturally occur in the human body generating small α-defensins-derived peptides [31]. These small α-defensins-derived peptides can exhibit distinct biological properties. Ehmann et al., in 2019 observed that full-length DEFA5 can be degraded by human duodenal fluid into fragments and showed various antimicrobial activities as well as modulation of the microbiota composition with maintaining the diversity [31]. DEFA5 (1–9) was therein capable of increasing the Akkermansia sp amount in the small intestine microbiota of treated mice for 2 weeks [31]. Moreover, in 2020, DEFA5 (1–9) was reported as an active anti-viral against primary and multi-resistant isolates of Human Cytomegalovirus (HCMV). DEFA5 (1–9) can prevent viral entry by inhibiting HCMV attachment to host cells [32]. In addition, Ehmann et al., in 2020 examined the other α-defensins member (DEFA4) and they reported the modified version of DEFA4 (1–11) was highly effective against multidrug-resistant bacteria more than DEFA4 full-length peptide [33].

DEFA1, 2, 3 account for up to 30% of the total protein content in azurophil granules of human neutrophils. In contrast, DEFA4 is considered much less abundant. DEFA4 constitutes only 1% to 2% of the total defensins found in neutrophils [34]. Little is known about DEFA4 compared to DEFA1, 2, 3 which have been extensively studied [30, 35]. To our knowledge, no prior reviews have focused on human DEFA4. Therefore, this review attempts to provide sufficient knowledge about human DEFA4 by discussing the most prominent reports about DEFA4 milestone discoveries from 1988 to date, along with the observed changes in DEFA4 expression in different diseases.

2. Study Design

Information is obtained from electronic databases including Medline, PubMed, ScienceDirect, Microsoft Academic. The search is limited to publications in the English language from 1988 until 2022. The search is applied based on keywords that included the gene and protein scientific names and symbols. The scientific terms used are Defensin Alpha4, Neutrophil Defensin4, Human neutrophil peptide-4, Human Neutrophil α-Defensin 4, Corticostatin, whereas the scientific symbols are: DEFA4, DEF4, HP4; HP-4; HNP-4.

3. Human Defensin Alpha 4 (DEFA4)

3.1. DEFA4 Milestone Discoveries. Human defensin alpha 4 was discovered and denoted for the first time as HP-4 in 1988 by Singh et al. This discovery came when they were trying to extract human neutrophil peptides that can exhibit anti-ACTH activity (corticostatic activity). They found that at nanomolar concentrations, the novel peptide has exhibited corticostatic activity on the rat adrenal cell suspension in vitro. Thus, due to this corticostatic activity, DEFA4 was also described as Corticostatin [9]. Later in 1989, Wilde et al. also announced the discovery of the same novel peptide that had been previously discovered in 1989 by Singh et al., and it was called Human Neutrophil Peptide-4 (HNP-4). Moreover, they observed the preferential ability of DEFA4 to kill Gram-negative bacteria more than Gram-positive bacteria [13]. In 1993, Palfree et al. found that DEFA4 was located on chromosome 8. In addition, at the sequence level, the complementary DNA (cDNA) of the DEFA4 gene was similar in approximately 72% of the nucleotide sequence identity of the DEFA1 gene. Nevertheless, the cDNA of the DEFA4 gene was different from the cDNA of the DEFA1 gene by an extra 83-base segment that appeared to be the result of a recent duplication within the coding region [4]. Due to DEFA4 scarcity in natural sources, previous studies have attempted to produce DEFA4 peptides [35, 36]. In 2004, DEFA4 was synthesized using the solid-
In 2005, DEFA4 antimicrobial ability was assayed compared to other α-defensins members, and the potency of DEFA4 against Gram-negative bacteria was confirmed [14]. Moreover, DEFA4 was investigated as an antiviral agent *in vitro*, and DEFA4 was observed to act effectively against human immunodeficiency virus type 1 (HIV-1) (detailed in DEFA4 functions) [15]. Later in 2006, the high-resolution X-ray structure of DEFA4 was presented by Szyk, and the similarity in the tertiary structure of DEFA4 with other members of α-defensins and β-defensin was demonstrated (detailed in DEFA4 protein structure). Additionally, DEFA4 anti-microbial activity has been assessed, confirming the strong ability of DEFA4 against Gram-negative [16]. In the context of production, antimicrobial peptides are an alternative to antibiotics to overcome the drug resistance-bacteria crisis. In 2015, transgenic chickens capable of expressing the DEFA4 protein in egg whites were generated [36]. In 2019, systematic mutational analysis was conducted to elucidate the molecular determinants underlying DEFA4 functions. All DEFA4 residues were individually mutated to alanine (Ala) except for the nine conserved residues (6xCys, 1xArg, 1xGlu, and...
1xGly). They have pointed to the important functional residues in DEFA4 (detailed in DEFA4 protein structure) [30]. In 2020 Ehmann et al. reported that the modified version DEFA4 (1–11) was highly potent and efficient against antibiotic-resistant bacteria, suggesting DEFA4 (1–11) can be used as a cost-effective therapeutic candidate [33]. DEFA4 has been identified as one of the top hub genes represented as nodes with a high degree of interaction in the network of differentially expressed genes in acute respiratory syndrome coronavirus (SARS-CoV) datasets. This suggests that DEFA4 may play an important role in SARS-CoV infection pathology [37, 38]. Hub genes produce proteins that can interact with a large number of other proteins (partners) [39]. The hub genes are considered functionally significant in the pathogenesis and progression mechanism of many diseases. Moreover, the hub genes may represent candidate biomarkers for diagnosis as well as be targeted in the therapeutic procedure. For example, DEFA4 was one of 6 genes constructed as a classifier that can predict the severity of different viral infections including the SARS-CoV 2 virus (COVID-19) [40]. All studies are summarized in Figure 2.

3.2. DEFA4 Gene Expression. Defensin Alpha4 gene (DEFA4) is a protein-coding gene that results in the production of Defensin Alpha4 peptides (DEFA4), also known as Human Neutrophil Peptide 4 (HP-4, HNP-4, HP4), Neutrophil defensin4, and Corticostatin. DEFA4 gene is located on chromosome 8, specifically 8p23.1 (Figure 3) [41]. DEFA4 gene consists of three exons, the first exon encoding a 5’ untranslated region. Therefore, only two exons are present in the final version of mature RNA (mRNA) as a protein-coding sequence [42]. At the transcription level, only one transcript is produced that is translated into the mature peptide containing only 33 residues [43]. In normal human tissues, DEFA4 is expressed in various tissues, but the highest DEFA4 expression rate was found in bone marrow and whole blood [41, 44].

3.3. DEFA4 Protein Structure. During the translation process, the mRNA of DEFA4 is translated as a 97-residue precursor. Cleavage of the DEFA4 precursor occurs at several sites resulting in mature peptides containing only 33 residues. DEFA4 mature sequence is: HN2-Val-Cys-Ser-(1–11) can be used as a cost-effective therapeutic candidate against antibiotic-resistant bacteria, suggesting DEFA4 version DEFA4 (1–11) was highly potent and efficient against Gram-negative bacteria, and whole blood [41, 44]. Out of the 33 residues, 1 Arg, 1 Glu, and 1 Gly residue. Moreover, the six Cys residues form intramolecular disulfide bonds. Arg (5) and Glu (13) form the invariant salt bridge, which has a role in cellular stability and biosynthesis (Figure 1(a)) [16]. Gly (17) is important for correct folding [30]. The folding of DEFA4 monomers is arranged into three antiparallel beta-sheets (B) (Figure 1(c)) [16]. The conserved residues are the most structurally conserved and rigid residues present in the peptide sequence and play roles in folding, proteolytic stabilizing, and chemotactic activity. Any changes or substitutions of these residues are structurally harmful [16]. Arg10, 11, 15 are important cationic residues that have a role in the elimination of bacteria, while Phenylalanine (phe26) is the key residue for most of the DEFA4 functions [30].

3.4. DEFA4 Functions

3.4.1. DEFA4 as Inhibitor of Corticosterone Production (Corticostatin). ACTH stimulates adrenocortical steroidogenesis to produce corticosterone (named Cortisol in humans) [47]. However, this stimulation action can be interfered with and inhibited by corticostatic activity (anti-ACTH). In 1988, when DEFA4 was discovered, DEFA4 ability to exert corticostatic activity was examined on rat adrenal cell suspensions in vitro. It was reported that, at nanomolar concentrations, DEFA4 can interfere with ACTH and inhibit corticosterone production. As a result of this DEFA4 functional property, DEFA4 was described and termed Corticostatin [9].

3.4.2. DEFA4 Anti-Microbial Activity. One of the most known features of DEFA4 is the preferential ability to kill Gram-negative bacteria more than Gram-positive bacteria. In 1989 Wilde et al. reported DEFA4 was 100 times more efficient against Gram-negative bacteria Escherichia coli (E. coli) and four times more against both Gram-positive bacteria Streptococcus faecalis (S. faecalis) and yeast Candida albicans (C. albicans) in comparison to DEFA1, 2, 3 mixture [13]. This finding was consistent with Ericksen’s results in 2005, when DEFA4 showed a stronger preference to kill Gram-negative bacteria E. coli and Enterobacter aerogenes (E. aerogenes) than other members (DEFA1, 2, 3) whereas, DEFA5 was comparable to DEFA4. Conversely, DEFA4 was less active against Gram-positive bacteria Staphylococcus aureus (S. aureus) and Bacillus cereus (B. cereus) [14]. In another study in 2006, DEFA4 was reported to be more potent against the Gram-negative bacteria E. coli than Gram-positive bacteria S. aureus, and yeast C. albicans [16]. This preferential ability of DEFA4 to kill Gram-negative bacteria is associated with its cationic properties and distinctive distribution of positively charged amino acids in the DEFA4 structure. DEFA4 has the extra positive charge (+4) compared to DEFA1, 2, 3 (+3). Moreover, DEFA4 has a positive cluster that is composed of three clustered cationic amino acids (Arg10, Arg11, and Arg15). The cationic cluster plays an important role in facilitating high-affinity interactions with negatively charged components lipopolysaccharide (LPS) in the outer membrane of Gram-negative bacteria (Figure 1(b)) [30].

On the other hand, the small α-defensins-derived fragments display distinctive biological properties that exceed the full-length peptide [31, 33]. Recently, a DEFA4-derived fragment (1–11) was generated by proteolytic digestion to investigate the antimicrobial activity of this fragment in vitro. Moreover, in aiming to enhance the stability against proteolytic digestion, a DEFA4 (1–11) modified version was generated by acetylation of the N-termi, amidation of the C-terminus, and exchanging the L-amino acids with D-amino acids. Results indicated that
the modified version DEFA4 (1–11) showed high potency as well as high efficacy in comparison with the full length of DEFA4 and the non-modified version DEFA4 (1–11) [33]. Interestingly, the modified version DEFA4 (1–11) was highly effective against Pseudomonas aeruginosa, Klebsiella pneumoniae and Acinetobacter baumannii that were considered the top pathogenic bacteria listed as multidrug-resistant bacteria according to Centers for Disease Control and Prevention (CDC) and the World Health Organisation (WHO) global priority pathogens list [33, 48, 49]. Thus, modified version DEFA4 (1–11) may serve as a promising alternative therapy to overcome the antibiotic-resistant bacteria crisis.

3.4.3. DEFA4 Antiviral Activity. The HIV-1 infection cycle begins when specific viral surface protein (viral envelope glycoprotein 120 (gp120)) recognizes and binds to specific T
cell receptors (the cluster of differentiation four receptor (CD4 receptor)) [50]. The α-defensins can block the initial phase of the HIV infectious cycle by acting as a lectin ligand of CD4 and HIV-1 gp120, preventing CD4-gp120 interaction. Besides, α-defensins can dramatically down-regulate the expression of CD4 receptors that are considered critical receptors in T-cell activation [51]. Wu et al. examined the antiviral ability of DEFA4 along with DEFA1, 2, 3 against both X4 and R5 strains of HIV-1 in vitro. DEFA4 was observed to be more effective than DEFA1, 2, 3 in protecting peripheral blood mononuclear cells (PBMCs) from infection by both strains of HIV-1 [15], although DEFA4 has a significantly weaker capacity in acting as a lectin ligand to gp120 and CD4. Thus, HIV-1 inhibition by DEFA4 is attributed to a lectin-independent property, but the exact mechanism underlying this effective inhibition is still unclear [15, 52].

As mentioned in the introduction, DEFA5-derived fragments served as promising therapeutic agents [31–33]. DEFA 5 (1–9) and DEFA5 (7–32) showed more anti-viral activity against HCMV than DEFA4 (1–11). Notably, DEFA5 (1–9) showed antiviral activity against HCMV primary and multiresistant HCMV strains with lower cytotoxicity. DEFA5 (1–9) can prevent HCMV infection through inhibition of HCMV attachment to various human cells. At the structure level, four amino acids showed functional importance for DEFA 5 (1–9) antiviral activity against HCMV, Cys3, and Cys5 that contribute to disulfide bridges formation as well as Arg6 and Arg9 which are important for membrane interactions [32].

3.5. DEFA4 Gene in Infectious and Inflammatory Diseases

3.5.1. Liver-Related Diseases. The primary function of DEFA4 is to eliminate pathogenic microorganisms such as viruses, bacteria, fungi, and yeast. Through the years, a strong connection between infectious diseases and DEFA4 has been established (Table 1 summarizes the studies that have investigated changes in DEFA4 gene expression related to infectious and inflammatory diseases). Several reports indicated the changes in the DEFA4 gene expression level are associated with liver pathology such as the different kinds of hepatitis infection including hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis E virus (HEV). A study was published by Zhou et al. to identify the pathogenesis of hepatitis B–related acute chronic liver failure (HBV-ACLF). This study concluded that DEFA4 is among one of the genes that could serve as potential biomarkers for HBV-ACLF detection [53]. Severe viral infection and increased inflammation could be significant risk factors for HBV-ACLF development. Thus, the high levels of DEFA4 may be attributed to its function as a host defense peptide in the inflammatory response [53]. Another study conducted by Qiu et al. aimed to analyze gene expression profiles of PBMCs from patients that failed to trigger effective immune response after HBV vaccination. DEFA4 was reported to be up-regulated in the non-responders [54]. A high level of DEFA4 can be produced by cytokines or the activation of toll-like receptors (TLRs) that can modulate adaptive immune response [54]. TLRs are pattern-recognition receptors that play a key role in the innate immune response. TLRs act as initiators of the innate immune response by enabling the host to identify pathogen-associated molecular patterns (PAMP). In general, defensins can prevent viral infection by directly acting on the virion or by affecting the target cell and hence interfering with viral infection indirectly [55].

Furthermore, the peripheral blood samples from patients with HCV submitted to liver transplant have been examined after seven days of the transplantation. The result indicated that DEFA4 was up-regulated in recipients [56]. In 2020 a study was conducted by Ramadasi and Arankalle among pregnant women with HEV to investigate the possible mechanisms that lead to the presence of this infection. The study examined women at the 2nd and 3rd trimesters, and DEFA4 was observed at a high level among these pregnant patients [57]. This DEFA4 up-regulation in pregnant women may be attributed to gene expression changes that naturally occur during pregnancy. According to Knight et al., DEFA4 was one of the genes that showed an increased expression throughout a healthy full-term pregnancy [58].

3.5.2. Respiratory-Related Diseases and Periodontitis. Additionally, numerous studies have revealed up-regulation of DEFA4 in various respiratory-related diseases. Interestingly, a study conducted by Overmyer et al. regarding COVID-19 on 102 COVID-19 patients sought to predict its severity and possible pathophysiology. The elevated level of DEFA4 expression was correlated with the status and severity of symptoms in COVID-19 patients [59]. Up-regulation of DEFA4 and neutrophil-related genes in patients with COVID-19 indicated an increased number of neutrophils and degranulation [59]. Consequently, neutrophils release the inflammatory mediators and contribute to cytokines storm [60]. In addition, neutrophils can release neutrophil extracellular traps (NETs) that can be defined as extracellular webs comprised of granule proteins, oxidant enzymes, and chromatin. These NETs may increase inflammation and microvascular thrombosis leading to organ damage [61, 62]. Moreover, it was reported that elevated levels of both Interleukin 6 (IL-6) and α-defensins were associated with the acceleration of clot formation and disease severity [62]. Furthermore, up-regulation of DEFA4 and neutrophil-related genes may reflect emergency myelopoiesis in fatal COVID-19 [63]. On the other hand, dexamethasone treatment was recommended for only those COVID-19 patients undertaking respiratory support; wherein, dexamethasone treatment was reported to reduce the deaths in ventilated patients and patients receiving oxygen therapy [64]. Dexamethasone is the synthetic version of cortisol hormone that exhibits the same anti-inflammatory effects of cortisol. Cortisol is naturally produced in the human body and plays a critical role in inhibiting inflammation through acting as a transcriptional factor and regulating the gene expression of inflammatory-related genes [65]. DEFA4 exhibited corticostatic effects at nanomolar concentrations [9]. Thus, another possible effect is that
### Table 1: List of studies that indicated an alteration in DEFA4 gene expression in respiratory-related diseases, periodontitis, liver-related diseases, autoimmune diseases.

| References | Diseases                          | Study aim                                                                 | Research contribution                                                                                                                                                                                                 |
|------------|-----------------------------------|---------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| [53]       | Hepatitis B virus (HBV)           | To identify the possible causes and pathogenesis of hepatitis B-related acute chronic liver failure (HBV-ACLF); the analysis of the transcriptome of PBMCs has been applied. | DEFA4 gene was indicated as one of the genes that could serve as a potential biomarker for HBV-ACLF detection.                                                                                                    |
| [54]       | Hepatitis B virus (HBV)           | Analysis of the transcriptome of PBMCs to recognize the gene expression pathways that failed to trigger effective immune response after a hepatitis B vaccination. | DEFA4 gene was up-regulated in the non-responders.                                                                                                                                                                     |
| [56]       | Hepatitis C virus (HCV)           | Gene expression profiles were examined on peripheral blood samples from liver transplant recipients with HCV (n = 6) before and after the seven-day liver transplantation | DEFA4 was detected to be one of the 97 up-regulated genes (>2-fold) in liver transplant recipients with HCV seven days after liver transplantation compared with before transplantation. |
| [57]       | Hepatitis E virus (HEV)           | RNAseq analysis was applied to understand the causes of HEV in: - Pregnant women (PR) at their 2nd and 3rd trimesters at two-phase acute and subclinical phases. - Non-pregnant women (NPR) also with two phases acute and convalescent, all compared with healthy subjects. | The result indicated that there was no presence of DEFA4 in NPR women at all phases while it was up-regulated in all phases for PR women.                                                                                     |
| [67]       | Asthma                            | To determine the differences between asthma and non-asthma groups, as well as stratify potential subgroups depending on gene expression of blood samples. | (i) DEFA4 was identified as one of the 19 up-regulated genes in neutrophil-predominant asthma compared with other asthma phenotypes. (ii) 40% of non-responders to corticosteroid treatment were suffering neutrophil-predominant asthma. |
| [68]       | Asthma                            | This study aimed to distinguish between different phenotypes of asthma in children through examining gene expression profiles of PBMCs. | (i) DEFA4 was one of the genes that showed significantly higher gene expression in patients with severe asthma compared with healthy individuals.                                                                 |
| [69]       | Idiopathic pulmonary fibrosis (IPF)| In this study, gene expression profile analysis of blood samples was performed to identify biomarkers that would enable determining the severity and monitoring the progression of IPF. | DEFA4 was reported as one of 13 differentially expressed genes that can distinguish between mild and severe IPF cases, wherein DEFA4 was up-regulated in severe cases.                                                                 |
| [70]       | Idiopathic pulmonary fibrosis (IPF)| This study aimed to examine the gene expression profiles from blood samples of IPF patients compared to controls at the baseline and longitudinal follow-up after (1, 3, 6) months as well as one year (if alive) to monitor changes in the gene expression over time. | DEFA4 was one of the top up-regulated genes in IPF subjects versus controls. Moreover, DEFA4 was one of the most significant genes that showed higher expression in IPF patients over 12 months. |
| [59]       | Coronavirus disease 2019 (COVID-19)| Multi-omic analysis was conducted on blood samples of COVID-19-positive patients (n = 102) and COVID-19-negative patients (n = 26) to provide insight into COVID-19 pathophysiology and prediction of its severity. | Increased DEFA4 expression was reported as one of 219 molecular features that significantly correlated to COVID-19 status and severity.                                                                                   |
| [73]       | Periodontitis                     | In order to understand the pathogenic process in periodontitis, by RT-qPCR, the expression levels of several AMPs genes were measured in gingival smears from 12 patients with moderate or severe chronic periodontitis and 11 healthy subjects. | (i) DEFA4 was one of three genes identified to be associated with periodontal health. DEFA4 was observed to be down-regulated in the periodontitis patients compared with healthy controls. (ii) Moreover, a significant positive correlation was recorded between the expression level of the DEFA4 gene and the presence of Porphyromonas gingivalis and Parvimonas micra. |
**Table 1: Continued.**

| References | Diseases | Study aim | Research contribution |
|------------|----------|-----------|-----------------------|
| [74]       | Systemic lupus erythematosus (SLE) | This study suggests that the low-density granulocytes (LDGs) have a substantial role in the pathogenesis of lupus erythematosus through the comparison with autologous normal-density neutrophils and control neutrophils. | DEFA4 gene was among the genes found to be up-regulated in LDGs. |
| [75]       | Systemic autoimmune diseases (SAID) | RNA microarray analysis has been applied to indicate the gene expression in PMBC from 20 monozygotic twin pairs discordant for multiple SAID as well as 40 unrelated control subjects. | DEFA4 gene was noticed to be up-regulated in subjects with SAID compared to unrelated, matched controls. |

DEFA4 may indirectly contribute to exacerbating the inflammatory state through interfering with Hypothalamic-Pituitary-Adrenal axis (HPA axis) activity and acting as anti-ACTH leading to inhibiting the production of cortisol. Consequently, the produced cortisol in COVID-19 patients may not meet the required bodily need to inhibit inflammation [66].

Obviously, DEFA4 has a significant influence on respiratory-related diseases such as asthma and idiopathic pulmonary fibrosis (IPF). In 2017, a study was conducted by Bigler et al. aiming to identify the differences between non-asthma and asthma patients through a gene expression analysis approach. It was found that DEFA4 was significantly associated with the severity of asthma. Chemotaxis, migration, and myeloid cell trafficking were found to be enhanced in patients with severe asthma, whereas B-lymphocyte development, hematopoietic progenitor cells, and lymphoid organ hypoplasia were found to be at a low level [67]. Moreover, another study published in 2018 targeted children with asthma to characterize the possible phenotypes of this disease. Among 19 up-regulated genes, DEFA4 was remarkably present in the neutrophil-predominant asthma phenotype compared to other asthma subtypes [68]. Childhood asthma phenotypes can be distinguished by either eosinophil-predominant or neutrophil-predominant inflammatory features. The great majority of differentially expressed genes in neutrophil-predominant asthma were related to corticosteroid response or anti-corticosteroid response [68].

Furthermore, DEFA4 was among 13 expressed genes that can distinguish between mild and severe IPF cases [69]. Another study regarding IPF demonstrated DEFA4 as one of the highly expressed genes in IPF patients [70]. One of the possible links between up-regulated DEFA4 and the severe forms of respiratory-related diseases is that up-regulation of DEFA4 may reflect an excess of NETs formation. Excess NETs can spread in the pulmonary alveoli leading to lung damage. Along with this, these NETs can contribute to endothelial and epithelial cell death [71, 72].

In oral-related diseases, DEFA4 was observed at low levels among periodontitis patients compared to the healthy subjects, whereas the low expression level of the DEFA4 gene could serve as a potential biomarker to detect periodontal conditions [73].

### 3.5.3. Autoimmune Diseases

Moreover, DEFA4 has an effective role in autoimmune diseases such as systemic lupus erythematosus (SLE). A study conducted by Villanueva et al. proposed that the low-density granulocytes (LDGs) have a potential role in the appearance of lupus erythematosus. The results showed a high level of DEFA4 in the LDGs. LDGs are distinct immature neutrophils subsets found in SLE patients with the ability to enhance the formation of Neutrophils extracellular traps (NETs) and up-regulate the expression of enzymes and neutrophil proteins implicated in the NETs formation. NETs are an important phenomenon in autointer modification and exposure to the immune system, as well as in the induction of tissue damage. NETs formation plays an important role in the autoimmune disease development and organ damage observed in chronic inflammatory disorders [74]. The results also showed DEFA4 is related to granulopoiesis genes that were found to be up-regulated in the LDGs in SLE patients [74]. Moreover, another study was applied in monozygotic twins discordant for multiple systemic autoimmune diseases (SAID), as well as compared to unrelated, matched controls. A high level of DEFA4 was observed in patients suffering from SAID [75].

### 3.6. Nervous System-Related Diseases and DEFA4

#### 3.6.1. Psychiatric and Neurodegenerative Diseases

Increasing evidence has indicated that an inflammatory component is involved in psychiatric disorders and different brain-related diseases. Many studies have investigated the levels of inflammatory mediators in patients with psychiatric disorders [76–79]. For example, plasma samples were used to measure protein levels of α-defensins from 21 monozygotic twins discordant for schizophrenia (SZ) and 8 unaffected twin pairs as controls. Elevated α-defensin levels have been identified in both affected and unaffected monozygotic twins discordant for SZ compared to healthy twin pairs. Notably, α-defensin levels in the unaffected discordant twins were at an intermediate level (more than healthy twin pairs but less than their schizophrenic twins). Thus, it was suggested that increased α-defensin levels might serve as an indicator of susceptibility to develop SZ [76]. On the other hand, DEFA4 showed increased levels in patients with different psychiatric disorders (Table 2). For instance, Gardiner et al. in 2013 found that DEFA4 was one of the 59 up-regulated genes in PBMCs samples in SZ patients and schizoaffective disorder patients compared with controls [77]. Furthermore, DEFA4 was observed as one of the eight proteins that showed increased levels in saliva samples of SZ and bipolar disorder (BD) patients versus the control group [78]. Recently, DEFA4 was observed to be up-regulated...

in Japanese women with post-traumatic stress disorder (PTSD) who have elevated levels of IL-6 compared with those who have normal levels of IL-6, suggesting that DEFA4 up-regulation appeared to be correlated with high levels of IL-6 [80]. Furthermore, altered DEFA4 gene expression was found in different neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) (Table 2). Recently, Cohen et al. reported that DEFA4 was one of the 50 genes that showed significant differences in gene expression

| References | Diseases | Study aim | Research contribution |
|------------|----------|-----------|-----------------------|
| [77]       | Schizophrenia (SZ) | Gene expression profiles of PBMCs samples were analyzed from 114 SZ and schizoaffective disorder patients versus 80 healthy controls. | DEFA4 was found to be one of the 59 genes that were significantly up-regulated in SZ patients and schizoaffective disorder patients versus controls. Moreover, a significant correlation was revealed between DEFA4 expression and gender. DEFA4 expression was therein higher in males than females. |
| [78]       | Bipolar disorder (BD) and schizophrenia (SZ) | The proteomics analysis was conducted on saliva samples from 32 SZ patients, 17 patients with BD compared to 31 healthy controls. | DEFA4 has been reported as one of the eight proteins that showed elevated levels in SZ and BD patients compared with healthy subjects. |
| [80]       | Post-traumatic stress disorder (PTSD) | Transcriptome profiles of blood samples from PTSD patients with high IL-6 levels (n=16) and PTSD patients with normal IL-6 levels (n=16) were compared with age-matched normal controls (n=16) (all participants were women). | DEFA4 was observed to be up-regulated in PTSD patients who have elevated levels of IL-6 compared with those who have normal levels of IL-6. A significant positive correlation was reported between DEFA4 expression and serum IL-6 levels. |
| [81]       | Alzheimer’s disease (AD) and mild cognitive impairment (MCI) | Gene expression analysis was conducted on blood samples of 200 subjects with a diagnosis of early AD, 400 individuals with MCI, and nearly 200 cognitively normal individuals as the control group. | DEFA4 was one of 50 genes that have shown the most significant differences between AD and MCI compared to the control group, wherein DEFA4 was up-regulated in both AD and MCI versus controls. |
| [82]       | Parkinson’s disease (PD) | Genes were analyzed to determine the genes involved in PD pathogenesis (both genetic PD or idiopathic PD) next-generation sequencing (RNA-seq) was conducted on blood samples of: (i) 20 PD patients with the G2019S mutation of the LRRK2 gene. (ii) 20 asymptomatic carriers of the mutation (iii) 20 subjects with idiopathic PD (iv) 20 controls | DEFA4 was one of the 13 common genes that were identified to have significant differential gene expression in G2019S-associated PD and idiopathic PD, wherein DEFA4 was up-regulated in both: (i) G2019S-associated PD compared with asymptomatic carriers (ii) between idiopathic PD compared with controls. |
| [83]       | Parkinson’s disease (PD) | To elucidate the molecular basis underlying PD, transcriptomic analyses were performed on blood samples of 72 PD patients compared to 22 healthy controls. | DEFA4 was reported as one of the top 20 aberrantly expressed genes in PD patients, DEFA4 down-regulation was found in PD patients compared to healthy controls. |
| [84]       | Multiple sclerosis (MS) | Gene expression analysis was conducted on blood samples from patients with MS in remission, relapsing and healthy controls, aiming to understand the molecular mechanisms underlying MS. | DEFA4 was one of the genes that up-regulated in females with MS in remission status compared with their controls. |
| [85]       | Multiple sclerosis (MS) | The study aims to analyze the post-mortem of normal-appearing white matter (NAWM) of MS patients to identify the possible gene expression pathways that affect the disease heterogeneity and HPA axis activity. | DEFA4 was one of the top ten genes that were highly up-regulated. |
| [86]       | Multiple sclerosis (MS) | This study aimed to identify the differentially expressed genes in CD4+ T lymphocytes of relapsing-remitting MS patients during relapse after 3–6 days of treatment with IVMP. DEFA4 was one of seven genes that were highly up-regulated. | 11 genes were found to be the most significantly differentially expressed in CD4+ T lymphocytes of patients after treatment with IVMP. DEFA4 was one of seven genes that were highly up-regulated. |
in blood samples of AD and mild cognitive impairment (MCI) versus healthy individuals. DEFA4 was therein observed to be up-regulated in both AD and MCI compared to individuals with the normal cognitive ability [81]. On the other side, DEFA4 was found as one of the 13 common genes that showed a significant differential gene expression in blood samples of both genetic-associated PD and idiopathic PD. Wherein DEFA4 was up-regulated in both genetic-associated PD compared with asymptomatic carriers, as well as between idiopathic PD compared with controls [82]. On the contrary, in another study, DEFA4 was reported as one of the top 20 genes with differential gene expressions in blood samples of PD patients. DEFA4 was therein down-regulated in PD patients compared to healthy controls [83].

It is unknown why DEFA4 showed differential gene expression in patients with various diseases related to the brain. This up-regulation of DEFA4 may be a consequence of using antipsychotics. According to Benedicco et al. (2019), DEFA4 up-regulation was observed after three months of treatment with antipsychotic medications [84]. On the other hand, DEFA4 up-regulation could reflect the inflammatory state underlying brain-related disorders, and not a result of using antipsychotics. This notion is supported by the aforementioned fact that elevated α-defensin levels were identified in unaffected and untreated monozygotic twins discordant for SZ compared to healthy unaffected twins [76].

From our knowledge of DEFA4, there are possible explanations. First, changes in DEFA4 gene expression may not be related to brain pathogenesis and could be a consequence of an inflammatory state in the body. As mentioned above in Hori et al., DEFA4 was up-regulated in PTSD patients who have high levels of IL-6 compared to other patients with normal levels of IL-6 [80]. This positive correlation between α-defensins and IL-6 was also recorded in other diseases such as COVID-19 [62]. This may indicate that the up-regulation of DEFA4 could be a consequence of increased IL-6 levels. It was therein reported that IL-6 can activate neutrophils and stimulate the release of α-defensins from human neutrophils in vitro [62]. High-level IL-6 was observed in blood samples of patients with different brain-related disorders [85–87]. Although the blood state does not necessarily reflect the brain state, IL-6 can cross the blood-brain barrier (BBB) [88]. The presence of IL-6 at excessive levels in the central nervous system (CNS) may contribute to the neuroinflammation that ultimately leads to brain-related disorders [86]. On the other hand, hypercortisolism was reported to be associated with changes in structure and function of the brain leading to impaired brain ability [89]. Thus the second possibility is that the up-regulation of DEFA4 as corticostatin may reflect the immunomodulatory role for the HPA axis in conditions of hypercortisolism [9, 90].

3.6.2. Multiple Sclerosis. Patients with Multiple sclerosis (MS) go through two main phases of remission and relapse. The relapsing phase is when the neurologic symptoms increase and appear as attacks, followed by the remitting phase when the patients recover partially or completely [91]. DEFA4 was found to be up-regulated in blood samples of MS females in remission status [92]. De Andres et al., 2018 reported that DEFA4 was one of seven highly up-regulated genes in CD4+ T lymphocytes of MS patients after 3–6 days treated by IVMP. Intravenous methylprednisolone (IVMP) is one of the known treatments for acute relapses of MS [93]. On the other hand, gene expression analysis was applied on the post-mortem of normal appearing white matter (NAWM) samples from MS patients and compared with controls. Different modules have been identified; one of these modules showed a strong positive correlation to both MS severity and HPA axis activity. DEFA4 was identified as one of the top ten genes strongly connected to this module [94]. As mentioned before, cortisol is produced by the HPA axis and classified as the primary corticosteroid in humans [47, 65]. Corticosteroids are commonly used as a treatment for MS patients as anti-inflammatory drugs [91]. DEFA4 as a corticostatin substance may be related to HPA axis activity and cortisol production which may affect disease progression. Future research is required to identify how DEFA4 is involved in pathways related to MS disorder (Table 2).

3.7. Cancer. In an attempt to detect cancer signature genes, bioinformatics analysis was applied on approximately 1500 microarray gene expression profiles from 26 cancer studies across 21 human cancer types. According to this analysis, DEFA4 has been concluded as one of 48 genes identified as common cancer signature genes [95]. Head and neck cancers are the most common cancer types that appear to be associated with changes in DEFA4 gene expression levels [96, 97]. Wenghoefer et al., reported an increased gene expression of DEFA4 (179.2 fold) in oral leukoplakia biopsies compared with healthy gingiva [97]. Leukoplakia can be described as a premalignant lesion in the oral cavity that occurs in an intermediate stage before malignant [98]. Thus, it was suggested that DEFA4 up-regulation may play a role in malignant transformation [97]. Later in 2012, Winter et al. provided further evidence supporting previous observations regarding DEFA4 up-regulation and its potential role in malignant transformation. Elevated level DEFA4 gene expression was observed in all tested subsets of salivary gland tumors. Conversely, a significant decrease in DEFA4 gene expression was reported in benign mixed tumors (pleomorphic adenomas) [96]. Altered DEFA4 gene expression has been observed in bone marrow-related cancers. Interferon-alpha2 is considered a therapeutic agent for Philadelphia-negative chronic myeloproliferative neoplasms (MPNs). In the context of using interferon-alpha2, gene expression of interferon-associated genes was analyzed using blood samples from patients with different phenotypes of MPNs: Essential thrombocytetemia (ET), Polycythemia vera (PV), Primary myelofibrosis (PMF). DEFA4 was reported as one of the top ten up-regulated genes in patients with PMF compared with controls [99]. Furthermore, DEFA4 up-regulation was one of the five distinct gene signatures that can be used as an indicator of disease transition from ET and PV to the aggressive phenotype or transform towards myelofibrosis [100] (Table 3).
The over-activation of epidermal growth factor receptor (EGFR) pathways plays a critical role in the progression of cancer cell proliferation and promotes tumorigenesis. In 2016 Hoppe et al., found DEFA4 (113%) stimulated proliferation on the (BHY) cell line. EGFR signaling pathways can be activated by DEFA4, promoting cyclin D1 activation, which ultimately leads to increased cell proliferation and tumor growth. DEFA4 ability to activate EGFR signaling pathways may be attributed to DEFA4 and epidermal growth factor (EGF) structural similarity, suggesting that DEFA4 may serve as a ligand for EGFR and may be involved in tumorigenesis [24].

### 4. Conclusion and Further Directions

This review set out to better understand the fourth member of the human α-defensin family by reviewing the available information about DEFA4. Based on the previous literature, the current review pointed to the potential role of **DEFA4** in two areas: First, the probable role of **DEFA4** in carcinogenesis that could occur through affecting the EGFR pathway. Future research is required to confirm whether **DEFA4** could be used as an early indicator of malignant transformation risk. Second, **DEFA4** may contribute to the modulation process of HPA activity. Further research is needed to assess the association between **DEFA4** expression, IL-6, HPA axis hormones in patients with brain-related disorders as well as in the other diseases treated by corticosteroids. Third, further research is recommended to discover the full therapeutic potential of **DEFA4** fragments generated by proteolytic digestion.

### 5. Limitations of This Study

A few limitations in the review should be considered: Many studies reported changes in **DEFA4** gene expression in different diseases. However, there was a lack of detail about **DEFA4**-related molecular pathways. Furthermore, regarding **DEFA4** function in immunity, we cannot completely rule out the impact of infection or the response to drugs that might be responsible for changes in **DEFA4** gene expression. Despite these limitations, this review has provided a general background on **DEFA4** and significant observations about **DEFA4**-related diseases that may open the door for further research.

### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

### References

[1] J.-M. Anaya, Y. Shoenfeld, A. Rojas-Villarraga, R. A. Levy, and R. Cervera, *Autoimmunity: From Bench to Bedside*, El Rosario University Press, Bogota, Colombia, 2013.
[32] R. Böffert, R. Businger, H. Preib et al., “The human α-defensin-derived peptide HD5 (1–9) inhibits cellular attachment and entry of human cytomegalovirus,” *Antiviral Research*, vol. 177, Article ID 104779, 2020.

[33] D. Ehmann, L. Koeninger, J. Wendler et al., “Fragmentation of human neutrophil α-defensin 4 to combat multidrug resistant bacteria,” *Frontiers in Microbiology*, vol. 11, p. 1147, 2020.

[34] J. E. Gabay, R. W. Scott, D. Campanelli et al., “Antibiotic proteins of human polymorphonuclear leukocytes,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 14, pp. 5610–5614, 1989.

[35] Z. Wu, B. Ericksen, K. Tucker, J. Lubkowski, and W. Lu, “Synthesis and characterization of human α defensins 4–6,” *The Journal of Peptide Research: Official Journal of the American Peptide Society*, vol. 64, no. 3, pp. 118–125, 2004.

[36] T. Liu, H. Wu, D. Cao et al., “Oviduct-specific expression of human neutrophil defensin 4 in lentivirally generated transgenic chickens,” *PLoS One*, vol. 10, no. 5, Article ID e0127922, 2015.

[37] P. Ramesh, S. Veerappapillai, and R. Karuppasamy, “Gene expression profiling of corona virus microarray datasets to identify crucial targets in COVID-19 patients,” *Gene Reports*, vol. 22, Article ID 100980, 2021.

[38] N. Hemmat, A. Derakhshani, H. Bannazadeh Baghi, N. Silvestris, B. Baradaran, and S. De Summa, “Neutrophils, crucial, or harmful immune cells involved in coronavirus infection: a bioinformatics study,” *Frontiers in Genetics*, vol. 11, p. 641, 2020.

[39] C.-J. Tsai, B. Ma, and R. Nussinov, “Protein–protein interaction networks: how can a hub protein bind so many different partners?” *Trends in Biochemical Sciences*, vol. 34, no. 12, pp. 594–600, 2009.

[40] L. Buturovic, Z. Hong, B. Tang et al., “A 6-mRNA host response classifier in whole blood predicts outcomes in COVID-19 and other acute viral infections,” *Scientific Reports*, vol. 12, no. 1, pp. 1–16, 2022.

[41] The National Center for Biotechnology Information, *DEF4A4 Defensin Alpha 4* [Homo sapiens (Human)], The National Center for Biotechnology Information, Bethesda, MD, USA, 2020.

[42] R. N. Cunliffe and Y. R. Mahida, “Expression and regulation of antimicrobial peptides in the gastrointestinal tract,” *Journal of Leukocyte Biology*, vol. 75, no. 1, pp. 49–58, 2004.

[43] Ensembl, *Gene: DEFA4 ENSG00000164821*, 2021, http://Apr2022.archive.ensembl.org/Homo_sapiens/Gene/Summary?dbcore=ENSG00000164821&r=8:6935820-6938306&t=ENS T0000029743.

[44] Genome Browser Gateway, *Gene Expression in 54 Tissues from GTEx RNA-Seq of 17382 Samples*. 948 Donors (8, 9 Aug 2019) (DEFA4), 2020, https://genome-euro.ucsc.edu/cgi-bin/hgcg很想is=287879719_bWYNyB2+BMrKDELDm6f14vru R7C&db=hg38&cr=chr8&ao=6935819&ro=69358306&so=693582 18&ts=6938338&g=gtexGeneV8&i=DEFA4.

[45] The Universal Protein Resource (UniProt), *ProtKB–P12838 (DEF4_HUMAN)*, 2021, https://www.uniprot.org/uniprot/P12838.

[46] UniProt, *U.K. B. - P12838 (DEF4_HUMAN)*, 2021, https://www.ebi.ac.uk/pdbe/entry/pdb/6dmm/protein/1.

[47] M. J. Allen and S. Sharma, “Physiology, adrenocorticotropic hormone (ACTH),” in *StatPearls*, StatPearls Publishing, St. Petersburg, FL, USA, 2019.

[48] Centers for Disease Control and Prevention, *Antibiotic Resistance Threats in the United States*, US Department of Health and Human Services, Centers for Disease Control and Prevention, Washington, DC, USA, 2019.

[49] E. Tacconelli, E. Carrara, A. Savoldi et al., “Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosi,” *The Lancet Infectious Diseases*, vol. 18, no. 3, pp. 318–327, 2018.

[50] J. A. Moss, “HIV/AIDS review,” *Radiologic Technology*, vol. 84, no. 3, 2013.

[51] L. Furci, F. Sironi, M. Tolazzi, L. Vassena, and P. Lusso, “Alpha-defensins block the early steps of HIV-1 infection: interference with the binding of gp120 to CD4,” *Blood*, vol. 109, no. 7, pp. 2928–2935, 2007.

[52] W. Wang, S. M. Owen, D. L. Rudolph et al., “Activity of α- and β-defensins against primary isolates of HIV-1,” *The Journal of Immunology*, vol. 173, no. 1, pp. 515–520, 2004.

[53] Q. Zhou, W. Ding, L. Jiang et al., “Comparative transcriptome analysis of peripheral blood mononuclear cells in hepatitis B-related acute-on-chronic liver failure,” *Scientific Reports*, vol. 6, no. 1, 2016.

[54] S. Qiu, P. He, X. Fang et al., “Significant transcriptome and cytokine changes in hepatitis B vaccine non-responders revealed by genome-wide comparative analysis,” *Human Vaccines & Immunotherapeutics*, vol. 14, no. 7, pp. 1763–1772, 2018.

[55] M. E. Klotman and T. L. Chang, “Defensins in innate antiviral immunity,” *Nature Reviews Immunology*, vol. 6, no. 6, pp. 447–456, 2006.

[56] K. Muffak-Granero, P. Bueno, C. Olmedo et al., “Study of gene expression profile in liver transplant recipients with hepatitis C virus,” *Transplantation Proceedings*, vol. 40, 2008.

[57] A. Y. Ramdas and V. A. Arankalle, “The expression patterns of immune response genes in the Peripheral Blood Mononuclear cells of pregnant women presenting with subclinical or clinical HEV infection are different and trimester-dependent: a whole transcriptome analysis,” *PLoS One*, vol. 15, no. 2, Article ID e0228068, 2020.

[58] A. K. Knight, A. L. Dunlop, V. Kilaru et al., “Characterization of gene expression changes over healthy term pregnancies,” *PLoS One*, vol. 13, no. 10, Article ID e0204228, 2018.

[59] K. A. Overmyer, E. Shiskova, I. J. Miller et al., “Large-scale multi-omic analysis of COVID-19 severity,” *Cell systems*, vol. 12, no. 1, 2021.

[60] L. H. A. Cavalcante-Silva, D. C. M. Carvalho, E. D. A. Lima et al., “Neutrophils and COVID-19: the road so far,” *International Immunopharmacology*, vol. 90, Article ID 107233, 2020.

[61] Y. Zuo, S. Yalavarthi, H. Shi et al., “Neutrophil extracellular traps in COVID-19,” *JCI insight*, vol. 5, no. 11, 2020.

[62] S. Abdeen, K. Bdeir, R. Abu-Fann et al., “Alpha defensins: risk factor for thrombosis in COVID 19 infection,” *British Journal of Haematology*, vol. 194, no. 1, pp. 44–52, 2021.

[63] A. J. Wilk, M. J. Lee, B. Wei et al., “Multi-omic profiling reveals widespread dysregulation of innate immunity
and hematopoiesis in COVID-19,” Journal of Experimental Medicine, vol. 218, no. 8, Article ID e20210582, 2021.

[64] R. C. Group, “Dexamethasone in hospitalized patients with Covid-19,” New England Journal of Medicine, vol. 384, no. 8, pp. 693–704, 2021.

[65] D. W. Cain and J. A. Cidlowski, “Immune regulation by glucocorticoids,” Nature Reviews Immunology, vol. 17, no. 4, pp. 233–247, 2017.

[66] A. S. Alzahrani, N. Mukhtar, A. Aljomaia et al., “The impact of COVID-19 viral infection on the hypothalamic-pituitary-adrenal axis,” Endocrine Practice: Official Journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists, vol. 27, no. 2, pp. 83–89, 2021.

[67] J. Bigler, M. Boedigheimer, J. P. R. Schofield et al., “A severe asthma disease signature from gene expression profiling of peripheral blood from U-BIOPRED cohorts,” American Journal of Respiratory and Critical Care Medicine, vol. 195, no. 10, pp. 1311–1320, 2017.

[68] M. W. Su, W. C. Lin, C. H. Tsai et al., “Childhood asthma clusters reveal neutrophil predominant phenotype with distinct gene expression,” Allergy, vol. 73, no. 10, pp. 2024–2032, 2018.

[69] J. V. Yang, L. G. Luna, J. Cotter et al., “The peripheral blood transcriptome identifies the presence and extent of disease in idiopathic pulmonary fibrosis,” PLoS One, vol. 7, no. 6, Article ID e37708, 2012.

[70] P. L. Molyneaux, S. A. G. Willis-Owen, M. J. Cox et al., “Host-microbial interactions in idiopathic pulmonary fibrosis,” American Journal of Respiratory and Critical Care Medicine, vol. 195, no. 12, pp. 1640–1650, 2017.

[71] B. N. Porto and R. T. Stein, “Neutrophil extracellular traps in pulmonary diseases: too much of a good thing?” Frontiers in Immunology, vol. 7, p. 311, 2016.

[72] M. Saffarzadeh, C. Juennemann, M. A. Queisser et al., “Neutrophil extracellular traps directly induce epithelial and endothelial cell death: a predominant role of histones,” PLoS One, vol. 7, no. 2, Article ID e32366, 2012.

[73] M. L. Jourdain, L. Pierrard, L. Kanagaratnam et al., “Anti-microbial peptide gene expression in periodontitis patients: a pilot study,” Journal of Clinical Periodontology, vol. 45, no. 5, pp. 524–537, 2018.

[74] E. Villanueva, S. Yalavarthi, C. C. Berthier et al., “Netting neutrophils induce endothelial damage, infiltrate tissues, and expose immunostimulatory molecules in systemic lupus erythematosus,” The Journal of Immunology, vol. 187, no. 1, pp. 538–552, 2011.

[75] T. P. O’Hanlon, L. G. Rider, L. Gan et al., “Gene expression profiles from discordant monozygotic twins suggest that molecular pathways are shared among multiple systemic autoimmune diseases,” Arthritis Research and Therapy, vol. 13, no. 2, pp. 1–13, 2011.

[76] R. M. Craddock, J. T. Huang, E. Jackson et al., “Increased α-defensins as a blood marker for schizophrenia susceptibility,” Molecular & Cellular Proteomics: MCP, vol. 7, no. 7, pp. 1204–1213, 2008.

[77] E. J. Gardiner, M. J. Cairns, B. Liu et al., “Gene expression analysis reveals schizophrenia-associated dysregulation of immune pathways in peripheral blood mononuclear cells,” Journal of Psychiatric Research, vol. 47, no. 4, pp. 425–437, 2013.

[78] F. Iavarone, M. Melis, G. Platania et al., “Characterization of salivary proteins of schizophrenic and bipolar disorder patients by top-down proteomics,” Journal of Proteomics, vol. 103, pp. 15–22, 2014.

[79] C. A. Köhler, T. H. Freitas, M. Maes et al., “Peripheral cytokine and chemokine alterations in depression: a meta analysis of 82 studies,” Acta Psychiatrica Scandinavica, vol. 135, no. 5, pp. 373–387, 2017.

[80] H. Hori, F. Yoshida, M. Itoh et al., “Proinflammatory status-stratified blood transcriptome profiling of civilian women with PTSD,” Psychoneuroendocrinology, vol. 111, Article ID 104491, 2020.

[81] D. Cohen, A. Pilozzi, and X. Huang, “Network medicine approach for analysis of Alzheimer’s disease gene expression data,” International Journal of Molecular Sciences, vol. 21, no. 1, p. 332, 2020.

[82] J. Infante, C. Prieto, M. Sierra et al., “Identification of candidate genes for Parkinson’s disease through blood transcriptome analysis in LRRK2-G2019S carriers, idiopathic cases, and controls,” Neurobiology of Aging, vol. 36, no. 2, pp. 1105–1109, 2015.

[83] Y. Fan, J. Li, Q. Yang et al., “ Dysregulated long non-coding RNAs in Parkinson’s disease contribute to the apoptosis of human neuroblastoma cells,” Frontiers in Neuroscience, vol. 13, p. 1320, 2019.

[84] C.-F. Benedicto, C. Prieto, and J. Sainz, “Altered gene expression in antipsychotic-induced weight gain,” NPJ Schizophrenia, vol. 5, no. 1, p. 7, 2019.

[85] S. Bradburn, J. Sarginson, and C. A. Murgatroyd, “Association of peripheral interleukin-6 with global cognitive decline in non-demented adults: a meta-analysis of prospective studies,” Frontiers in Aging Neuroscience, vol. 9, p. 438, 2017.

[86] N. M. Lyra e Silva, A. G. Rafaela, A. P. Tharick et al., “Pro-inflammatory interleukin-6 signaling links cognitive impairments and peripheral metabolic alterations in Alzheimer’s disease,” Translational Psychiatry, vol. 11, no. 1, pp. 1–15, 2021.

[87] H. K. Al-Hakeim, D. A. Al-Rammahi, and A. H. Al-Dujaili, “IL-6, IL-18, sIL-2R, and TNFα proinflammatory markers in depression and schizophrenia patients who are free of overt inflammation,” Journal of Affective Disorders, vol. 182, pp. 106–114, 2015.

[88] W. A. Banks, A. J. Kastin, and E. G. Gutierrez, “Penetration of interleukin-6 across the murine blood-brain barrier,” Neuroscience Letters, vol. 179, no. 1-2, pp. 53–56, 1994.

[89] M. Piasecka, E. Papakokkinou, E. Valassi et al., “Psychiatric activity and susceptibility to endogenous hypercortisolism,” Journal of Internal Medicine, vol. 288, no. 2, pp. 168–182, 2020.

[90] G. Gatti, R. G. Masera, A. Bateman et al., “Effects of CRH, acth and the corticostatin HP-4 on the spontaneous NK cell activity and susceptibility to endogenous modifiers,” Archives of Gerontology and Geriatrics, vol. 15, pp. 159–171, 1992.

[91] K. M. Myhr and S. I. Miegren, “Corticosteroids in the treatment of multiple sclerosis,” Acta Neurologica Scandinavica—Supplement, vol. 120, no. 189, pp. 73–80, 2009.

[92] H. Irizar, M. Munoz-Culla, L. Sepulveda et al., “Trancriptomic profile reveals gender-specific molecular mechanisms driving multiple sclerosis progression,” PLoS One, vol. 9, no. 2, Article ID e90482, 2014.

[93] C. De Andres, M. I. Garcia, H. Goicoechea et al., “Genes differentially expressed by methylprednisolone in vivo in CD4 T lymphocytes from multiple sclerosis patients:
potential biomarkers,” The Pharmacogenomics Journal, vol. 18, no. 1, pp. 98–105, 2018.
[94] J. Melief, M. Orre, K. Bossers et al., ”Transcriptome analysis of normal-appearing white matter reveals cortisol-and disease-associated gene expression profiles in multiple sclerosis,” Acta neuropathologica communications, vol. 7, no. 1, pp. 60–19, 2019.
[95] L. Xu, D. Geman, and R. L. Winslow, ”Large-scale integration of cancer microarray data identifies a robust common cancer signature,” BMC Bioinformatics, vol. 8, no. 1, p. 275, 2007.
[96] J. Winter, A. Pantelis, D. Kraus et al., ”Human α-defensin (DEFA) gene expression helps to characterise benign and malignant salivary gland tumours,” BMC Cancer, vol. 12, no. 1, p. 465, 2012.
[97] M. Wenghoefer, A. Pantelis, T. Najafi et al., ”Gene expression of oncogenes, antimicrobial peptides, and cytokines in the development of oral leukoplakia,” Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology & Endodontics, vol. 110, no. 3, pp. 351–356, 2010.
[98] F. Mohammed and A. T. Fairozekhan, Oral Leukoplakia, 2017.
[99] V. Skov, T. S. Larsen, M. Thomassen et al., ”Whole blood transcriptional profiling of interferon inducible genes identifies highly upregulated IFI27 in primary myelofibrosis,” European Journal of Haematology, vol. 87, no. 1, pp. 54–60, 2011.
[100] H. C. Hasselbalch, V. Skov, T. Stauffer Larsen et al., ”Transcriptional profiling of whole blood identifies a unique 5-gene signature for myelofibrosis and imminent myelofibrosis transformation,” PLoS One, vol. 9, no. 1, Article ID e85567, 2014.