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Original Article

IL-4 and CCR7 play an important role in the development of keloids in patients with a family history

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Abstract: Background: The occurrence of keloids tends to show family clusters and more severe symptoms in patients with a family history of the condition, but their pathological mechanisms remain unclear. In this study, we aimed to investigate the differences in genetic susceptibility between keloid patients with a family history of keloids and sporadic patients with sporadic keloids and explore potential therapeutic targets of keloids at the molecular level. Methods: High-throughput sequencing data were obtained from normal skin tissue of patients with a family history of keloids (FN group) and normal skin tissue from sporadic patients (N group). Bioinformatics analysis was employed to identify hub genes. Promising hub genes were identified using RT-qPCR, immunohistochemistry and immunofluorescence assays, and Western blotting. GO and KEGG pathway enrichment analysis was performed to determine the main functions between the two groups. Results: Patients with a family history of keloids had more severe clinical symptoms ($P = -0.749$, $P < 0.001$). The expression of IL-4 and CCR7 was significantly different between patients with a family history of keloids and those with sporadic keloids. The high expression of IL-4 and the low expression of CCR7 in the FN group may be of key importance in explaining why keloids run in families ($P < 0.05$). Conclusion: Having a family history of keloids is a risk factor for increased severity of keloids. IL-4 and CCR7 play an important role in the development of keloids in patients with a family history of the condition and may represent new targets for the treatment of keloids.

Keywords: Keloids, family history, differentially expressed genes, bioinformatics

Introduction

Keloids are a tumor-like, fibroproliferative skin disease, characterized by aggressive growth [1]. The condition generally occurs following a skin injury; it has only been observed to occur in humans. Keloids are caused by the accumulation of excessive extracellular matrix (ECM), particularly the excess formation of collagen [2]. Keloids exceed the scope of the original wound and invade the surrounding normal skin tissue, causing severe discomfort to the patient, both in appearance and function. Patients can suffer severe physical and mental pain. Although keloids affect all ethnic groups, there is a higher incidence in people of African American and Asian descent. Black people are nearly 15-times more likely to develop this disease than white people [3]. The mechanism of the occurrence and development of keloids remains unclear. Genetic factors are considered to be an important cause of the onset of keloids, and keloid patients often show a family history of the condition [4]. Furthermore, clinical observations suggest that keloid patients with a family history tend to have more severe symptoms and respond less well to treatment [5]. So far, the evidence for the inheritance of keloids is inconclusive. Therefore, clarifying the genetic pathogenesis and determining the genes linked with susceptibility to the condition could have a very positive impact on guiding both basic theoretical research and disease treatment.

In recent years, the rapid development of high-throughput sequencing technology has provided important data to act as a basis for susceptibility gene research [6]. Genetic studies of keloids have mostly focused on the expression levels of keloid-related genes, in particular the P53, FAS, c-myc, c-fos, ras, Bcl-2 family, ICE

Table 1. Clinical characteristic of keloid's patients for sequencing

Patients	Age of onset (years)	Gender	The number of keloids	The area of keloid	mVSS
N7	24	female	2	2 cm × 4 cm	11
N8	32	male	1	2 cm × 2 cm	10
N9	37	female	1	2 cm × 2 cm	10
N14	21	male	1	2 cm × 1 cm	9
N24	22	male	2	2 cm × 3 cm	9
N25	26	male	1	3 cm × 2 cm	8
N36	30	female	1	1 cm × 2 cm	10
FNTwinN1	20	male	2	3 cm × 3 cm	12
FNTwinN2	20	male	1	4 cm × 2 cm	12
FN36	22	female	3	3 cm × 7 cm	12
FN39	24	female	4	4 cm × 8 cm	14
FN40	30	female	2	3 cm × 5 cm	10
FN42	27	female	3	4 cm × 2 cm	13

family, HLA, and TGF-β/Smad signaling pathways [7-13]. This work is still in the exploratory research stage. So far, no consensus has been formed. By investigating different families with keloids, some researchers have suggested that keloids are inherited via different modes of inheritance, such as autosomal dominant inheritance and autosomal recessive inheritance. Most researchers consider that keloids are inherited in an autosomal dominant manner, rather than by simple Mendelian single-gene inheritance [14, 15]. Others think that the disease essentially follows autosomal dominant inheritance, with incomplete clinical penetrance and diverse manifestations [15]. While the occurrence of keloids can be familial, there are also many sporadic cases. However, patients with a family history of keloids tend to have more severe symptoms than sporadic patients [5].

Why do the symptoms tend to be more severe in patients with keloids who have a family history of the condition? What are the genetic susceptibility genes associated with this? What are the environmental susceptibility factors related to keloid patients? Based on the tumor immune-related genes analysis database, this study compared the differentially expressed genes (DEGs) of patients with a family history of keloids and patients with sporadic keloids and explored the role of genetic susceptibility genes in the development of keloid disease. Using bioinformatics methods to mine genetic susceptibility genes, we further conducted experimental verification, combined with clinical data. We sought to explore the molecular mechanisms and potential therapeutic targets of keloids at the molecular level.

Methods

Patients

This study was approved by the Medical Ethics Committee of Peking Union Medical College Hospital, China. All participants provided written informed consent. From March 2019 to June 2021, a total of 256 patients were included in this study, who were diagnosed with keloids. Thirteen normal skin tissues from around keloids were used for high-throughput sequencing (**Table 1**), and 17 samples were used for experimental verification (Supplementary Table 1). The clinical epidemiological characteristics of 256 patients diagnosed with keloids were recorded (**Table 2**), and the modified Vancouver Scar Scale (mVSS) [16] was used to assess the condition of each patient's keloid(s) according to their manifestation. The more severe the clinical symptoms of patients with keloids, the higher the mVSS value. None of the patients had any systemic diseases, were on medication, or received treatment, so as not to affect the results of the study. All samples were taken from the chest. The normal skin samples from around keloids were taken from the normal skin tissues that must be removed during a keloid resection. The distance between the surrounding normal skin tissue and the keloid tissue was 2-3 mm.

We collected normal skin tissues from both patients with a family history of keloids and sporadic patients, to better represent any abnormal gene expression during the initial stages of the disease.

The collected samples were divided into two groups: normal skin tissue from patients with a family history of keloids (FN group) and normal skin tissue from patients with sporadic keloids (N group). There were six skin samples collect-

Table 2. Clinical characteristics of 256 keloid's patients

Variables	Total n	mVSS	
		Pearson's correlation coefficient	
		ρ[a]	*P*
Gender		-0.045	0.471
Male	96 (37.5%)	/	/
Female	160 (62.5%)	/	/
Genetic background		-0.749	< 0.001*
family history	61 (23.8%)	/	/
sporadic patient	195 (76.2%)	/	/
Age		0.117	0.062
0-15	14 (5.5%)	/	/
16-25	73 (28.5%)	/	/
26-45	118 (46.0%)	/	/
46-65	40 (15.6%)	/	/
> 65	11 (4.3%)	/	/
Occupation		-0.015	0.807
student	45 (17.5)	/	/
peasantry	7 (2.7%)	/	/
worker	172 (67.1%)	/	/
others	32 (12.5%)	/	/

[a]Pearson's correlation coefficient between the modified Vancouver Scar Scale (mVSS) and relevant characteristics; ρ: Pearson's correlation coefficient; *Significant variables: P < 0.05.

ed from the FN group and seven skin samples collected from the N group.

Confirmation of family history

A patient was considered to have a family history of keloids if a first-, second-, or third-degree relative of the case had keloids. First-degree relatives refer to parents, children, and siblings of an individual, whose genes have a 50% chance of being the same as those of the individual concerned. Second-degree relatives include grandparents, uncles, aunts, nieces, nephews, and grandchildren, whose genes have a 25% probability of being the same as those of the individual concerned. Third-degree relatives include cousins and great-grandparents and share one in eight of their genes with the individual concerned.

Identification of differentially expressed genes

The expression of tumor immune-related genes was analyzed in 13 samples. The Oncomine Immune Response Research Assay kit (OIRRA, Thermo Fisher, USA) was used for high-throughput sequencing. This is an RNA-based sequencing kit that can measure the expression levels of 395 genes related to immune responses. Thresholds were set to a *P*-value < 0.05 and log2 fold-change (FC) > 1.5 or < -1.5. The R package was used for visualization using volcano mapping.

Screening hub genes and functional annotation

DEGs were input to the STRING database (https://string-db.org/) to construct a network map of protein-protein interactions (PPI) [17]. The PPI file was imported into Cytoscape for visual analysis [18]. The CytoHubba plug-in was used to further screen out hub genes. MCODE was used to calculate information for each node in the PPI network diagram and highlight important modules. The R package was used to find corresponding genes in the GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) database. Visual analysis was performed.

Gene set enrichment analysis (GSEA)

GSEA [19] is a method for analyzing genome-wide expression profile microarray data by comparing genes with predefined gene sets. A molecular label database was constructed based on existing information about gene locations, properties, functions, and biological significance. By analyzing gene expression profile data, the expression status in specific functional sets could be determined.

Metascape

Metascape [20] integrates more than 40 bioinformatics databases, providing biologists with easy access to comprehensive data analysis using a simple interface for one-click quick analysis. It not only contains enrichment analyses of biological pathways, structural analyses of PPI networks, and rich gene-annotation functions but also presents the results in a high-quality graphical language that biologists can easily understand.

Hematoxylin-eosin staining (HE)

Tissue slices were placed in xylene I for 10 minutes, xylene II for 10 minutes, anhydrous ethanol I for 5 minutes, anhydrous ethanol II for 5

Table 3. Primers and their sequences for PCR analysis

Primer	Sequence (5'-3')
GAPDH-F	GGAAGCTTGTCATCAATGGAAATC
GAPDH-R	TGATGACCCTTTTGGCTCCC
CCR7-F	TGAGGTCACGGACGATTACAT
CCR7-R	GTAGGCCCACGAAACAAATGAT
CD40LG-F	ACATACAACCAAACTTCTCCCCG
CD40LG-R	GCAAAAAGTGCTGACCCAATCA
IL-4-F	CCAACTGCTTCCCCCTCTG
IL-4-R	TCTGTTACGGTCAACTCGGTG
CD80-F	GGCCCGAGTACAAGAACCG
CD80-R	TCGTATGTGCCCTCGTCAGAT
IL-10-F	TACCACCTCCCGAAAATGTCA
IL-10-R	CCCAGTCTGAATGCTCATCTG
CTLA-4-F	GCCCTGCACTCTCCTGTTTTT
CTLA-4-R	GGTTGCCGCACAGACTTCA
CD86-F	CTGCTCATCTATACACGGTTACC
CD86-F	GGAAACGTCGTACAGTTCTGTG
IL-2-F	ACCGCTTTGCGGAATCTCA
IL-2-R	AGGTCAGGGAAACATCAGGGA
IL-6-F	ACTCACCTCTTCAGAACGAATTG
IL-6-R	CCATCTTTGGAAGGTTCAGGTTG
STAT3-F	CAGCAGCTTGACACACGGTA
STAT3-R	AAACACCAAAGTGGCATGTGA

minutes, 95% alcohol for 5 minutes, 90% alcohol for 5 minutes, 80% alcohol for 5 minutes, and 70% alcohol for 5 minutes. They were then washed with distilled water. Hematoxylin stains the nuclei and eosin stains the cytoplasm. The slices were dehydrated. The slices were removed from the xylene to dry a little and then mounted with neutral gum. A microscope was used for image acquisition and analysis.

Immunocytochemistry

The paraffin sections were deparaffinized, hydrated, and then rinsed three times with PBS, for 3 minutes each time. The PBS solution was shaken off and 50 μl primary antibody was added to each slice. The slices were incubated for 60 minutes at room temperature and then rinsed with PBS for 5 minutes. Rinsing three times with PBS, 3 minutes each time. Then, 100 μl of freshly prepared DAB/AEC chromogenic solution was added to each slice, and they were observed under the microscope for 3-10 minutes. The slices were then rinsed in distilled water and re-stained with hematoxylin.

IF

Each tissue block was cut into 0.5 cm^2 and the tissue was sectioned and fixed. Samples were washed with PBS three times, for 5 minutes each time. Sections were added to permeability agent for 15 minutes, washed with PBS, and sealed for 30 minutes. The sections were incubated with primary antibody at room temperature for 1 hour then rinsed with PBS. The sections were incubated with secondary antibody at room temperature for 1 hour, then the sections were sealed by dropping sealing tablets (fluorescent sealing tablets, Thermo Fisher, USA).

RT-qPCR

Skin tissues (FN and N tissues) were extracted from the same patient for PCR analysis. The primers used in this study are shown in **Table 3**. Tissue samples (200 mg) were mixed with Trizol reagent (Thermo Fisher, USA) to extract total RNA using a one-step method. A reverse transcription kit was used to obtain cDNA (HiScript III 1st Strand cDNA Synthesis Kit, Beijing, China). Then, a qPCR fluorescence kit (Taq Pro Universal SYBR qPCR Master Mix, Beijing, China) was used to quantitatively analyze the expression of the target genes. The reaction system comprised 10 μl 2 × Taq Pro Universal SYBR qPCR Master Mix + 0.4 μl Gene Specific Primer Forward (10 μM) + 0.4 μl Gene Specific Primer Reverse (10 μM) + 1 μl cDNA + 9.2 μl RNase-free ddH20. The reaction conditions were 95°C for 30 sec during pre-denaturation; 95°C for 3 sec; and 60°C for 10 s, 40 cycles; 95°C for 15 sec; 60°C for 60 sec; and 95°C for 15 sec. An amplification curve was obtained at the end of the run.

Western blotting (WB)

The tissue was ground at 4°C and a BCA kit (BCA Protein Assay Kit, Thermo Fisher, USA) was used for protein quantification. The protein sample was boiled to denature it. The sample was then added to the electrophoresis apparatus. When the sample was in the concentrate glue, the voltage was set to 80 V; when it was in the separating glue the voltage was set to 120 V. The protein was transferred from the gel to a polyvinylidene fluoride (PVDF) membrane, the primary antibody was added dropwise, and the samples were incubated overnight at 4°C. The secondary antibody was added, and samples

were incubated at room temperature for 2 hour. GAPDH was used as an internal reference. CCR7 polyclonal antibody (dilution rate = 1:1000, 25898-1-AP, Proteintech, USA) was used to detect CCR7, while the secondary antibody was a goat anti-mouse monoclonal antibody (dilution rate = 1:5000, ab205719, Abcam, Cambridge, UK). IL-4 monoclonal antibody was used to detect IL-4 (dilution rate = 1:1000, 66142-1-Ig, Proteintech, USA). A secondary antibody was applied (dilution rate = 1:5000).

Statistical analysis

SPSS (version 21) was used for clinical data analysis. Pearson correlation analysis was used to explore correlations between clinical characteristics and the severity of keloids. A *P*-value less than 0.05 was considered statistically significant.

Results

Clinical characteristics of keloid patients

From the perspective of their clinical manifestations, the clinical symptoms of keloids in patients with a family history of the condition are more serious than those that occur in sporadic cases, with larger areas covered by tissue lesions. Some patients present multiple sporadic lesion (**Figure 1A**, **1B**). We carried out a retrospective study of 256 patients with keloids; the maletofemale ratio was 1:1.67 (**Table 2**). There were 61 (23.8%) patients with a family history of keloids and 195 sporadic patients. The youngest patient had an age of 5 years and the oldest 79 years (33.3 ± 15.0 years). Patients with a family history of keloids had more severe clinical symptoms ($\rho = -0.749$, $P < 0.001$). Through clinical information analysis, we found that the symptoms of the patients with family history were more serious, and we then explored the underlying causes of this phenomenon at the molecular level.

HE staining characteristics of patients with keloids of different genetic backgrounds

There were no significant morphological differences between the normal skin around keloid tissue from the family history (FN) and the sporadic keloids (N) groups (**Figure 1C**, **1D**). Both showed an abundance of collagen fibers and additional blood vessels.

Identification of DEGs

Principal component analysis (PCA) is used to project data into a low-dimensional subspace to achieve a reduction in dimensionality. The data from the FN and N groups were divided into two distinct clusters on the principal component 1 (PC1) and PC2 axes (**Figure 1E**), indicating that the data from the two groups were different at the molecular level. The threshold was set to a *P*-value < 0.05 and a log2 fold-change (FC) > 1.5 or < -1.5. The results showed that 5 genes were upregulated and 73 were downregulated (**Figure 1F**).

Identification of hub genes

DEGs were used to construct a PPI network to clarify the interactions between DEGs; hub genes are highlighted in the figure (**Figure 2A**). The Cytoscape software MCODE plug-in was used to further refine important molecules (**Figure 2B**). The first ten hub genes are shown in **Figure 2C**, which need to be further verified.

Functional annotation of DEGs

Biological processes (BP) in which DEGs were enriched include T-cell activation, regulation of lymphocyte activation, and regulation of immune effector process (**Figure 2D**). Variations in DEGs of cell components (CC) were markedly enriched in external side of plasma membrane, lysosomal membrane, and lytic vacuole membrane (**Figure 2E**). Molecular functions (MF) in which DEGs were enriched include peptide binding, amide binding, and cytokine receptor binding (**Figure 3A**). The KEGG pathway was enriched most in Th17 cell differentiation (**Figure 3B**). GSEA was also used to enrich the KEGG pathway, which showed that there were genes involved in cytokine-cytokine receptor interactions and chemokine signaling pathways (**Figure 3C-F**). Metascape was used to enrich GO analysis, and the regulation of T-cell activation came first (**Figure 3G**). Matescape was used to plot a rich network of terms shaded with cluster IDs (**Figure 4A**). Terms that contained more genes tended to have more significant *P*-values (**Figure 4B**).

Validation of hub genes

Compared with the N group, CCR7, CD40LG, IL-10, CD80, and CD86 showed a tendency to decrease in the FN group (**Figures 4C**, **4D**, **4F**,

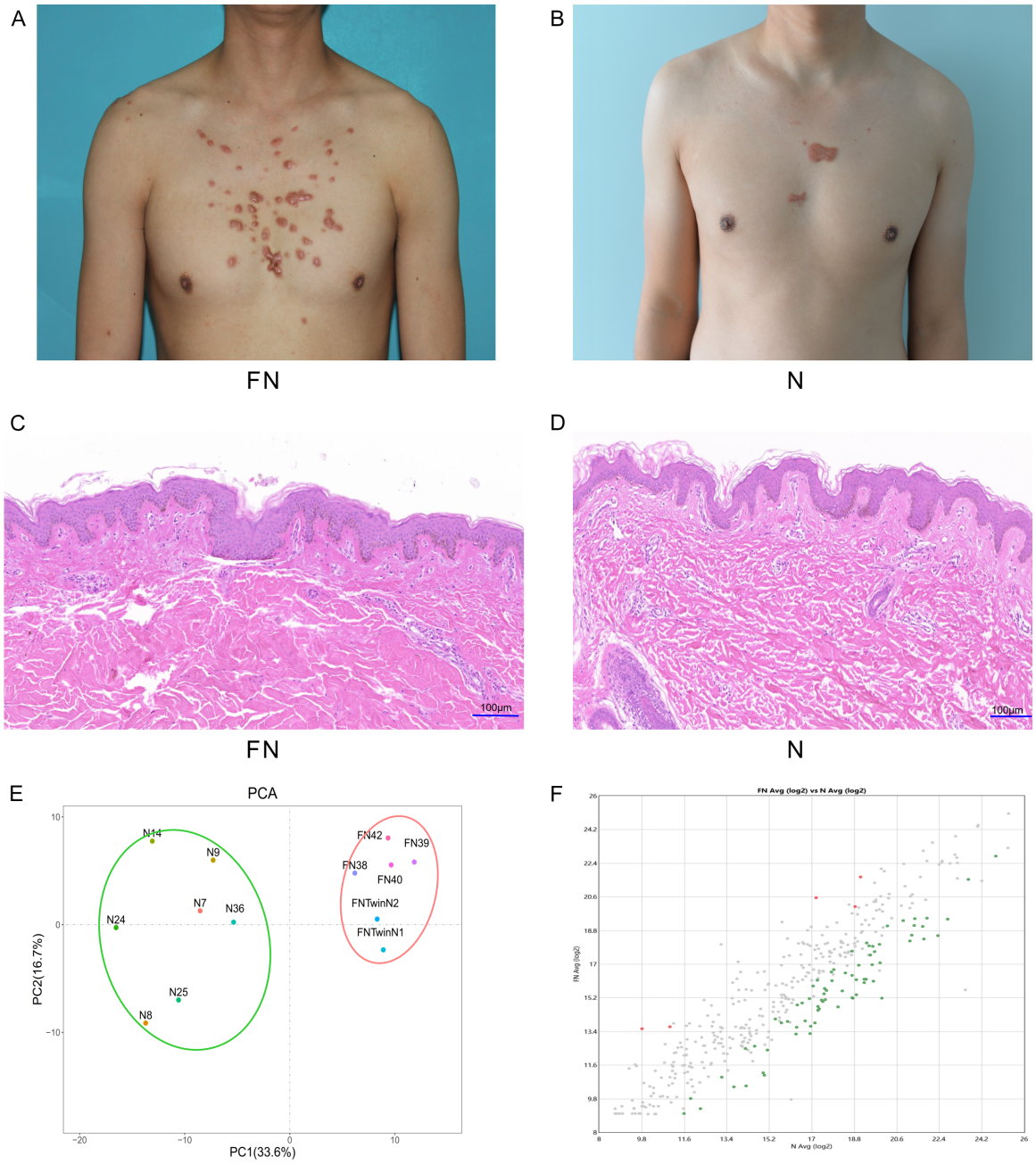

Figure 1. A. Photograph of the chest of a keloid patient with a family history of keloids (FN group). B. Photograph of the chest of a patient with sporadic keloids (N group). C. HE staining of the FN group, × 100. D. HE staining of the N group, × 100. E. Principal component analysis (PCA) of samples between the FN and N groups. In the figure, principal component 1 (PC1) and principal component 2 (PC2) are used as the X-axis and Y-axis, respectively, to draw the scatter diagram, where each point represents a sample. In such a PCA diagram, the farther the two samples are from each other, the greater the difference is between the two samples in terms of gene expression patterns. F. A scatter plot between the FN and N groups. The horizontal and vertical coordinates represent the average value of each gene that is differentially expressed between the FN and N groups. The cutoffs of log2 fold change > 1.5 or < -1.5 and P < 0.05 were used as the screening criteria. Significantly upregulated DEGs are shown in red, and significantly downregulated DEGs are shown in green.

4G, **5A**). The expression of CCR7 was significantly different between the two groups (P < 0.05) (**Figure 4C**). Compared with the N group, IL-4, CTLA-4, IL-2, IL-6, and STAT3 showed a tendency to increase in the FN group (**Figures 4E**, **4H**, **5B-D**). The expression of IL-4 was signifi-

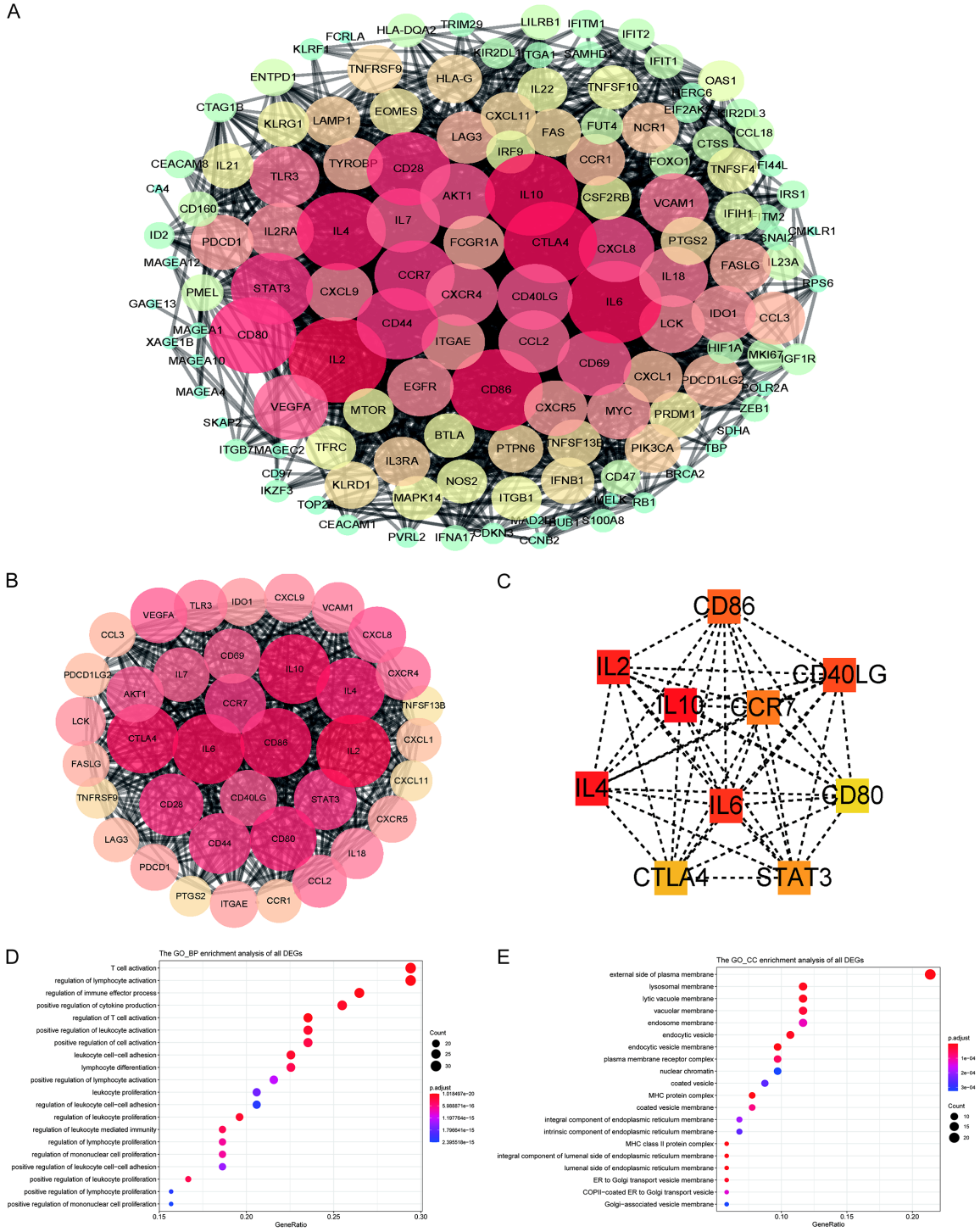

Figure 2. A. The PPI network of DEGs in the FN and N groups. B. MCODE was performed for the FN and N groups. Protein complexes are represented as graphs, using the spoke model. The vertices represent proteins, and the edges represent experimentally determined interactions. The criteria for MCODE were: MCODE score > 5, degree cutoff = 2, k-score = 2, maximum depth = 100, and node score cutoff = 0.2. When the degree ≥ 10, hub genes were identified. C. The hub genes were identified from the PPI network in the FN and N groups. D, E. GO enrichment analyses of biological processes (BP) and cellular components (CC) between the FN and N groups.

cantly different between the two groups ($P < 0.01$) (**Figure 4E**). CCR7 and IL-4 may be promising biomarkers to account for the differences in clinical manifestations of keloids seen

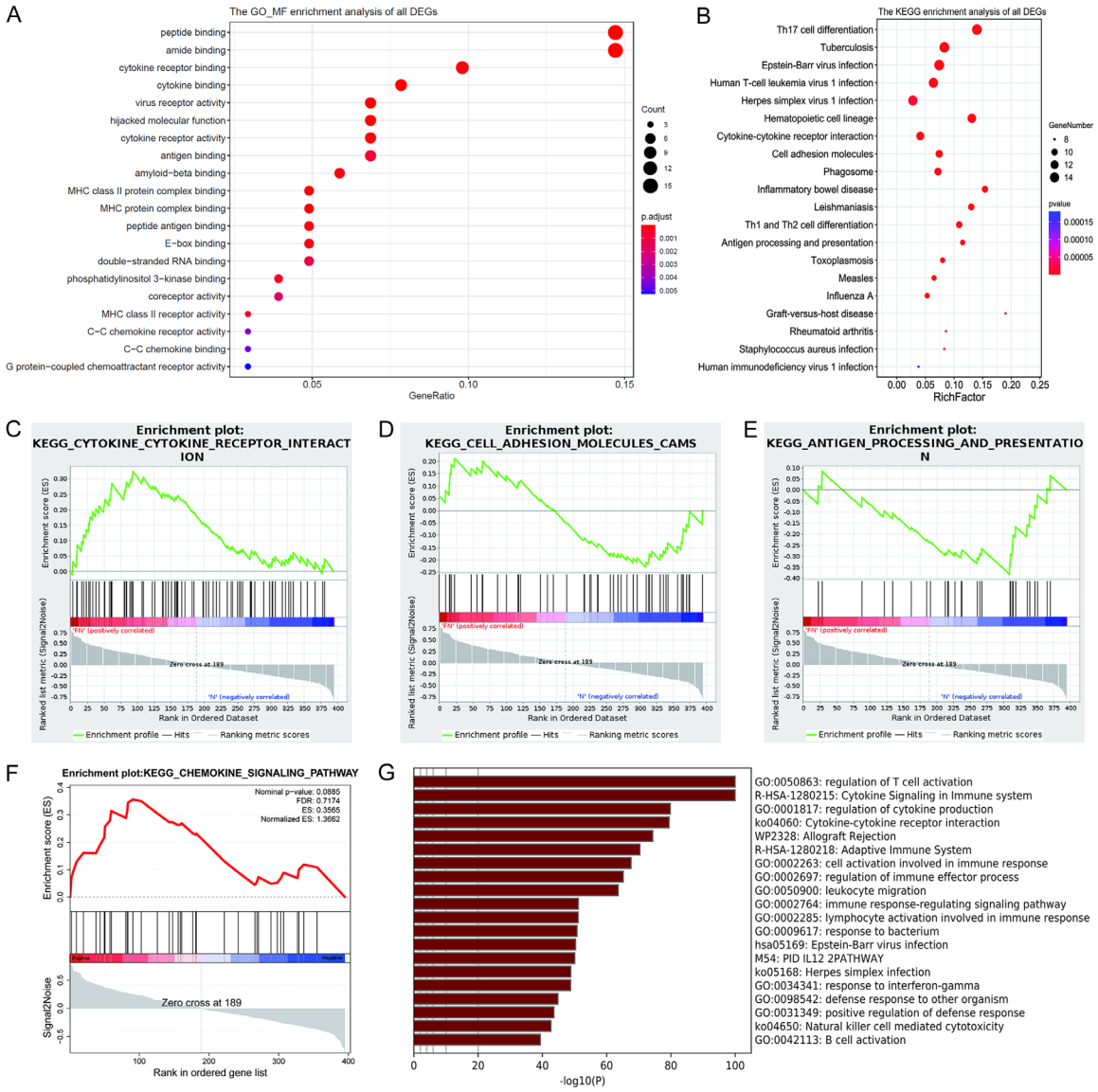

Figure 3. A. GO enrichment analyses of molecular functions (MF) of DEGs between the FN and N groups. B. KEGG pathway analysis of DEGs between the FN and N groups. The cutoffs for log2 fold change > 1.5 or < -0.5 and P < 0.05 were used as the screening criteria. C-F. Gene set enrichment analysis (GSEA) was used to analyze the KEGG pathway obtained from the DEGs enrichment between the FN and N groups. G. Enrichment analysis for GO using Metascape.

between cases who have a family history of the condition and sporadic cases, which were further validated in our research.

The differences of IL-4 and CCR7 expression in patients with different genetic backgrounds

The protein of IL-4 was significantly increased in the FN group compared with its level in the N group (P = 0.0348), while the protein of CCR7 was significantly decreased in the FN group compared with its level in the N group (**Figure 5E**, P = 0.0263), as did the immunohistochemistry results (**Figures 5F**, **6A**). The immunofluorescence result showed that the expression of IL-4 was significantly increased in the FN group, while decreased in N group (**Figure 6B**, **6D**, P < 0.05). CCR7 was significantly decreased in FN group, while increased in N group (**Figure 6C**, **6E**, P < 0.05).

Discussion

Keloids are a fibroproliferative disease caused by excessive collagen deposition [21]. The condition tends to occur in susceptible people fol-

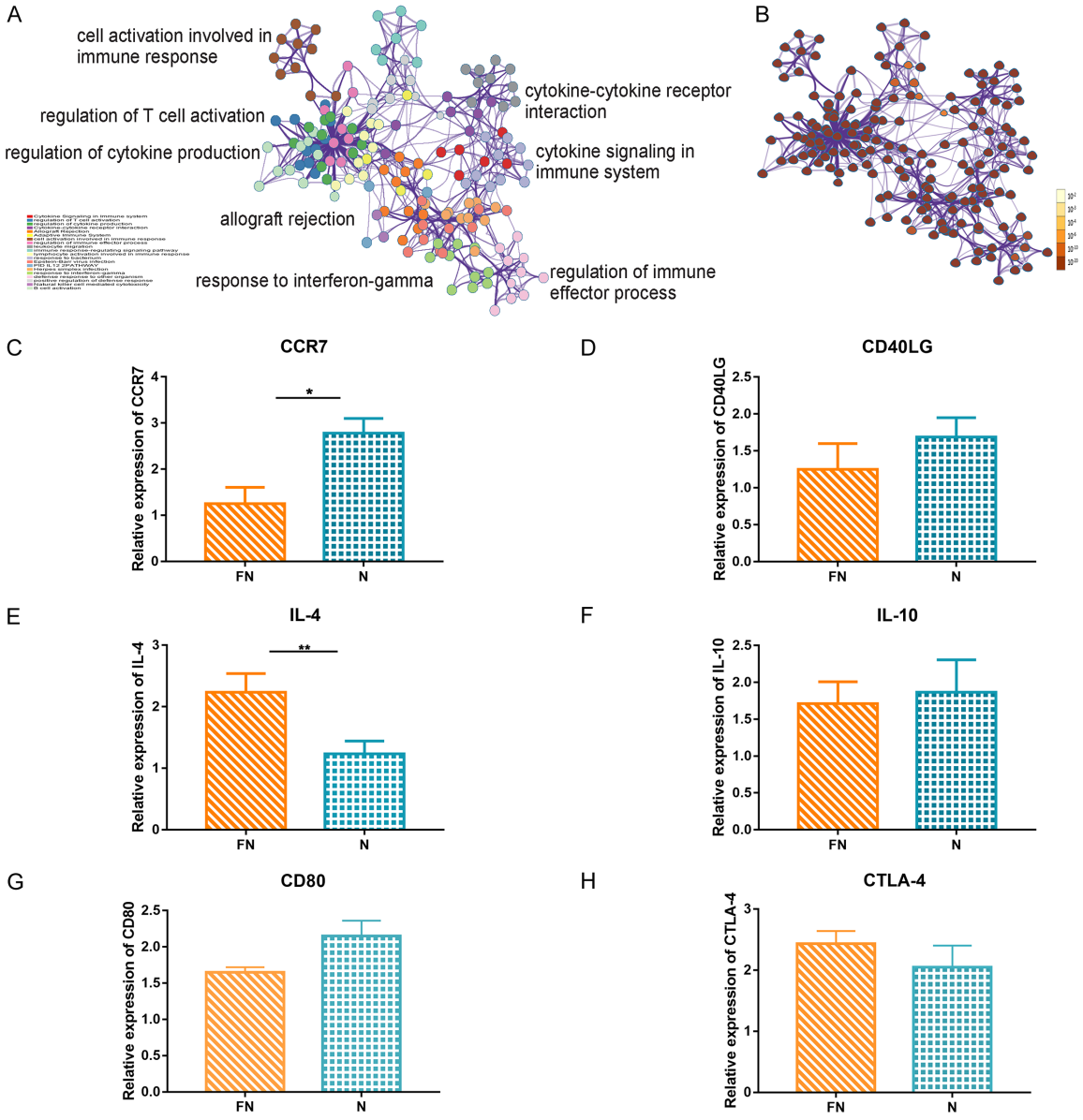

Figure 4. A. The network of enriched terms colored by cluster ID, where nodes that share the same cluster ID are typically close to each other. B. The network of enriched terms colored by *P*-values, where terms containing more genes tend to have a more significant *P*-value. C-H. Relative expression of CCR7, CD40LG, IL-4, IL-10, CD80, and CTLA-4 by RT-qPCR analysis.

lowing a trauma, where the skin lesions extend beyond the original injury into the surrounding normal skin [2]. Keloids can occur at any age, but are most common in young people. They can occur in any ethnic group, but people with darker skin are thought to be more susceptible [4]. The skin is initially damaged by a small, solid red rash, which slowly increases. It is raised to the skin, and crab feet to the outward stretch. The early skin is red with pain, rubber hardness, and the surface can be expanded. During their resting stage, keloids are less strong than before, and their itchiness is reduced [22]. Keloid tissue is commonly found in the sternum area and can also occur on the shoulder, face, neck, and ear. Patients with a severe case not only suffer from serious disfigurement to their appearance but are also affected by somatic movement, which can cause great physical pain and heart damage to patients. The prevalence of keloids differs in different ethnic groups, with a higher preva-

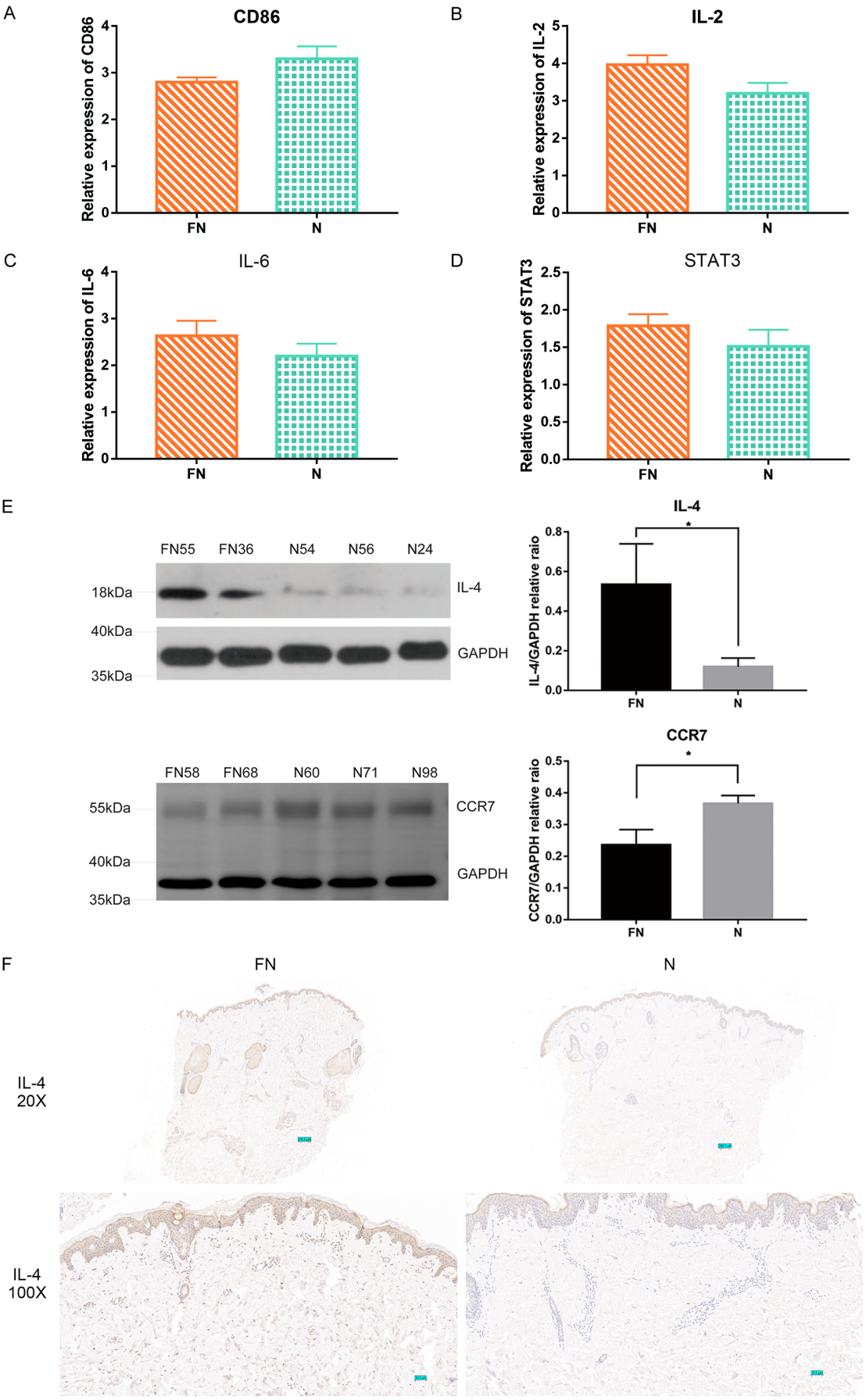
A
CD86
Relative expression of CD86
FN
N
B
IL-2
Relative expression of IL-2
C
IL-6
Relative expression of IL-6
D
STAT3
Relative expression of STAT3
E
FN55 FN36 N54 N56 N24
18kDa
40kDa
35kDa
IL-4
GAPDH
IL-4
IL-4/GAPDH relative raio
FN58 FN68 N60 N71 N98
55kDa
40kDa
35kDa
CCR7
GAPDH
CCR7
CCR7/GAPDH relative raio
F
FN
N
IL-4
20X
IL-4
100X

Figure 5. A-D. Relative expression of CD86, IL-2, IL-6, and STAT3 by RT-qPCR analysis. E. Western blotting analysis showed that the expression of IL-4 and CCR7 proteins between the FN and N groups. F. The expression of IL-4 between the FN and N groups was investigated using immunohistochemistry, × 20 and × 100.

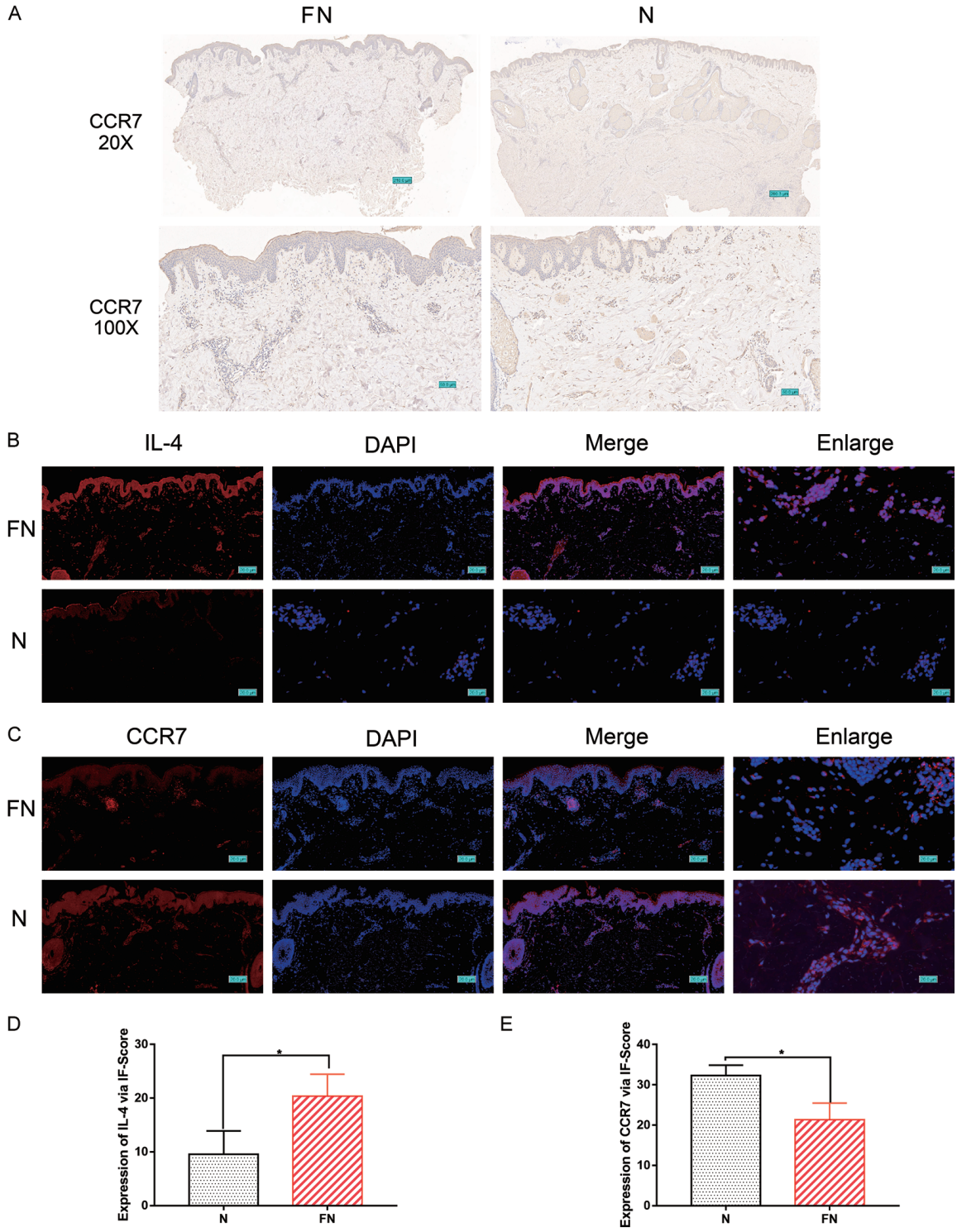

Figure 6. A. The expression of CCR7 between the FN and N groups was investigated using immunohistochemistry, × 20 and × 100. B. The expression of IL-4 was investigated between the FN and N groups using immunofluorescence, × 100 and × 400 (enlarge). C. The expression of CCR7 between the FN and N groups was investigated using immunofluorescence, × 100 and × 400 (enlarge). D. The mean fluorescence intensity of IL-4 was analyzed by Image-Pro Plus 6.0. E. The mean fluorescence intensity of CCR7 was analyzed by Image-Pro Plus 6.0.

lence among darker-skinned people [23]. Researchers believe that the incidence of keloid tissue is the result of a combination of genetic and environmental factors, with genetic factors being the internal cause of keloids and environmental factors being the external cause of keloids [24]. According to previous studies, there are many possible genetic patterns, including an explicit autosomal genetic pattern [25], an autosomal recessive genetic pattern, [15] and autosomal dominant inheritance, which is not completely explicit [14]. However, there is no definite single gene that causes the scarring, and there is no specific evidence that the scar tissue is inconsistent with a multi-gene accumulation pattern.

While some patients' keloids are sporadic, a large number of patients tend to show a family history of the condition [26]. Patients with a family history tend to have more severe symptoms than sporadic patients [5]. Shaheen et al. found that 19.3% (50/259) of patients had a family history and that 76% (38/50) of keloids were located in the same anatomical site as those of their relatives [27]. In our study, 61 patients (23.8%) had a family history of keloids and showed a similar pattern as patients with a family history of keloids that had more severe clinical symptoms (P = -0.749, $P < 0.001$). The occurrence of keloids exhibits a clear familial tendency and suggests that genetic factors may play a major role in the development of the disease. Exploring the relationship between keloids and heredity is more conducive to the exposition of molecular genetics of keloids. In this study, we investigated the differences in genetic susceptibility genes between patients with a family history of keloids and sporadic patients with sporadic keloids. We also validated IL-4 and CCR7 at the molecular level, which show promise as a research direction and as therapeutic targets in the molecular mechanism of keloids.

IL-4 is a glycoprotein secreted by a short α-helix bundle, encoded by the cytokine gene cluster on chromosome 5q [28]. IL-4 is mainly produced by Th2 polarized T cells and can also be secreted by natural killer T cells to activate mast cells, eosinophils, basophils, macrophages, and dendritic cells [29]. During skin wound healing, IL-4 promotes the chemotaxis and proliferation of fibroblasts, the differentiation of myofibroblasts, and the production of collagen and ECM macromolecules [30]. Maeda et al. found that IL-4 induces the expression and secretion of periosteum protein, which in turn induces the secretion of TGF-β1, mediated by the RhoA/ROCK pathway. Secreted TGF-β1 further induces the production and secretion of periosteum, thereby promoting the formation of abnormal scarring [31]. There have been no studies of the role of IL-4 in different genetic backgrounds of keloid patients. In our study, it was found that the expression level of IL-4 in patients with a family history of keloids was significantly higher than its expression level in sporadic patients (**Figure 4E**, $P < 0.01$), which may indicate that IL-4 is an important therapeutic target for patients with a family history of keloids. This may also be one of the factors contributing to the severity of symptoms in patients with a family history of keloids.

CCR7, a member of the chemokine receptor superfamily, is an alpha-helical transmembrane structure with seven hydrophobic amino acids, which regulates signal transduction through heterotrimer G protein and its downstream molecules [32]. CCR7 is expressed in semi-mature and mature dendritic cells (DCs), primary B-cells, T cells, Treg cells, central memory T cells, and other non-immune cells (such as tumor cells) [33]. Under the stimulation of infection and inflammation, immature DCs and their precursors circulating in the blood enter the site of inflammation. Antigens in the inflammation environment are absorbed through receptor-mediated endocytosis and other actions, then gradually mature and strengthen. The expression of MHC II molecules, co-stimulatory molecules (such as CD80, CD86, and CD83), and CCR7 is upregulated [33, 34]. Subsequently, antigen-loaded DCs, mediated by CCR7, enter lymph nodes through the input lymphatic vessels and migrate to the T-cell-rich region to meet the T cells and present the antigen [32]. Native T cells are activated to initiate an immune response. Few studies have detailed the role of CCR7 in keloid pathogenesis. In our study, CCR7 expression was significantly reduced in patients with a family history of keloids (**Figure 4C**, $P < 0.05$). CD80 and CD86 also tended to decrease in the FN group, but there was no significant difference. This may explain the low expression of CCR7 in keloid patients with a family history of keloids,

which results in insufficient T-cell activation, leading to more severe symptoms in these patients. This may therefore represent a therapeutic target during the initial stages of the onset of keloids. We used several functional enrichment algorithms to predict promising research directions to explore molecular mechanisms in the cytokine-cytokine receptor interactions and T-cell activation in the FN group compared with the N group. This is also consistent with the process by which CCR7 mediates DCs to enter lymph nodes, activating native T cells [32, 35].

Our study has some limitations. For example, IL-4 and CCR7 were not further validated in their predicted pathways at the cellular level. It is very interesting to give more insight into the mechanisms by which the IL-4 and CCR7 expression is dysregulated. In vivo validation could not be performed due to the lack of an in vivo model for keloids. In the future, we will further study the role of IL-4 and CCR7 in the development of keloids in patients with a family history of the condition.

Conclusion

In this study, bioinformatics methods were used to analyze high-throughput sequencing data and identify DEGs between keloid patients with a family history of the condition and those with sporadic keloids. RT-PCR was initially used to validate ten hub genes (CCR7, CD40LG, IL-10, CD80, CD86, IL-4, CTLA-4, IL-2, IL-6, and STAT3). We found that the expression of IL-4 and CCR7 was significantly different between the two groups; this was repeatedly verified by immunohistochemistry, Western blotting, and immunofluorescence. IL-4 and CCR7 are important differential genes between patients with a family history of keloids and those with sporadic patients. The high expression of IL-4 and the low expression of CCR7 in the FN group may be a key factor in explaining the family history of keloids. IL-4 and CCR7 play an important role in the development of keloids in patients with a family history of the condition, and they may provide new targets for the treatment of keloids.

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Disclosure of conflict of interest

None.

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Supplementary Table 1. Clinical characteristics of keloid's patients

Patients	Age of onset (years)	Gender	mVSS
N1	20	male	8
N2	23	female	8
N3	23	male	6
N24	40	female	7
N54	32	male	6
N56	28	male	9
N60	33	female	10
N71	29	male	11
N98	42	female	11
FN1	26	male	12
FN2	42	female	13
FN3	23	female	11
FN4	36	female	14
FN36	30	male	10
FN55	28	male	9
FN58	41	female	13
FN68	20	female	15