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Modern diagnostic techniques in dermatology (clinical lecture)

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The article provides an overview of current diagnostic techniques (including morphological and functional methods for assessment of skin structure and condition) used to verify skin and subcutaneous fat diseases.

Keywords: diseases of the skin and subcutaneous fat, microscopy, immunohistochemistry, immunofluorescence, enzyme immunoassay, biomarkers, molecular diagnostics, sequencing and functional testing methods, non-invasive diagnosis.

Clinical diagnosis of the skin and subcutaneous fat diseases within the competence of dermatologists and venereologists is a pressing issue due to, on the one hand, high incidence and, on the other hand, negative effects of these diseases on human health.

In Moscow, the capital city of the Russian Federation, high incidence of skin and subcutaneous fat diseases is registered over the past few years in adult and child population. Thus, skin and subcutaneous fat diseases accounted for 72.5% of the total illness pattern in 2016 (70.3% in 2013, 68,55% in 2014, 73.11% in 2015). The incidence of dermatoses was 3583.7 per 100 thousand population, and the highest incidence rate of this disease was observed in adolescents aged 15—17 years (2016 — 9515.2 cases per 100 thousand population) (Fig. 1).

It should be noted that there are increasingly more diseases that are torpid to conventional therapy and often lead to disability and even death.

By 2016, there has been considerable increase in the incidence of skin tumors (mostly benign, 99.4%), by 431% compared to the data as of 2009 (Fig. 2).

It is known that skin and subcutaneous fat diseases and skin tumors are diagnosed by dermatovenerologists, and visual examination is a primary diagnostic technique, which is based on the observation of primary and secondary morphological elements that ultimately match the clinical presentation of a certain disease. However, in some cases (such as unclear and slight clinical symptoms or similar clinical presentation of some diseases), additional verification is required for the diagnosis.

Diagnostic techniques used to verify the diagnosis of skin and subcutaneous fat diseases can be divided into two large groups: methods for morphological evaluation of skin structure, which in turn are divided into invasive and non-invasive techniques, and functional methods for assessment of skin condition (Fig. 3).

Pathomorphological (histological) study is still the basic laboratory method for verification of the diagnosis of dermatoses and skin tumors**.** Pathomorphological examination of skin bioptates **enables examination of** skin structure and detection of changes (patterns) characteristic of a certain dermatosis; it is highly helpful when the differential diagnosis is required. Apart from the conventional light microscopy, **phase contrast, interference, luminescence, polarization, stereoscopic, ultraviolet, and infrared microscopy,** which are based on various properties of light, are used to study biological objects, including the skin.

***In vitro* confocal microscopy,** an optical laser scanning, which enables obtaining complete high-resolution image of the skin layers in three dimensions (height, width, and depth), is one of the modern pathological method [1].

When talking about the verification of the diagnosis of dermatoses, we cannot ignore **electron** microscopy, a precise ultrastructural method, which, in turn, may be divided into **transmission, scanning, and immunoelectron.** In this method, images of studied objects are obtained due to the directed electron flow. However, the analyzed biological material must be prepared in the form of very thin sections because of the low penetrating ability of electrons. However, only electron microscopy can provide direct information about the molecular organization of cells and assess the ultrastructural changes that occur in the skin under the influence of various therapeutic effects [2—5].

Histochemistry, which studies localization of various substances and their metabolites in tissues by dye binding to specific chemical components of cells, is a good supplement to a pathomorphological examination. This method can be used for differential staining of fat, glycogen, nucleic acids, nucleoproteins, enzymes, and other chemical components of *cells.* Quantitative assessment of the results of reactions is also possible. The use of histochemistry in dermatology can be exemplified by determining the amount of **melaniferous cells** in the basal and spinous layer of epidermis in vitiligo patients [6]. The presence of **glycogen** is a common diagnostic criterion of

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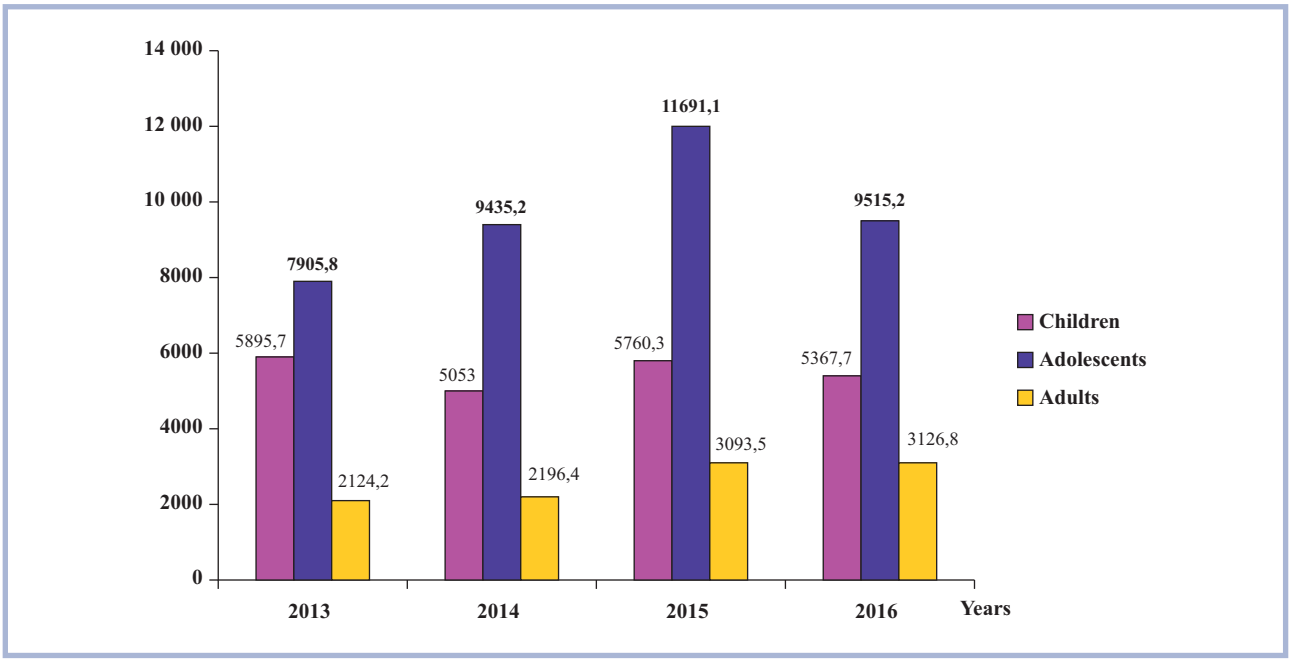

Fig.1. **The incidence of skin and subcutaneous fat diseases in Moscow (2013—2016).**

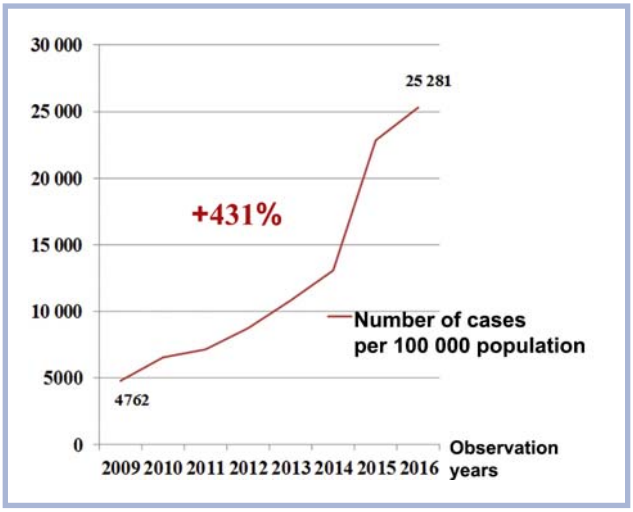

Fig. 2. **The incidence of skin tumors in Moscow (2009—2016).**

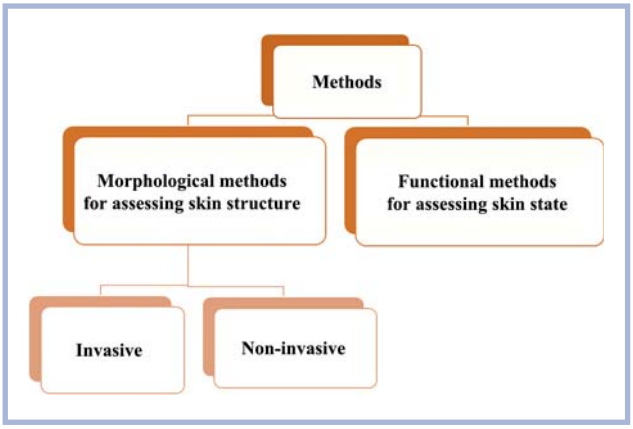

Fig. 3. **Diagnostic techniques used to verify the diagnosis in patients with skin and subcutaneous fat diseases.**

hair follicle tumors (clear cell gidroadenoma and eccrine poroma); in this case, all cells of the epithelial sheath and sweat glands contain large quantities of glycogen. **Neutral mucopolysaccharides** are detected in patients with Paget's disease [7].

Immunohistochemistry is an immuno-microscopy of tissues, which detects specific substances (antigens). It is currently a popular method for diagnosing skin dermatoses and tumors. **The direct method** is based on direct specific binding of labeled antibodies to an antigen being detected (Fig. 4).

The indirect method is two-staged (Fig 4). At the first stage, *unlabeled* primary antibodies bind to a target antigen. At the second stage of the study, the target antigen is detected by interaction between the secondary, *labeled,* antibodies and primary antibodies, which play a role of antigens for secondary antibodies.

Immunohistochemistry is rapidly becoming a standard procedure in many laboratories and it is available in the form of fluorescence *in situ* hybridization (FISH), chromogenic *in situ* hybridization (CISH), and silvering *in situ* hybridization (SISH).

The use of immunohistochemistry in dermatology can be exemplified by its application for diagnosis of *mycosis fungoides*. Thus, the *immunophenotype of tumor lymphoid cells* corresponding to mature memory T cells (βF1+ CD3+ CD4+ CD5+ CD7+ CD8– CD45RO+) [8], *aberrant phenotype of lymphocytes* (decrease in expression of CD2, CD3, CD5, CD7 markers, the loss of pan-T cell antigens) [9], and the *number and maturity of*

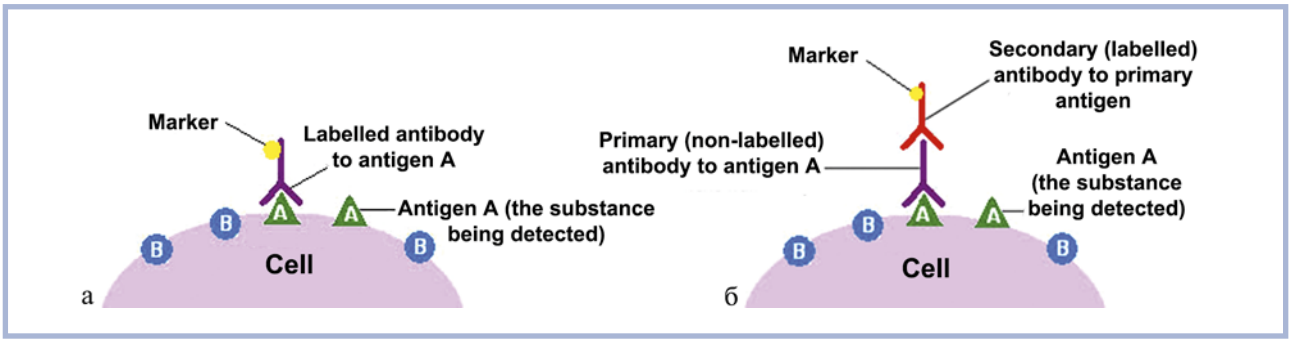

Fig. 4. **The principle of direct (A) and indirect (B) immunohistochemistry method.**

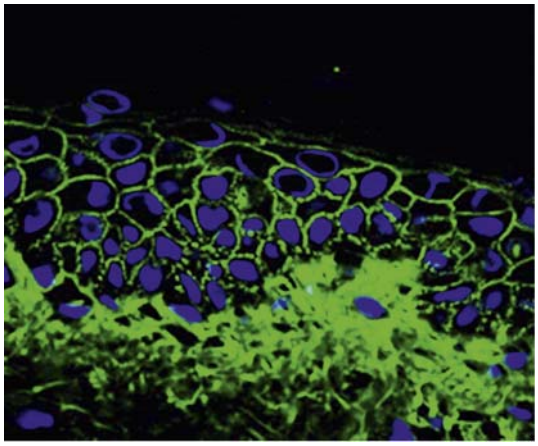

Fig. 5. **Fixation of *IgG* in the epidermal intercellular spaces of a patient with pemphigus vulgaris.**

dendritic cells [10] can be assessed using appropriate markers.

Immunofluorescence analysis, an immunological method for detection of surface and intracellular antigens in samples of tissue sections, as well as cell suspensions and blood, is conceptually close to the immunohistochemistry. The method is based on the visualization of antigen using specific antibodies labeled with fluorescent markers. Similarly to immunohistochemistry, immunofluorescence method can be either direct or indirect, and in the latter case it is two-staged. In dermatology, this method is most often used to confirm the diagnosis of pemphigus. In the presence of *pemphigus vulgaris,* direct immunofluorescence reaction shows fixation of complement (C3) and *Ig* (G, A, or *M)* in the intercellular spaces of the epidermis, and the disease is diagnosed based on this fact (Fig. 5) [11,12].

Among the major trends in the development of modern dermatology in the near future, a special role is played by the direction associated with the development and implementation of non-invasive methods for *in vivo* examination of skin structures [13—20].

These methods include the following.

dermoscopy is based on visual assessment of skin lesions for fast verification of the diagnosis (it enables recognition of morphological structures that are invisible to the naked eye**).**

In vivo **confocal microscopy** using optical scanning laser produces 3D real-time high-resolution images of the skin layers (height, width, and depth).

Optical coherence tomography uses an optical near-infrared radiation to probe biological tissue; the method is characterized by high resolution, contrast, and depth of investigation of 1.5 mm**.**

Ultrasound examination of the skin is a highly accurate and reproducible noninvasive method for skin examination, which enables the differentiation of the skin layers, assessment of vascularization state, and operating with "echogenicity" and "echostructure" concepts.

There are a number of methods to assess the functional condition of the skin. Some of these methods enable evaluation of the lipid balance in the skin **(sebumetry** evaluates the activity of the sebaceous glands), others **(mexametry, chromametry)** determine the color characteristics of the skin. Skin hydration can be assessed by **corneometry** and **vapometry. Tevametry** assesses the barrier properties of the stratum corneum, **reviscometry** assesses the state of connective tissue fibers of the dermis; **cutometry** and **ballistometry** assess deformation and elastic properties of the skin, measurement of **skin pH** assesses the status of acid-base equilibrium (Table 1).

Methods for assessing the functional state of the skin are currently widely used in dermatology and cosmetology for examination of patients with acne, rosacea, and other skin diseases and cosmetic defects [21—26].

A so-called **marker diagnosis** is an important direction in the diagnosis of dermatitis**. Biomarker** is an analyte (specific protein, metabolite, gene, RNA), whose characteristics significantly correlate with the physiological, pathological, or clinical manifestations of the disease. The main purpose of targeted *"marker"* diagnostics is to identify certain molecules or their complexes, which are present *only in the diseased tissues or cells* and are secreted to the external or internal environment of the body in the case of disease.

Depending on the methodologies used for detection, markers can be divided into "biochemical" and "clinical" (detectable by biochemical and clinical methods), "immunological" (detectable by immunological methods), "molecular or molecular genetic" (detectable by molecular methods), "proteomic" (detectable by proteomic analysis techniques), etc.

Biochemical and clinical methods can be used to detect:

rheumatoid factor (*IgM* autoantibodies interacting with F_c-fragment of *IgG),* which are often observed in patients with systemic lupus erythematosus (SLE), having a pronounced articular syndrome [27—28];

lupus anticoagulant (LA) belongs to *IgG. L*A is a parameter of coagulogram, a group of antibodies to phospholipids comprising cell membranes. On the average, LA is detected in 60% of SLE patients. It was first detected in a SLE patient, which determined its name. The presence of LA in the blood is indicative of the predisposition to thrombosis, whose mechanism is not fully understood [29];

LE-cells (lupus erythematodes cells) are the morphological manifestation of the immunological phenomenon characteristic of SLE. The death of neutrophils is an important characteristic of this phenomenon**.** Complex autoimmune processes associated with SLE result in cell destruction and formation of structureless spherical entities (hematoxylin bodies) from their nuclear substance (depolymerized DNA), which are then phagocytosed by neutrophilic leukocytes. Thus, LE-cells are the polymor-

Table 1. **The methods for evaluation of the functional state of the skin**

Method	Parameter	Evaluation principle
	Evaluation of the lipid balance in the skin	
Sebumetry	Sebaceous gland activity	Photometric
	Measurements of the color characteristics of the skin	
Mexametry	Assessment of pigmentation level (melanin) and erythema (hemoglobin)	Absorption of various wavelengths of light by the skin in the red, green, and infrared spectral ranges. It is used to study the processes associated with photo-aging of the skin and to develop skin lightening agents
Chromametry	Evaluation of skin color	According to the "Commission Internationale l'Eclairage" (CIE) system, which is recommended for evaluation of skin color, each color corresponds to the numeric characteristic. The scale of values that describes skin pigmentation and vascularization is used. It can be used to evaluate the antioxidant effect of cosmetics
	Evaluation of skin hydration	
Corneometry	Evaluation of skin hydration	Dielectric properties of the skin vary depending on the amount of moisture in the stratum corneum. Skin conductivity is tested. The higher conductivity, the higher corneometry index
Vapometry	Assessment of transepidermal water loss (TEWL)	Measurement of the density gradient of the water vapor evaporating from the skin per unit time
	Evaluation of other properties of the skin	
Tevametry	Assessment of barrier properties of the stratum corneum	Calculation of the gradient of water vapor density
Reviscometry or reviscosimetry	Assessment of dermal connective tissue fibers, skin anisotropy and heterogeneity of its properties	Acoustic method determines skin anisotropy by means of an acoustic wave penetrating the skin in different directions. Skin anisotropy increases with age due to accumulation of cross-linked (glycated) collagen molecules in the dermis. There is a significant reduction of the acoustic wave velocity in the direction perpendicular to the preferential orientation of dermal fibers (Langer lines).
Cutometry	Assessment of deformation and elastic properties of the skin	Skin suction under vacuum
Ballistometry	Assessment of viscoelastic properties of the skin	Study of the depth of pressing-in, and the attenuation coefficient
pH measurement	Skin pH	pH measurement
Cohesiometry	Skin desquamation test	Transparent adhesive bands are used so that corneocytes stick to the band when it is applied to the skin. Bands are stained and photographed in transmitted light. Then, the image is processed and desquamation index is calculated.
	Assessment of skin topography changes	
Analysis of silicone replicas (casts) of the skin	Assessment of wrinkle density, their length and depth	Silicon is prepared and applied to the skin followed by cast removal, scanning of the surface relief, digitization, and data analysis.
Optical profilometry		Replicas (casts) are analyzed using optical profilers.
Analysis of skin images		Images are obtained using high-resolution digital cameras, visioscopes or dermatoscopes.

Table 2. **Immunological markers used for diagnosis of autoimmune diseases**

Disease	Marker
SLE	Anti-double-stranded native DNA antibodies, histones, Sm-antigen, cardiolipin, and β2-glycoprotein 1
Dermatomyositis	Auto antibodies to cytoplasmic proteins and ribonucleic acids in muscle tissue (aminoacyl-tRNA-synthetases, JO-1-synthetase, Mi-2, factor I-a, SRP-antigen)
Scleroderma	Antibodies to centromere B and topoisomerase I (Scl-70) are the diagnostic indicators of focal and diffuse systemic scleroderma, respectively
Systemic vasculitis	Antibodies to neutrophilic cytoplasmic antigens (ANCA) (cytoplasmic (c-ANCA), perinuclear (p-ANCA)), including antibodies to MPO and serine protease from the a-granules of neutrophils (PR3) are the serological markers of primary systemic vasculitis

phonuclear neutrophils (more rarely, eosinophils or basophils) with phagocytosed cell nucleus or its individual fragments. On the average, these cells are detected in 70 to 80% of SLE patients [30];

"Muscle decay enzymes" (creatine phosphokinase, lactate dehydrogenase, alanine, and aspartate aminotransferase, AST, aldolase) in patients with dermatomyositis [31-33].

Immunological methods are used to detect:

total *IgE* in the serum of patients with atopic dermatitis (enzyme immunoassay, ELISA);

specific *IgE/IgG4* antibodies to food, domestic antigens, vegetable, animal, and chemical antigens, detectable in patients with atopic dermatitis (ELISA);

antibodies to type 1 and 3 desmogleins in patients with pemphigus (ELISA);

***IgG*-antibodies** to bullous pemphigoid antigens (230 kD; BPA-1; 60-180 kD; BPA-2) (ELISA) [34—41].

A number of autoimmune diseases, such as SLE, dermatomyositis, scleroderma, and systemic vasculitis are verified by identifying autoantibodies using immunological methods (ELISA, protein microarrays). In this case, anti-double-stranded native DNA antibodies, histones, Sm-antigen cardiolipin, and β_2-glycoprotein 1 [42—46] are used as immunological markers. Antibodies against cytoplasmic proteins and ribonucleic acids of the muscle tissue (to aminoacyl-tRNA-synthetases, JO-1-synthetases, Mi-2, Factor I-a, and SRP-antigen) are determined for verification of dermatomyositis [47]. Antibodies to centromere B and topoisomerase I (Scl-70) are considered as markers of scleroderma [48—51].

Systemic vasculitis is verified by detecting antibodies to neutrophilic cytoplasmic (c-ANCA) and perinuclear (p-ANCA) antigens, including determining antibodies to myeloperoxidase (MPO) and serine protease from the a-granules of neutrophils (PR3) [52—55] (Table 2).

There are developments in the field of highly sensitive detection of **proteins using proteomic analysis** of the skin, blood serum, and interstitial fluid, which enabled detection of up to 21 candidate proteins in psoriasis (including squamous cell carcinoma antigen-2, cytokeratin 14, 17, glutathione S transferase, heat shock protein 27, RhoGDP-dissociation inhibitor 1, etc.) [56], up to 46 candidate proteins in atopic dermatitis (including NCC27) [57], up to 185 candidate proteins in chronic hand eczema, which mostly belong to barrier proteins, such as filaggrin (FLG), FLG-2, hornerin; antimicrobial peptides S100A7 and S100A8/A9, small proline protein 2B and S100A11, etc. [58].

Dermatoses may be verified using molecular analysis methods, such as polymerase chain reaction (PCR), including a real-time one, hybridization techniques (DNA arrays, qualitative and quantitative analysis: expression microarrays), sequencing techniques from Sanger sequencing to new generation sequencing (NGS). These methods have a number of advantages such as high information content, high speed of the tests, relatively low invasiveness (blood or buccal mucosa imprint are tested), the possibility of simultaneous study of the groups of genes and loci and genome-wide sequencing.

Molecular techniques (PCR) are used to determine clonality of T-lymphocytes (based on the genes of g- and β-chains of T-cell receptor) and B-cells (based on the genes of the heavy chain of *IgH* immunoglobulins) for the differential diagnosis of tumor (skin lymphoma) and reactive lymphocyte proliferation (parapsoriasis, pseudolymphoma of the skin, etc.). In this case, detection of monoclonal proliferation is indicative of malignant nature of the disease [59—62].

Timely identification of a comorbid disease, which can significantly burden the course of the underlying disease, is an important approach to the management of patients with dermatoses. For this purpose, high-performance systems for biochemical, hematologic, enzyme immunoassay, ion-selective, atomic absorption analysis, and chemiluminescence are currently widely used based on the large-scale implementation of automation processes. At the same time, there is the trend towards significant increase in the sensitivity of analytical methods due to change in the analyte detection limits (ceptomoles — 1×10^{-21}; yoctomoles — 1×10^{-24} per liter), which is indicative of a multifaceted "technological revolution" in laboratory analysts [63]. In the context of the possibility of rapid detection of various pathologies in patients with dermatoses, the high-precision multi-parameter microbead immunoassays (MBIA) should be mentioned. This method is based on the use of flow fluorimetry of polystyrene microspheres labeled with red and infrared fluorophores followed by laser detection and digital signal processing. Analyzed substrates include serum/blood plasma, extracts of skin bioptates, synovial fluid, saliva, biological secrets, and other body fluids. The research

Table 3. **Molecular predictors of some genodermatosis**

The name of genodermatosis	Defective gene/mutation	Reference
Epidermolysis bullosa: simplex, dystrophic, junctional, and Kindler syndrome	*COL7A1, COL17A1, KRT14, KRT5* and genes encoding the laminin *332*	[77]
Incontinenia pigmenti, Bloch-Sulzberger syndrome	NEMO	77]
Neurofibromatosis	*NF1 gene*	[77]
Palmoplantar keratoderma FPPK	*KRT6C*	[78]
Acrokeratosis verruciformis of Hopf	*ATP2A2*	[79]
Porokeratosis of Mibelli	*ENPP1*	[80]
Ichthyosis (vulgar, X-linked)	*KRT10; ELOVL4; STS*	[81, 82]

subjects include the markers of diabetes mellitus, acute phase, angiogenesis, apoptosis and sepsis, skin markers (keratins -6, -1, 10, 11, involucrin, fibronectin), bone and carbohydrate metabolism, cytokines (panels of 8 to 27 and more cytokines), immunoglobulin isotypes, markers of cardiovascular diseases; system for determining cellular signals. Moreover, this method can be used to study gene mutations (SNP-genotyping), proteins, nucleic acids, transcription factors. Thus, this analytic method can be used in medical practice and for research.

Personalized, predictive, preventive medicine that studies the possibility of predicting diseases in a particular person on the basis of the revolutionary achievements of the XXI-century biology, which is called a "high-dimensional biology", is one of the most promising areas of modern molecular medicine, relying on laboratory methods.

The term "personalized medicine" was first used by K.K. Jain [64]. *Personalized medicine* is an integrative medicine, which includes the development of personalized treatments, testing for predisposition to diseases, prevention, and diagnosis in combination with treatment and monitoring of treatment.

Methodology of predictive personalized medicine is based on the identification of genes, whose certain alleles predispose, or conversely, prevent the development of various diseases, using sequencing (determining the nucleotide sequence) as the basic technique. [65].

The available molecular genetic methods can be used to study the characteristic features of the genotypes of sick and healthy individuals at the level of gene fragments (analysis of mutation and *single nucleotide polymorphisms* (SNPs); the study of abundance of a gene fragment), the whole gene (full-length sequencing), genome (genome-wide sequencing), and also to study the level of gene expression (transcriptomics).

To date, the structure of genetic risk factors for development of psoriasis (*PSORS* loci, *TNF-A* gene, *RAGE* gene, *IL-10* gene; *rs1800871, IL12B* gene; *rs7709212, RUNX3* gene; *rs7536201; rs1886734,* genes of apoptosis and inflammatory mediators *(DR4 Glu228Ala, CasplO Ile479Leu, rs12188300 IL 12B A/T,* and *TRAF3IP2 Trp74Arg);* psoriatic arthritis *(IL23R* gene; *rs7530511; rs11209026; IL12B* gene; *rs3212227;* rs6887695; G (Arg) allele of *DR4 Lys441Arg* gene, and T allele of *rs12188300* polymorphism of *IL 12B A/*T gene), atopic dermatitis (mutations in filaggrin gene, *rs1800795* of *IL6* gene, *DEFB1* gene), and SLE *(TNFSF4, CD44, IRF8, MIR-146A, TMEM39A* genes*)* was partially solved owing to the so-called Genome-Wide Association Studies, as well as more local research using sequencing techniques [66—76].

Currently, the genetic basis of many genodermatoses, in particular, epidermolysis bullosa, Bloch-Sulzberger syndrome, and a number of others are known due to the use of molecular methods and sequencing techniques, which enable verification of the diagnosis in disputable cases (Table 3).

Molecular markers can be used to personalize the early diagnosis (for example, identification of histocompatibility antigens HLA-B8 and HLA-DR3, linked with the production of myositis-specific antibodies in patients with dermatomyositis), prediction of metastasis of malignant tumors of the skin, the so-called preventive genomics and micro RNA-omics [83—85].

It is well known that therapy of dermatosis can result in pronounced therapeutic response, have no clinical effect, or cause complications. Pharmacogenomics addresses the issues of prediction of the efficacy and safety of treatment and studies gene polymorphism, which determines the variability of the individual response of the human body to the pharmacotherapy. For example, Russian and international publications describe the predictive value of determining *TNF-R-II* gene polymorphism, expression level of some chemokines, their receptors, and CCR9 protein, and the presence of HLA-Cw6 antigen in psoriasis patients in terms of efficacy of therapy with genetically engineered drugs (infliximab, ustekinumab, etc.) [86—88].

Therefore, the current repertoire of the Russian and international dermatology includes both conventional and innovative, invasive and non-invasive analytic methods, which enable clinicians to verify the diagnosis in questionable cases, carry out early diagnosis, predict the course of the disease and pharmacological response to therapy. Automation of routine procedures, miniaturization of the study format, and combining various modules into integrated multifunction systems lead to rapid increase in productivity of biological studies in dermatology and their rise to a new level.

There is no conflict of interest

ЛИТЕРАТУРА/REFERENCES

1. Petrachi T, Lotti R, Palazzo E et al. Organ culture and Reflectance Confocal Microscopy as new integrated tools for barrier rescue studies in inflammatory skin diseases. *Exp Dermatol*. 2015 Dec;24(12):980-982.
2. Stokes DJ. 2003. Investigating biological ultrastructure using environmental scanning electron microscopy (ESEM). *Sci Technol Ed Microsc* Overview. 564-570.
3. Kaliyadan F, Gosai BB, Al Melhim WN, et al. Scanning Electron Microscopy Study of Hair Shaft Damage Secondary to Cosmetic Treatments of the Hair. *Int J Trichology*. 2016 Apr-Jun;8(2):94-98.
4. Wawzy MM, El Maadawi ZM, Hegazy RA, El Fatah NS. Vitiligo — The story from within: A transmission electron microscopic study before and after narrow-band ultraviolet B. *Ultrastruct Pathol*. 2016 Sep-Oct;40(5): 265-275.
5. Kumar A, Al-Ghamdi A, et al. Ultrastructural effects of different low-level lasers on normal cultured human melanocytes: an in vitro comparative study. *Lasers Med Sci*. 2016 Dec;31(9):1819-1825.
6. Прошутинская Д.В. *Влияние узкоспектрального средневолнового ультрафиолетового излучения на образование меланина в коже больных витилиго*.Тезисы X Всероссийского съезда дерматовенерологов. М.: 2008. [Proshutinskaya DV. *Influence of narrow spectral midwave ultraviolet radiation on the formation of melanin in the skin of vitiligo patients.* Abstracts of the 10th All-Russian Congress of Dermatovenerologists. M. 2008. (In Russ.)].
7. Цветкова Г.М., Мордовцев В.К. *Патоморфологическая диагностика заболеваний кожи*. М. 1986. [Tsvetkova GM, Mordovtsev VK. *Pathomorphological diagnostics of skin diseases.* M.: 1986. (In Russ.)].
8. Жуков А.С., Белоусова И.Э., Самцов А.В. Роль иммуногистохимического метода в диагностике грибовидного микоза. *Вестник дерматологии и венерологии*. 2014;2:38-46. [Zhukov AS, Belousova IE, Samtsov AV. The role of the immunohistochemical method in the diagnosis of fungal mycosis. *Vestn dermatol venereol.* 2014;2:38-46. (In Russ.)].
9. Burg G, Kempf W. *Cutaneous Lymphomas* (Basic and Clinical Dermatology). Informa Health-Care. 2005;592.
10. Berger CL, Hanlon D, Kanada D, et al. The growth of cutaneous T-cell lymphoma is stimulated by immature dendritic cells. *Blood.* 2002 Apr 15;99(8):2929-2939.
11. Mutasim DF, Adams BB, Cincinnati. Immunofluorescence in dermatology. *J Am Acad Dermatol* . 2001 December;45(6):803-822.
12. Parlowsky T, Welzel J. Neonatal pemphigus vulgaris: IgG4 autoantibodies to desmoglin 3 induce skin blisters in new born. *J AM Acad Dermatol*. 2003;48:623-657.
13. Maher NG, Solinas A, Scolyer RA, et al. Detection of desmoplastic melanoma with dermoscopy and reflectance confocal microscopy. *J Eur Acad Dermatol Venereol*. 2017 Jun 2. https://doi.org/10.1111/jdv.14381 [Epub ahead of print]
14. Jin H, Yang MY, Kim JM, et al. Arborizing vessels on dermoscopy in various skin diseases other than basal cell carcinoma. *Ann Dermatol*. 2017 Jun;29(3):288-294.
15. Alessandrini A, Starace M, Piraccini BM. Dermoscopy in the evaluation of nail disorders. *Skin Appendage Disord*. 2017 May;3(2):70-82.
16. Zhou W, Chen Z, Yang S, Xing D. Optical biopsy approach to basal cell carcinoma and melanoma based on all-optically integrated photoacoustic and optical coherence tomography. *Opt Lett*. 2017 Jun 1;42(11):2145-2148.
17. Manfredini M, Greco M, Farnetani F, et al. Acne: morphologic and vascular study of lesions and surrounding skin by means of optical coherence tomography. *J Eur Acad Dermatol Venereol*. 2017 May 30. https://doi.org/10.1111/jdv.14369 [Epub ahead of print]
18. Taghavikhalilbad A, Adabi S, Clayton A, et al. Semi-automated localization of dermal epidermal junction in optical coherence tomography images of skin. *Appl Opt*. 2017 Apr 10;56(11):3116-3121.
19. Wang L, Yan F, Yang Y. et al. Quantitative assessment of skin stiffness in localized scleroderma using ultrasound shear-wave elastography. *Ultrasound Med Biol*. 2017 Jul;43(7):1339-1347.
20. Greenlund LJS, Merry SP, Thacher TD, Ward WJ. Primary care management of skin abscesses guided by ultrasound. *Am J Med*. 2017 May;130(5):e191-e193.
21. Luebberding S, Krueger N, Kerscher M. Skin physiology in men and women: in vivo evaluation of 300 people including TEWL, SC hydration, sebum content and skin surface pH. *Int J Cosmet Sci*. 2013 Oct;35(5):477-483.
22. Cannavò SP, Guarneri F, Giuffrida R, Aragona E, Guarneri C. Evaluation of cutaneous surface parameters in psoriatic patients. *Skin Res Technol*. 2017 Feb;23(1):41-47.
23. Rudolph R, Kownatzki E. Corneometric, sebumetric and TEWL measurements following the cleaning of atopic skin with a urea emulsion versus a detergent cleanser. *Contact Dermatitis*. 2004 Jun;50(6):354-358.
24. Губанова Е.И. *Инволюционные изменения кожи нижней трети лица у женщин (клинико-функциональное исследование):* Дисс. ... д-ра мед. наук. М. 2010. [Gubanova EI. *Involutional changes in the skin of the lower third of the face in women (clinical and functional study)*. The dissertation author's abstract on competition of a scientific degree of the doctor of medical sciences. M. 2010. (In Russ.)]. http://medical-diss.com/docreader/326347/a#?page=41
25. Эрнандес Е.И., Губанова Е.И., Парсагашвили Е.З., и др. *Методы эстетической медицины в косметологии*. М. 2010. [Hernandez EI, Gubanova EI, Parsagashvili EZ, et al. *Methods of aesthetic medicine in cosmetology.* M. 2010. (In Russ.)].
26. Панова О.С., Губанова Е.И., Лапатина Н.Г., и др. Современные методы оценки гидратации и биомеханических свойств кожи. *Вестник дерматологии и венерологии*. 2009;2:80-87. [Panova OS, Gubanova EI, Lapatina NG et al. Modern methods for assessing hydration and biomechanical properties of the skin. *Vestn dermatol venereol.* 2009;2:80-87. (In Russ.)].
27. Белов Б.С., Балабанова Р.М., Манукян С.Г., Полянская М.В. Коморбидные инфекции при ревматических заболеваниях: современное состояние проблемы. *Научно-практическая ревматология*. 2006;3:62-66. [Belov BS, Balabanova RM, Manukyan SG, Polyanskaya MV. Comorbid infections in rheumatic diseases: the current state of the problem. *Scientific Pract Rheumatol.* 2006;3:62-66. (In Russ.)].
28. Gladman D, Hussian F, Ibanez D, et al. The nature and outcome of infection in systemic lupus erythematosus. *Lupus.* 2002;11:234-239.
29. Riley R, Friedine J, Rogers J. Antiphospholipid antibodies: standarttization and testing. *Clin Lab Med.* 1997;17:395-399.
30. Насонова В.А., Астапенко М.Г. *Клиническая ревматология*: *руководство для врачей*. М.: Медицина. 1989;592. [Nasonova VA, Astapenko MG. *Clinical rheumatology*: *a guide for doctors*. M.: Medicine. 1989;592. (In Russ.)].
31. Fernandez-Sola J, Pou JC, Junyent JM, Urbano-Manquez A. Normal creatine kinase activity in idiopathic inflammatory myopathy: does it have prognostic implications? *J Rheumatol*. 1991 Oct;18(10):1640-1641.
32. Tsung SH, Huang TY, Han D, Loh WP. Total creatinine kinase and isoenzyme MB activity in serum and skeletal muscle of a patient with dermatomyositis. *Clin Chem*. 1980 Dec;26(13):1912-1913.
33. Kroll M, Otis J, Kagen L. Serum enzyme, myoglobin and muscle strength relationships in polymyositis and dermatomyositis. *J Rheumatol*. 1986 Apr;13(2):349-355.
34. Okamoto S, Taniuchi S, Sudo K, Hatano Y, Nakano K, Shimo T, Kaneko K. Predictive value of IgE/IgG4 antibody ratio in children with egg allergy. *Allergy Asthma Clin Immunol*. 2012 Jun 7;8(1):9.
35. Sasamoto A, Saito S, Uchiyama H, et al. Study of IgE.IgG4 antibodies on eczema in infants. II. Clinical characteristics of infants with egg white. RAST 4+. *Arerugi*. 1991 Nov;40(11):1399-1406.
36. Calkhoven PG, Aalbers M, Koshte VL, et al. Relationship between IgG1 and IgG4 antibodies to foods and the development of IgE antibodies to inhalant allergens. II. Increased levels of IgG antibodies to foods in children who subsequently develop IgE antibodies to inhalant allergens. *Clin Exp Allergy*. 1991 Jan;21(1):99-107.
37. Mutasim D., et al. Immunofluorescence in dermatology. *J Am Acad Dermatol*. 2001;12:803-822.
38. Rani Z., et al. Immunofluorescence in immunobullous diseases *J of Pakistan Association of Dermatol*. 2003;13:76-88.
39. *Diagnostic significance of immunofluorescent tests in dermatology* [Electronic resource]. 2013. Mode of access: http://www.ncbi.nlm.nih.gov/pubmed/20491380
40. Kneisei A, et al. Autoimmune bullous skin diseases. Part 2: diagnosis and therapy. *JDDG*. 2011;9:927-947.
41. *Autoimmune Diseases of the Skin. Pathogenesis, Diagnosis, Management*. M.: Hertl. 3rd ed. Springer. 2011;469.
42. Al Arfaj AS, Chowdhary AR, Khalil N, Ali R. Immunogenicity of singlet oxygen modified human DNA: implications for anti-DNA antibodies in systemic lupus erythematosus. *Clin Immunol*. 2007 Jul;124(1):83-89.
43. Khan MA, Alam K, Zafaryab M, Rizvi MMA. Peroxynitrite-modified histone as a pathophysiological biomarker in autoimmune diseases. *Biochimie*. 2017 Jun 12. pii: S0300-9084(17)30150-5
44. Nojima J, Motoki Y, Hara K, Sakata T, Tsuneoka H, Ichihara K. Detection of antibodies against domain 1 of β₂-glycoprotein I is key in predicting thromboembolic complications in patients with systemic lupus erythematosus. *Thromb Res*. 2017 May;153:83-84.
45. Mahler M. Sm peptides in differentiation of autoimmune diseases. *Adv Clin Chem*. 2011;54:109-128. (Review).

46. Yang HO, Zhang XQ, Fu QH. Evaluating Anti-SmD1-amino-acid 83-119 peptide reactivity in children with systemic lupus erythematosus and other immunological diseases. *Chin Med J* (Engl). 2016 Dec 5;129(23):2840-2844.

47. Trăilă D, Mlădinescu OF, Oancea C, Tudorache V. Interstitial lung disease as first clinical manifestation within the antisynthetase syndrome—dermatomyositis. *Pneumologia*. 2015;64(2):37-40.

48. Dixit S, Kalkur C, Sattur AP, et al. Scleroderma and dentistry: Two case reports. *J Med Case Rep*. 2016 Oct 24;10(1):297.

49. Patterson KA, Roberts-Thomson PJ, Lester S, et al. Interpretation of an extended autoantibody profile in a well-characterized Australian systemic sclerosis (scleroderma) cohort using principal components analysis. *Arthritis Rheumatol*. 2015 Dec;67(12):3234-3244.

50. Kuwana M. Circulating anti-nuclear antibodies in systemic sclerosis: utility in diagnosis and disease subsetting. *J Nippon Med Sch*. 2017;84(2):56-63.

51. Henes J, Glaeser L, Kötter I, et al. Analysis of anti-topoisomerase I antibodies in patients with systemic sclerosis before and after autologous stem cell transplantation. *Rheumatology (Oxford)*. 2017 Mar 1;56(3):451-456.

52. Segelmark M, Baslund B, Wieslander J. Some patients with anti-myeloperoxidase autoantibodies have a C-ANCA pattern. *Clin Exp Immunol*. 1994 Jun;96(3):458-465.

53. Kallenberg CG, Tervaert JW. What is new with anti-neutrophil cytoplasmic antibodies: diagnostic, pathogenetic and therapeutic implications. *Curr Opin Nephrol Hypertens*. 1999 May;8(3):307-315. Review.

54. Bossuyt X, Rasmussen N, van Paassen P, et al. A multicentre study to improve clinical interpretation of proteinase-3 and myeloperoxidase anti-neutrophil cytoplasmic antibodies. *Rheumatology (Oxford)*. 2017 Jun 20. https://doi.org/10.1093/rheumatology/kex266

55. Panda R, Krieger T, Hopf L, et al. Neutrophil extracellular traps contain selected antigens of anti-neutrophil cytoplasmic antibodies. *Front Immunol*. 2017 Apr 13;8:439.

56. Carlén LM, Sánchez F, Bergman AC, et al. Proteome analysis of skin distinguishes acute guttate from chronic plaque psoriasis. *J Invest Dermatol*. 2005 Jan;124(1):63-69.

57. Park YD, Jang HS, Kim SY, et al. Two-dimensional electrophoretic profiling of atopic dermatitis in primary cultured keratinocytes from patients. *Proteomics*. 2006 Feb;6(4):1362-1370.

58. Molin S, Merl J, Dietrich KA, et al. The hand eczema proteome: imbalance of epidermal barrier proteins. *Br J Dermatol*. 2015 Apr;172(4):994-1001.

59. Cotta AC, Cintra ML, Macedo de Souza E, et al. Reassessment of diagnostic criteria in cutaneous lymphocytic infiltrates. *Sao Paulo Medical Journal* 2004;122(4):161-165.

60. Furmanczyk PS, Wolgamot GM, Kussick SJ, et al. Diagnosis of mycosis fungoides with different algorithmic approaches. *J Cutan Pathol* 2010;37:8-14.

61. Грознова А. *Роль молекулярно-генетических методов определения Т- и В-клеточной клональности в диагностике злокачественных лимфом кожи.* Ссылка активно на 14.04.2014 [Groznova A. *The role of molecular genetic methods for determining T- and B-cell clonality in the diagnosis of malignant skin lymphomas.* Accessed April 14 2014 (In Russ.)]. http://doktorgroznova.ru/t-b-

62. Молочков В.А., Молочков А.В., Федоровская А.В. Роль молекулярно-генетических методов определения Т- и В-клеточной клональности в диагностике злокачественных лимфом кожи. *Вестник дерматологии и венерологии.* 2011;3:52-57. [Molochkov VA, Molochkov AV, Fedorovskaya AV. The role of molecular genetic methods for determining T- and B-cell clonality in the diagnosis of malignant skin lymphomas. *Vestn dermatol venereol.* 2011;3:52-57. (In Russ.)].

63. Старцева О.Н., Хоровская Л.А., Эмануэль В.Л. *Клиническая лабораторная диагностика* 2003;11:25-32. [Startseva ON, Horovskaya LA, Emanuel VL. *Clinical laboratory diagnostics* 2003; 11: 25-32. (In Russ.)].

64. Jain KK. From molecular diagnostics to personalized medicine. The IBC Workshop, London, UK, 1st May, 2002. *Expert Rev Mol Diagn*. 2002 Jul;2(4):299-301.

65. Щербо С.Н., Тогузов Р.Т. Тенденции развития современной лабораторной медицины (лекция). *Клиническая лабораторная диагностика.* 2009;3:25-32. [Shcherbo SN, Toguzov RT. Trends in the development of modern laboratory medicine (lecture). *Clin lab diagnost.* 2009;3:25-32. (In Russ.)].

66. Мордовцев В.Н., Бутов Ю.С., Мордовцева В.В. Псориаз. Под ред. Скрипкина Ю.К., Бутова Ю.С. *Клиническая дерматовенерология. Руководство для врачей.* В 2 т. М. 2009;2:212-233. [Mordovtsev VN, Butov YuS, Mordovtseva VV. *Psoriasis*. Pod red. Skripkin YuK, Butov YuS. *Clinical dermatovenereology. A manual for doctors*. 2 vol. M. 2009;2:212-233. (In Russ.)].

67. Шульман А.Я. *Изучение полиморфизма RAGE-гена как фактора предрасположенности к развитию псориаза:* Дисс. ... канд. мед. наук. М. 2008. [Shulman AYa. *Study of the polymorphism of the RAGE gene as a predisposition factor to the development of psoriasis:* The dissertation author's abstract on competition of a scientific degree of the candidate of medical sciences. Moscow. 2008 (In Russ.)].

68. Максимова Ю.В., и др., Полиморфизм некоторых генов иммунного ответа при атопическом дерматите. *Клиническая дерматология и венерология.* 2015;14:5:24-27. [Maksimova YuV, et al. Polymorphism of some immune response genes in atopic dermatitis. *The Russian Journal of Clinical Dermatology and Venereology.* 2015;14: 5:24-27. (In Russ.)].

69. Максимова Ю.В., Свечникова Е.В., Максимов В.Н., Лыкова С.Г., Хорошевская Я.А. Клинический полиморфизм атопического дерматита и вульгарного ихтиоза при мутациях в гене филаггрина. *Клиническая дерматология и венерология.* 2015;14:5:157-165. [Maksimova YuV, Svechnikova EV, Maksimov VN, Lykova SG, Khoroshevskaya YaA. Clinical polymorphism of atopic dermatitis and vulgar ichthyosis with mutations in the filaggrin gene. *The Russian Journal of Clinical Dermatology and Venereology.* 2015;14:5:157-165. (In Russ.)].

70. Хайрутдинов В.Р. *Роль полиморфных генов программируемой клеточной гибели, Т-лимфоцитов и дендритных клеток в патогенезе псориаза:* Дисс. ... д-ра мед. наук. Санкт-Петербург. 2012. [Khayrutdinov VR. *The role of polymorphic genes of programmed cell death, T-lymphocytes and dendritic cells in the pathogenesis of psoriasis:* . Diss. ... d-rao med. nauk. Sant-Petersburg. 2012. (In Russ.)].

71. Wong HK, Wilson AJ, Gibson HM, et al. Increased expression of CTLA-4 in malignant T-cells from patients with mycosis fungoides — cutaneous T cell lymphoma. *J Invest. Dermatol*. 2006 Jan;126(1):212-219.

72. Nedoszytko B, Szczerkowska-Dobosz A, Zablotna M, et al. Associations of promoter region polymorphisms in the tumour necrosis factor-alpha gene and early-onset psoriasis vulgaris in a northern Polish population. *Br J Dermatol*. 2007 Jul;157(1):165-167.

73. Reich K, Hüffmeier U, Konig IR et al. TNF polymorphisms in psoriasis: association of psoriatic arthritis with the promoter polymorphism TNF*-857 independent of the PSORS1 risk allele. *Arthritis Rheum*. 2007 Jun;56(6):2056-2064.

74. Prado-Montes de Oca E, García-Vargas A, Lozano-Inocencio R, et al. Association of beta-defensin 1 single nucleotide polymorphisms with atopic dermatitis. *Int Arch Allergy Immunol*. 2007;142(3):211-218.

75. Filer C, et al. Investigation of association of the IL12B and IL23R genes with psoriatic arthritis. *Arthritis Rheum*. 2008 Dec;58(12):3705-3709.

76. Indhumathi S, et al. T helper-2 cytokine/regulatory T-cell gene polymorphisms and their relation with risk of psoriasis in a South Indian Tamil cohort. *Hum Immunol*. 2017 Feb;78(2):209-215.

77. Wertheim-Tysarowska K, Gos M., Niepokój K., Kowalewski C. Inherited skin diseases — a review of selected genodermatoses. *Med Wieku Rozwoj*. 2012 Jul-Sep;16(3):183-195.

78. Bowden PE. Mutations in a keratin 6 isomer (K6c) cause a type of focal palmoplantar keratoderma. *J Invest Dermatol*. 2010 Feb;130(2):336-338.

79. Dhitavat J, Macfarlane S, Dode L, et al. Acrokeratosis verruciformis of Hopf is caused by mutation in ATP2A2: evidence that it is allelic to Darier's disease. *J Invest Dermatol*. 2003;120(2):229-232.

80. Eytan O, Morice-Picard F, Sarig O, et al. Cole Disease results from mutations in ENPP1. *Am J Hum Genet*. 2013;93(4):752-757.

81. Diociaiuti A, Fortugno P, El Hachem M, et al. Early immunopathological diagnosis of ichthyosis with confetti in two sporadic cases with new mutations in keratin 10. *Acta Derm Venereol*. 2014.

82. Mir H, Raza SI, Touseef M, et al. A novel recessive mutation in the gene ELOVL4 causes a neuro-ichthyotic disorder with variable expressivity. *BMC Med Genet*. 2014;Feb 26:15:25.

83. Greenberg ES, Chong KK, Huynh KT, Tanaka R, Hoon DS. Epigenetic biomarkers in skin cancer. *Cancer Lett*. 2014 Jan 28;342(2):170-177.

84. Mueller DW, et al. miRNA expression profiling in melanocytes and melanoma cell lines reveals miRNAs associated with formation and progression of malignant melanoma. *Journal of Investigative Dermatology*. 2009;129:1740-1751.

85. Lupu M, et al. Gene expression and proteome analysis as sources of biomarkers in basal cell carcinoma. *Dis Markers*. 2016;2016:9831237.

86. Лесная И.Н., Фриго Н.В., и соавт. Молекулярные маркеры в прогнозировании клинической эффективности инфликсимаба у больных псориазом. *Вестник дерматологии и венерологии.* 2010;1:57-67. [Lesnaya IN, Frigo NV, et al. Molecular markers in predicting the clinical efficacy of infliximab in patients with psoriasis. *Vest dermatol and venereol.* 2010;1:57-67. (In Russ.)].

87. Talamonti M, et al. Pharmacogenetics of psoriasis: HLA-Cw6 but not LCE3B/3C deletion nor TNFAIP3 polymorphism predisposes to clinical response to interleukin 12/23 blocker ustekinumab. *Br J Dermatol*. 2013 Aug;169(2):458-463.

88. Koga A, et al. Enhanced CCR9 expression levels in psoriatic skin are associated with poor clinical outcome to infliximab treatment. *J Dermatol*. 2016 May;43(5):522-525.