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# Molecular Diagnostics for Dermatology

## Practical Applications of Molecular Testing for the Diagnosis and Management of the Dermatology Patient

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# Abbreviations

| | |
|---|---|
| A | Adenine |
| ACGH | Array-based comparative genomic hybridization |
| AD | Autosomal dominant |
| ADCC | Antibody-dependent cell cytotoxicity |
| ADE | Adverse drug event |
| AFB | Acid-fast bacilli |
| AFH | Angiomatoid fibrous histiocytoma |
| AIN | Anal intraepithelial neoplasia |
| AJCC | American Joint Committee on Cancer |
| AKT1 | v-akt murine thymoma viral oncogene homologue 1 |
| ALCL | Anaplastic large cell lymphoma |
| ALL | Acute lymphoblastic leukemia |
| AMA | American Medical Association |
| AML | Acute myeloid leukemia |
| AMP | Association for Molecular Pathology |
| APL | Acute promyelocytic leukemia |
| AR | Autosomal recessive |
| ARMS | Amplification refractory mutation system |
| ATRA | All-trans retinoic acid |
| AVL | Atypical vascular lesion |
| BA | Bacillary angiomatosis |
| BAC | Bacterial artificial chromosomes |
| BAP1 | BRCA1-associated protein 1 |
| BCL-2 | B-cell lymphoma 2 |
| BCL-6 | B-cell lymphoma 6 |
| bDNA | Branched deoxyribonucleic acid amplification |
| BP | Base pair |
| BRAF | v-raf murine sarcoma viral oncogene homologue B1 |
| BRIM | BRAF-in-melanoma |
| C | Cytosine *or* constant (domain) |
| CADMA | Competitive amplification of differentially melting amplicons |
| CAMTA1 | Calmodulin-binding transcription activator 1 |
| CAP | College of American Pathologists |
| CCS | Clear cell sarcoma |
| CD | Cluster of differentiation |
| CDC | Centers for Disease Control and Prevention *or* complement-dependent cytotoxicity |

| | |
|---|---|
| CDK4 | Cyclin-dependent kinase 4 |
| CDKN2A | Cyclin-dependent kinase N2A |
| CE | Capillary electrophoresis |
| CEA | Carcinoembryonic antigen |
| CF | Cystic fibrosis |
| CGH | Comparative genomic hybridization |
| CISH | Chromogenic in situ hybridization |
| CLIA | Clinical Lab Improvement Act |
| CLL | Chronic lymphocytic leukemia |
| CML | Chronic myelogenous leukemia |
| CMML | Chronic myelomonocytic leukemia |
| COSMIC | Catalogue of Somatic Mutations in Cancer |
| CPE | Cytopathic effect |
| CPT | Current procedural terminology |
| CR | Conserved region (domain) |
| CREB | cAMP response element binding protein |
| CSD | Cat scratch disease |
| CSF | Cerebrospinal fluid |
| CTCL | Cutaneous T-cell lymphoma |
| CTLA-4 | Cytotoxic T-lymphocyte antigen 4 |
| CVS | Chorionic villus sampling |
| CYP | Cytochrome p450 |
| D | Diversity (as in V-D-J) |
| DAPI | 4′,6-Diamidino-2-phenylindole |
| ddNTP | dideoxynucleotide triphosphate |
| DFA | Direct fluorescent antibody |
| DFSP | Dermatofibrosarcoma protuberans |
| DGGE | Denaturing gradient gel electrophoresis |
| DIHS | Drug-induced hypersensitivity syndrome |
| DNA | Deoxyribonucleic acid |
| dNTP | Deoxynucleotide triphosphate |
| DOE | Department of Energy |
| DRESS | Drug rash with eosinophilia and systemic symptoms |
| DTIC | Dacarbazine |
| EBV | Epstein-Barr virus |
| EDV | Epidermodysplasia verruciformis |
| EGFR | Epidermal growth factor receptor |
| EHE | Epithelioid hemangioendothelioma |
| EHK | Epidermolytic hyperkeratosis |
| EORTC | European Organization for Research and Treatment of Cancer |
| EPCAM | Epithelial cell adhesion molecule |
| ERK | (aka MAPK) mitogen-activated protein kinase |
| ETS | E-twenty-six (gene family) |
| EWS | Ewing sarcoma |
| FAMM | Familial atypical mole melanoma (syndrome) |
| FDA | United States Food and Drug Administration |
| FET | Fus-Ewsr1-Taf15 (gene family) |
| FFPE | Formalin fixed and paraffin embedded |

| | |
|---|---|
| FISH | Fluorescence in situ hybridization |
| FR | Framework region (domain) |
| FRET | Fluorescence resonance energy transfer |
| G | Guanine |
| GCF | Giant cell fibroblastoma |
| GIST | Gastrointestinal stromal tumor |
| GMS | Gömöri methenamine silver |
| GNA11 | Guanine nucleotide-binding protein subunit α-11 |
| GNAQ | Guanine nucleotide-binding protein G(q) subunit α |
| GWAS | Genome-wide association studies |
| H&E | Hematoxylin and eosin |
| HCCC | Hyalinizing clear cell carcinoma |
| HCV | Hepatitis C virus |
| HHV-8 | Human herpesvirus 8 |
| HIV | Human immunodeficiency virus |
| HLA | Human leukocyte antigen |
| HNPCC | Hereditary nonpolyposis colon cancer |
| HPV | Human papillomavirus |
| HRAS | v-Ha-ras Harvey rat sarcoma viral oncogene homologue |
| HRSA | Health Resources and Services Administration (US Department of Health) |
| HSP | Heat shock protein |
| HSV | Herpes simplex virus |
| HTLV-1 | Human T-cell leukemia virus type 1 |
| ICD | International Statistical Classification of Diseases and Related Health Problems (codes) |
| Ig | Immunoglobulin |
| IGH | Immunoglobulin heavy chain |
| IGK | Immunoglobulin light chain kappa |
| IGL | Immunoglobulin light chain lambda |
| IHC | Immunohistochemistry |
| ISCL | International Society for Cutaneous Lymphoma |
| ISCN | International System for Human Cytogenetic Nomenclature |
| ISH | In situ hybridization |
| IVD | In vitro diagnostic |
| J | Joining (as in V-D-J) |
| JBAIDS | Joint Biological Agent Identification and Diagnostic System (anthrax detection) |
| JM | Juxtamembrane (domain) |
| JMML | Juvenile myelomonocytic leukemia |
| KIT | v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homologue |
| KOH | Potassium hydroxide |
| KRAS | v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue |
| KS | Kaposi sarcoma |
| KSHV | Kaposi sarcoma herpesvirus |
| LANA-1 | Latency-associated nuclear antigen 1 |
| LCA | Leukocyte common antigen |

| | |
|---|---|
| LCH | Langerhans cell histiocytosis |
| LCR | Ligase chain reaction |
| LDT | Lab-developed test |
| LGFMS | Low-grade fibromyxoid sarcoma |
| LYP | Lymphomatoid papulosis |
| MALT | Mucosa-associated lymphoid tissue (lymphoma) |
| MAP | MUTYH-associated polyposis |
| MAPK | Mitogen-activated protein kinase (pathway) |
| MART-1 | Melanoma antigen recognized by T cells 1 |
| MC1R | Melanocortin-1 receptor |
| MCC | Merkel cell carcinoma |
| MCV | Merkel cell polyomavirus (or MCPyV) |
| MDM2 | Mouse double minute 2 (gene/protein) |
| MEK | (aka MAP2K) mitogen-activated protein kinase kinase |
| MET | (aka HGFR) hepatocyte growth factor receptor |
| MF | Mycosis fungoides |
| MFH | Malignant fibrous histiocytoma |
| MGMT | O(6)-methylguanine DNA methyltransferase |
| miRNA | microribonucleic acid |
| MiTF | Microphthalmia transcription factor |
| MLH1 | Human homologue of *E. coli* MutL 1 |
| MLPA | Multiplex ligation-dependent probe amplification |
| MMR | Mismatch repair |
| MOTT | Mycobacteria other than tuberculosis |
| mRNA | messenger ribonucleic acid |
| MRSA | Methicillin-resistant *Staphylococcus aureus* |
| MSH | Melanocyte-stimulating hormone |
| MSH2 | Human homologue of *E. coli* MutS 2 |
| MSH6 | Human homologue of *E. coli* MutS 6 |
| MSI | Microsatellite instability |
| MSMD | Mendelian susceptibility to mycobacterial diseases |
| MSS | Microsatellite stable |
| mtDNA | Mitochondrial deoxyribonucleic acid |
| MTOR | Mechanistic target of rapamycin (gene/protein) |
| MTS | Muir-Torre syndrome |
| MUTYH | mutY homologue (gene/protein) |
| N | Nucleotide |
| NCI | National Cancer Institute |
| NER | Nucleotide-excision repair |
| NGS | Next-generation sequencing |
| NIH | National Institutes of Health |
| NK | Natural killer (cells) |
| NPV | Negative predictive value |
| NRAS | Neuroblastoma rat sarcoma viral oncogene homologue |
| NSCLC | Non-small cell lung cancer |
| NSE | Neuron-specific enolase |
| NTM | Nontuberculous mycobacteria |
| OMIM | Online Mendelian Inheritance in Man |

| | |
|---|---|
| PAS | Periodic acid-Schiff |
| PBP | Penicillin binding protein |
| PCFCL | Primary cutaneous follicle center cell lymphoma |
| PCMZL | Primary cutaneous marginal zone B-cell lymphoma |
| PCR | Polymerase chain reaction |
| PD-1 | Programmed cell death 1 |
| PEL | Primary effusion lymphoma |
| PET-FISH | Paraffin-embedded tissue fluorescence in situ hybridization |
| PGDFR | Platelet-derived growth factor receptor |
| PIK3CA | Phosphatidylinositol-4,5-bisphosphate 3-kinase |
| PLC | Pityriasis lichenoides chronica |
| PLEVA | Pityriasis lichenoides et varioliformis acuta |
| PMS-2 | Postmeiotic segregation increased, *S. cerevisiae*, 2 (gene/protein) |
| PNET | Primitive neuroectodermal tumor |
| PPK | Palmoplantar keratoderma |
| PPV | Positive predictive value |
| PTEN | Phosphatase and tensin homologue |
| RAF | Rapidly accelerated fibrosarcoma (gene family) |
| RAPID | Ruggedized advanced pathogen identification device |
| RAS | Rat sarcoma (gene family) |
| RB | Retinoblastoma (gene/protein) |
| RFLP | Restriction fragment length polymorphism |
| RMSF | Rocky Mountain spotted fever |
| RNA | Ribonucleic acid |
| ROC | Receiver operating characteristic (curve) |
| ROS | Reactive oxygen species |
| RR | Relative risk |
| RSS | Recombination signal sequences |
| RSV | Respiratory syncytial virus |
| RTK | Receptor tyrosine kinase |
| RT-PCR | Reverse transcription polymerase chain reaction |
| SALT | Skin-associated lymphoid tissue (lymphoma) |
| SCC | Squamous cell carcinoma |
| SCC*mec* | Staphylococcal cassette chromosome |
| SCF | Stem cell factor |
| SCLC | Small cell lung carcinoma |
| SCPLTCL | Subcutaneous panniculitis-like T-cell lymphoma |
| SDA | Strand displacement amplification |
| siRNA | Small interfering ribonucleic acids |
| SJS | Stevens-Johnson syndrome |
| SLL | Small lymphocytic lymphoma |
| SMRT | Single molecule real time |
| SNP | Single nucleotide polymorphism |
| SOD | Superoxide dismutase |
| SPA | Staphylococcal protein A |
| SS | Sézary syndrome |
| SSCP | Single-strand conformation polymorphism |
| T | Thymine |

| | |
|---|---|
| TB | Tuberculosis |
| TCR | T-cell receptor |
| TEN | Toxic epidermal necrolysis |
| TERT | Telomerase reverse transcriptase |
| TM | Transmembrane (domain) |
| TMA | Transcription-mediated amplification |
| TMZ | Temozolomide |
| TNF | Tumor necrosis factor |
| TNM | Tumor-node-metastasis (staging) |
| TNMB | Tumor-node-metastasis-blood (staging) |
| tRNA | Transfer ribonucleic acid |
| TTF-1 | Thyroid transcription factor 1 |
| Tyrp-1 | Tyrosinase-related protein 1 |
| U | Uracil |
| V | Variable (as in V-D-J) |
| VEGFR | Vascular endothelial growth factor receptor |
| VIN | Vulvar intraepithelial neoplasia |
| VZV | Varicella zoster virus |
| WGS | Whole-genome sequencing |
| WHO | World Health Organization |
| XLD | X-linked dominant |
| XLR | X-linked recessive |
| XP | Xeroderma pigmentosum |
| YAC | Yeast artificial chromosomes |

# Contents

# Introduction 1

## Content



For many, understanding molecular medicine is like standing at the tip of a long oceanic pier, gazing out. This vast, boundless body of information is enticing to some, overwhelming to most. If we choose to ignore it, at the very least, we will be lesser providers of care. We can choose to accept it or, better, embrace it, and we will not only benefit our patients but elevate the quality of modern medicine, entering new diagnostic and treatment frontiers.

Over a century of research on nucleic acids has led to step-by-step advancements in the understanding of their role in inheritance and disease. The uncovering of the double helix structure of DNA by James Watson and Francis Crick in 1953 was instrumental, beginning an era of manipulating these genetic building blocks to predict, diagnose, and manage disease, spawning the discipline of molecular diagnostics (Fig. 1.1). The completion of the Human Genome Project in 2003 was another notable leap. As part of this project, the entire 3.2 gigabase human genome was sequenced [1]. Since then, more genomes have been sequenced, including those from research organisms such as *Drosophila melanogaster* (fruit fly) and *Caenorhabditis elegans* (roundworm), pathogens such as *Haemophilus influenzae*, and, of course, more humans, including James Watson himself. Out of the Human Genome Project, we learned of the approximately 25,000 human genes, a surprisingly low total capable of orchestrating our development and every menial and complex task. We confirmed that all humans are >99.9 % genetically alike,

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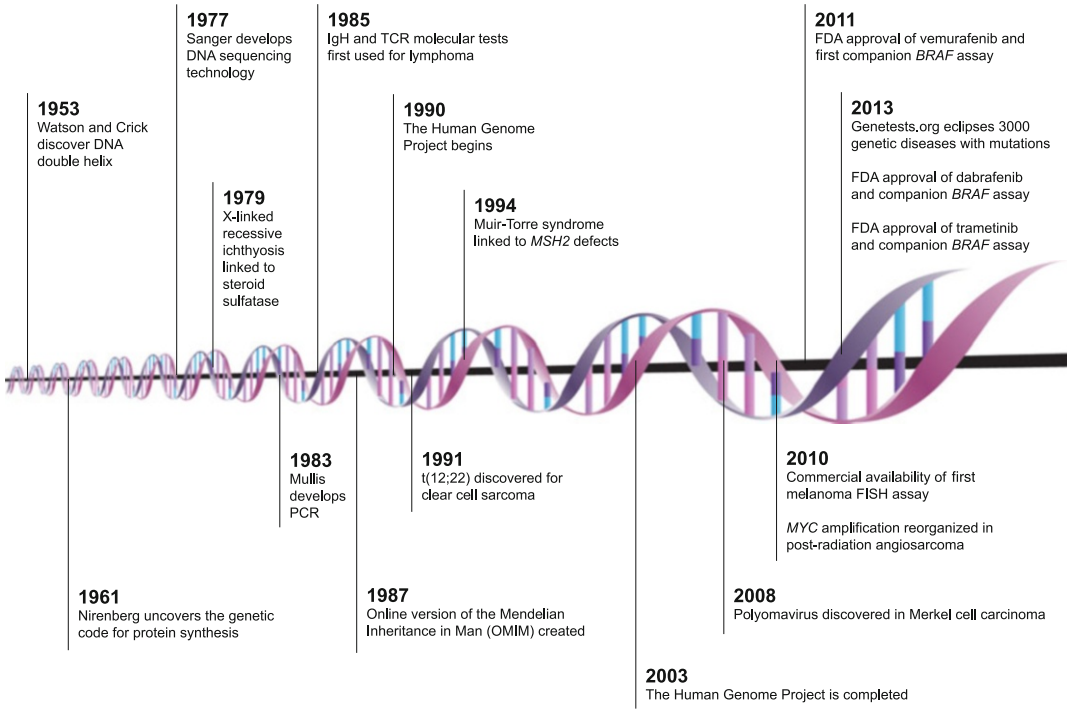


**Fig. 1.1** Timeline of significant events in molecular diagnostics. There have been innumerable impactful events in the history of molecular diagnostics over the past half century. Several, including some in the field of dermatology, are highlighted here

even at the base pair level, with the other <0.1 % holding the mystery to all of our individual differences and genetic sources of disease. And, perhaps most importantly, the human genome became accessible to the entire investigative world, providing an unprecedented template for molecular research. The field of molecular medicine became poised to explode. Molecular diagnostics has captivated medicine in a "Gangnam Style" fashion—fresh, new, and unavoidable. But unlike the popular song, molecular diagnostics has staying power.

In contrast to more conventional diagnostic tools such as histology, cultures, and biochemical assays, molecular diagnostics is traditionally defined by the use of DNA-based (or RNA-based) tests for the diagnosis of human disease. The field has evolved, however. Molecular diagnostics is no longer limited to mere *diagnostics*, separating itself from other ancillary tests in its ability to predict disease behavior and a patient's response to therapeutic targets. In colon cancer, for example, the diagnosis is usually not in question, but molecular testing—*KRAS* mutational analysis, for example—is ordered to predict whether or not the tumor will respond to a specific therapy—cetuximab. Now, the trifecta of "molecular diagnostics" includes *diagnostics* (identifying and classifying disease), *prognostics* (predicting disease course), and *theranostics* (predicting response to therapy), with the latter arguably the most rapidly growing area. And the field refuses to stay stagnant, as applications continue to reach new areas, such as risk assessment and therapeutic monitoring.

In dermatology, the incorporation of molecular diagnostics has admittedly lagged behind other disciplines, with only few and focused practical applications. This narrative is beginning to change, however, with recent important advancements and exciting new applications, touching all

areas of the above italicized trifecta. As examples, molecular tests are now used to help identify germline mutations in the genodermatoses, somatic mutations in tumors such as melanoma and various sarcomas, and the presence of certain cutaneous infectious agents, just to name a few. For melanoma and lymphoma, testing can potentially predict tumor behavior and modify patient staging. And, regarding theranostics, there is no better impactful example in dermatology than the recent observation that targeted therapy to the mutated B-Raf$^{V600E}$ in a subset of melanoma patients dramatically reduces tumor burden and, in rare cases, leads to apparent cure. The entire treatment paradigm for melanoma and other cancers is evolving. "Excision and pray" approaches are being replaced by personalized medicine. Treatment regimens are now being tailored to the individual based on their genome and their tumor's genome. In cases of relapse, second and third rounds of targeted therapy may induce second and third rounds of remission, respectively. Ultimately, in patients unable to achieve a cure, therapy may evolve to constant tumor genome surveillance with molecularly based fine-tuning of treatments, transforming cancer, as we currently know it, into a chronic illness not unlike HIV and diabetes.

With every new test comes hope for revolutionizing applications. In their wake, however, we often struggle with how to implement them. For example, there is a great tendency to overuse new diagnostic tests, supplanting conventional means. Molecular diagnostic tests are like any other ancillary test, dependent on the prevalence of the disease in the population tested. Testing a large number of samples in a population of low disease prevalence will increase the number of false positives and result in a poor predictive value for the assay. Molecular testing is designed to shape a diagnosis for the pathologist, not be a crutch for the "parapathologist" (see Fig. 1.2 for further development of this concept). New tests may also introduce unanticipated practical or ethical problems. We are now able to generate immense patient and/or tumor genetic data, most of which we do not understand. We must resist the temptation of testing just because we can, without an evidence-based infrastructure. A recent Supreme Court decision on gene patenting and the new practice of linking specific molecular tests to the FDA approval of therapy have opened avenues and introduced new wrinkles, respectively, for laboratories interested in test development.

Indeed, this is an exciting time in dermatology, and our goal as authors is to present this current (and near-future) state of affairs of molecular testing as it pertains to the dermatology patient, recognizing that this is in constant flux. In the following chapters, we begin with a basic introduction to molecular biology and commonly used methods for molecular diagnostics. We continue by covering practical applications of molecular diagnostics over a cross section of dermatologic disease, including melanoma, lymphoma, soft tissue tumors, genodermatoses, and infectious disease. Throughout the text, we emphasize the role of the dermatopathologist in test selection, preparing the sample, and interpreting results. And as molecular assays trend toward the generation of thousands of data points in a single reaction, we underline the importance of critically evaluating data in the context of the individual patient, often requiring input by the entire care team. We offer some practical advice, to those ordering molecular tests as well as to those considering performing such tests, with the following chapters serving as a potential template for a comprehensive dermatologic molecular diagnostic test menu. Our focus is on current, practical applications, but we also take several opportunities to look ahead, exploring the future of molecular diagnostics in dermatology and its potential impact on later generations. So as we pull off the fresh seal of the molecular peanut butter jar, exposing its contents with that initial scoop, we hope that all readers—clinicians, pathologists, laboratorians, or other inquisitive minds—independent of their level of molecular expertise, can find some nugget that will provoke thought or perhaps even change their practice.

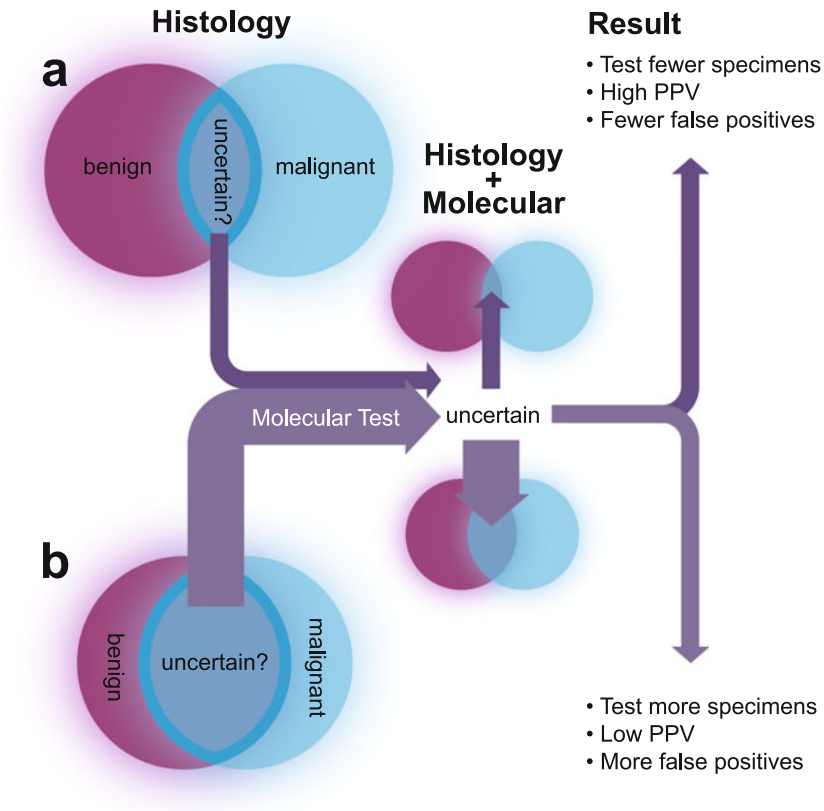


**Fig. 1.2** Conceptual schematic of the role of a new diagnostic test. With every new diagnostic test, there is a positive or negative result. The power of the test, or its ability to distinguish the presence or absence of disease, is dependent on its performance characteristics, including but not limited to sensitivity and specificity. This concept can be applied to a biochemical assay, a molecular test, or even looking through the microscope. Using melanoma as an example, the experienced pathologist may look at an H&E section through the microscope and be able to distinguish melanoma from nevus in most cases, with a small but significant overlapping area corresponding to ambiguous lesions or lesions with indeterminate biology (**a**). The "parapathologist" will have a different starting point, less able to distinguish benign from malignant, with virtually overlapping *circles* (**b**). With the use of a molecular or other ancillary test, the goal is to pull those circles apart, minimizing the overlapping area. The *blue bold lines* along the edges of the overlapping *circles* represent a narrow population of cases with the highest (positive and negative) pretest probability. In (**b**), there is overutilization (more area in intersection of *circles* leading to additional testing) with many of the tested cases having a low pretest probability and, thus, higher numbers of false-positive and false-negative results. Ancillary tests are designed to supplement conventional tests and rarely completely eliminate interpretive overlap. *PPV* positive predictive value

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# Basics of Nucleic Acids and Molecular Biology

# 2

## Contents



**Key Points**

- Nucleic acids are essential for all forms of life.
- The human genome is composed of approximately three billion base pairs of DNA, which are organized into two copies of each of 22 autosomes (non-sex chromosomes) and one pair of sex chromosomes (either XX or XY), for a total of 46 chromosomes.
- In humans, DNA stores the genetic code of life. It is the blueprint, or recipe, for producing all of the proteins needed to carry out cellular functions.
- RNA carries out many diverse and highly specialized cellular functions. These functions primarily involve the processes of transcription and translation, which lead to the production of proteins. RNA functions not only to produce proteins but also to regulate the production process.
- The term "gene expression" is used to indicate the production of RNA and/or protein from a gene. A gene may be silent (no expression) or may be highly expressed. The expression level of genes results in the phenotype of a cell.
- DNA can be altered from normal (wild type) in a wide variety of ways including chromosomal number alterations,

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structural alterations, and sequence alterations.
- DNA alterations are either inherited (germline) or acquired (somatic). Germline mutations result in inherited diseases. Somatic mutations are important drivers of neoplasia.
- The structural and chemical properties of nucleic acids can be exploited to develop molecular diagnostic tests with an array of clinical utilities.

## 2.1 Introduction

Many think of Watson and Crick's description of the double-stranded helix as the beginning of nucleic acid research, while in fact, nucleic acids were first discovered almost 100 years prior (1869) by Swiss scientist Friedrich Miescher. As indicated by the name nucleic acid, initial work discovered these molecules in the nucleus of cells and determined that they had acidic properties. Early work also determined that there are two basic types of nucleic acids, *d*eoxyribo*n*ucleic *a*cid (DNA) and *r*ibo*n*ucleic *a*cid (RNA). Although these basic properties were understood, it would take decades to reveal the structure and function of these molecules. Around the same time (1865), the Austrian monk Gregor Mendel established the idea that physical characteristics are passed from one generation to the next by discrete units, later to be called genes. Over the next several decades, the parallel research into the function of nucleic acids and the mechanism of inheritance started to converge. The microbiologist Oswald Avery and his colleagues at the Rockefeller Institute in New York are largely credited with the collision of these two areas, establishing that DNA, not proteins as many had hypothesized, was the carrier of genetic information [1].

James Watson and Francis Crick, along with significant contributions from Rosalind Franklin, determined the structure of DNA in 1953. This historic discovery is considered the beginning of the development of modern genetics. Understanding the structure of DNA provided an almost immediate understanding of how DNA was replicated and how it might be passed from one generation to the next. In their landmark publication, Watson and Crick wrote "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material" [2]. The discovery of the double-helix structure of DNA also laid the foundation for the development of molecular biology methods and tools, further accelerating research and discovery.

It is now well established that nucleic acids are found in all living cells and in viruses and are essential for all forms of life. Also well established is the concept that while the structures of DNA and RNA are similar, their function and some important chemical characteristics are very different. The sequencing of the entire human genome and the rapid advances in the fields of genetics and molecular biology have set the stage for a much greater understanding of human disease. Application of this knowledge is leading to improvements in making diagnoses and identifying effective treatments. The concept that nucleic acid alterations resulted in inherited diseases was obvious early on. It was not until the early 1990s, however, that researchers began to appreciate the genetic nature of cancer [3].

This discussion relates specifically to the structure and function of human DNA and RNA. It is not the purpose of this chapter to provide a comprehensive review of nucleic acids and molecular biology. There are entire textbooks devoted to these topics. Rather, the purpose of this chapter is to review basic concepts in molecular biology and nucleic acid chemistry to provide an understanding of the nomenclature and vocabulary required to comprehend molecular testing and its impact on patient care. The normal structure and cellular functions of nucleic acids are reviewed, providing a foundation for the discussion of molecular methods used in the clinical molecular laboratory (Chap. 3). In addition, this chapter begins the discussion of how deviations from normal structure and function result in disease, with special attention given to dermatologic disease.