Basic Principles of Human Genetics: A Primer for Oral Medicine

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Humans have known for several 1000 years that heredity affects health. However, it was only 150 years ago when Gregor Mendel first described the mechanism by which genotype results in phenotype. It was less than 100 years ago when Garrod began to apply genetic knowledge to human diseases and disorders. Ironically, for most of the 20th century, clinicians viewed genetics as a somewhat esoteric academic specialty until rather recently with the completion of the Human Genome Project (HGP) in October 2004.[1–3] Meanwhile, rapid advances in high throughput gene sequencing and related bioinformatics have realized that the human genome can be achieved for $1000 per person thereby introducing a tool for diagnostics and prognosis that emphasizes individualized or *personalized* health care.[4–6]

Despite enormous public interest in *genomics* and the thousands of articles published about the completion of the human genome, neither medicine nor dentistry would abruptly change or transform. Medicine and dentistry have not been *gene free* for the last 100 years. Increasingly, a growing and evolving body of knowledge and information has significantly expanded how we think about and how we use human genetics in medicine and dentistry to address epidemiology, public health and risk assessment, single and multiple predictive and prognostic gene-based diagnostics, and pharmacogenomics and pharmacogenetics with customized drug selection specific for individualized metabolism. We are experiencing an expanding knowledge base for Mendelian inheritance, complex

human diseases (multifactorial diseases and disorders), and bioinformatics.[7–15] Further, the 21st century has heralded the introduction of inborn errors of development which provides the molecular basis of clinical disorders of human development.[16]

This chapter is a primer for the emerging field of human genetics, and the era of *-omics* (genomics, transcriptomics, proteomics, metabolomics, diseasomics, phenomics) with oral medicine. The authors and readers acknowledge that the pace of transformation for oral medicine and clinical dentistry and medicine in general is limited not only by the pace of scientific discovery but also by the need to educate practicing dentists, physicians and allied health professions and our patients about the uses and shortcomings of human genetic knowledge and information.[17–22] Human genetic variation is associated with many, if not all, human diseases and disabilities, including the common chronic diseases of major public health impact. Genetic variation interacts with environment and sociocultural influences to modify the risk of disease.

As we look into the future, we must anticipate the logical and rapid advances of human and microbial genomics. This extraordinary progress is already shaping how we consider the etiology and progression of diseases and disorders, how we reach diagnostically useful information, and even how we select therapeutics for particular patients and communities. Today we appreciate the unique interrelationships between the microbiome and the human genome. The average adult consists of 13 trillion cells that coexist with 130 trillion bacterial cells. Our 21,000 human functional genes coexist with 5–8 million bacterial genes in what has recently been termed the *microbiome* or the *second human genome*. In tandem, these advances will also become integrated into the continuum of dental and medical education—predoctoral, doctoral, postdoctoral, residency, and lifelong continuing professional education.

BASIC HUMAN GENETIC PRINCIPLES

The general principles of genetics have been appreciated since the dawn of agriculture some 10,000 years ago when ancient farmers engaged in domestication of plants and animals. The British biologist William Bateson gave the science of inheritance a name—genetics—as recently as 1909. More recently, the use of cytogenetic techniques heralded the technology that enabled cells, intracellular organelles, and chromosomes to be visualized using light microscopy with specific histopathology stains. Karyotyping enabled visualization of the number and fidelity of human chromosomes, and this enabled better diagnostics for chromosomal disorders such as trisomy 21 or Down syndrome.

In the early 1950s, James Watson and Francis Crick discovered the molecular structure of deoxyribonucleic acid (DNA).[7] Thereafter, it became increasingly evident that DNA was arranged in a double-helical structure as an exceedingly long chain of only four units called nucleotides

or bases (adenosine, A; thymidine, T; cytosine, C; and guanosine, G). Three of these nucleotides form codons (e.g., UUA for leucine; U [uracil] substitute for T, thymidine, in ribonucleic acids [RNAs]) and thereby represent the information or code for the ordering of amino acids into forming polypeptides; the so-called *genetic code* was established.

During the last three decades of the 20th century, the fundamental science of DNA accelerated and applications of human genetics rapidly advanced so that a *genetic paradigm* for human diseases and disorders was embraced to a limited extent in many US medical and dental schools.[8–22] Terms such as *susceptibility* versus *resistance* were readily incorporated into the language of health professionals engaged in patient care. Simultaneously, there was also a major acceleration in the study of genes, proteins, and their functions during the human lifespan.[1–15]

The HGP was initiated in 1988 and was completed as of October 2004.[1–6] Exhaustive analyses of the enormous database (the *instruction book* of life) representing the HGP revealed that humans contain 21,000 functional genes and 19,000 nonfunctional pseudogenes within the nucleus of each somatic cell, and another nine genes that are encoded within mitochondria found in all human somatic cells. Genes are discrete units of information encoded within DNA, which, in turn, is localized within chromosomes found in the nucleus of each somatic cell or within mitochondria dispersed through the cytoplasm of all cells. Each cell contains 3.2×10^9 nucleotide pairs per haploid genome in the nucleus and 16,569 nucleotide pairs in each mitochrondrion.[1–15]

Even before a gene's function in disease is fully understood and treatment is available, diagnostic applications can be useful in minimizing or preventing the development of health consequences. The discovery of mutations in the *BRCA1* gene associated with early breast cancer is such an example.[10] The DNA tests used for the presence of disease-linked mutations are proving to be very useful for clinicians. Such tests can assist in the correct diagnosis of a genetic disease, foreshadow the development of disease later in life, or identify healthy heterozygote carriers of recessive diseases. Testing for genetic diseases can be performed at any stage in the human life span.

Importantly, the Mendelian rules that govern the inheritance of many human traits are useful for rare human diseases with highly penetrant changes in a single gene. What is much more difficult is to tease out of the human genome are the multiple genes that are functionally related to complex human diseases such as diabetes, heart disease, oral cancer, periodontal disease, most cleft lip and palate patients, autoimmune disorders (e.g., Sjögren's syndrome), and psychiatric conditions. The challenge is to find multiple, low-penetrance variants, which in the aggregate account for the vast majority of chronic diseases and disorders. This requires new strategies of conceptualizing multifactorial diseases.

There are 46 chromosomes found in every nucleus of every diploid somatic cell in the human body. These

chromosomes contain approximately 6 ft in length of the double-stranded DNA and associated proteins (histones and nonhistone chromosomal proteins). These chromosomal proteins insulate and regulate genes and gene expression during the human life span. Specifically, methylation, acetylation, and deacetylation of these proteins are significant posttranslational mechanisms that regulate gene functions and contribute to the relatively new field termed *epigenetics*.

Of the 46 chromosomes that contain DNA, 44 are termed autosomes that exist in homologous pairs (numbered 1–22, with 1 being the largest and the remaining chromosome pairs numbered in descending order of size) and the remaining two chromosomes (designated X and Y) are termed sex chromosomes. In addition, the maternally inherited mitochondria also contain mitochondrial deoxyribonucleic acid (mtDNA), as mentioned.

In either case, whether it is chromosomes in the nucleus of every somatic cell or mtDNA localized within mitochondria, DNA is a polymer macromolecule that is composed of recurring monomeric units called *nucleotides* or *bases*. There are 3.2 billion nucleotides in the haploid genome. Each monomer or base has three components: (1) a phosphate group linked to (2) a five-carbon atom cyclic sugar group, which, in turn, is joined to (3) a *purine* or *pyrimidine* nucleotide or base. The four nucleotides are the purines adenosine (A) and guanosine (G) and the pyrimidines thymidine (T) and cytosine (C). Permutations and combinations of the four nucleotides constitute the DNA sequence within which the genetic code is embedded. Permutations and combinations of A, C, T, and G result in a DNA sequence, and this sequence becomes highly informative within regulatory or structural regions of human genes. Interestingly, only 1%–2% of the entire human genome encodes 21,000 genes, with the vast majority being apparently noninformative or not-as-yet-informative expanses of nucleotides that include repetitive DNA sequences, pseudogenes, and additional DNA whose function is yet unknown.[2,5,6]

The analysis of X-ray diffraction patterns of purified DNA led James Watson and Francis Crick, based on earlier data from Rosalind Franklin on adenoviral DNA, to build three-dimensional models of DNA that represented a right-handed double helix.[7] They showed that the best fit of the data would be two antiparallel chains in a structure that resembles a spiral staircase. Further, they asserted that within these two strands of DNA, A binds to T and C to G—so-called base pairing or hybridization. These rules apply to DNA found within all living organisms, such as microbes (virus, bacteria, and yeast), plants, animals, and people. Watson and Crick conclude the following:

> It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.[7]

Genes contain the information for proteins. Genes represent hereditary blueprints. All hereditary information is transmitted from parent to offspring through the inheritance of genes, which are identified as the nucleotide sequences within DNA that produce a functional protein product. The largest genetic variance has been determined to be 0.1% between any two people on Earth (or 3 million nucleotides of 3.2 billion found in the haploid human genome), or, from a different perspective, any two humans show 99.9% genetic identity or homology.[1,2]

Genetic variation has phenotypic relevance when considering the impact of this variation on the protein sequence encoded. When comparing protein sequences (of two functionally related proteins or those from two different species), the term *homology* implies that the corresponding amino acid residues in homologous proteins are also homologous. They are derived from the same ancestral residue and, typically, inherit the same function. If the residue in question is the same in a set of homologous sequences, it is assumed that it is evolutionarily conserved. Importantly, protein structure is conserved during evolution much better than protein sequence. For example, lysozyme, the enzyme that hydrolyzes bacterial cell walls, shows little sequence similarity across species but readily adopts similar protein structures, contains identical or related amino acid residues in the bioactive site of the enzyme, and retains a similar catalytic mechanism.[23] Such shared features support the concept that despite low sequence similarities, such proteins are homologous.

Since the completion of the HGP in October 2004,[1,2] numerous other species have also been sequenced with over 300 eukaryotic and nearly 20,000 bacterial genomes in the list (www.genomesonline.org). These genomes include vertebrates such as chimpanzee,[24] rat,[25] mouse,[26] chicken,[27] and dog[28], as well invertebrates such as microbes,[29] malarial parasites,[30] *Anopheles* mosquito,[31] roundworm,[32] fruit fly,[33] and mustard plant.[34] This has been followed by subsequent sequence analysis, comparisons, and interpretations related to structure, function, and evolutionary conservation.[24] Genomic comparisons between human and fruit fly sequences demonstrated that 60% of the human disease genes are conserved between fruit flies and humans.[35] Curiously, two-thirds of the human genes known to be involved in cancers have counterparts in the fruit fly. It would seem counterintuitive that the fruit fly animal organism offers unique opportunities to explore the onset and progression of many human diseases and disorders such as early-onset Parkinson's disease.[36,37] It is now estimated that the number of microbes associated with oral/dental, skin, vaginal, gut, and nasal/lung surfaces is 10-fold greater than the trillions of human somatic cells that comprise an individual adult human being (The International Human Microbiome Consortium, http://www.human-microbiome.org/).[38–47]

It is now accepted that profound similarities or homology exists among genomes of the earth's organisms—microbes (virus, bacteria, yeast), plants, animals, and humans—and that genomes can differ by variations in nucleotide sequences and through duplications or deletions of DNA, through combinations that rearrange the order of genes, and/or by

insertions of DNA that may be derived from microbes.[28–37] In humans, the process of sexual reproduction generates new combinations of genes across multiple generations, constituting the fundamental process of evolution.

Surveys of the human genetic code reveal approximately 10 million variations of nucleotides encoded within the DNA found in human chromosomes—about 0.1% variation between two people. Millions of single nucleotide polymorphisms (SNPs) have been well characterized and enable scientific assessment of extremely small variations between people in health and in disease.[48,49] SNPs and haplotype maps enable genetic linkage with specific diseases and disorders using a process referred to as genome-wide association study (GWAS) illustrated in Figure 25-1. These human genetic variants are closely linked with many diseases and disorders, a person's susceptibility or resistance to disease, and individual responses to therapeutics.[48,49] For example, nucleotide variants within genes encoding opioids and opioid receptors can explain why people differ in their responses to pain or pain stimuli.

Briefly, genes function through a complex series of processes. First, encoding sequences of DNA are *transcribed* to messenger ribonucleic acids (mRNAs). These are, in turn, *translated* into proteins. Another class of RNAs termed transfer or tRNAs are guided and instructed by DNA-derived specific mRNAs to assemble amino acids into
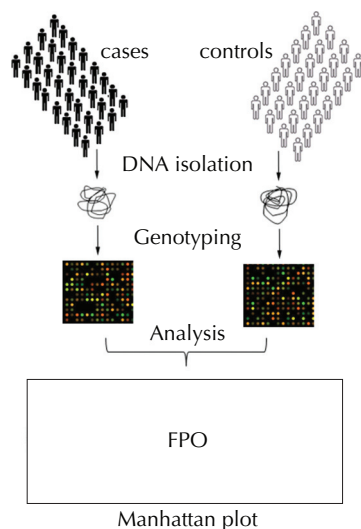
FIGURE 25-1 Genome-wide association study. DNA is isolated from each individual within an age and gender-matched cohort consisting of cases and controls. Genotypes are determined on a microarray that represents up to 2 million SNPs and subjected to statistical analysis to determine if specific alleles at one or more SNP loci are overrepresented in either cases or controls. A representative resulting "Manhattan" plot is shown at the bottom of the figure. Each dot represents a SNP with the horizontal axis indicating each SNP's chromosomal location, while the vertical axis indicates the statistical significance of disease association; SNPs positioned higher on the plot have stronger association with the disease/trait under study with a SNP yielding a P value $<5 \times 10^{-8}$ (horizontal line) considered as being significant at the genome-wide level.

the correct sequence to produce the functional protein on the ribosomes located in the cytoplasm of cells.

A generic gene (sequence of nucleotides A, C, T, and/or G) begins with a *start sequence* in which the mRNA transcription begins. The region before or *upstream* from this site or location contains the *switches* that turn on the gene and also constitute the gene's promoter *sequence*. Further upstream in a region, typically 2000 nucleotides in length, there are additional control elements that regulate the rates, amplitudes, or quantity of transcription. These elements can be enhancers or repressors that respond to DNA-binding proteins, hormones, certain types of vitamins such as retinoic acid, or growth factors. The body of the gene contains discrete coding sequences that give rise to a protein product; these are called *exons*. These are separated by noncoding sequences termed *introns*. Genes terminate with a *stop sequence*. DNA can be transcribed into RNAs (mRNA) by RNA polymerase II enzyme or can be replicated by DNA polymerase enzyme into copy strands of DNA (as required in mitosis or cell division). These two major processes, *transcription of DNA* into mRNA or *replication of one strand of DNA to a copy strand of DNA*, are enormously important for biologic activities (Figure 25-2).

A process termed *alternative splicing* can modify or alter significantly the gene sequence that is transcribed into mRNAs by producing splices and rearrangements between exons that result in as many as 8–10 different isoforms or variants of the gene. Alternative splicing is a regulatory mechanism by which variations in the incorporation of exons encoded within DNA into mRNAs during transcription can produce different isoforms of the same protein. About 15% of human genetic diseases appear to be caused by point mutations at or near splice junctions located within or between introns and exons that control the fidelity of alternative splicing.

Nucleic acid sequences that encode genes within DNA represent *structural proteins* (e.g., genetically different types of collagens, multiple genes for keratins, globins, amelogenins, enamelins, metalloproteinases, albumins, dentin sialoglycoproteins, dentin phosphoproteins) or *regulatory proteins* (e.g., transcriptional factors, signal transduction–related proteins, growth factors).

How Genes Function

How is the information encoded in DNA—sequences of nucleotides—converted into a protein with bioactivity? The process begins with several events: (1) combinations of multiple transcription factors bind to one another (i.e., protein–protein binding) and through binding to DNA (i.e., protein–DNA binding); (2) methylation of cytosine within nucleic acid sequences is highly informative for transcription; and (3) an enzyme, RNA polymerase II, attaches to a specific sequence within DNA and is then followed by the transcription process (DNA to mRNA), followed eventually by translation (mRNA to protein amino acid sequence) on ribosomes physically located within the cytoplasm of cells.
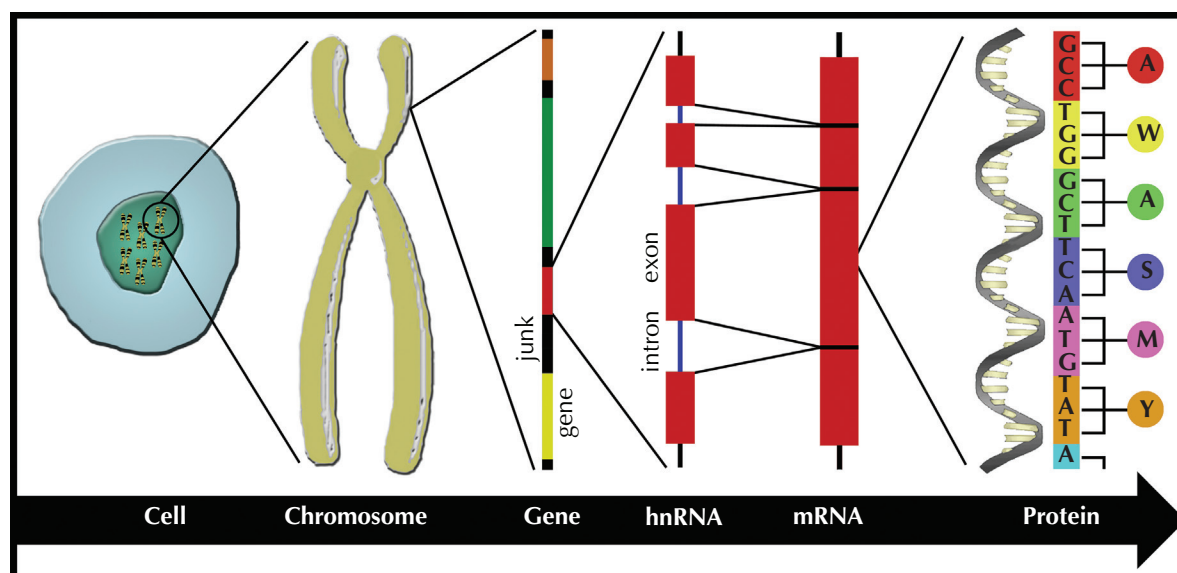
FIGURE 25-2   From gene to protein. Chromosomes are found in the nucleus of the cell. Composed of DNA, these chromosomes contain thousands of genes (colored blocks) which are separated by noncoding, or *junk*, DNA (black blocks). Each gene is composed of both exons (coding DNA; red) and introns (noncoding DNA; blue) which are copied or transcribed (expressed), for the purposes of constructing the protein it codes for, or encodes. There is an intermediary stage between the gene and the protein, where a temporary copy of the gene blueprint is produced as a heterogeneous ribonucleic acid molecule, or hnRNA. The hnRNA consists of both the exons, which are pieced together to form the messenger RNA, or mRNA, and the introns, which are discarded. The mRNA is used as the template for the synthesis of the protein from amino acid building blocks. For this, the DNA is read in three letter blocks, called codons, each of which signifies a single amino acid (colored circles).

Transcription defines the process by which genes encoded within the DNA template are copied into mRNAs that, in turn, leave the nucleus and migrate into the cytoplasm. The number and variation of transcripts from a single discrete gene are created by alternative splicing. In addition, genes that encode ribosomal RNA and tRNA are transcribed and also migrate to the cytoplasm, where they participate in the process of protein synthesis.

For example, tooth formation is a complex developmental process that results from a sequence of epithelial–mesenchymal interactions.[50–55] Mutations in one or more transcription factors (e.g., *MSX1*, *MSX2*, *DLX5*, *PAX9*) may inhibit, arrest, or retard tooth development, and these are clinically diagnosed as tooth agenesis (oligodontia or hypodontia).[56–60] Mutations in regulatory genes such as growth factors and their cognate receptors related to signal transduction and/or transcription factors cause a wide variety of abnormalities in tooth number, position, and shape.[50–70]

Translation is the process that defines mRNA being translated into a precise sequence of amino acids termed *polypeptide* or *protein*. Genetic information is stored as the *genetic code*. Each member of the genetic code consists of three bases or nucleotides that represent a *codon* designating a specific amino acid. The three nucleotides within the codon are determined from four possibilities (A, C, T, and G). Therefore, there are 4^3 or 64 different codons, and all but

three specify an amino acid. The functional codons designate 20 different amino acids. Since the alternative splicing of the human 21,000 functional genes is common, the proteome that reflects the human genome is measured in many thousands of distinct proteins well beyond the number of genes in the human genome. For example, the major protein of forming enamel extracellular matrix is amelogenin derived from the AMELX or AMELY gene. In human and other mammals, the number of different amelogenin isoforms is six to eight proteins of varying molecular weights and all cross-reactive with anti-amelogenin rabbit antibodies. Historically, these multiple amelogenins were assumed to be generated by posttranslation enzymatic steps associated with enamel biomineralization. More recently, it was discovered that AMEL gene produces multiple and different mRNAs through a process termed alternative splicing; one gene produces multiple transcripts which in turn are translated into multiple and different proteins.[14,19,22,71]

Another example is found in dentinogenesis. Dentin formation during tooth development represents secretory odontoblasts engaged in the synthesis, translation, and posttranslation (e.g., glycosylation, phosphorylation) of a number of structural proteins that form the extracellular matrix and control the process of tissue-specific biomineralization. Odontoblasts synthesize and secrete type I collagen and a number of noncollagenous and

highly specialized glycoproteins and phosphoproteins. These extracellular matrix structural proteins control the size, shape, and structure of the minerals that engage in biomineralization. The dentin sialophosphoprotein gene (*DSPP*) is located on chromosome 4 (4q21.3) and encodes two different noncollagenous proteins: (1) dentin sialo-protein and (2) dentin phosphoprotein.[61,62] Mutations in type I collagen and/or *DSPP* produce five different patterns of inherited dentin defects termed *dentinogenesis imperfecta* types.[61,62]

Regulation of Gene Expression

The central problem in human genetics is the temporal and spatial expression of the 21,000 functional genes—the organization of the two-dimensional DNA encoded genetic information into the dynamic three-dimensional morphogenesis and development, cell determination, cell fate, and cytodifferentiation (i.e., growth, development, maturation, senescence). It is now well known that there are *master regulatory genes* (homeotic genes) that control the geometry of body forms.

The most significant level for control is at the level of mRNA production termed *transcriptional control*. Transcriptional control is performed by proteins that bind to DNA, either by modifying cytosine methylation or by *transcription factors* binding to a specific sequence or motif within DNA. A complex of multiple transcription factors often binds to the *TATA box* (a sequence of 8–10 T and A bases) physically located upstream to the formal start sequence of the gene. Other regulatory units encoded within the nucleic acid sequence include the *CAAT box* (a sequence of C, A, and T) and the *GC box* (GGGCGG). Promoters define when and where genes will be expressed, and enhancers define the levels of expression (i.e., copies of mRNA per unit time per cell). In addition to these, molecular tools for regulation, steroids, lipophilic vitamins, and trace elements, also function to control protein–protein and protein–nucleic acid interactions.[1–10]

A number of morphoregulatory or master genes have been identified that are highly conserved from fish to humans. These genes encode highly conserved transcription factors such as *HOX* (homeotic) genes, *PAX* genes, and *T-Box* genes.[50–53] Each of these three types of gene clusters encodes master control genes that regulate the body plan for invertebrates and all vertebrates, including humans.[50–53] Further, each of these three types of transcription factors is transcribed and translated into DNA-binding proteins with high affinities for specific nucleic acid sequence motifs.

For example, two of the morphoregulatory genes are the *FOXC1* and the *PITX2* homeobox genes (Figure 25-3). Mutations in either of these contribute to Axenfeld–Rieger syndrome (ARS), an autosomal dominant developmental disorder that represents a spectrum that involves anomalies of the anterior segment of the eyes, iris hypoplasia, tooth anomalies, craniofacial dysmorphogenesis, cardiac defects,

FIGURE 25-3   Mutations in PITX2 (pituitary homeobox transcription factor 2) and/or FOXC1 (forkhead box transcription factor C1) results in Axenfeld-Rieger Syndrome (ARS) which represents a spectrum of diseases and disorders including those of the dentition (i.e., extreme dental hypoplasia as shown in this figure). Courtesy of Dr. Carl Allen.

limb anomalies, pituitary anomalies, mental defects, and neurosensory defects.[63] Mutations in the *PITX2* gene or the *FOXC* gene have been identified in 40% of ARS.[63]

Cell Division

The cell cycle is the process by which the somatic cell divides to form two daughter cells. This process maintains the 46 chromosomes. A complete cell cycle consists of four phases: G_1, S, G_2, and M (mitosis). Progression through these phases is energy dependent and requires phosphorylation and dephosphorylation steps mediated by kinase enzymes. Gene products called cyclins regulate each of these four phases by specific interactions with kinase phosphatases. Loss of cell cycle controls is the signature for carcinogenesis and many birth defects. One of the major conceptual advances in the last decade is the recognition that cancer is largely a genetic disease and that neoplastic cells display a diverse array of genetic rearrangements, point mutations, and gene amplifications.

Epigenetic Controls

Epigenetic controls, molecular controls that are chromosomal protein posttranslational modifications (e.g., methylation, acetylation) and clearly not intrinsic to the nucleic acid sequence within DNA, provide the multiple gene–gene and gene–environment regulatory influences of the human condition. During embryogenesis, fetal development, infancy, childhood, adolescence, and thereafter, multiple combinations of genes are transcribed and translated into protein products that inform, regulate, and build the human organism.[50–53]

Monozygotic twins share an identical genome or genotype. Yet, as monozygotic twin pairs develop and age they

present several types of phenotypic discordance, such as differences in susceptibilities to diseases and disorders and even a range of anthropomorphic features. One current explanation for these phenotypic variances is epigenetic control differences. To address this issue, a number of studies examined global and gene or locus-specific differences in DNA methylation and histone acetylation of a large cohort of monozygotic twins. These studies report that monozygotic twins are epigenetically indistinguishable during the early years of life, yet older twins (i.e., fourth decade and beyond) exhibited striking differences in their overall content and genomic distribution of 5-methylcytosine DNA and histone acetylation, affecting their gene expression profile. These findings indicate how an appreciation of epigenetics has been a missing link towards our understanding of how different phenotypes (e.g., arthritis, osteoporosis, periodontal disease, fibromyalgia, Alzheimer's disease and other forms of dementia) can be originated from the identical genotype.

MUTATION AND GENETIC HETEROGENEITY

Mutation is defined broadly as any change in the sequence of nucleic acids within DNA. Mutations or *misspellings* can be silent without clinical symptoms or can be profound, such as a single point mutation in a single nucleotide within one of the codons found in one of the exons for the globin gene that can result in sickle cell anemia. In humans, the mutation rate ranges from 1 to 10 million per gene per cell cycle. Importantly, mutations can be fundamental drivers for evolution as organisms adapt to various environs, or they can become clinically relevant as they delete, inhibit, or truncate specific gene expression during human development from conception through senescence.

Mutations can cause disease by a variety of means. The most common is *loss of function mutations*, resulting in a decrease in the quantity or function of a protein. Other mutations cause disease through *gain of function mutations*, such as the dominantly inherited Huntington's disease.

Single-Gene Mutations

Point mutations affect only one nucleotide with the substitution of one for another (Figure 25-4) (e.g., GAG is codon for glutamic acid in the sixth exon of the β-hemoglobin(HbB) gene; a point mutation or substitution of the A for a T in the codon changes meaning to valine and results in sickle cell anemia). *Missense mutation* describes a point mutation that results in the change of a codon, resulting in a change in the primary structure of the protein product resulting from translation. This is clinically observed in select examples of hemoglobinopathies such as sickle cell anemia (globin), craniosynostosis (e.g., fibroblast growth factor receptor), osteogenesis imperfecta (collagen), and amelogenesis imperfecta (AI; amelogenin). *A silent mutation* is a point mutation that has no effect on transcription or translation.

Mutations that abolish protein expression or function are termed *null alleles*. Mechanisms that produce null alleles include mutations that interfere with transcription in general, termination of transcripts, or mutations within splice sites related to alternative splicing. Human carriers of a null allele are often asymptomatic or can have clinical phenotypes if and when the mutations directly inhibit structural protein structure and function. Hypodontia involving primarily molar teeth is associated with mutations in the *PAX9* gene and a repertoire of mutations ranging from point mutations of the missense type to a small insertion within the exon to deletion of the entire gene result in the same clinical phenotype.[59,60]

Chromosomal Mutations

Mutations that involve large alterations in chromosome structure are readily visible microscopically by karyotypic analysis. These macromolecular mutations include deletions, duplications, inversions, and translocations from one chromosome to another. These chromosomal mutations affect large numbers of genes encoded in specific regions of DNA.

Single Nucleotide Polymorphism

One of the derivatives from the federally funded HGP (1988–2004) is a comprehensive catalog of common human sequence polymorphisms. Every person, with the exception of monozygotic twins, has a unique genome with the variance between any two people on the planet being 0.1% or we are 99.9% identical. That reality still leaves many millions of differences among the 3.2 billion base pairs. Present evidence has confirmed that within these several million bases resides what explains our differences with respect to risk or susceptibility to a variety of diseases and disorders. Most of these differences are actually in the form of single nucleotide or base and are termed *single nucleotide polymorphisms* (SNPs). Today the international SNP map working groups have identified and mapped several million SNPs and also discovered a mutation rate of 2×10^{-8} per base pair per generation within the total 6 billion bases that are found in an individual human genome. These tools, still in their infancy, will enable critical dissection of human genetic variance in health as well as in disease. The reader is encouraged to follow this area of inquiry and to appreciate that genetics is the study of variation, and the prospect of carrying it on this level of resolution is heady while being remarkable.

GENETIC DISEASES AND DISORDERS

Approximately 2%–3% of all newborns are born with a serious congenital anomaly, and an additional 2%–3% of infants and children are found to have birth defects by the age of 5 years. Genetics plays a role in 40%–50% of childhood
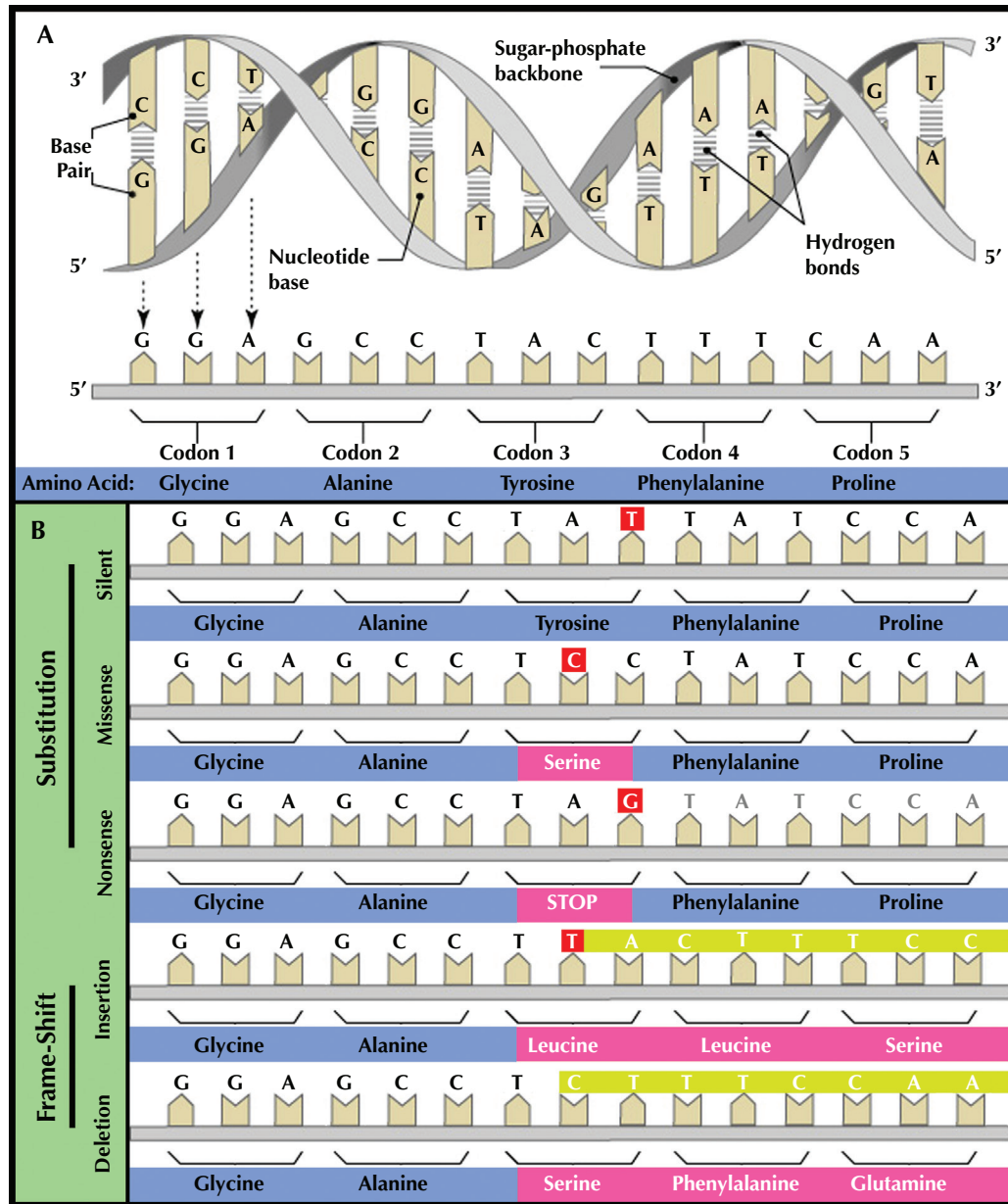
Figure 25-4   (*A*) The structure of DNA, which is composed of just four chemical, or nucleotide, building blocks; A, C, G and T. Adenine always associates with thymine whilst guanine always associates with cytosine. To form individual genes, these nucleotide bases are placed in a unique order, or sequence, upon a poly-sugar backbone that determines their unique characteristics. This is sectioned into triplet nucleotide groups, called codons, which code for the different amino acids. (*B*) The five major types of mutation. Silent mutations cause no change in the encoded amino acid sequence due to redundancy in the codons. Missense mutations lead to a single change in the encoded amino acid sequence. Nonsense mutations result in the formation of one of the three translation stop codons (TAA, TAG and TGA) that result in truncation of the encoded amino acid sequence. Frame-shift mutations (insertions or deletions) result in a change in the encoded amino acid sequences from the point of the mutation as they shift the nucleotide positions in each triplet codon such that they form different codons. Mutated nucleotides are highlighted in red, affected nucleotides are highlighted in yellow and affected amino acids are highlighted in pink.

deaths, 5%–10% of common cancers, and >50% of the older population's medical problems. About 4% of human genes contribute to disease in a major way. Of the over 5000 genetic syndromes known, over 700 have dental-oral-craniofacial defects and over 250 have associated clefting.[50–55,61–70] Facial

clefting is a clinical phenotype associated with Mendelian gene inheritance (single-gene mutation), and facial clefting is also presented in complex human diseases that are polygenic, involving multiple genes and multiple environmental factors.[72,73] Mutations in many different classes of

TABLE 25-1 Examples of Genetic Disorders in Newborn Screening Programs		
Genetic Disorder	**Screening Method**	**Treatment**
Phenylketonuria	Guthrie bacterial inhibition assay	Diet restricting phenyl alanine
	Fluorescence assay	
	Amino acid analyzer	
	Tandem mass spectrometry	
Congenital hypothyroidism	Measurement of thyroxine and thyrotropin	Oral levothyroxine
Hemoglobinopathies	Hemoglobin electrophoresis	Prophylactic antibiotics immunization against *Diplococcus pneumonia* and *Haemophilus influenza*
	Isoelectric focusing	
	High-performance liquid chromatography	
	Follow-up DNA analysis	
Galactosemia	Beutler test	Galactose-free diet
	Paigen test	
Maple syrup urine disease	Guthrie bacterial inhibition assay	Diet restricting intake of branched-chain amino acids
Homocystinuria	Guthrie bacterial inhibition assay	Vitamin B12
		Diet restricting methionine and supplementing cystine
Biotinidase deficiency	Colorimetric assay	Oral biotin
Congenital adrenal hyperplasia	Radioimmunoassay	Glucocorticoids
	Enzyme immunoassay	Mineralocorticoids
		Salt
Cystic fibrosis	Immunoreactive trypsinogen assay followed by DNA testing	Improved nutrition
	Sweat chloride test	Management of pulmonary symptoms

All of these genetic screening tests for newborn infants are well established.

Tests are offered throughout the United Sates. The other tests are available in a limited number of states.

genes cause craniofacial-dental-oral defects, and these genes include those encoding transcription factors, hormone receptors, cell adhesion molecules, growth factors and their receptors, G proteins, enzymes, transporters, and collagens.[50–55,59–71]

Genetic disorders are broadly categorized as (1) single-gene or Mendelian disorders that are typically rare and familial (e.g., hemophilia); (2) chromosomal anomalies that are typically sporadic (e.g., Down syndrome); (3) multifactorial disorders or complex human diseases in which multiple genes are involved with a role played by environmental factors (e.g., many congenital craniofacial malformations, arthritis, hypertension, many cancers, diabetes, osteoporosis, temporomandibular disorders); and (4) acquired somatic genetic disease (e.g., many cancers) (Tables 25-1 and 25-2).

Mendelian Diseases and Disorders

The Online Mendelian Inheritance of Man (OMIM) catalogues approximately 11,000 monogenic or Mendelian traits. These inherited human diseases, typically caused by a mutation in a single gene, can be transmitted within families in a dominant or recessive mode. A dominant disease results if one copy of the two copies of a given gene bears a deleterious mutation. Examples of dominant diseases include achondroplasia (or short-limb dwarfism), myotonic dystrophy, and neurofibromatosis. Certain forms of hypodontia involving molar or premolar teeth also display autosomal dominant inheritance.[54–71] Even though one copy of the gene is normal, the abnormal copy of the gene is able to override it, causing disease. Dominant diseases can be traced through family pedigrees and appear to spread vertically because everyone carrying a dominant mutant allele (form of the gene) generally shows the disease symptoms. Individuals with disease are present in successive generations. There are an equal number of males and females with disease, and each affected individual has only one parent with disease. Individuals mating with an unaffected individual rarely have an affected offspring. Over 200 autosomal dominant diseases are known and can manifest in any organ system and occur at different frequencies.

A disease displays a recessive inheritance pattern when two abnormal copies of the gene are present for the individual

TABLE 25-2   Selected Examples of Genetic Tests for Neurological Diseases, Connective Tissue Diseases, Cancer, and Renal Diseases

Condition	Genes	Testing Utility
Neurologic		
Spinocerebellar ataxias	*SCAI, SCA2, SCA3, SCA6, SCA7, SCAI0, DRPLA*	Diagnostic, predictive
Early-onset familial Alzheimer's disease	*PSENI, PSEN2*	Diagnostic, predictive
Canavan's disease	*ASPA*	Diagnostic, prenatal
Nonsyndromic inherited congenital hearing loss (without other medical complications)	*GJB2*	Diagnostic, prenatal
Fragile X syndrome	*FMRI*	Diagnostic, prenatal
Huntington's disease	*HD*	Diagnostic, predictive, prenatal
Neurofibromatosis type 1	*NFI*	Prenatal
Neurofibromatosis type 2	*NF2*	Predictive, prenatal
Connective tissue		
Ehlers–Danlos syndrome, vascular type	*COL3AI*	Diagnostic, prenatal
Marfan's syndrome	*FBNI*	Diagnostic, prenatal
Osteogenesis imperfect types I–IV	*COLIAI, COLIA2*	Diagnostic, prenatal
Oncologic		
Familial adenomatous polyposis	*APC*	Diagnostic, predictive
Hereditary nonpolyposis colorectal cancer	*MLHI, MSH2, PMS2, MSH3, MSH6*	Diagnostic, predictive
von Hippel–Lindau disease	*VHL*	Diagnostic, predictive
Li–Fraumeni syndrome	*TP53*	Diagnostic, predictive
Hematologic		
β-Thalassemia	*HbB*	Carrier detection, prenatal diagnosis
Hemophilia A	*F8C*	Prognostic, carrier detection, prenatal
Hemophilia B	*F9C*	Carrier detection, prenatal
Renal		
Nephrogenic diabetes insipidus	*AVPR2, AQP2*	Diagnostic, carrier detection, prenatal
Polycystic kidney disease (autosomal dominant and autosomal recessive)	*PKDI, PKD2, PKHD I*	Predictive, prenatal

This table is intended to be illustrative, not exhaustive. Most entries are based on information from GeneTests-GeneClinics at <http://www.geneclinics.org>. This Web site includes a comprehensive list of available molecular genetic tests and further clinical information about these and other genetic conditions.

to be affected. Over 900 autosomal recessive diseases that manifest in a wide range of organs are known. Examples of recessive diseases include phenylketonuria, cystic fibrosis, Tay–Sachs disease, and Gaucher's disease. Recessive diseases that are rare are seen more often in communities in which consanguineous marriages are quite common since there is a high probability of mating between two carriers of the mutant gene. Parents of the affected individual show no symptoms even though they carry one mutant copy of the gene. If both parents are carriers of the gene, the child has a one in four chance of receiving a recessive allele from each parent and inheriting the disease. The distinctive features of an autosomal recessive disease are unaffected parents having affected offspring, equal numbers of affected males and females, all offspring being affected when both parents are affected, and, frequently, the presence of consanguinity or origin of the population from a small group of founders.

Common population traits can be recessive, such as the blood group O; it may be brought into a pedigree by two parents independently and may appear dominant, but it is really pseudodominant and is a recessive trait. Therefore, it occurs in successive generations. Many dominant diseases may appear to skip a generation owing to a phenomenon referred to as nonpenetrance. Thus, even though a dominant disease should be apparent in all gene carriers, this is true only when the disease is 100% penetrant. The molecular basis of incomplete penetrance is unclear but is likely due to the effect of modifier genes that have an

impact on the disease-causing mutation. Many psychiatric diseases, such as schizophrenia and bipolar mental disease, show incomplete penetrance owing to the effect of environmental factors and modifier genes. Late-onset diseases such as spinocerebellar ataxias demonstrate an age-related penetrance, with gene carriers being symptom free until midlife. This is the result of slow cell death—the inability to restore the normal cellular state on environmental damage or accumulation of a toxic product over time. In contrast to penetrance, which refers to the all-or-one state with respect to disease phenotype, variable expressivity is the variable expression of the disease phenotype within the same family. Many dominant diseases (e.g., Charcot–Marie–Tooth disease, neurofibromatosis) display variable expressivity, and the phenomenon is attributed to the effect of modifier genes since each member of the family who carries the same disease mutation can have a unique complement of genes other than those related to the disease that do interact with the disease gene.

Clinicians know that a major feature of gingivitis and periodontitis is the destruction of the collagenous matrix of the connective tissues by microbe-derived and/or host enzymes. Proteolytic cathepsins B, D, and L are biomarkers for the progression of disease.[63] What was quite surprising to many clinicians was the discovery that cathepsin C mutations appear to cause Papillon–Lefèvre syndrome.[74,75]

Sex-linked or X-linked diseases arise when there is a mutation in 1 of more than 285 genes that are located on the X chromosome. In X-linked dominant disease, both males and females are affected, although the females are usually less severely affected. This is because females have two X chromosomes, and during development, one of the two X chromosomes is selected at random and inactivated to allow X-chromosome gene dosage between males and females to be balanced. Thus, in some cells of the body, the X chromosome carrying the disease allele is inactivated, and in others, the *normal* X chromosome is inactivated. An example of an X-linked dominant disease is anhidrotic ectodermal dysplasia characterized by the absence of sweat glands, abnormal teeth, and sparse hair.[50] X-linked dominant inheritance can manifest in either sex, with more affected females than males. Females are more mildly affected than males. All female children of an affected male are affected, and all children of an affected female have a 50% chance of being affected. Most importantly, there is no male-to-male transmission of the disease since males receive their only X chromosome from their mother.

In X-linked recessive inheritance, only males are affected. Females are typically carriers with no symptoms or very mild symptoms. Affected males are usually born to unaffected parents, and the mother is normally an asymptomatic carrier. There is no male-to-male transmission of the disease. Occasionally, females may be affected if by misfortune most cells in a critical tissue have inactivated the normal X (referred to as nonrandom X-inactivation). Examples of such diseases are Duchenne muscular dystrophy and fragile X syndrome.

One example of a clinical phenotype that appears to be caused by either X-linked or autosomal inherited mutations is AI. There are three types of this genetic disorder: (1) type 1, hypoplastic AI; (2) type 2, hypocalcified AI; and (3) type 3, hypomaturation AI.[64–70] AI also shows three types of inheritance patterns: X-linked, autosomal dominant and autosomal recessive with mutations in any of nine genes to date, attributed to the disorder. X-linked AI is associated with mutations in the *AMELX* gene (amelogenin), the most prevalent protein in forming enamel extracellular matrix with genes located on both the X and Y chromosomes.[71] In most cases, males with X-linked AI are more severely affected than affected females. Autosomal dominant AI is associated with mutations in either the *ENAM* gene (enamelin) encoding the second most prevalent protein in the forming enamel matrix, or in the FAM83H gene whose function is unknown. Autosomal recessive AI is associated with mutations in both copies of the genes encoding *ENAM, MMP20, KLK4, FAM20A, C4orf26 or SLC24A4* in each ameloblast cell. Of these, *MMP20* and *KLK4* encode proteases that allow time- and position-specific protein degradation related to calcium hydroxyapatite crystal formations[68–71] while *SLC24A4* encodes a calcium transporter that mediates calcium transport to developing enamel during tooth development. The function of the remaining genes is presently unknown. Additional genes yet unidentified are expected to bear mutations leading to AI.

Y-linked inheritance implies that only males are affected. An affected male transmits his Y-linked trait to all of his sons but none of his daughters. Deletions of genes on the Y chromosome have been linked to infertility owing to azoospermia (or absence of sperm in semen) in males.

Chromosomal Diseases and Disorders

Chromosomal disorders are categorized into three general areas. The first is incorrect chromosomal number such as trisomy 21 (Down syndrome) of chromosome 21; this type is termed *aneuploidy*. Trisomies of chromosomes 13 and 18 are additional examples. Turner's syndrome occurs in women who acquire only one X chromosome. Klinefelter's syndrome occurs in men who receive two X chromosomes in addition to one Y chromosome. The second type is large chromosomal structural defects including microdeletions. DiGeorge syndrome is characterized by T-cell immunodeficiency and cardiac anomalies and is caused by a microdeletion of chromosome 22. The third type of anomaly is uniparental disomy, which refers to the presence of two copies of a chromosome (or part of a chromosome) from one parent and none from the other parent. One example of an adverse outcome of a uniparental disomy is the consequence of genetic imprinting. This term is used to describe when a genetic trait is inherited only when transmitted by the mother in some diseases, such as Beckwith–Wiedmann syndrome,

or the father in others, such as glomus tumors. Genetic imprinting can be described as *parent of origin differences* in the expression of inherited genetic traits. Other examples of such genetic imprinting are presented with Prader–Willi and Angelman's syndromes, caused by a deficiency of paternal and maternal contributions, respectively, to a segment of the long arm of chromosome 15.

Mitochondrial Diseases and Disorders

Mitochondria are exclusively inherited from the mother since only maternal mitochondria are transmitted to the forming zygote in early embryogenesis. mtDNA is a small circular piece of DNA consisting of 16,569 nucleotides that encodes nine genes. Each mitochondrion normally contains multiple copies of mtDNA, whose total amount per cell is typically in the range of 40–2,000 copies. Most of these genes encode information for oxidative phosphorylation and energy production for the individual somatic cell type. Mitochondria contain a small fraction of the genes required for mitochondrial functions. Therefore, the remainder of genes is those found within the nucleus. Curiously, several codons are used for mtDNA differently from codons used for nuclear DNA.

Mitochondrial diseases frequently affect organs that are dependent on relatively high levels of energy, such as the nervous system, muscle, and beta cells in the pancreas. Each somatic cell contains different mixtures of mutant or partially deleted mitochondria that exist along with normal mitochondria. This interesting condition is known as heteroplasmy. The hallmark of mitochondrial inheritance, aside from maternal origin, is a broad spectrum of symptoms within a family segregating the same mitochondrial mutation, extreme variability in severity, and delayed onset with age. Examples of mitochondrial genetic diseases include mitochondrial encephalomyopathy or myoclonic epilepsy with ragged red muscle fibers, Leber's hereditary optic neuropathy with bilateral loss of central vision, and Kearns–Sayre syndrome, which presents retinal disease and cardiac disease (Tables 25-3 and 25-4).

Complex Human Diseases and Disorders

Complex human diseases or *multifactorial genetic disorders* are the most common forms of human genetic disease; they do not present a well-delineated Mendelian pattern of inheritance but *tend to run in families*. These disorders include many types of craniofacial malformations, tooth decay, periodontal disease, atherosclerosis, cardiovascular disease, osteoporosis,

TABLE 25-3 Craniofacial Dysmorphology Associated With Chromosomal Abnormalities		
Syndrome	Chromosomal Defects	Craniofacial Features
Disorders affecting the autosomes		
Down syndrome	Trisomy 21	Mongolism, brachycephaly, mental retardation, upslanting palpebral fissures, epicanthus, iris brushfield spots, small mouth, protruding tongue, small ears, folded helix, short nose, flat nasal bridge, short neck
Edwards' syndrome	Trisomy 18	Prominent occiput, small chin, narrow palpebral fissures, occasional cleft lip, mental defect
Patau's syndrome	Trisomy 13	Cleft lip and palate, broad nasal bridge, sloping forehead, mental retardation, microcephaly, occasional holoprosencephaly, microphthalmia, forehead hemangiomas
Partial trisomy 22	Trisomy 22, partial	Microcephaly, hypertelorism, epicanthus, coloboma, preauricular pits and tags, mental retardation
9p Trisomy	Trisomy 9p	Microcephaly, small deep-set eyes, thin protruding upper lip, bulbous nose, mental retardation
Killian–Pallister mosaic syndrome	Mosaic tetrasomy of short arm of chromosome 12	Coarse facial features, broad forehead, hypertelorism, saggy cheeks, droopy mouth, prominent upper lip, sparse hair, mental retardation
Wolf–Hirschhorn syndrome	Deletion of short arm of chromosome 4	Frontal bossing, high hairline, prominent glabella, short prominent philtrum, occasional cleft lip, mental retardation
13q Syndrome	Deletion of long arm of chromosome 13	Minor dysmorphic face, trigonocephaly, microcephaly, broad nasal root, mental retardation, retinoblastoma
18q Syndrome	Deletion of long arm of chromosome 18	Midface hypoplasia, deep-set eyes, preauricular pits, short philtrum, carp-shaped mouth, narrow or atretic external auditory canal
Disorders affecting the sex chromosomes		
Turner's syndrome	45(XO)	Minor dysmorphic face, narrow maxilla, small chin, curved upper lip, straight lower lip, prominent ears, neck webbing
Females with multiple X chromosomes	47 (XXX) 48(XXXX)	Resemble Down syndrome features, broad flat nose, hypertelorism, epicanthus, prominent jaw, mental retardation

TABLE 25-4 Selected Examples of Craniofacial-Oral-Dental Mendelian Genetic Diseases and Disorders

Type	Gene Name	Gene Symbol	Chromosomal Location	Omim Number for Gene	Syndrome	OMIM Number for Syndrome	Inheritance	Description of Craniofacial-Oral-Dental Features*
ECM	Collagen, type I, alpha-1 chain	COL1A1	17q21.31-q22.05	120150	Osteogenesis imperfecta, type I	166200	AD	Hypoplasia of dentin and pulp, translucent teeth with yellow or blue–gray coloration, delayed tooth eruption, irregular placement of teeth, susceptibility to caries, wormian bones, occasional deafness, otosclerosis, blue sclerae
					Osteogenesis imperfecta, type III	259420	AR	Dentinogenesis imperfecta, macrocephaly, trianglar facial appearance, wormian bones, occasional deafness
					Osteogenesis imperfecta, type IV	166220	AD	Dentinogenesis imperfecta, multiple caries, wormian bones, occasional hearing loss, otosclerosis
					Ehlers-Danlos syndrome, type VII	130060	AD	Narrow maxilla, small mandible, occasional hypodontia and microdontia, wide nasal bridge, epicanthus
ECM	Collagen, type I, alpha-2 chain	COL1A2	7q22.1	120160	Osteogenesis imperfecta, type I	166200	AD	Hypoplasia of dentin and pulp, translucent teeth with yellow or blue–gray coloration, delayed teeth eruption,irregular placement of teeth, susceptibility to caries, wormian bones,occasional deafness, otosclerosis, blue sclerae
					Osteogenesis imperfecta, type III	259420	AR	Dentinogenesis imperfecta, macrocephaly, triangular facial appearance, wormian bones, occasional deafness
					Osteogenesis imperfecta, type IV	166220	AD	Dentinogenesis imperfecta, multiple caries, wormian bones, occasional hearing loss, otosclerosis
ECM	Collagen, type III, alpha-1 chain	COL3A1	2q31	120180	Ehlers-Danlos syndrome, type IV	130050	AD	Narrow maxilla, small mandible, occasional hypodontia and microdontia, wide nasal bridge, epicanthus, large eyes, pinched nose, thin lips
ECM	Collagen, type VII, alpha-1 chain	COL7A1	3p21.3	120120	Epidermolysis bullosa dystrophica	226600	AR	Defective tooth enamel, lingual adhesions, microstomia, bullae of conjunctiva and cornea

(Continued)

TABLE 25-4 Selected Examples of Craniofacial-Oral-Dental Mendelian Genetic Diseases and Disorders (Continued)

Type	Gene Name	Gene Symbol	Chromosomal Location	Omim Number for Gene	Syndrome	OMIM Number for Syndrome	Inheritance	Description of Craniofacial-Oral-Dental Features*
ECM	Collagen, type XI, alpha-2 chain	COL11A2	6p21.3	120290	Stickler syndrome, type II	184840	AD	Cleft palate, micrognathia, glossoptosis, severe myopia, flat facies, dental anomalies, deafness
ECM	Keratin 16	KRT16	17q12-q21	148067	Pachyonychia congenita, Jadassohn–Lewandowsky type	167200	AD	Oral leukoplakia, neonatal teeth, early loss of secondary teeth
ECM	Keratin 17	KRT17	17q12-q21	148069	Pachyonychia congenita, Jackson–Lawler type	167210	AD	No oral leukoplakia, neonatal teeth, early loss of secondary teeth
ECM	Amelogenin	AMELX	Xp22.3-p22.1	301200	Amelogenesis imperfecta 1, hypoplastic type	301200	XD	Hypoplastic-type amelogenesis imperfecta, very hard enamel, thin enamel, small teeth, rough tooth surface
ECM	Dentinogenesis imperfecta 1 gene	DGI1	4q13-q21	125490	Dentinogenesis imperfecta 1	125490	AD	Dentinogenesis imperfecta, blue-gray or amber brown opalescent teeth, enamel splitting, teeth have bulbous crowns, narrow roots with small or obliterated pulp chambers and root canal
ECM	Fibrillin 1	FBN1	15q21.1	134797	Marfan's syndrome	154700	AD	Dolichocephaly, high arched palate, narrow palate, crowded teeth, micrognathia
					Shprintzen-Goldberg syndrome	182212	AD	Craniosynostosis, microcephaly, maxillary and mandibular hypoplasia, palatal shelf soft tissue hypertrophy, cleft palate, prominent nose, narrow palpebral fissures
ENZ	Alkaline phosphatase, liver/bone/kidney type	ALPL	1p36.1-p34	171760	Hypophosphatasia, infantile	241500	AR	Generalized lack of ossification, craniostenosis, microcephaly, leptomeningeal hemorrhage, absent bony cranial vault, poorly formed teeth
ENZ	Iduronate 2-sulfatase	IDS	Xq28	309900	Mucopolysaccharidosis, type II (Hunter's syndrome)	309900	X	Scaphocephaly, macrocephaly, frontal bossing, coarse facies, enlarged tongue, deafness
ENZ	Galactosamine (N-acetyl)-6-sulfate sulfatase	GALNS	16q24.3	253000	Mucopolysaccharidosis, type IVA (Morquio's syndrome)	253000	AR	Dense calvarium, broad mouth, wide-spaced teeth, thin enamel

IS	Guanine nucleotide-binding protein, alpha-stimulating activity polypeptide 1	GNAS1	20q13.2	139320	Pseudohypoparathyroidism, type Ia	103580	AD	Round face, low nasal bridge, short neck, cataracts, delayed tooth eruption, enamel hypoplasia
					McCune-Albright syndrome	174800	AD	Cranial foramen impingement, craniofacial hyperostosis, facial asymmetry, prognathism
IS	Retinoblastoma 1	RB1	13q14.1-q14.2	180200	Retinoblastoma	180200	AD	Cleft palate, high forehead, prominent eyebrows, broad nasal bridge, bulbous tip of the nose, large mouth with thin upper lip, long philtrum, prominent earlobes
IS	Cyclin-dependent kinase inhibitor 1C	CDKN1C	11p15.5	600856	Beckwith-Wiedemann syndrome	130650	AD	Coarse facial features, linear earlobe creases, posterior helical indentations, macroglossia, midface hypoplasia
NP	Small nuclear ribonucleoprotein polypeptide N	SNRPN	15q12	182279	Prader-Willi syndrome	176270	AD	Narrow bitemporal head dimension, thin upper lip, down-turned corners of mouth, viscous saliva
NP	Werner's syndrome gene	WRN	8p12-p11.2	277700	Werner's syndrome	277700	AR	Wide face, prematurely aged face, beaked nose
NP	CREB binding protein	CREBBP	16p13.3	600140	Rubinstein-Taybi syndrome	180849	AD	Microcephaly, hypoplastic maxilla, beaked nose, slanted palpebral fissures, hypertelorism, short upper lip, pouting lower lip
SEC	Sonic hedgehog	SHH	7q36	600725	Holoprosencephaly, type 3	142945	AD	Cyclopia, ocular hypotelorism, proboscis, midface hypoplasia, single nostril, midline cleft upper lip, premaxillary agenesis
NP	Eyes absent 1 gene	EYA1	8q13.3	601653	Branchio-otorenal Syndrome	113650	AD	Branchial cleft fistulae; external, middle, and inner ear malformations; hearing loss
TM	Fibroblast growth factor receptor 1	FGFR1	8p11.2-p11.1	136350	Pfeiffer's syndrome	101600	AD	Mild craniosynostosis, flat facies, acrocephaly
TM	Fibroblast growth factor receptor 2	FGFR2	10q26	176943	Crouzon's craniofacial dysostosis	123500	AD	Craniosynostosis, parrot-beaked nose, short upper lip, hypoplastic maxilla, relative mandibular prognathism, shallow orbit
					Jackson-Weiss syndrome	123150	AD	Craniosynostosis, midfacial hypoplasia Craniosynostosis, brachysphenocephalic acrocephaly, flat facies, high narrow palate

(Continued)

TABLE 25-4 Selected Examples of Craniofacial-Oral-Dental Mendelian Genetic Diseases and Disorders (*Continued*)

Type	Gene Name	Gene Symbol	Chromosomal Location	Omim Number for Gene	Syndrome	OMIM Number for Syndrome	Inheritance	Description of Craniofacial-Oral-Dental Features*
					Apert's syndrome	101200	AD	
					Pfeiffer's syndrome	101600	AD	Mild craniosynostosis, flat facies, acrocephaly
					Beare-Stevenson cutis gyrata syndrome	123790	AD	Craniosynostosis, cloverleaf skull, cleft palate or uvula, craniofacial anomalies
TM	Fibroblast growth factor receptor 3	FGFR3	4p16.3	134934	Achondroplasia	100800	AD	Frontal bossing, megalencephaly, midfacial hypoplasia, low nasal bridge
					Hypochondroplasia	146000	AD	Normocephaly or occasional brachycephaly, mild frontal bossing
					Thanatophoric dysplasia	187600	AD	Megalencephaly, small foramen magnum, cloverleaf skull, depressed nasal bridge
					Crouzon's disease with acanthosis nigricans	134934	AD	Craniosynostosis
					Craniosynostosis, nonsyndromic	134934	AD	Craniosynostosis
TM	Insulin receptor	IR	19p13.2	147670	Leprechaunism, insulin-resistant diabetes mellitus with acanthosis nigricans	147670	AD	Bitemporal skull narrowing, supernumerary teeth, severe premature caries, prominent lower canines and upper incisors, thickened lips, prominent ears
TM	Parathyroid hormone receptor	PTHR	3p22-p21.1	168468	Metaphyseal chondrodysplasia, Murk Jansen type	156400	AD	Sclerosis of cranial base, wide cranial sutures, supraorbital hyperplasia, prominent supraorbital ridges, frontonasal hyperplasia, micrognathia, high arched palate, deafness
TM	RET oncogene	RET	10q11.2	164761	Neuromata, mucosal, with endocrine tumors	162300	AD	Neuromata of lips and tongue, conjunctival and nasal mucosa neuromas, diffuse lip hypertrophy, high arched palate, coarse facies
TM	Ectodermal dysplasia gene, anhidrotic	EDA	Xq12.2-q13.1	305100	Ectodermal dysplasia, anhidrotic	305100	X	Absent teeth, small pointed incisors, saddle nose, sparse hair, prominent forehead, prominent lips

	Gene name	Symbol	Locus	Gene OMIM	Syndrome	OMIM	Inheritance	Clinical features
TM	Patched	PTC	9q22.3	601309	Basal cell nevus syndrome (Gorlin's syndrome)	109400	AD	Macrocephaly, broad facies, frontal and biparietal bossing, mild mandibular prognathism, odontogenic keratocysts of jaws, misshapened and/or carious teeth, cleft lip and palate, ectopic calcification of falx cerebri
TF	Msh homeobox homolog 1	MSX1	4p16.1	142983	Tooth agenesis, familial	142983	AD	Hypodontia
TF	Msh homeobox homolog 2	MSX2	5q34-q35	123101	Craniosynostosis, type 2	123101	AD	Craniosynostosis, forehead retrusion, frontal bossing, turribrachycephaly, Kleeblattschaedel deformity (cloverleaf skull, trilobular skull anomaly)
TF	Microphthalmia-associated transcription factor	MITF	3p14.1-p12.3	156845	Waardenburg's syndrome, type IIA	193510	AD	Wide nasal bridge, short philtrum, cleft lip or palate, deafness
TF	GLI-Kruppel family member 3 oncogene	GLI3	7p13	165240	Greig's cephalopoly-syndactyly syndrome	175700	AD	Peculiar skull shape, expanded cranial vault, high forehead and bregma, frontal bossing, macrocephaly, hypertelorism
					Pallister-Hall syndrome	146510	AD	Short nose, flat nasal bridge, multiple buccal frenula, microglossia, micrognathia, cleft palate, malformed ears
TF	Paired box homeotic gene 3	PAX3	2q35	193500	Waardenburg's syndrome, type I	193500	AD	Wide nasal bridge, short philtrum, cleft lip or palate, occasional deafness, dystopia canthorum
					Waardenburg's syndrome, type III	148820	AD	Microcephaly, wide nasal bridge
					Craniofacial-deafness-hand syndrome	122880	AD	Flat facial profile, hypertelorism, hypoplastic nose and maxilla, slitlike nares, hearing loss
TF	Solurshin	RIEG	4q25-q26	601542	Rieger's syndrome, type I	180500	AD	Maxillary hypoplasia, mild prognathism, protruding lower lip, short philtrum, microdontia, hypodontia, cone-shaped teeth
TF	Core-binding factor, runt domain, alpha subunit 1	CBFA1	6p21	600211	Cleidocranial dysplasia	119600	AD	Brachycephaly, frontal and parietal bossing, wormian bones, persistent open anterior fontanel, midfacial hypoplasia, delayed eruption of deciduous and permanent teeth, supernumerary teeth

(Continued)

TABLE 25-4 Selected Examples of Craniofacial-Oral-Dental Mendelian Genetic Diseases and Disorders (*Continued*)

Type	Gene Name	Gene Symbol	Chromosomal Location	Omim Number for Gene	Syndrome	OMIM Number for Syndrome	Inheritance	Description of Craniofacial-Oral-Dental Features*
TF	Twist	TWIST	7p21	601622	Saethre-Chotzen syndrome	101400	AD	Craniosynostosis, acrocephaly, brachycephaly, flat facies, thin long pointed nose, cleft palate, cranial asymmetry, ptosis, malformed ears
UNK	DiGeorge's syndrome chromosome region	CATCH22	22q11	188400	DiGeorge's syndrome	188400	AD	Low-set ears, short ears, small mouth, submucous or overt palatal cleft, cleft lip, bulbous nose, square nasal tip, short philtrum, micrognathia
					Velocardiofacial syndrome	192430	AD	Pierre Robin syndrome, cleft palate, small open mouth, myopathic facies, retrognathia, prominent nose with squared-off nasal tip
UNK	Treacle	TCOF1	5q32-q33.1	154500	Treacher Collins mandibulofacial dysostosis	154500	AD	Malar hypoplasia, cleft palate, mandibular hypoplasia, macrostomia, malformed ears, sensorineural deafness, coloboma of lower eyelid

AD, autosomal dominant; AR, autosomal recessive; ECM, extracellular matrix protein; ENZ, enzyme; IS, intracellular signaling protein; NP, nuclear protein; SEC, secretory protein; TF, transcription factor; TM, transmembrane protein; UNK, unknown; X, X-linked; XD, X-linked dominant.

ªThe following description is a summary of the craniofacial-oral-dental features of the diseases and disorders. For detailed information regarding defects in other affected tissues and organs, refer to Online Mendelian Inheritance in Man (OMIM) at <http://www.ncbi.nlm.nih.gove/omim/>.

autoimmune disorders, hypertension, emphysema, diabetes, peptic ulcers, numerous mental diseases, and numerous birth defects, such as clefting, spina bifida, limb deformities, and congenital heart disease (Figure 25-5).[10,15] These conditions are caused by multiple genes with environmental factors and appear to cluster in families over multiple generations. Further, these examples are more prevalent in females versus males or males versus females, depending on the specific disease or disorder. For example, autoimmune diseases (e.g., Sjögren's syndrome, lupus) are more prevalent in females. These and other areas of interest have led to the emerging field of *gender biology* and *gender medicine*.

This section focuses on selected examples of complex human diseases and disorders that demonstrate multigene and multigene–environment interactions and that are of importance to oral health care providers in the everyday practice of dentistry.

The etiology, pathogenesis, manifestations, management, and treatment outcomes of *complex human diseases* or *multifactorial genetic disorders* represent a dynamic interplay between regulatory and structural genes and environmental and behavioral factors. The results from international, multicenter studies of cleft lip and/or palate suggest that many genes (e.g., *MSX1*, interferon regulatory factor 6 [*IRE6*]) and their expression are coupled with several environmental factors.[73] Protein–calorie malnutrition, vitamin deficiencies such as those related to folic acid and retinoic acid, and alcohol and tobacco consumption are a few of the environmental factors that relate to specific sets of genes essential for morphogenesis.[10,15,50–53]

The sex chromosomes were previously assumed to be the determinants of the sex of a child. Currently, it is believed that gender identity vis-à-vis sex chromosomes not only influences gender sexuality but also has profound influences on multigene and complex human disorders. Individual gene

FIGURE 25-5   Multiple mutations in multiple genes, genes often associated with cell cycle regulation during mitosis and/or tumor suppressor genes, are implicated in squamous cell carcinoma as presented in numerous head and neck cancers (i.e., advanced oral cancer as shown in this figure). (Courtesy Dr. Parish Sedghizadeh.)

expression, multiple gene–gene interactions, and multigene–environment interactions are fundamentally different between men and women.

Gene networks that regulate metabolism, drug absorption, and drug use differ between genders. Gene circuits in the immune and endocrine systems show gender variance. These emerging observations may also reflect physiologic effects influenced by genes encoded within the two X chromosomes of the female, albeit with only one of the two active in any given cell, versus the one X and one Y chromosome of the male. Multiple gene–gene and gene–environment interactions demonstrate significant differences in many aspects of growth, development, maturation, and senescence between genders. As increasing numbers of men and women live longer within industrial nations, data are emerging that demonstrate gender differences in the prevalence and incidence of many complex conditions, such as cardiovascular diseases, diabetes, periodontal diseases, osteoporosis, and pulmonary diseases. Risk factors such as diet, lack of exercise, and stress seem to have different influences on the incidence, onset, and progression of cardiovascular diseases between men and women. Examples involve relationships such as low birth weight premature infants and periodontal diseases,[76,77] osteoporosis, and cardiovascular diseases.[78–80] Women's diseases are no longer viewed as those limited to diseases of the reproductive system and related hormones (e.g., estrogen and progesterone).

The function of multiple genes encoded within both sex chromosomes and autosomes, the specific gender of the individual (i.e., XX versus XY), and multiple environmental influences serve as the foundation for increased susceptibility to different manifestations of diseases. Further, gender-based genetic differences are also implicated in pharmacogenomics and individual responses to the absorption, diffusion, use, and metabolism of many therapeutics, including analgesics for pain management.[81,82] Gender differences in analgesic absorption, diffusion, and binding to specific sets of receptors suggest strong gender-specific differences, and these are important in oral medicine and other disciplines. Collectively, these and many other scientific discoveries herald the new fields of *gender biology* and *gender medicine*. A primer in modern human genetics for health professionals must include *gender oral medicine*.

Respiratory Diseases

Asthma is an inflammatory disease of the small airways of the lung. Adult-onset asthma is more commonly seen in women than in men. In the United States, since 1990, mortality from chronic lower respiratory diseases remained relatively stable for men, whereas it increased for women. Genetic susceptibility and environmental factors weigh equally in contributing to this finding. Asthma affects one child in seven in some societies and approximately 15 million

individuals worldwide. Although identification of all asthma genes is incomplete, five asthma susceptibility genes have been identified by positional cloning: *ADAM33*, *PHF11*, *DPP10*, *GRPA*, and *SPINK5*. Approximately a third of the genetic predisposition to asthma has been uncovered. Genetic findings will lead to the development of new therapy and new treatments for individuals with severe asthma who do not respond to commonly available steroid inhalers.[83–86]

Cardiovascular Disease

Cardiovascular disease is one of the leading causes of death in the world. The 2002 World Health Report demonstrated a higher prevalence and incidence of cardiovascular diseases (rheumatic heart disease, hypertension, cerebrovascular disease, and inflammatory heart disease) in women than in men.[87] The risk for cardiovascular diseases also increases with age at a faster rate in women than in men. Genetic and environmental factors and biologic and anatomic differences have been considered as possible contributory factors to these differences. A genetic predisposition to cardiovascular disease results from gene mutations that alter the biologic function expressed by the original gene(s) and increase an individual's risk for cardiovascular diseases. A region on chromosome 13 in Caucasians and on chromosome 19 in African–Americans has been linked to hypertension and stroke.[88–90]

In addition to gene mutations that directly increase susceptibility, there are multiple genes that indirectly increase the risk of cardiovascular diseases. These indirect predispositions are in the form of genes that are related to unhealthy behaviors, such as metabolic pathways related to tobacco use and alcohol consumption. For example, the complications associated with the angiotensin-converting enzyme (*ACE*) gene in ischemic cerebrovascular disease were investigated in smoking and nonsmoking patients. The *ACE* gene mutation was a risk factor only in individuals who smoked but did not appear to behave as a risk factor in those individuals who did not smoke.[91,92]

Endocrine Disease

Oral diseases and disorders are associated with systemic diseases.[93–95] Oral infections are closely linked with diabetes, and management of diabetes is related to the management of oral infections.[78–80,93–95] At present, there is evidence that more than 20 regions of the genome may be involved in susceptibility to type 1 diabetes.[96] The genes in the human leukocyte antigen region of chromosome 6 are currently considered to have the highest influence on susceptibility to type 1 diabetes. To date, more than 50 genes have been studied for their possible association with type 2 diabetes in different populations worldwide. The most noteworthy genes are *PPAR8*, *ABCC8*, *KCNJ11*, and *CALPN10*.[96–98] For more information on diabetes, see Chapter 21, "Diabetes Mellitus and Endocrine Diseases."

The prevalence of type II diabetes is significantly higher in populations within Mexico and Latin America. A newly discovered gene sequence mutation or variant in *SLC16A11* revealed disease risk alleles in Mexican and other Latin American populations using a panel of SNPs (analysis of 9.2 million SNPs) and no risk alleles in other populations.[99] Currently, the variant gene for *SLC16A11* produces a mRNA that is localized to hepatocytes and the protein product functions in lipid metabolism causing an increase of intracellular triacylglycerol levels leading to the type II diabetic phenotype.[99]

Autoimmune Diseases

Women are 2.7 times more likely than men to acquire an autoimmune disease. Women have enhanced immune systems compared with men, which increases women's resistance to many types of infection but also makes them more susceptible to autoimmune diseases.[100–102] Men appear to have higher levels of natural killer cell activity than women. This difference in bioactivity may be associated with reduced levels of autoimmune disease in men. The plasma activity level of phospholipase A_2, a key enzyme in causing chronic inflammatory diseases, is significantly higher in Caucasian and Asian Indian women than in their male counterparts.[103–105] A molecule involved in reducing the inflammatory response, interleukin (IL) 1 receptor II, is present in higher concentrations in blood fractions from men than from women.[100–105]

The most striking sex differences for complex human diseases are observed in Sjögren's syndrome, lupus, autoimmune thyroid disease (Hashimoto's thyroiditis, Graves' disease), and scleroderma; these represent a spectrum of diseases in which the patient population is greater than 80% female. In rheumatoid arthritis, multiple sclerosis, and myasthenia gravis, the sex distribution is 60%–75% female.[100–102,106]

In addition to increased susceptibility to autoimmune disorders, women experience certain viral infectious conditions that affect their immune system disproportionately when compared with men.[106,107] For example, human immunodeficiency virus (HIV) infection that was more prevalent in men in the early 1980s is currently affecting women at an alarming rate.[106] Women account for almost 50% of the 40 million people living with HIV-1 worldwide, with an even higher percentage in developing countries. In the United States, the estimated number of acquired immune deficiency syndrome (AIDS) cases increased 15% among women and only 1% among men from 1999 to 2003.[106] The major burden of the disease was in young women, particularly African–American and Hispanic women.[106]

Heterosexual transmission is now the most commonly reported mode of HIV transmission in women. Women's increased susceptibility has been linked to physiologic factors such as hormonal changes, vaginal microbial ecology, and a higher prevalence of sexually transmitted diseases. These

factors, in combination with other factors, such as gender disparities, poverty, cultural and sexual norms, lack of education, and sexual and domestic violence, make women vulnerable to this and other viral infections.[107,108]

Women develop signs and symptoms of AIDS, including oral manifestations, at a lower HIV viral load than men.[109] They also seem to benefit from the initiation of antiretroviral therapy at a lower HIV viral load than men.

Cancer

The role of genetics in cancer is widely recognized. The prevalence of cancer is projected to increase 50% worldwide within the next 20 years. According to the World Cancer Report, 10 million new cancer cases are diagnosed annually. Cancer contributes to 12.6% of the global mortality rate.[110] All cancers have genetic determinants. Cancers are usually caused by a sequence of multiple genetic mutations, and this is highlighted by neoplastic diseases of the head and neck.[111–116] There are three categories of cancer: inherited, familial, or sporadic. *Inherited* cases of a dominant type are often caused by direct mutations of genes that are passed successively from parents to offspring throughout generations. These are often regulatory genes required for the control of the cell cycle and cell division, as well as genes that regulate tumor suppression. *Familial* cases involve mutations of multiple susceptibility genes that increase an individual's risk for cancer (so-called multigene–gene and gene–environment interactions). *Sporadic* cancer cases are those in which an individual randomly develops cancer in the absence of any familial pattern, such as chronic exposure to carcinogenic substances.

Three of the most studied cancers with susceptibility genes are breast, colorectal, and prostate cancers. *BRCA1*, *BRCA2*, *TP53* (breast cancer), *hMLH1*, *hMLH2*, *IGF2* (colorectal), *BRCA1*, *BRCA2*, *hMLH1*, *hMLH2* (ovarian), *CYP1A1* (lung), and *HPC2* (prostate) are among the genes of interest.[117–119] Further, molecular studies of development has revealed that gene products associated with embryonic pattern formation and morphogenesis in most organs can also be causative to the process of neoplasia. One example is found associated with Wnt signaling that may lead to cancer. Severe permanent tooth agenesis (oligodontia) and colorectal neoplasia are found to segregate with dominant inheritance. Both oligodontia and predisposition to cancer are caused by a nonsense mutation, Arg656Stop, in the Wnt-signaling regulator *AXIN*$_2$.[120]

Tumor suppressor genes are involved in regulating the cell cycle and activate cell apoptosis or cell death.[111,112] The gene mutation results in the production of altered protein that is no longer capable of initiating apoptosis. Although great progress is being made in the discovery of genetic susceptibility to cancer, little attention had been paid to gender and sex disparities in cancer. For most common cancers, men seem to have a higher incidence than women. Men and women are predisposed to different anatomic, biochemical,

and genetic features, and these factors may play a role in the susceptibility to and onset of cancer. Men and women respond differently to stress-inducing environments, which may make them more susceptible to certain behaviors (e.g., tobacco use and alcohol consumption) that might enhance their susceptibility to neoplastic diseases, such as oral and pharyngeal cancers (see Figure 25-5).[112–116]

Neurodegenerative Diseases and Mental Diseases

Research in neurodegenerative and mental diseases and disorders is focusing on tracing positions of gene mutations coupled with environmental factors that lead to the causes of these profound chronic disease conditions. Alzheimer's disease constitutes about two-thirds of all cases of dementia. Alzheimer's disease is a progressive neurologic disease that results in irreversible loss of neurons, particularly in the cortex and hippocampus.[121,122] There are missense mutations in three genes in families with early-onset autosomal dominant Alzheimer's disease: beta-amyloid on chromosome 21, presenelin 1 on chromosome 14, and presenilin 2 located on chromosome 1. The *APOE* gene on chromosome 19 has been linked to late-onset Alzheimer's disease, which is the most common form of the disease. This gene has three different forms: *APOE2*, *APOE3*, and *APOE4*. *APOE3* is the most common form in the general population. *APOE4* occurs in 40% of all late-onset Alzheimer's disease patients but is not limited to those whose families have a history of Alzheimer's disease. Patients with no known family history (sporadic Alzheimer's disease) are also more likely to have an *APOE4* gene.

Parkinson's disease is the second most common neurodegenerative disorder after Alzheimer's disease. The exact cause of Parkinson's disease remains unknown, but genetic factors have been identified as potential contributing factors in the onset and severity of the disease.[123] Studies with monozygotic twins show a very high level of concordance in the early-onset (before age 50 years) type of Parkinson's disease. The early onset version appears to be autosomal dominant in families of the Mediterranean and German regions and has several missense mutations in the gene coding for α-synuclein located on chromosome 4q21.

Genetics studies are also contributing toward enhanced understanding of mental diseases and disorders. For example, men are more likely to express depression or severe unhappiness through an *externalizing pathway* of physical behaviors, including drinking, drug abuse, and violence, whereas women are more likely to *internalize*, leading to depression and anorexia. This sex difference is more prominent during puberty. Epidemiologic studies have shown that in families of women with bulimia, the men often have alcoholism and other addictions. Men may cope with disasters by drinking. In some cultures, it is not acceptable for women to drink, and they may cope with disasters by developing anxiety disorder and depression. More than half of all female suicides worldwide take place in China.[117–119,124]

It has been have proposed that sex hormones are responsible for a higher incidence of mental disorders in women.[125] A comprehensive evaluation of women's mental health is currently ongoing in 28 countries as part of an epidemiologic study sponsored by the World Health Organization.[87] New emerging information by genetic epidemiologists reveals a number of structural and functional differences between men's and women's brains. Men's brains are more lateralized, whereas women's brains are not. This makes it more likely for women than for men to overcome language deficits resulting from strokes in the left hemisphere, where language is centered. This structural difference has also been linked to the lower likelihood of childhood developmental and mental disorders in girls than in boys. Sex differences are also present in substance abuse. Female substance abusers are more likely to report psychiatric symptoms before the onset of substance abuse, whereas male substance abusers are more likely to report depression and other psychiatric symptoms after chronic substance abuse. Recently, bioimaging studies have provided information about the neural processes underlying differences in the manifestations of substance abuse.[118,119,124]

GENETICS, GENDER, AND TREATMENT RESPONSES (PHARMACOGENOMICS)

Human genetic variance, the 200–300 million base differences (SNPs) between human genomes, can explain why all patient responses to therapy are not always the same.[12,81,82] For example, despite appropriate management of hypertension and accomplishment of target blood pressure, some hypertensive patients still develop myocardial infarction or stroke, and, despite appropriate management of these conditions, some patients survive and some do not. Similarly, not all patients respond to behavioral modifications in the same way. It is known that regular exercise alleviates hypertension in some patients but not all. In summary, human genetic variations contribute to these differences. Recent investigations have identified five sources of genetic variation between multiple families receiving medications for the management of cardiovascular diseases.[126]

The birth and evolution of pharmacogenetics and pharmacogenomics have contributed significantly to a better understanding of individual variations in therapeutics. The genetics of pharmacokinetics and pharmacodynamics and physiologic regulation that is influenced by ethnicity, age, and gender all affect an individual's discrete response to a drug therapy. Men and women may respond to the same drug differently. For example, intake of certain antibiotics, antihistamines, antiarrhythmics, and antipsychotics places women at a higher risk than men for drug-induced arrhythmias.[127,128]

The differences in responses to drugs have been missed in the past because women were not always included in clinical trials; if they were, the data were not broken down

by sex.[129,130] Recently, based on a long-term clinical trial, it was shown that aspirin, which protects men against heart attack but not stroke, has exactly the opposite effect in women. Evidence shows that men and women differ in the activity of liver enzymes that metabolize drugs. Women are, on average, smaller in size and have a higher percentage of body fat. Therefore, women may absorb and/or excrete drugs more slowly or may retain fat-soluble drugs longer than men. Furthermore, women were reported to have a significantly higher likelihood than men of being admitted to a hospital as a direct consequence of adverse drug reactions.[131]

Traditionally, health care providers have used a combination of history, clinical evaluation, and diagnostic tests as a basis for their diagnoses and patient management. Often multiple patients who presented with similar histories and had similar clinical and laboratory findings received the same treatment. Today, with the availability of different genetic tests, the level of risk for or susceptibility to different diseases can be identified. This will change the practice of medicine and dentistry in the future because risk factors will be the driving force for treatment selection, not merely the clinical signs and symptoms. Patients with clinical risk factors will be treated differently even though the presenting signs and symptoms are the same.

Advances in genomics and molecular tests for assessments and diagnoses, as well as pharmacogenomics and pharmacogenetics, will change the future of the practice of medicine and dentistry.[8,10,14,15,51–55,61–73,76–81,94,132–134] Efforts should be made to incorporate *genomic* information in continuing education programs for current practitioners and in medical and dental school curriculums for future health care providers.[111,135,136] The interpretation of genetic test results, the provision of appropriate counseling, and the solutions to potential ethical and confidentiality-related issues need adequate training and expertise involving all health care providers.

PHENOTYPIC (PHENOMICS) AND GENETIC HETEROGENEITY (GENOMICS) IN DISEASES AND DISORDERS

Human disorders are heterogeneous as the result of complex interactions between multiple genetic loci and environmental factors during the life span resulting in phenotype. This interpretation is true for human diseases that segregate as simple Mendelian traits as well as for non-Mendelian multifactorial conditions. There are many examples of single gene disorders in which identical mutations result in widely different clinical phenotypes, referred to as variable expressivity. For example, monozygotic twins, two siblings with identical genetic inheritance, show phenotypic heterogeneity with age and varying environments.[137,138] This

nonintuitive realization has become the rapidly advancing field of *epigenetics*, which refers to the posttranslational alteration of gene function through methylation, acetylation, sulfation, or phosphorylation of histone and non-histone chromosomal proteins without altering the nucleic acid sequence of DNA.

Single-gene mutations can have pleiotropic or multiple effects. For example, patients who present with xeroderma pigmentosum are unusually sensitive to sunlight, and patients with α_1-antitrypsin deficiency often have a predisposition to developing emphysema and are more sensitive to tobacco smoke.[139] Single-gene mutations may also be associated with serious and inappropriate responses to certain therapeutics. Mendelian and non-Mendelian multifactorial diseases may also present clinical complexities with drug use, absorption, and metabolism.[12,15,48,49,81,82] For example, glucose-6-phosphate dehydrogenase deficiency is inherited as an X-linked recessive disorder and can induce hemolytic anemia in response to various drug therapies.[139] Mendelian and non-Mendelian multifactorial diseases and pharmacogenomics are remarkably linked to genetic variations in drug metabolism in a growing array of patients. Distinct alleles of the cytochrome P-450 network of genes that function in drug metabolism have an impact on drug efficacy and toxicity.[81,82,140]

In addition to genetic heterogeneity resulting from gene–gene and gene–environment interactions, other factors serve to enhance or increase the phenotypic heterogeneity of human disorders, such as *penetrance*. The varying degree of severity and incomplete penetrance of gene expression need to be appreciated by all clinicians. Many autosomal dominant diseases often display varying severity owing to variable expressivity and incomplete penetrance. Examples of disorders with reduced penetrance are tuberous sclerosis and Marfan's syndrome.

It is important to recognize the phenomenon of locus heterogeneity wherein mutations in different genes can cause remarkably similar clinical phenotypes. One classic example is that forms of hemophilia can be caused by genetic mutations in either the gene for factor VIII (so-called classic hemophilia) or the gene for factor IX (Christmas disease). Both of these two genes are located on the X chromosome, and both of these diseases or conditions are inherited as X-linked recessive disorders. Similarly, the enamel disorder AI can be caused by mutations in *AMELX*, *ENAM*, or *MMP20*.[63–71] In addition, different point mutations in the same gene can result in very different clinical phenotypes. One example is the gene fibroblast growth factor receptor 2 (*FGFR2*). Different point mutations in *FGFR2* result in very different craniofacial dysmorphogenesis syndromes with craniosynostosis.[51–53,135,138] Similarly, severe mutations within the dystrophin gene, such as deletion of large portions of the gene, result in Duchenne muscular dystrophy, whereas milder mutations, such as certain point mutations, result in the milder Becker dystrophy.

THE "OMICS" REVOLUTION AND A SYSTEMS BIOLOGY APPROACH TO DISEASE

Within the fields of molecular biology and genetics, the *genome* is the entirety of an organism's hereditary information. It is encoded either in DNA or, for some viruses, in RNA. The genome includes both genes, pseudogenes, and the noncoding nucleic acid sequences of DNA and RNA. The literature describes the human genome as inclusive or highlights the mitochondrial genome (mitDNA) in juxtaposition to the nuclear genome (nuclear DNA + mitochondrial DNA = human genome). More recently, the human genome term also implies the inclusion of the total bacterial genomes that coexist with the human condition (*microbiome*).

The advent of genomics and other *–omics* technologies have begun to revolutionize biomedical research and clinical practice. The term *genomic medicine* or *personalized medicine* that refers to a clinical decision guided by knowledge of an individual's DNA sequence is ever expanding in scope and now includes information from derivatives of genomes that include RNAs (*transcriptome*), proteins (*proteome*), and metabolites (*metabolome*). The progress in part has been the result of inexpensive high-throughput sequencing and array-based solutions that address genomic complexity. From a clinical perspective, a patient's nuclear and mitochondrial genome can be completed within 24 hours at a cost less than $5000 using leading-edge technology.[4–6,9] The curious reader is encouraged to study Keith Davies provocative book *The $1,000 Genome: The Revolution in DNA Sequencing and the New Era of Personalized Medicine*. Soon to be added to this compendium will be a detailed profile of an individual's *epigenome*, that reflects modifications that occur in the genome that do not change the sequence of the bases in the DNA an individual is born with but that can change the DNA conformation and as a consequence, change the expression of genes. Exact descriptions of a person's every physical and behavioral characteristic constitutes his *phenome*. Compilation of phenome data on humans is underway but is much more challenging than it is for model organisms such as the mice, rats or yeast as the same symptoms may have different descriptors such as *caries* or *tooth decay* or *cavity* which could make construction of databases akin to those with other -omic data more difficult. Astute clinical observations and annotations coupled with standardized electronic patient records that fully integrate a patient's total health information are imperative to advance phenomics.

The advent of next-generation (or third-generation) sequencing (NGS) has greatly enhanced the ability to examine the genomes and transcriptomes of humans and other species at a pace and cost that was not possible with earlier sequencing technologies. These rapid advances were made possible through federal funding from the HGP (1988–present) as well as industry and venture capital investments

that has resulted in the development, manufacture and marketing of life science tools and integrated systems for large-scale analysis of human, viral, bacterial and yeast genomes.[4–6,9] DNA sequencing using the NGS approach has focused on either only exons of genes (*exome*) representing 1%–2% of the genome or the entire genome (*whole genome*).[6] The choices are largely driven by cost and the extent of data storage required with costs for the latter often surpassing the cost of the actual sequencing! Exome sequencing has had a great impact on rare Mendelian diseases, particularly autosomal recessive ones, by enabling the identification of a gene underlying a disease by identification of candidate mutations by the analysis of a single affected individual. For instance, at least four novel genes associated with recessive amelogenesis imperfecta have been identified using exome sequencing of single patients within unrelated families.

The 1000 Genomes Project was launched in 2008, to establish a detailed catalog of human genetic variation by sequencing the whole genomes of at least 1000 anonymous participants from a number of different ethnic groups. Although Mendelian diseases are considered rare, genetic mutations are estimated to occur at a rate of 40–82 per 1000 live births. This catalog has been an invaluable resource for GWA studies (Figure 25-1) on common diseases as knowledge about the frequency and types of sequence variants and their order on chromosomes in different populations is necessary for the success of these studies. The data have also been instructive on tracing the history of world populations by examination of the nature and pattern of variants amongst different world populations.

Knowledge of DNA variants however, is not sufficient to understand disease mechanism ultimately needed for design of both prevention as well as treatment regimens. The approach for achieving these goals is a systems-based approach which is a type of analysis that examines changes across the expanse of the entire genome at the DNA, RNA, and protein level and the interactions between these elements that result in changes in biochemical molecules (e.g., Cytokines) and clinical outcomes (e.g., Hypertension). Systems Biology encompasses studies that aim to define the interactions between molecules within a cell on a large-scale (*interactome*) as well as those that relate genomic, transcriptomic, and metabolomic data to each other and to phenome data at the level of the whole organism such as yeast or a whole organ level such as liver (Figure 25-1).

SALIVARY DIAGNOSTICS

Saliva as a screening and/or diagnostic tool has received significant visibility in recent years due to the advancements in biomedical, translational, and clinical sciences. Salivary biomarkers have a promising future and may eventually be used routinely for risk assessment, disease prevention, and identification of systemic and oral diseases in clinical settings.[145–148] Several laboratory-based tests are currently available to assess the presence of microbial components, viruses, drugs and hormones in saliva.[149] In addition, scientists are utilizing salivary biomarkers to assess genetic risk factors for the development of common oral diseases such as dental caries, periodontal disease, and oral cancer.[150,151]

The term salivaomics was introduced to the literature in 2008 as a result of the rapid knowledge that was emerging based on the study of salivary biologic molecules.[152] Since then terms such as salivary proteome, transcriptome, microRNA (mi RNA), metabolome, and microbiome have been added to the nomenclature associated with salivary-related research. The metabolome is the complete set of small molecules found in a biologic sample. Salivary transcriptome consists of 180 mRNAs. The core salivary proteome contains 1,166 proteins, as determined by the collective efforts of three scientific groups with support from the National Institute of Dental and Craniofacial Research.[145,152,154] Scientists are utilizing salivary metabolome for early detection of oral and systemic diseases. Potential variation in the salivary microbiome has recently been used as a vehicle for detection of early resectable pancreatic cancer. Two microbial markers (*Neisseria elongate* and *Streptococcus mitis*) have been successfully used in one study to identify those individuals with early-stage resectable pancreatic cancer from study participants without cancer.[155] Scientists are developing informatics and statistical models to determine the most discriminatory combination of salivary biomarkers for specific diseases.

Abundant salivaomics data has been generated utilizing evolving high-throughput technologies in recent years.[156–159] In order to facilitate access to information, compare data sets from different studies, and support salivary diagnostic research, the Salivaomics Knowledge Base (SKB) data management (*UCLA WEB SITE ACCESSED 2013*) system and Web resource was developed by a group of scientists at the University of California at Los Angeles (UCLA).[160] Ontologies are controlled structured vocabularies designed to provide consensus-based means to ensure consistent descriptions of data by scientists working in different domains. The Saliva Ontology (SALO) is used in an attempt to facilitate salivaomics data retrieval and integration across multiple fields of research together with data analysis and data mining. The SKB and the SALO initiatives are devoted to the translation of saliva data into saliva knowledge, and to the task of enabling saliva data and knowledge sharing in the broadest possible communities of researchers. The SALO is being created through cross-disciplinary interaction among saliva experts, protein experts, diagnosticians, and ontologists.[161]

In 2004, the US Food and Drug Administration (FDA) approved an over-the-counter saliva-based test for HIV antibody screening that can be used in the comfort of one's home.[162] There is growing interest in the development of saliva-based rapid tests for screening and/or diagnosis of hepatitis C virus (HCV) and human papilloma virus (HPV) infection. However, no test as yet has received FDA approval.[163]

Several other saliva-based laboratory tests are commercially available for the detection of periodontal pathogens and genetic risks of developing periodontal diseases. Infection, inflammation and tissue destruction are cause and consequences of periodontal diseases. Biological phenotypes could be of value to determine disease progression and response to therapy at the individual patient level. Emerging data support the predictive value of genetic microbial and protein saliva-based byproducts in gingival inflammation and periodontal bone destruction. Salivary biomarkers of periodontal diseases in the presence of comorbidities such as rheumatoid arthritis,[164,165] as well as patients' responses to therapies such as periodontal surgery combined with matrix metalloproteinase (MMP) inhibition or to oral tissue engineering, have also been topics of investigations by scientists in recent years.[166–168]

Furthermore, wound repair biomarkers have been utilized to determine the tissue-healing response of patients undergoing intraoral soft tissue transplant procedures.[169] Matrix metalloproteinase-8 (MMP-8) is an enzyme for tissue destruction. In 2010, a commercial test with reasonable sensitivity and specificity became available for the identification of active or stable periodontal lesions in smokers and nonsmokers.[170] More recent scientific approaches have focused on the utilization of multiple salivary biomarkers including MMP-8, microbial factors, viruses, and pro-inflammatory cytokines such as IL-1B[171] or IL-17 from a single saliva sample to predict diseases.[172–174] It is estimated that variations in more than 70 genes are associated with periodontitis.[175] In assessing risks for the development and progression of periodontal disease, a patient's DNA can be captured through a saliva-based oral rinse sample and analyzed for the genotypic status of IL-1. This test can be further complemented by other laboratory tests that focus on the microbial biofilm in the patient's dental plaque. Collectively these sources of information can enhance the prediction of risk assessment based on the patient's genetic make-up modulated by the microbial infection.

A new saliva test for caries risk assessment was introduced by scientists in recent years.[176] The test integrates a variety of host factors to predict individual risk levels that are tooth-group specific. These various host factors correlate with caries history, decayed and filled teeth (DFT), or decayed and filled surfaces (DFS) in young adults. The test is based on the pattern of genetically determined oligosaccharides present on salivary glycoproteins. The mechanism behind the test is believed to be centered on the specific oligosaccharides that either facilitate bacterial attachment and colonization at the surface of teeth or protect against colonization by promoting agglutination and removal of free bacteria. It is the ratio of the two classes of oligosaccharides that is very strongly correlated with the numerical range of DFS or DFT observed in a young adult population. Sensitive DNA-based methods such as DNA–DNA hybridization, genomic finger printing, 16S rRNA gene cloning and sequencing, or Terminal Restriction Fragment Length Polymorphism are also being investigated in identification and classification of dental caries microbiota.[177] The real-time quantitative polymerase chain reaction technique is believed to be more sensitive for enumeration of cariogenic salivary pathogens as compared to the traditional culture-based methods.[178]

Salivary biomarker panel inclusive of thioredoxin, IL-8, SAT, ODZ, and IL-1b has been used successfully in the detection of oral cancer. Breast cancer, Alzheimer disease, myocardial necrosis, and stomach cancer are other areas of interest and investigation.[179,180]

PROSPECTUS: HUMAN GENETICS, PHENOMICS, GENOMICS, MICROBIOME, AND ORAL MEDICINE

It was 61 years ago (1953) when Watson and Crick published a one-page presentation of the structure of DNA with biological implications. It was 39 years ago when the international recombinant DNA conference was held at Asilomar, California, that established regulations for biotechnology using viral, bacterial, plant, animal, and human DNA. It was 26 years ago when the US Congress authorized and funded the National Institutes of Health to initiate the ambitious HGP. It was 14 years ago when a 95% draft of the complete human genome was published (under budget and under time) and 10 years ago the HGP was completed revealing the functional and pseudogenes as well as noncoding regions of the human genome. Last year the FDA authorized the first high throughput DNA and RNA sequencer that can complete a patient's genome within 24 hours at a cost less than $5000.

It is readily apparent that genetics will continue to dominate dental and medical education, health care, industry. and continuing health professional education in the 21st century. Human and microbial genomics, proteomics, and metabolomics, coupled with pharmacogenomics, will continue to shape the future in the health professions.[4–6,10–22,132–137,148,151,152] Mendelian and non-Mendelian patterns of inheritance are clinically important. Genetic screening assays for individual and multiple genes as found in multifactorial conditions will become more specific, more sensitive, faster, and cheaper.

High-throughput phenotype testing on single cells is increasingly important for assessment of viruses, bacteria, yeast, and animal as well as human cells. Phenotype microarrays now make it possible to quantitatively identify and measure many thousands of cellular phenotypes all at the same time. Phenotype microarrays enable simultaneous assays of the phenotype of living cells in response to environmental challenges. This and many other revolutionary advances in molecular assays further enhance the astute clinician with clinical diagnostics and prognostics to improve clinical outcomes.

Our future is even brighter when we consider the emerging opportunities to be found within phenomics, which expands the role of astute clinical observations and evaluations coordinated with genomics. A number of currently employed innovations in imaging and bioassays have already advanced the precision of comprehensive health care. Genotype to phenotype correlations are advancing. In order to realize *personalized health care* that includes mental, vision, and oral health care, significant progress will be made from analysis of large data bases that align and further coordinate genotype with phenotype. In this sense, clinicians are crucial to increase the study power while decreasing observational or measurement errors. Phenomics is the systematic measurement and analysis of qualitative and quantitative traits, including physical observations, vital signs, blood, urine and saliva chemistries, and biochemical real-time metabolic data that collectively define phenotype.[181–183]

Diagnosis will encompass the cardinal features of the all-inclusive clinical phenotype, differential diagnosis, and sensitive and specific tests for the detection of one or more biomarkers (e.g., genes and/or gene products). Risk assessment will increasingly be used, coupled with patient, family and community patterns of disease, environmental insults, and carrier detection within individuals and populations. Molecular epidemiology will emerge as increasingly useful for individuals, families, and populations to define people at risk for disease as well as for the precise diagnosis of diseases and disorders. Moreover, increasing evidence indicates that the human microbiome plays a major role in health ranging from obesity, infectious oral diseases to a patient's susceptibility to cancer or diabetes. Genetic counseling will encompass the human genome, human microbiome as well as the psychosocial management issues. Legal, regulatory, and ethical issues will continue to surface related to genetic screening, privacy and confidentiality, disclosure of unexpected and unwanted findings, and obligations to identify and communicate difficult issues.

The field of genetics should no longer be limited to syndromes of the head and neck as a chapter in a textbook. It should be considered as the essential primer for all aspects of health care and should become encoded within medicine, dentistry, pharmacy, nursing, and the allied health professions and beyond. Oral medicine specialists are instrumental in closing the gap between medicine and dentistry. These specialists serve as a resource to the medical and dental communities in the detection, prevention, and management of conditions that affect systemic and oral health. Greater interaction is necessary among all health professionals in planning systemic and oral care for patients.

Molecular microbial studies led to the emergence of the concept of biofilm formation on tooth surfaces, mucosal surfaces, stents, catheters, medical and dental implants, and even water lines.[141] Biofilms contain microbial species within a three-dimensional structure. Immunology and targeted pharmaceutical developments must address antimicrobial resistance within biofilms, coupled with an aging population in the industrial nations of the world.[140] DNA microarray technology is useful for the purposes of disease diagnosis and drug development.[142] The emerging information on molecular epidemiology will facilitate the assessment of an individual's risk profile based on the host's susceptibility genotype and risk behaviors (smoking, drinking, diet), as well as exposure to common pathogens (bacterial, viral, fungal) and other environmental factors (heavy metals, allergens).

An individual's response to foreign chemicals (xenobiotics) is genetically controlled. This explains why some individuals are at risk for oral conditions such as oral cancer, dental caries, periodontal disease, and soft tissue disorders. Understanding the molecular pathogenesis of squamous cell carcinoma will provide the basis for improved risk assessment, as well as diagnostic and therapeutic approaches.[142,143] Infectious pathogens and dietary factors influence dental caries and periodontal diseases, but genetic variance also contributes to host susceptibility in different populations. Tooth morphology, tooth structure, salivary composition, response to microbial pathogens, and response to fluoride and other anti-caries (tooth decay) products, as well as susceptibility to head and neck cancers and cardiovascular, respiratory, and autoimmune disorders, are all genetic in nature. Recent studies analyzing the genetic and epigenetic changes in preneoplastic head and neck squamous cell carcinoma (HNSCC) indicated that 46% of HNSCC tumors demonstrate mutations in mtDNA.[144]

Except for monozygotic twins, each person's genome is unique. All dentists and physicians need to understand the concept of genetic variability, its interactions with the environment, and its implications for patient and population health care. In the near future, genomics will become a fundamental tool for health professionals to enhance prevention, diagnostics and therapeutics. As nucleic acid sequencing technology becomes *faster, smarter, cheaper* and becomes fully integrated into health care, can we ensure the availability of genetic counseling to guide patients through complex decision making? What role will oral health professionals play in genomics and counseling? How can we be educated and trained to address the potential reality that whole genome sequencing may produce unexpected findings with as yet unknown clinical significance?

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Web-based Resources

The reader is encouraged to access the expanding human, animal, plant, and microbial genomic databases using the Internet: American Society of Human Genetics http://www.ashg.org/genetics/ashg/ashgmenu.htm

Arachaea and *Eubacteria* microbial genome projects sorted by taxonomic groups, present in GenBank, annotation in progress and "in progress" http://www.ncbi.nlm.nih.gov/Taxonomy/

Ethical, legal, and social implications of genome research on privacy/confidentiality http://www.ornl.gov/hgmis/elsi/elsi.html

GenBank, current status of human, animal, plant, and microbial genomics http://www.ncbi.nlm.nih.gov/Genbank/index.html

Gene expression in teeth http://bite-it.helsinki.fi/

Human Genome Map compiled by the National Center for Biotechnology Information http://www.ncbi.nlm.nih.gov/gen-emap/

Human Microbiome Project http://commonfund.nih.gov/hmp/

Genome Programs of the US Department of Energy Office of Science http://www.doegenomes.org/

National Coalition of Health Professional Education in Genetics http://www.nchpeg.org

OMIM, Online Mendelian Inheritance in Man http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM

Agency for Healthcare Research and Quality http://www.ahrq.gov

Centers for Disease Control and Prevention http://www.cdc.gov

GeneTests-GeneClinics http://www.genetests.org/

National Human Genome Research Institute http://www.nhgri.nih.gov

National Institute of Dental and Craniofacial Research http://www.nidr.nih.gov

National Institutes of Health http://health.nih.gov

National Center for Biotechnology Information projects with Cancer Genome Anatomy Project data http://www.ncbi.nlm.nih.gov/ncicgap/

The International Human Microbiome Consortium http://www.human-microbiome.org/

For the full reference list, please refer to the accompanying CD ROM.

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