

# The Genome Projects: Implications for Dental Practice and Education

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*Abstract:* Information from the Human Genome Project (HGP) and the integration of information from related areas of study and technology will dramatically change health care for the craniofacial complex. Approaches to risk assessment and diagnosis, prevention, early intervention, and management of craniofacial conditions are and will continue to evolve through the application of this new knowledge. While this information will advance our health care abilities, it is clear that the dental profession will face challenges regarding the acquisition, application, transfer, and effective and efficient use of this knowledge with regards to dental research, dental education, and clinical practice. Unraveling the human genomic sequence now allows accurate diagnosis of numerous craniofacial conditions. However, the greatest oral disease burden results from dental caries and periodontal disease that are complex disorders having both hereditary and environmental factors determining disease risk, progression, and course. Disease risk assessment, prevention, and therapy, based on knowledge from the HGP, will likely vary markedly for the different complex conditions affecting the head and neck. Integration of information from the human genome, comparative and microbial genomics, proteomics, bioinformatics, and related technologies will provide the basis for proactive prevention and intervention and novel and more efficient treatment approaches. Oral health care practitioners will increasingly require knowledge of human genetics and the application of new molecular-based diagnostic and therapeutic technologies.

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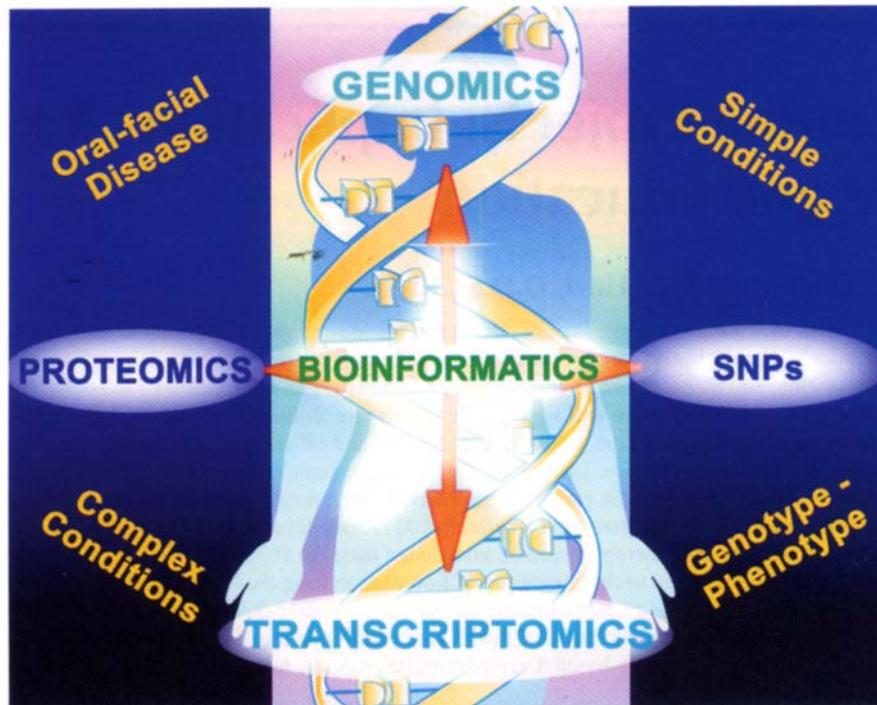
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**R**ough draft publication of the human genome in February 2001 was heralded as the greatest scientific achievement of humankind.<sup>1</sup> While it can be argued whether deciphering the human genome is our greatest scientific achievement, there is no question that knowledge of the human and other genomes will have broad and diverse ramifications for health care and society at large. As we move into the post-genomics era and information from the genome projects is put to practical use, it will become increasingly important for dental practitioners to understand human genetics and the rapidly changing methods for selectively and appropriately applying this newly acquired and vast amount of knowledge. The purpose of this paper is to review how current information is being amassed from the Human Genome Project (HGP) and emerging technologies, and provide a framework for the likely future directions and challenges that will be faced by the oral health profession.

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## Integration of Information from Genome Projects and Related Fields

The HGP goals of identifying all the genes coded in human DNA and sequencing the entire 3 billion base pair human genomic code are nearing completion and, even more, are providing massive amounts of information. This explosion of information coupled with the rapid development of molecular biological and computational technologies is fueling a number of related fields of study and research. Integration of the information derived from these diverse yet related fields of study (Figure 1) will provide impetus for change in fundamental approaches to certain aspects and areas of oral health care.



**Figure 1.** Information from initiatives in genomics, proteomics, transcriptomics, and SNPs will be integrated to advance our diagnosis and treatment of both simple and complex hereditary conditions that affect the craniofacial complex.

Nearing completion of the sequencing of the human genome, researchers are now faced with understanding the function, interaction (gene/gene; gene/environment), and regulation of genetic material and the role of these factors in determining health and disease. While recent estimates suggest there are approximately 50,000 genes, there are thought to be three times that many proteins as a result of alternative splicing and post-translational modification.<sup>2</sup> There are now major initiatives in the area of proteomics (study of protein expression and function) to identify and understand the function of the diverse proteins produced from the genome.<sup>3</sup> Understanding protein function and interaction will advance our knowledge of genotype-to-phenotype relationships and how changes in the genome produce the tremendous variability of expression seen in many conditions. As our knowledge of genomics and proteomics advances, it is becoming increasingly clear that even simple hereditary traits must be viewed as complex conditions when trying to define the mechanisms determining the marked variability of severity and expression. Just having the primary sequence of human DNA alone will not provide the full understanding needed to revolutionize diagnostic and treatment approaches in health care.

Indeed our basic understanding and definition of disease are changing as we unravel the complex relationships among genotype, environmental influence, and phenotype. For example, disease can be defined as the clinical presence of symptoms, or alternatively it may be characterized as having a predisposing cancer gene that has a high probability of resulting in tumorigenesis.<sup>4</sup> Diseases are now being defined as a genotype and/or phenotype that have the potential for adverse consequences, whereas they were previously classified solely on phenotype. This has significant ramifications for conditions such as head and neck cancer where there are known mutations that increase the risk for developing cancer. Detailed clinical studies are required to determine which genetic mutations are associated with disease risk and the level of increased risk associated with specific mutations or polymorphisms (variations in the DNA sequence that exist as a stable component of the population's genome).

Because the major oral disease burden of modern humans results from complex disorders involving multiple genes and environmental interactions, it will be necessary to understand genetic variability at an even greater level of detail than required for simple hereditary conditions. Finding the multiple

genes involved in complex conditions is difficult at best. The use of genomic locations where a single base in the DNA sequence is altered could provide a powerful tool for identifying genes associated with complex diseases. These single-nucleotide polymorphisms (SNPs) are referred to as “snips” and occur about every 1,000 bases along the genome.<sup>5</sup> Evaluating the tremendous amount of information generated by SNP studies to identify complex disease-associated genes has been daunting, but there have been successes (Crohn’s disease, for example). New technologies for genotyping and statistical analysis to separate disease-related SNPs from normal genetic variation could make this approach useful for identifying genes associated with complex traits such as dental caries and periodontal disease.

The massive amounts of data being generated by the genome and related projects raise the question of how to effectively analyze this information. Bioinformatics is a critically important emerging field that integrates computer science and molecular biology. Bioinformatics integrates data from multiple sources such as genomic sequences, the temporal and spatial expression of mRNA, proteomics, protein-protein interaction, and clinical outcomes.<sup>6</sup> Ultimately, advancement in disease diagnosis and treatment is and will be determined by our growing ability to acquire new data (driven by innovations in molecular biology) and to efficiently analyze it (driven by development of computer technology).

Other areas of study that will further our understanding of the molecular determinants of health and pathology include transcriptomics (large-scale analysis of messenger RNAs transcribed from active genes), comparative genomics (comparison of human DNA sequence and patterns to other organisms), and genome studies of other species. Not surprisingly, as new knowledge is acquired, bringing with it new technologies and approaches, there are multitudes of ethical, legal, and social issues that must be addressed. The HGP established the ELSI (ethical, legal, and social issues) Program as an integral and funded part of the project to address these issues. This program also deals with and has requested applications for proposals on how best to educate oral health care professionals in these new fields of study and keep practitioners optimally knowledgeable in translational technologies and molecular biology-related health care issues.

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## Other Genome Projects

While on the surface, sequencing the entire genomes of multiple organisms might not seem critically important, this knowledge will directly influence our understanding of human gene function, provide the foundation for critical research models, and markedly advance our understanding of species-pathogen interactions.<sup>7</sup> For example, the mouse genome is approximately the same size as the human genome (approximately 3.1 billion base pairs) and comprised of essentially the same genes. Therefore, the mouse has become an invaluable tool for studying the function of human genes and for the study of pathogenesis and the application of new treatments and novel therapies.

Microbial genome projects not only provide a means of identifying specific pathogens, but also the potential for developing genetically engineered strains that are less virulent or non-pathogenic and can compete with pathogenic wildtype strains.<sup>8</sup> Molecular-based detection of oral pathogens is being used to identify new bacterial stains and help define their role in pathogenesis of dental caries and periodontal disease.<sup>9</sup> While the utility of these tests in determining clinical disease status or risk remains limited, the ability to understand the microbial genes involved in biofilm production and maintenance, virulence, and pathogenicity will likely allow more accurate assessment of disease risk, to efficiently identify microbial pathogens and to develop more effective treatment strategies.

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## Genetic Disorders

To illustrate how knowledge derived from these different fields of study is and will influence oral health care, we can consider hereditary head and neck pathologies in a traditional sense as being either simple or complex conditions. Simple conditions are those resulting from a single gene that has a major effect, while complex conditions result from a collection of altered genes interacting with environmental influences. Oral health care practitioners are routinely faced with both simple and complex hereditary conditions. During the past two decades our understanding of the genes that control craniofacial growth and development, including many of its specialized

tissues such as teeth, bone, and salivary glands, has dramatically increased. Characterization of the molecular control for normal developmental and pathological processes can, for at least some craniofacial conditions, provide the means for innovative diagnostic and management approaches. The following sections illustrate our rapidly changing molecular-based understanding of the most prevalent oral health-related conditions that are commonly encountered by dental practitioners.

## Simple Hereditary Conditions

Normal development and maintenance of the craniofacial complex is highly regulated at the molecular level. Therefore, it is not surprising that there are numerous simple hereditary conditions that affect the craniofacial structures.<sup>10</sup> Tooth development also is highly regulated at the molecular level with hundreds to possibly several thousand genes being involved in the complex and exquisitely orchestrated process of odontogenesis.<sup>11,12</sup> Developmental defects of teeth can occur as isolated genetic traits, be associated with a chromosomal abnormality or syndrome, or be inherited as a complex trait with genetic and environmental interactions.

**Congenitally Missing Teeth.** Congenitally missing teeth (hypodontia) is the most common simple hereditary trait affecting the oral cavity having a prevalence that varies between races and the specific tooth type. Approximately 5 percent of Caucasians will have a congenitally missing permanent maxillary lateral incisor or premolar (the most commonly missing teeth excluding third molars), while only 1 percent of African Americans will have a congenitally missing tooth.<sup>13</sup> Congenitally missing primary teeth is less prevalent than missing permanent teeth with the mandibular central incisor being the most commonly missing primary tooth. The variable prevalence of missing teeth between racial groups, primary and permanent teeth, and tooth types occurs because this is a genetically heterogeneous group of conditions. Mutant gene frequencies likely vary between populations, accounting for prevalence differences between populations. Differences in the prevalence of hypodontia between dentitions and tooth types likely result from different mutations in the multiple genes that are combinatorially expressed to determine tooth type, location, and time of formation.<sup>14</sup> The clinical and genetic heterogeneity of hypodontia illustrates the complexity of this common simple trait.

**Table 1. Simple hereditary traits associated with abnormal tooth formation**

Condition	Inheritance	OMIM Number	Gene
<i>HYPODONTIA</i>			
Hypodontia – Premolar, 3 <sup>rd</sup> Molar	Autosomal Dom	106600	<i>MSX1</i>
Oligodontia – Incisor, Molar	Autosomal Dom	604625	<i>PAX9</i>
<i>SYNDROME/HYPODONTIA</i>			
Hypohidrotic Ectodermal Dysplasia	X-Linked Rec	305100	<i>EDA</i>
Hypohidrotic Ectodermal Dysplasia	Autosomal Dom -Rec	129490 - 224900	<i>DL</i>
Incontinentia Pigmenti	X-linked Dom	308300	<i>NEMO</i>
Witkop/Tooth & Nail Syndrome	Autosomal Dom	189500	<i>MSX1</i>
Reiger Syndrome Type I	Autosomal Dom	180500	<i>RIEG1</i>
Ellis Van Creveld Syndrome	Autosomal Rec	225500	<i>EVG</i>
Ectodermal Dysplasia, Cleft, Syndactyly	Autosomal Rec	225000	<i>PVRL1</i>
<i>ENAMEL</i>			
Amelogenesis Imperfecta	X-Linked	301200	<i>AMELX</i>
Amelogenesis Imperfecta	Autosomal Dom	104530	<i>ENML</i>
Junctional Epidermolysis Bullosa	Autosomal Rec	226700	<i>LAMA1, LAMB3, LAMC2, ITGB4</i>
Tuberous Sclerosis	Autosomal Dom	191100	<i>TSC1</i>
Tricho-Dento-Osseous Syndrome	Autosomal Dom	1903320	<i>DLX3</i>
Mucopolysaccharidosis Type IVA	Autosomal Rec	2353000	<i>GALNS</i>
<i>DENTIN</i>			
Dentinogenesis Imperfecta Type I (with Osteogenesis Imperfecta)	Autosomal Dom & Rec	166240 166220	<i>COL1A1</i> <i>COL1A2</i>
Dentinogenesis Imperfecta Type II	Autosomal Dom	125490	<i>DSPP</i>
Vitamin D-Resistant Rickets	Autosomal Rec	277440	<i>VDR</i>
Ehlers-Danlos Syndrome Type I	Autosomal Dom	1330000	<i>COL5A2</i>

Several genetic mutations resulting in hypodontia have been identified (Table 1). A missense mutation in the *MSX1* gene (gene coding for a transcription factor) causes an autosomal dominant trait of variably missing lateral incisors, second premolars, and third molars.<sup>15</sup> More recently a mutation in the transcription factor gene *PAX9* has been associated with a variable and unusual pattern of hypodontia consisting of missing mandibular incisors, premolars, and molars.<sup>16</sup> Individuals with missing teeth can receive genetic testing to determine if the molecular basis of their condition is known and to establish the mode of inheritance and recurrence risk.

Numerous hereditary syndromes are associated with congenitally missing teeth. In some instances there can be only a few or no missing teeth (for example, Down syndrome) or, as in the case of a group of conditions known as the ectodermal dysplasias (ED), there can be multiple missing teeth (Table 1). There are over a hundred clinically and genetically diverse conditions classified as ED, with many having abnormal tooth development.<sup>17</sup> Hypohidrotic ED (OMIM# 305100), one of the better recognized forms of ED, is characterized by a decreased ability to sweat (hypohidrosis), sparse hair (hypotrichosis), and missing or malformed teeth. Any or all of the teeth can be missing, and there are frequently conical shaped incisors.<sup>18</sup> The numerous genes associated with the ectodermal dysplasia are rapidly being identified.<sup>19,20</sup> Knowledge of the molecular basis of isolated missing teeth and the ED conditions now allows individuals with these, often clinically similar, conditions to be definitely delineated.

**Simple Enamel and Dentin Traits.** There are hundreds of pathological conditions that negatively affect normal development of the structure and composition of teeth.<sup>21</sup> Both environmental influences and/or genetic mutations can affect a variety of developmental processes causing aberrant tooth formation. The diverse etiology of these pathological conditions and phenotypic overlap often makes establishing an accurate diagnosis by phenotype alone difficult. For example, hereditary enamel defects can occur as part of a generalized condition or syndrome or it may occur as a defect involving only enamel.<sup>22,23</sup> Amelogenesis imperfecta (AI) represents a group of hereditary conditions that manifest enamel defects without evidence of generalized or systemic disorders.<sup>24</sup> These conditions are clinically and genetically diverse, and fourteen distinct subtypes have been recognized.<sup>24</sup> Autosomal dominant and recessive as

well as X-linked inheritance patterns have been reported for AI.<sup>25</sup>

The clinical phenotypes range from thin enamel that is normal in color to enamel that is severely hypomineralized and readily abrades from the teeth as they erupt into the oral cavity.<sup>26</sup> Depending on the AI type, the teeth can be extremely sensitive to thermal and chemical stimuli. Although the molecular defects remain unknown for many forms of AI, the rapid pace of discovery suggests they will likely be identified in the near future. Twelve different mutations have been identified in the *AMEL X* gene that codes for amelogenin, the most abundant enamel matrix protein.<sup>27-32</sup> The phenotypes resulting from these mutations are diverse, ranging from enamel hypoplasia to enamel of normal thickness that is hypomineralized.<sup>33</sup> Understanding the genotype/phenotype relationship of the different AI conditions will allow clinicians to accurately diagnose the different AI types, better predict prognosis, and select optimal treatment approaches by understanding the tissue defects (for example, treatment using bonding versus crowns).

Many hereditary disorders of the ectodermal and ectodermal/mesenchymal types, such as the tricho-dento-osseous syndrome (OMIM #190320), incontinentia pigmenti (OMIM #3080300), tuberous sclerosis (OMIM #191100), and junctional epidermolysis bullosa (EB) (OMIM #226700), can have marked enamel involvement (Table 1).<sup>22</sup> Enamel defects associated with syndromic conditions vary substantially depending on the molecular defect and the gene's role in tooth formation. For example, in the tricho-dento-osseous syndrome (TDO), an autosomal dominant disorder caused by a mutation in the *Distal-less 3* homeobox gene, the teeth have smooth or pitted enamel hypoplasia as well as taurodontism.<sup>34,35</sup> Individuals with this condition also have kinky curly hair at birth and develop dense and/or thickened bone and have frequently been misdiagnosed as amelogenesis imperfecta.<sup>36</sup> In contrast, individuals with junctional epidermolysis bullosa (EB) have variable expression of generalized enamel hypoplasia, skin fragility, and blistering.<sup>37</sup> The molecular defects causing junctional EB involve genes that produce proteins essential for maintaining dermal/epidermal integrity (for example, laminin V) and are also important in normal functioning of the ameloblasts and enamel formation.<sup>37,38</sup>

**Dentin Defects.** Dentin malformations that severely affect the form and function of teeth occur in

numerous syndromic and non-syndromic hereditary conditions (Table 1). The most common Mendelian traits affecting dentin have historically been classified based on phenotype and histological features.<sup>24,39</sup> Dentinogenesis imperfecta has been subdivided based on its association with osteogenesis imperfecta (OI) (Type I) (OMIM# 166240) or not (Type II) (OMIM# 125490), or being associated with the Brandywine triracial isolate (Type III) (OMIM# 125500).<sup>40</sup> The molecular defects in OI include numerous mutations in the pro-alpha chains of collagen type I that result in a phenotype characterized by increased bone fragility.<sup>41</sup> Although the dental phenotypes of DI types I and II appear very similar, the latter disorder is not associated with any of the non-dental phenotypic features of osteogenesis imperfecta. Recently, in certain families, DI type II has been associated with mutations in the dentin silaophosphoprotein gene (*DSPP*).<sup>42,43</sup> Identification of the molecular basis for DI types I and II now allows delineation of families that may have mild OI with DI from those with DI type II.<sup>44</sup>

All DI types have teeth characterized by a variable, opalescent, blue-gray to yellow-brown discoloration due to the defective, abnormally colored dentin shining through the translucent enamel.<sup>24</sup> The lack of support provided by the poorly mineralized dentin often results in enamel fracturing and loss, leading to rapid wear and attrition of the teeth. Dentin dysplasia type II (OMIM# 125420) is also inherited as an autosomal dominant trait. The primary dentition in dentin dysplasia type II appears virtually identical to dentinogenesis imperfecta (DI) type II presenting with yellow-brown to blue-gray discoloration of the teeth and pulpal obliteration. Although the molecular defect is unknown for DD type II, it has been linked to the same region as DI type II on chromosome 4q21, consistent with it being an allelic *DSPP* mutation.<sup>45</sup>

There are many systemic conditions associated with abnormal dentin formation as a result of the molecular defect altering dentin developmental pathways. For example, conditions with molecular defects that influence mineralization such as hypophosphatasia (alkaline phosphatase defect) and vitamin D-resistant rickets (vitamin D metabolism defect) can have significant dentin involvement.<sup>46</sup> Children with the later condition often develop dentoalveolar abscesses due to large pulps with extensive pulp projections (pulp horns) that become exposed to the oral environment and allow bacterial invasion

into the tooth.<sup>47</sup> Other systemic conditions with dentin involvement include Ehlers Danlos syndrome, mucopolysaccharidoses, and tumoral calcinosis.<sup>46</sup>

## Complex Hereditary Oral Health Conditions

The major oral disease burden in modern humans results from complex diseases that involve infectious microbial agents coupled with hereditary and environmental risk factors. The two most common oral diseases that afflict large segments of the population are dental caries and periodontal disease. Head and neck cancer represents a third category of disease that has a substantial genetic determinant and that will likely show marked advances in diagnosis and treatment as a result of molecular biology-derived knowledge. The multifactorial nature of these and other important head and neck conditions (cleft lip/cleft palate, for example) makes identifying the hereditary contribution more difficult compared with simple hereditary condition (Table 2). However, due to the tremendous population burden presented by these conditions, the potential public health benefit could be much greater.

**Dental Caries.** Dental caries is the most common chronic disease of childhood and is unequally distributed in the population with most of the disease occurring in 20 percent of children.<sup>48</sup> While dental caries is considered an infectious disease, there are numerous host resistance and risk factors that are genetically determined.<sup>49</sup> Although molecular markers have not been identified for specific hereditary factors that contribute to increased risk for or resistance to developing dental caries, there is substantial evidence that heredity plays an important role in this multifactorial disease process (see Shuler 2001 for recent review).<sup>50,51</sup> Hereditary factors contribute to many of the caries risk/resistance factors including pit and fissure morphology, enamel structure and composition, tooth eruption time, salivary flow and composition, arch form, dental spacing, immunologic function, and dietary preference. Studies of monozygotic twins suggest that approximately 50 percent of the variance in dental caries can be attributed to hereditary factors.<sup>52</sup>

Identifying the genetic factors contributing to caries risk and resistance will provide clinicians with new tools for targeting individuals and/or populations for more efficient and effective preventive therapies. For example, individuals with certain types of

**Table 2. Simple and complex hereditary conditions associated with periodontal disease, clefting, and head and neck cancer**

Condition	Inheritance	OMIM Number	Gene
<i>PERIODONTITIS</i>			
Papillon Lefevre Syndrome	Autosomal Rec	245000	<i>CTSC</i>
Haim Munk Syndrome	Autosomal Rec	245010	<i>CTSC</i>
Ehlers-Danlos IV	Autosomal Dom	130050	<i>COL3A1</i>
Leukocyte Adhesion Deficiency I	Autosomal Dom	116920	<i>ITGB2</i>
<i>CLEFT LIP/PALATE</i>			
Oral Facial Digital Syndrome 1	X-Linked	311200	<i>CXORFS</i>
Cleft Palate with Ankyloglossia	X-Linked	303400	<i>TBX22</i>
Hay-Wells Syndrome	Autosomal Dom	106260	<i>p63</i>
Hypothyroidism, Athyroidal with Spiky Hair and Cleft Palate	Autosomal Rec	241850	<i>FKHL15</i>
<i>HEAD AND NECK CANCER</i>			
Squamous Cell Carcinoma	Complex	601400	<i>TNFRSF10B</i>
Cowden Disease	Autosomal Dom	158350	<i>PTEN</i>
Basal Cell Nevus Syndrome	Autosomal Dom	109400	<i>PTCH</i>
Bloom Syndrome	Autosomal Rec	210900	<i>RECQL3</i>
Beckwith-Wiedemann Syndrome	Autosomal Dom	130650	<i>CDKN1C</i>

epidermolysis bullosa (EB) (that is, recessive dystrophic and junctional EB types) have a markedly increased dental caries risk compared with unaffected people.<sup>53</sup> It is likely that this increased risk results from soft tissue and subsequent dietary and microbial changes in recessive dystrophic EB and from enamel defects in junctional EB.<sup>53</sup> Understanding the human hereditary traits contributing to dental caries coupled with genetic knowledge of the virulence and pathogenicity associated with cariogenic bacteria will allow new diagnostic and novel therapeutic approaches to be applied in the management of this disease. However, it is not clear whether genetic testing to determine the multiple factors that collectively determine 50 percent of an individual's risk for developing caries will be cost-effective. It is indeed probable that traditional public health approaches (such as fluoride and sealants) for managing dental caries will remain the most efficient and effective preventive measures for the next decade.

**Periodontal Pathology.** Periodontal diseases are a heterogeneous group of diseases characterized by inflammation and destruction of the periodontium.<sup>54</sup> Although moderate-severe periodontal destruction affects approximately 10-15 percent of the U.S. population, this risk is not shared equally by all individuals.<sup>55</sup> While the importance of a microbial etiology in periodontitis is well established, and specific microbes appear to be associated with certain forms of periodontitis, in most cases microorganisms

alone are not sufficient to cause disease.<sup>56,57</sup> Significant data now supports an important role for heredity in susceptibility to a variety of types of periodontitis.<sup>58-60</sup>

A direct cause and effect relationship for a specific gene defect and periodontitis susceptibility can be illustrated by the association of severe periodontitis with a number of genetic diseases. Over the past decade, the genetic basis for several syndromic forms of periodontitis has been identified (Table 2). Mutations of the cathepsin C gene are responsible for Papillon Lefevre syndrome, Haim Munk syndrome, and at least a portion of Prepubertal Periodontitis cases.<sup>61-63</sup> *CHS* gene mutations cause Chediak Higashi syndrome, and beta-2 integrin (a cell surface receptor) gene mutations are responsible for leukocyte adhesion deficiency type 1.<sup>64,65</sup> Identification of these gene mutations clearly illustrates how a variety of different gene defects can influence disease susceptibility. Identification of these gene mutations permits genetic testing and determination of a definitive diagnosis in these conditions.

While specific genes have been identified for several syndromic forms of periodontitis, the genetic basis for the more prevalent (~1-2 percent) forms of aggressive periodontitis are less well characterized. Formal genetic studies of aggressive periodontitis suggest that susceptibility is inherited as a simple genetic trait, but it is unclear how many genes may be involved in these non-syndromic forms of peri-

odontitis.<sup>66,67</sup> While the major genes responsible for aggressive periodontitis remain elusive, several genes that may modify the clinical expression of this disease form have been identified, including interleukin-4 polymorphisms,<sup>68</sup> Fc gamma receptor genotype,<sup>69</sup> vitamin D receptor polymorphisms,<sup>70</sup> and immunoglobulin allotypes.<sup>71</sup> In addition to a significant genetic etiology for aggressive periodontitis, there is substantial evidence that environmental factors such as smoking and microbial agents (virulence factors) interact with modifying genes to determine the final disease trajectory.<sup>71-74</sup> In contrast to the relatively simple genetic etiology of syndromic forms of periodontitis, it is clear that aggressive periodontitis has a more complex etiology, and the final disease phenotype is modified by multiple genetic and environmental factors.

Heredity also appears to be a significant factor in the etiology of the most common form of periodontitis: chronic periodontitis. Results of studies of periodontitis in human twins suggests approximately 50 percent of susceptibility may be attributable to genetic factors.<sup>59</sup> However, the etiology of chronic periodontitis is likely to be even more complex than that of syndromic and aggressive periodontitis. There is no evidence of a simple pattern of genetic transmission that would support an etiological role for a single gene mutation in chronic periodontitis. This has important implications for disease-associated gene discovery.

Chronic periodontitis is likely to result from the additive effect of multiple genes, which may each contribute to disease susceptibility individually, as well as through interactive effects with other gene products and through modulation by environmental factors.<sup>60</sup> In contrast to gene mutations that cause syndromic forms of periodontitis, naturally occurring genetic variants (genetic polymorphisms) are likely to impart genetic risk in chronic periodontitis. Each individual gene polymorphism may contribute a relatively small part of susceptibility; none alone is sufficient to cause nor predictive of disease risk. As a result, it is unlikely that analysis of genes for mutations will provide diagnostic and prognostic value. In contrast to the relatively simple gene mutation-phenotype correlations described for syndromic periodontitis, genetic association studies are performed to assess the strength of the relationship between the presence of one or more gene polymorphism and a disease phenotype. As a consequence, the statistical issues are more complex and contro-

versial, and it is more difficult to utilize genetic information for diagnostic and prognostic testing. These are issues that will gain more attention as it is generally realized that the ability to test for genetic polymorphisms exists before the meaning of test results is fully understood and the value of such testing for clinical management of periodontitis is known.

**Head and Neck Cancer.** Cancer involves the progressive loss of control of normal cellular homeostasis. A progressive accumulation of genetic damage has been associated with the histological changes characteristic of neoplastic progression. In contrast to germ line mutations that cause genetic syndromes, head and neck squamous cell carcinoma (HNSSC) are characterized by genetic alterations in somatic cells such as loss of heterozygosity (LOH), mutations, amplification (and hence overexpression) of certain genetic regions and genomic rearrangements. These genetic changes appear to be early and essential events in tumor development.<sup>75</sup> Genetic alterations consistently correlated with histological stages of head and neck squamous cell carcinoma have been used to develop molecular progression models for the disease. In this sense, cancer is a genetic disease. A number of environmental factors also appear to be important in HNSSC risk. Epidemiological studies indicate that tobacco, alcohol, and human papilloma viruses are significantly associated with HNSSC risk.<sup>76</sup> While these agents have been clearly associated with HNSSC risk, not everyone exposed to them develops cancer. Genetic factors also appear to modulate risk.

The genetic model for HNSSC susceptibility appears to be complex, involving gene-gene and gene-environment interactions. For the majority of HNSSC cases, risk is not determined by the presence of a single gene mutation, but rather the cumulative effect of multiple different gene polymorphisms and environmental exposures to a variety of agents.<sup>77</sup> Consequently, risk assessment will require information about an individuals' genetic background as well as environmental exposures and behavioral patterns. In some cases, genetic predispositions for certain environmental risk factors are being identified and evaluated. The presence of specific alcohol dehydrogenase gene polymorphisms has been determined to confer differential susceptibility to the effect of alcohol risk in certain populations.<sup>78</sup> Genetic polymorphisms of several xenobiotic metabolizing enzymes including the cytochrome P450 1A1 (*CYP1A1*), glutathione S-transferase genes (*GSTM1* & *GSTT1*), and

UDP-glucuronosyltransferase 1A7 (*UGT1A7*) genes may confer increased risk for tobacco-related HNSCC.<sup>79-80</sup> Understanding the spectrum of genetic susceptibility for HNSCC may help identify those agents that increase risk for disease and permit identification of those individuals at greatest risk.

Delineation of molecular progression models of genetic damage in HNSCC may be of value in several ways. First, understanding the molecular progression may help understand why cancer progresses and help identify specific biological points appropriate for intervention strategies. For example, alterations of the tumor suppressor gene p53 are commonly reported in a variety of cancer types.<sup>81</sup> This physiologically important gene may also be affected by human papilloma viruses, which appear to have a causal association with a subset of head and neck cancers.<sup>76</sup> Vitamin E may help inhibit cancer formation by stimulating the expression of the cancer suppressor gene p53.<sup>82</sup> Altered expression of a number of genes has been reported for HNSCC, and in many cases, a specific role in the disease process has been proposed. Altered expression of several of the matrix metalloproteinases and their inhibitors may be important determinants of the invasiveness and ability to metastasize seen in HNSCC.<sup>83</sup>

For certain cancers, identification of specific genetic changes provides helpful information for management and prognosis. The presence of the Philadelphia chromosome rearrangement in leukemia patients indicates a better prognosis. In the case of HNSCC, high levels of expression of certain genes, for instance, the p65 subunit of the transcription factor kappa B (NF-kappaB) and IkappaB kinase, may contribute to malignancy.<sup>84</sup> Lack of the tumor suppressor gene PTEN may be an important prognostic indicator in squamous cell carcinoma of the tongue, while overexpression of the hepatocyte growth factor MET oncogene is involved in invasive-metastatic behavior of HNSCCs.<sup>85,86</sup> High expression of the epidermal growth factor receptor (EGFR) and the proliferating cell nuclear antigen (PCNA) have been correlated with short patient survival and are thus indicators of poor prognosis.<sup>87</sup> Cyclin D1 (*CCND1*) is a cell cycle regulatory factor that modulates a critical step in cell cycle control. *CCND1* is overexpressed in a significant proportion of HNSCC and correlates with aggressiveness, early recurrence, and poor prognosis.<sup>88,89</sup> Overexpression of *CCND1* has also been correlated to radiosensitivity and may be a useful predictor of the effectiveness of radiation

therapy on HNSCC.<sup>90</sup> Antisense cyclin D1 may be useful, particularly in combination therapy, for instance with cisplatin, in treatment of HNSCC.<sup>88</sup>

It is likely that genetic characterization will be combined with more traditional histological staging of HNSCCs to develop classifications of better clinical utility.<sup>91</sup> Identification of genetic alterations that occur in a specific form of HNSCC may provide the basis for novel, highly specific, and sensitive diagnostic tests. Such testing may be extended to identify a recurrence before lesions are histologically visible. Based on the theory of the clonal initiation of cancer, methods are being developed to identify specific genetic changes in very few cells. With this technology, it should be possible to test for and identify a recurrence in a specific individual by testing a blood, stool, or saliva sample.<sup>92</sup> Such assays should permit more effective surveillance and, when indicated, earlier intervention. Research efforts are also being directed to the identification of specific genetic alterations that may help guide treatment decisions and render certain interventions as more effective. Although the pathophysiological changes that occur in cancer are complex, emerging technologies are able to utilize gene sequence information on a variety of platforms such as micro-array chip analysis to detect the concomitant changes in expression profiles of many genes at different stages of malignancy.<sup>93</sup>

In addition to studies in humans, understanding the genome of other species will also be important. Studies of environmentally induced oral cancer in animal models can provide a better understanding of the molecular progression of cancer, which may have parallels in humans. Studies of rats demonstrate that genetically different strains have very different susceptibility to carcinogen induced cancer, and such studies have been able to identify specific genes that confer susceptibility.<sup>94</sup> Ultimately, a comprehensive cataloging and understanding of the molecular progression of genetic changes that occur in HNSCC will provide a robust framework for the development of diagnostic, prognostic, and intervention strategies that may significantly enhance management of these devastating conditions.<sup>95</sup> Dentists will need to be familiar with a variety of genes, such as xenobiotic metabolizing enzymes, which are highly polymorphic in many populations and be able to understand and interpret data from a variety of studies to evaluate the veracity of claims suggesting association with disease such as HNSCC.<sup>96</sup>

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## Future Directions and Implications for Dental Education

Clearly, future clinicians will have at their disposal new diagnostic and treatment approaches that have had their genesis from knowledge derived from the genome projects. As we move into the post-genomic era, clinicians will increasingly be called upon to apply molecular-based diagnostic and treatment approaches in their practices.

It also is evident that the oral health profession will be faced with several profound issues as a result of this new knowledge and emerging technologies. For example, it is probable that our ability to perform genetic testing will greatly exceed our ability to perform meaningful analyses and obtain useful information. So will it be productive to use SNP approaches to determine genetic risk for dental caries so that preventions can be better targeted to specific individuals? The dental community will ultimately need to determine how to efficiently obtain and effectively apply this information. However, dental professionals are not formally trained to determine what types of molecular information will be most valuable in optimizing oral health care. They are also not versed in how to effectively interpret or apply the tremendous amounts of molecular information that will likely be available for their patients in the future.

Dental curricula generally have very limited time devoted to genetics and molecular biology. However, it is critical that dental practitioners be able to appropriately diagnose, refer for further evaluation, and eventually apply molecular-based treatments to their patient populations. This will require a basic working knowledge of molecular biology and human genetics. Initially, this information can be integrated into traditional disciplines such as oral pathology, periodontology, and biochemistry.<sup>97</sup> However, as molecular-based knowledge continues to expand and new molecular-based diagnoses and interventions become the routine in dental practice, more novel, integrated, and comprehensive teaching approaches will become beneficial. Similarly, novel approaches to keep existing and future practitioners current through continuing education will be critical. Offerings for continuing education in this area are currently very limited.

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## Conclusions

While information gleaned from the HGP is already allowing accurate diagnosis and risk assessment of numerous simple hereditary conditions, knowledge derived from the study of proteomics and gene expression will further advance the diagnosis and treatment of craniofacial pathology. Although more difficult, it is likely that these same molecular approaches could provide useful tools for managing at least some complex conditions that commonly affect the head and neck. Information on the molecular control of bone, periodontal, salivary gland, and tooth development will lead to new and novel treatment approaches well beyond those of our current surgical-based techniques. Tissue engineering approaches are already making significant strides in cell manipulation and developing tissues such as skin, bone, and cartilage. Similarly, advances in drug delivery, gene therapy, and biopharmaceuticals will present new therapeutic opportunities.

The post-genomic era will present many opportunities for improvement in oral health care and a multitude of challenges. The dental profession will be faced with determining how best to incorporate this knowledge and the resulting new technologies into our health care system and how to effectively educate oral health care providers and keep them abreast of the rapidly changing field of molecular biology.

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