Research Article

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Genomics: Paradigm Shift in Diagnostics and Therapeutics

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ABSTRACT

Background: Genomic paradigm can be thought of as the foundation upon which a scientific structure is providing new findings and insights. In Dentistry and Orthodontics, at present, we are on the threshold of a paradigm shift that changes the fundamental and conceptual understanding of orthodontics, and with it, the traditional emphasis is on diagnosis and treatment planning. Formerly, the emphasis was on the dental and skeletal components; then the soft tissue components and now, greater attention to the genomic aspects of orthodontics is required. With the increase in knowledge of the human genome and its DNA sequence, many numbers of disease genes can now be examined using DNA analysis. Various genes attributed for craniofacial deformities are identified. The recent advances of genome-editing technologies have enabled a new hope in which the sequence of the human genome can be precisely manipulated to achieve a therapeutic effect. Fundamental concepts of genetics and applied translational research in orthodontics are opening new horizons in the procedures of gene mapping and gene therapy which provide a foundation for a new emphasis on precision orthodontics.

Keywords: Genomics, therapy, dentistry.

INTRODUCTION

Genetics is the word which is derived from the Ancient Greek word *Genetikos*. It is the science of genes, heredity, and variation in living organisms. Genetics provides an insight into what makes us humans and what distinguishes each of us as individuals [1]. Awareness that physical constitution and especially facial appearance are passed from one generation to the next, or are somehow inherited [2].

In dentistry we encounter numerous differences in the dentofacial characteristics of individuals, even among family members [3-5]. The three most common problems in dentistry today remain dental caries, periodontal diseases and malocclusion. All these conditions have multifactorial etiology, where in both genetic and environmental contributions add variability [6-10].

The purpose of this article is to highlight the evolutionary concepts in development of malocclusion and correlate with the discoveries and advances in genetics. Attention will be placed on discussions about heredity and genetics that have appeared as primary etiological factor in malocclusion. DNA analysis as pre-symptomatic diagnosis of inherited conditions and the detection of carriers following initial diagnosis by more conventional laboratory tests. Gene therapy which has been defined as the introduction of normal genes into cells in place of missing or defective ones in order to correct genetic disorders. On the other hand, the recent advances in gene-editing technologies has given a new beginning in which

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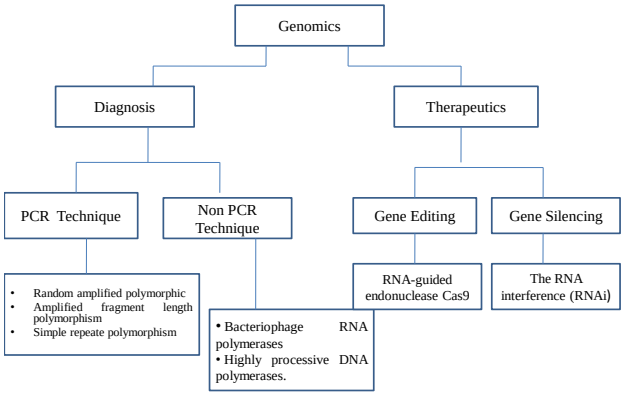

Fig 1: overview of Genomic, Diagnosis and Therapeutics.

GENE ARCHITECTURE

The gene is the fundamental unit of inheritance and the ultimate determinant of all phenotypes. The DNA of a normal human cell contains an estimated 30,000 to 120,000 genes, but only a fraction of these are expressed in any particular cell at any given time. Every gene consists of several functional components, each involved in a different facet of the process of gene expression. Broadly speaking, however, there are two main functional units: the '*promoter*' region and the '*coding*' region.

The promoter region controls when and in what tissue a gene is expressed. In the DNA of the gene's promoter region, there are specific structural elements, '*nucleotide sequences*' that permit the gene to be expressed only in an appropriate cell (Fig 2).

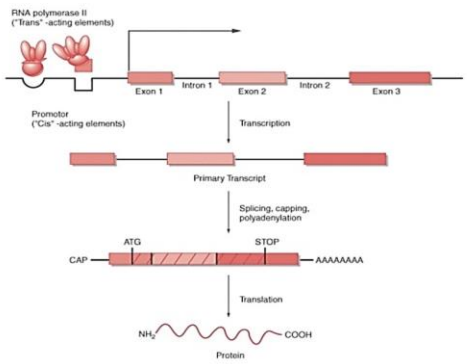

Fig 2: Architecture of gene.

These are the elements in the globin gene that instruct an erythroid cell to transcribe globin mRNA from that gene. These structures are referred to as *cis*-acting elements because they reside on the same molecule of DNA as the gene. In some cases, other tissue type-specific *cis*-acting elements, called *enhancers*, reside on the same DNA molecule, but at great distances from the coding region of the gene. In the appropriate cell, the *cis*-acting elements bind protein factors that are physically responsible for transcribing the gene. These proteins are called *trans*-acting factors because they reside in the cell's nucleus, separate from the DNA molecule bearing the gene. For example, brain cells would not have the right *trans*-acting factors that bind to the β-globin promoter, and therefore brain cells would not express globin. They would, however, have *trans*-acting factors that bind to neuron-specific gene promoters (Fig 3).

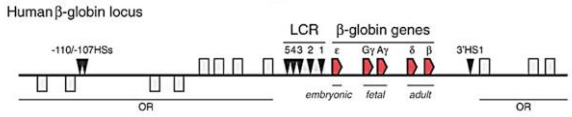

Fig 3: Structure of β-globin promotor in Human DNA.

The structure of a gene's protein is specified by the gene's coding region. The coding region contains the information that directs an erythroid cell to assemble amino acids in the proper order to make the β-globin protein. DNA is a linear polymer consisting of four distinguishable subunits called *nucleotides* (Fig 4).

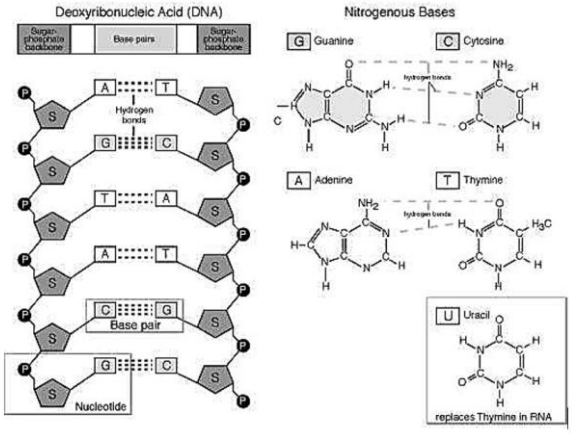

Fig 4: DNA subunits (nucleotides).

In the coding region of a gene, the linear sequence of nucleotides *encodes* the amino acid sequence of the protein. This genetic code is in triplet form so that every group of three nucleotides encodes a single amino acid. The 64 triplets that can be formed by 4 nucleotides exceed the 20 distinct amino acids used to make proteins (Fig 5).

First Position	Second Position				Third Position
	U	C	A	G	
U	Phe	Ser	Tyr	Cys	U
	Phe	Ser	Tyr	Cys	C
	Leu	Ser	Stop	Stop	A
	Leu	Ser	Stop	Trp	G
C	Leu	Pro	His	Arg	U
	Leu	Pro	His	Arg	C
	Leu	Pro	Gln	Arg	A
	Leu	Pro	Gln	Arg	G
A	Ile	Thr	Asn	Ser	U
	Ile	Thr	Asn	Ser	C
	Ile	Thr	Lys	Arg	A
	Met	Thr	Lys	Arg	G
G	Val	Ala	Asp	Gly	U
	Val	Ala	Asp	Gly	C
	Val	Ala	Glu	Gly	A
	Val	Ala	Glu	Gly	G

Fig5: Determination of nucleotides for a base pairing

This makes the code degenerate and allows some amino acids to be encoded by several different triplets. The nucleotide sequence of any gene can now be determined. By translating the code, one can derive a predicted amino acid sequence for the protein encoded by a gene (Fig 6) [11].

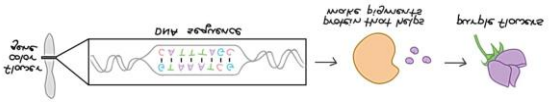

Fig 6: Example for Code Translation in DNA.

Table 1: Specific Genes Responsible For Craniofacial Deformities.

CRANIOFACIAL STRUCTURE	GENES RESPONSIBLE and RELATED DEFORMITY
DENTITION	1) MSX1 causes premolar and 3rd molar agenesis. 2) Pax9 causes hypodontia(lateral incisor missing) 3) TGF-A causes dental agenesis 4) Axin2 causes tooth agenesis 5) KLK4 causes amelogenesis imperfecta involves both deciduous and permanent dentition. 6) COL1A1 or COL1A2 causes dentinogenesis imperfecta. 7) Pax9 gene causes transposition of teeth.
PALATE	Interferon regulatory factor 6 (IRF6),SHH causes palatal deformation.
MAXILLA	1) Fibroblast growth receptor 2-FGFR 2 causes maxillary retrusion. 2) SOBP gene causes anterior maxillary excess. 3) RUNX2 gene causes maxillary hypoplasia.
MANDIBLE	1) IGF1, HOXC, COL2A1, EPB41, MATN1, TGFB3, LTBP2, DUSP6 causes mandibular prognathism. 2) 14q32.13 causes mandibular hypoplasia.
CRANIUM	1) Sonic Hedgehog-SHH-causes Holoprosencephaly 2) 6p24,2p13, 19q13,4q, FOXE1, IRF6, causes orofacial clefting 3) FGFR 2 causes craniosynostosis
OTHERS	MMP9 causes skeletal anterior open bite(due to vertical excess).

Table 2: Specific Genes Responsible For Skeletal Maloccusion.

MALOCCLUSION	GENES RESPONSIBLE
Class I malocclusion	SNP rs6504340, EDA (rs3764746 and rs3795170), XEDAR (rs372024), and BMP2 (rs1005464) [12].
Class II malocclusion Division 1	SNP (g.4861609G, rs186861426) [13].
Class II malocclusion Division 2	3q31.2, 12q13, and 19p13.2 [5].
Class III malocclusion	1p22.3, 1p35-36and 19p13.2, 1p36 [5].
External Root Resorption	IL-1B allele "1" [5].
Canine Impaction (palatal)	RUNX2 and CX43 [14].

GENE MAPPING

Numerous disease genes can now be examined using DNA analysis. The polymerase chain reaction (PCR) is widely used in genomic DNA analysis. One of its main purpose has been in the development of DNA markers for map construction, which are useful in taxonomy, evolution, gene cloning and breeding. Gene mapping mainly includes 2 techniques, one is PCR and other is non PCR technique.

PCR technique

This technique involves denaturation of the double strands of DNA to form single stranded DNA. This procedure is done at 92^0C-96^0C for two minutes. Following which annealing of primer to each strand is done at 45^0C-55^0C, followed by DNA polymerase which adds dNTPs at 3' primer end at 72^0C. Several PCR marker and hybridization systems are available varying in complexity, reliability and information generating capacity. The techniques available for genetic analyses includes, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat polymorphism (SSR) and a few others [15].

The alternative to PCR for nucleic acid amplification is isothermal transcription-based amplification (ITA), based on either bacteriophage RNA polymerases or a group of highly processive DNA polymerases.

Transcription-based systems are used for RNA detection and produce an RNA amplicon rather than DNA amplicon. Because RNA amplicons are more labile than DNA, this helps to reduce the possibility of carry-over contamination. A single round of transcription can produce 10–1000 RNA copies per cycle as compared with PCR that produces only two copies per cycle. DNA polymerase-based non-PCR amplification systems are used for the detection of dsDNA molecules and require an initial denaturation step prior to isothermal amplification. Rolling-circle amplification (RCA) and strand displacement amplification (SDA) rely on the activity of DNA polymerase to invade a nicked double-stranded molecule and displace the downstream DNA strand during elongation. Both strategies resemble rolling circle replication used by small plasmids, viroids, and bacteriophages. A key to the adaptation of this technology is the development of a method to permit the cleavage of one strand of a double-stranded DNA molecule [16].

GENOMIC THERAPEUTICS:

Gene therapy is defined as the addition of new genes to human cells. The recent advances of genome-editing technologies has enabled a new hope in which the sequence of human genome can precisely be manipulated to achieve a therapeutic effect. This includes the correction of mutations that cause disease, the addition of therapeutic genes to specific sites in the genome, and the removal of deleterious genes or genome sequences.

Mechanisms of gene editing

The field of gene editing wherein DNA double strand breaks (DSBs) could be used to stimulate the endogenous cellular repair machinery. Breaks in the DNA are typically repaired through one of two major pathways: a) Homology-directed repair (HDR) b) Non-homologous end-joining (NHEJ) [17].

HDR relies on strand invasion of the broken end into a homologous sequence and subsequent repair of the break in a template-dependent manner [18].

The RNA-guided endonuclease Cas9 has emerged as a versatile genome-editing platform. However, the size of the commonly used Cas9 from Streptococcus pyogenes (SpCas9) limits its utility for basic research and therapeutic applications that use the highly versatile adeno-associated virus (AAV) delivery vehicle. The Cas9 which is derived from staphylococcus aureus are as efficient as SpCas9, which is more than 1 kilobase shorter. Cas9, an RNA-guided endonuclease, formulated from the type II CRISPR-Cas bacterial adaptive immune system, [19-25] has been used for genome editing and holds immense assurance for biomedical research [26,27].

Vectors can also be used for gene therapy, which are classified into two types (Fig 7).

1. Viral vectors

2. Non-viral

Table 3: Classification of vectors.

VIRAL VECTOR	NON VIRAL VECTOR
Adenovirus	Lipid complex
Retrovirus	Liposomes
Adeno-Associated virus	Peptide/Protein
Lentivirus	Polymers
Vaccinia	
Herpes Simplex virus	

The direct gene transfer methods are i) electroporation and ii) gene gun are used to transfer genes into target cells [28].

Gene silencing

The functional approach is to edit the atypical epigenetic state of key drivers to reprogram the etio-pathology of the disease. Cytosine methylation in mammalian DNA is regarded as a key epigenetic modification controlling essential processes such as imprinting, silencing of retro transposons and cell differentiation [29]. In addition to DNA methylation (DNAme), repressive histone post-translational modifications reinforce gene inactivation, resulting in the formation of inactive chromatin, namely heterochromatin [30].

The RNA interference (RNAi) technique has been widely used as a tool for gene-functional studies and also has great potential for developing therapies against viral infection, genetic disorders and cancers [31–35]. Several RNAi therapies have been used clinically, while many more are in clinical trials [36].

Related studies

Today foetal surgeries allow early treatment of certain congenital anomalies that can be easily diagnosed with high-resolution ultrasound [37]. Open foetal surgery has been performed only in a few centers around the world. Maximum information was gathered at the Foetal Treatment Centre of the University of California in San Francisco (UCSF), directed by Dr Michael Harrison [38–42].

Fetoscopy allows the diagnosis of certain human congenital malformations at the early gestational age of almost 9 weeks. Pregnant sheep have been widely used as an animal model to understand foetal etiophysiopathology and to study experimental foetal surgery. For this animal model, special small diameter cannulas with flexible balloon-tipped shafts have been developed for feto-endoscopic procedures [43,44].

One of the first interventions reported was feto-endoscopic suturing for repair of surgically created fetal cleft lip and palate in the sheep model [45]. Several other reports show widespread acceptance and application of the technique, employed in animal models as well as in the treatment of life threatening malformations in humans [46,47,48,49,50–57].

The first feto-endoscopic treatment in the human foetus was an endoscopic vesicostomy carried out by MacMahon and his team. Quintero et al. recently performed intrauterine cystoscopy through a

suprapubic access [54]. Unfortunately, foetal morbidity and mortality rates remain excessively high after such interventions, due to (a) direct operative trauma to the myometrium and foetal membranes; and (b) effects of postoperative premature membrane rupture, uterine flow decrease and the increase of uterine contractions. The latter are almost always observed after foetal surgery and may lead to premature labour and foetal demise [58].

CONCLUSION

Application of fundamental concepts of genetics and applied translational research in orthodontics will open new horizons in the procedures of gene mapping and gene therapy which provide a foundation for a new emphasis on precision orthodontics, which will establish a modern genomic basis for major improvements in the treatment of malocclusion and dentofacial deformities as well as many other areas of concern to orthodontists through the assessment of gene variants in each individuals.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

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