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Review Article **Orthodontics**

Precision Orthodontics: How Much Can Genomics and Gene Therapy Help?

Ranjana Ravindran[1*], Roopesh Ramakrishnan[1], Deepu Leander[1], Madhav Manoj K[1], Ravindran Ankathil[2*]

[1]Department of Orthodontics, PMS College of Dental Science and Research, Kerala University of Health Sciences, Kerala, India
[2]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150, Kubang Kerian, Kelantan, Malaysia

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***Corresponding author:** Ranjana Ravindran[1], Ravindran Ankathil[2]
[1]Department of Orthodontics, PMS College of Dental Science and Research, Kerala University of Health Sciences, Kerala, India
[2]Human Genome Centre, School of Medical Sciences, Universiti Sains Malaysia, Health Campus, 16150, Kubang Kerian, Kelantan, Malaysia

Abstract

With the newly emerging concepts and advancements in genetic/genomic technologies, information on causative or candidate gene(s), transcription factors or growth factors responsible for orthodontic cases such as craniofacial dysmorphologies, morphogenesis and differentiation of craniofacial tissues, midfacial defects, crowding of teeth, prognathism, genetic variations associated with susceptibility of developing and/or heterogeneity in treatment response of malocclusions are emerging. Promising results have been accrued on utilization of genetic testing for management of monogenic traits such as primary failure of eruption (PFE), and Class III malocclusion. As the impact of genetic/genomic factors on orthodontic treatment outcome is emerging, gene therapy as a novel approach is being explored for effective and precise orthodontic treatment. Despite certain challenges and biosafety issues, the emergence of some promising success stories and ongoing research in gene therapy provides an optimistic future in the field of precision orthodontics. However, in order for precision orthodontics to be implemented in daily practice, more exquisite research is warranted. This review encompasses a conspectus on the potentials of genomics and gene therapy in achieving precision orthodontics.

Keywords: Gene therapy, Precision orthodontics, Genomics, Modulation of growth, Personalised medicine, Genetics.

INTRODUCTION

Generally, appliances that mechanically modify bone size and shape and also bring about dentoalveolar changes are used for interceptive and comprehensive orthodontic treatment. However, use of these orthodontic appliances in bone modifications and tooth movements are not only complex, painful and require long periods of retention, but also are subject to significant amounts of relapse. Hence, there still exists a quest for optimization of the mechanics, materials and aesthetics of orthodontic appliances that could make orthodontic treatment more predictable and amenable to patients. Therapeutic adjuncts that may optimize bone remodelling during orthodontic treatment and improve hard tissue adaptation after treatment completion, were given less importance.

The conclusion of the Human Genome Project (HGP) in 2003 and subsequent developments in genomic technologies had resulted in accumulation of vast information on the role of genes and genomic variations in the etio-pathogenesis of several diseases and abnormal conditions. Not only that, this information has also provided clues on the role of individual genomic variations that account for differences in susceptibility risk and treatment response among patients with the same disease and/or abnormal conditions. The field of genetics is making its own prominence in the field of orthodontics also as a result of its newly emerging concepts day by day. This review summarizes the current knowledge of the role of genes and genomic variations in the etiopathogenesis of selected orthodontic cases and also discusses the potentials of gene therapy in precision orthodontics.

Genetics, Genomics and Orthodontics

The field of genetics is making its own prominence in the field of orthodontics as a result of its newly emerging concepts day by day. Genetics in

simple words deals with the mechanisms of heredity whereby biological characteristics or traits are transmitted from cell to cell, parents to offsprings and hence from generations to generations. The basic unit of genetics is the gene which is composed of specific DNA sequences. These DNA sequences in turn code for a specific protein. However, not all genes encode enzymes, some genes encode proteins with other functions while some genes do not encode proteins at all but produce functional RNA molecules (Primrose and Twyman, 2006). The uniquity of each individual can be attributed to genes and genetic variations. For this same reason, any defect of genes can result in disruption of normal cell activities and also abnormalities in cell structure and function.

Most oral, dental, craniofacial variations in growth and development, diseases and disorders such as malocclusions arise from complex interactions of genetic, biological, behavioural and environmental factors. Orthodontists regularly observe a great deal of variation between the facial growth responses of different patients to various appliances such as those used for mandibular protrusion. Genomics which is the study of all of a person's genes, investigates the complex biological details of an individual and the use of these for effective diagnosis and tailor-made treatment. Combination and interaction of genetic, genomic and environmental (including treatment) factors (nature and nurture together) that influence the treatment response of orthodontic patients is fundamental to the evidence based practice of orthodontics.

The comparative influence of genomics and environmental factors on the etiology of malocclusions has earlier been a matter of debate, deliberation and controversy in orthodontic literature (Carlson, 2015). However, in this current era, orthodontic researchers have begun to consider genomic information to improve diagnosis and treatment of dental disorders and developmental deformities in orthodontic patients. Multiple factors and processes contribute to the response of orthodontic treatment. Furthermore, more emphasis is given on the genetic/genomic factors underlying clinical problems that are seen more regularly in orthodontic practices such as malocclusion, tooth movement, and dental crowding rather than on less common, genomically less complicated craniofacial anomalies.

The onset of the orthodontics-genomics era at the start of the 21st century marked the start of a paradigm shift in orthodontic research, characterized most readily by the use of genetic basis of the specific molecular pathways underlying dentofacial development and deformities. Advanced genetic research studies that focused towards genomic basis of craniofacial growth and genetic variants of dentofacial abnormalities, had clearly shown that mandibular prognathism (class III) and cleft lip and palate are primarily genetic in origin (Carlson, 2015; Xue *et al.*, 2010) and also certain number of them are environmental in origin. Class III malocclusion involves expression of certain genes that encode specific growth factors (Indian hedgehog homolog, parathyroid hormone-like hormone, insulin-like growth factor-1, vascular endothelial growth factor) and harbour genes accountable for class III malocclusion. Whereas, trans-membrane protein-1 and GAD1 are responsible for occurrence of cleft lip and cleft palate. Likewise, genetic variants in REF, IRF6, PVRL1 and MSX1 are involved in the formation of syndromic associated cleft lip and palate (Cox, 2004; Suzuki *et al.*, 2004b; van den Boogaard *et al.*, 2000). A genetic role for orthodontic tooth movement influencing the remodelling is a clear possibility. Studies revealed that local osteoprotegerin (OPG) transfer inhibits the relapse of orthodontic tooth movement. Genes like SOX-9, PTHɤP and IHH, RANKL, MLCSF and RANKL-OPG pathways play significant role in avoiding ankylosis of tooth within and during orthodontic tooth movement. These pathways are supported by IL-1, αIL-6, IL-11, TNF-α, BMP2, BMP7, TGFβ and FGF (Siddharth, 2018).

In association with mechanical factors derived from orthodontic treatment, genetic predisposition as a result of inter-individual genetic variation has been reported to confer susceptibility or resistance to external apical root resorption (EARR) (Nieto-Nieto *et al.*, 2017). Several genes such as IL-1 gene cluster, P2RX7, CASP1, OPG, RANK, osteopontin, TNFα, vitamin D receptor, TNSALP and IRAK1 have been implicated to be influential factors for developing EARR secondary to orthodontic treatment (Sharab *et al.*, 2015).

Malocclusion seen in most of the instances is of polygenic/multifactorial cause (Graber *et al.*, 2012). The genome-wide association studies (GWAS) have associated many genetic markers on various chromosomes with class III malocclusion. However, analysis on malocclusion families using the latest next generation sequencing (NGS) technology had yielded growing list of genetic variants in key pathways of jaw development to be associated with Class III malocclusion in those families (Manocha *et al.*, 2019). The fact that most families have had a different gene involved in the etiology of malocclusion emphasizes the genetic heterogeneity of Class III malocclusion. Different variants in the same gene were also reported to be associated with class III or Class II malocclusion (Zebrick *et al.*, 2014). This warrants the need for further investigations of the genetic variants associated with malocclusion. These advancements led to the utilization of an individual's genomic evidence and clinical data to anticipate the predisposition for malocclusion(s), to implement treatment plans in a precision-based manner and thereby to customize orthodontics in a personalized

manner. This developing field is personalized orthodontics or precision orthodontics.

What is Precision Orthodontics?

Genetics, anatomy and past environmental exposure influence how each patient responds to treatment, either favourable response or unfavourable response. This phenomenon is known as heterogeneity of treatment effects (HTE). Precision medicine, or otherwise personalised medicine, has been defined by The Precision Medicine Initiative Cohort Program at the NIH, as an emerging approach for disease treatment and prevention which considers variations in genes, environment, and lifestyle for each individual. Precision medicine optimizes treatment for the individual rather than the average patient typically described in clinical trials (Zanardi *et al.*, 2012). Advances in genetics/genomics, proteomics, metabolomics, molecular biology and molecular medicine along with clinical profile and other relevant data are used in precision medicine to personalize/individualize treatment. The precision medicine approach appears to be relevant in the field of dentistry, especially in the field of orthodontics.

Due to its heavy dependence on appliances and forces to modulate tooth movement and to facilitate craniofacial orthopaedics, orthodontics definitely stands apart from other medical and dental fields. Therefore, it has to be assumed that precision orthodontics by necessity extends beyond the classic definition of precision medicine of the patients' individual biology and lifestyle to include customized care though well-planned treatment approaches. Precision orthodontics (PO) refers specifically to the tailoring of medical treatment to the individual characteristics of each patient considering maturity, physical features and genetic data as well. In other words, PO is the application of a specific type of treatment to an individual because that person belongs to a subpopulation of patients who are expected to develop disease and/or respond to treatment differently than the rest of the overall population based on his/her genetic/genomic profile (Vogenberg *et al.*, 2010). Neverthless, precise prediction of orthodontic treatment outcome is not tenable because of the heterogeneous complexity of facial and dental development, the physiology of tooth movement and the occurrence of EARR. Investigations are ongoing on many genetic factors and how they may relate to orthodontic treatment outcome. The following section discusses the potentials of gene therapy on orthodontic treatment.

Gene therapy and orthodontics

The original premise behind gene therapy (GT) in the 1990s was the belief that if a defective gene resulting in a specific disease could be replaced by a healthy gene, then the disease could be cured. The concept of gene therapy involves the deliberate introduction of exogenous gene/DNA/RNA into somatic cells of body organs, express its protein and produce a desired therapeutic effect and thereby correct the cellular dysfunction or provide a new desired cellular function (Rashid and Ankathil, 2020). Gene therapy is designed to introduce nucleotides into the cells to compensate for mutated genes or to restore a normal protein that is missing in an individual. If mutation causes a crucial protein to be defective or missing, gene therapy may be able to introduce a normal copy of a mutated gene to restore the normal function of the protein. Once the gene that encodes the target protein is integrated into a patient's genetic machinery, the required protein can be constantly produced at high therapeutic level (Wirth *et al.*, 2013).

GENE THERAPY FUNDAMENTALS

Gene therapy techniques involve identification and isolation of the target gene coding for the protein of interest, transfer of this gene into an appropriate production host and finally expression of the gene (Primrose and Twyman, 2006). Different methodological approaches are available for the introduction of DNA or RNA into different cell types to produce recombinant proteins, or replace or repair the target gene of interest in gene therapy. Corrective gene therapy where a normal gene is inserted into a non-specific location to replace a non-functional gene and thereby correcting the genetic makeup, is the most common approach. This approach is applicable for dominant disorders especially when the defective gene produces a disease causing protein or an interfering substance. Other approaches are swapping of abnormal gene by a normal gene through homologous recombination, repairing a defective gene through selective reverse mutation and thereby regain normal function of the gene, altering the regulation (the degree to which a gene is turned on or off) of a particular gene, knocking out or inactivating a mutated gene that is functioning improperly, and replacing of mitochondrial DNA by spindle transfer that changes entire mitochondria (Chatterjee *et al.*, 2013; Patil *et al.*, 2012; Rashid and Ankathil, 2020). Gene transfer or gene replacement involves direct delivery of DNA into nucleus, released from its complex and rendered competent for expression and/or interaction with the host genome. Gene therapy approaches require viral or non-viral vectors. Vectors are carrier systems or messengers used to transfer the desired genetic material across the cell membrane and preferably into the cell nucleus. Viral vectors commonly used are retrovirus, adenovirus, adeno-associated virus, lentivirus and herpes simplex virus. Non-viral vector methods of gene delivery involve physical or chemical methods (Rashid and Ankathil, 2020). The potential role of GT as a clinical tool has expanded and it is no longer limited to the replacement of defective genes, but rather has become a tool for producing as well as delivering individual proteins to specific tissues and cells.

ROLE OF GENE THERAPY IN ACHIEVING PRECISION ORTHODONTICS

The dynamic treatment mechanisms of gene therapy have been advancing by quantum leaps. These advancements are transforming the conventional approaches into more precise and preventive ones that may limit the need of using the former. Gene therapy as a genetic engineering technique has the potential to make possible the prevention of many antenatal, congenital and postnatal genetically induced dentofacial anomalies, including dental malocclusion. The gene therapy experiments in orthodontic treatment are still nascent and limited to cell cultures or animal experiments (Abraham and Maliekal, 2017). In orthodontics, gene therapy has started carving its niche mainly to i) modulate orthodontic tooth movement, ii) alleviate pain associated with orthodontic mechanotherapy and temporomandibular disorders and iii) modify growth.

GENE THERAPY FOR TOOTH MOVEMENT MODULATION

Orthodontic tooth movement has its foundation laid upon remodelling of periodontal ligament and alveolar bone. This requires the relay of mechanical loading to biological signals by alveolar bone (AB) cells such as osteoblasts, osteocytes and osteoclasts and periodontal ligament (PDL) (Atsawasuwan and Shirazi, 2018). The remodelling process has its reins handled by osteoclasts and osteoblasts. Precursors of osteoclasts and osteoblasts are hemopoietic cells and stromal cells respectively. Osteoclastic maturation and activation require interaction with cells from the osteoblastic lineage. The molecular mediators for such interactions are the receptor activator of the nuclear factor kappa B (RANK) or receptor activator of nuclear factor kappa-B ligand (RANKL) (Boyce and Xing, 2007b; Yamaguchi, 2009). Osteoclastic precursors are converted into multinucleated giant cells by interaction of RANK with RANKL. Osteoprotegerin (OPG), a soluble receptor produced by osteoblasts, is a competitive analogue with the RANK receptor binding to RANKL. Upon binding with RANKL, it inhibits osteoclastogenesis, thus obstructing the process of bone resorption (Boyce and Xing, 2007a; Boyce and Xing, 2007b; Yamaguchi, 2009). A balance between OPG and RANKL determines normal bone turnover and stable bone marrow. ATP/P2XR7/IL-1β inflammatory signalling pathway and the RANKL/RANK/OPG bone modelling and remodelling pathways are the two important pathways that influence orthodontic tooth movement and external apical root resorption (Neela *et al.*, 2021).

Gene therapy has been implicated to be beneficial in tooth movement. Two experimental studies (Kanzaki *et al.*, 2006; Kanzaki *et al.*, 2004) were conducted by using gene therapy with OPG and RANKL to decelerate and accelerate orthodontic tooth movement respectively, in a rat model. The gene transfer approach used a hemagglutinating virus of Japan (HVJ) envelope vector carrying mouse OPG messenger RNA (mRNA). RANKL gene was transferred locally to the periodontal tissue. It gave results that point towards accelerated orthodontic tooth movement by approximately 150% after 21 days, without evoking any systemic effects, thereby reducing the time of treatment (Kanzaki *et al.*, 2006). It was suggested that local RANKL gene transfer might be a useful tool not only for abbreviating the duration of orthodontic treatment, but also for treating ankylosed teeth**.** In contrast to RANKL, local OPG gene transfer inhibited tooth movement by about 50% after 21 days of application of force (Kanzaki *et al.*, 2004). These findings with gene therapy could cause a paradigm shift in orthodontic treatment by reducing treatment tim*e* with improved results.

Iglesias-Linares *et al.*, (2011) (Iglesias-Linares *et al.*, 2011) experimentally tested the efficiency of selective gene therapy with RANKL as an alternative to corticotomy surgery. This study utilized a hemagglutinating virus of Japan envelope vector containing mouse RANKL mRNA in rats for 32 days. The results showed increased level of RANKL protein by 3 folds in the gene therapy group and 2 folds in the corticotomy group after 10 days. This finding was however accompanied by evidence that pointed out the level of RANKL protein was maintained in the gene therapy group but not in the corticotomy group. The study concluded that gene therapy is more effective in terms of accelerating tooth movement compared to the latter.

In PDL and alveolar bones, the expression and roles of several microRNAs (miRNAs) have been reported (Irwandi and Vacharaksa, 2016; Luan *et al.*, 2017; Wang *et al.*, 2018; Zhou *et al.*, 2016). MicroRNAs are a class of non-coding RNA molecules, 20-23 nucleotides long that function as post-transcriptional regulators of gene expression in biological processes by repressing and fine tuning protein output (Li *et al.*, 2021). By binding to complementary target mRNA, the miRNAs play a central role in cell differentiation, proliferation and survival and thereby result in mRNA translational inhibition or degradation (Bartel, 2009). Selected miRNAs have the ability to target multiple mRNAs that are altered in disease conditions. This makes miRNAs interesting candidates as therapeutics or as targets of therapeutics (Iorio and Croce, 2012; Li *et al.*, 2014). In the last decade, endogenous miRNAs have been utilized as powerful tools for targeted gene delivery in gene therapy approaches. Gene therapy using endogenous miRNAs involve selection of candidate miRNAs, design of expression cassettes if constant expression is needed, selection of delivery carrier and finally evaluation of system in cell lines, animal models and clinical trials. miRNAs could be mechanosensitive and hence regulators of bone-remodelling process (Chen *et*

al., 2016). In PDL, osteoblasts and osteoclasts, miRNA-21 has been shown to have critical roles (Chen *et al.*, 2016; Li *et al.*, 2012; Li *et al.*, 2015; Sugatani *et al.*, 2011; Wei *et al.*, 2015; Wei *et al.*, 2014; Wei *et al.*, 2017). Aditionally, delayed tooth movement via inhibition of osteoclastogenesis was demonstrated in mice with deficit of miRNA-21 compared to the control mice. In human PDL, expression of miRNA-29 was reported to be up-regulated under compression but down-regulated under stretch force orientation (Chen *et al.*, 2015). These encouraging results suggest that gene therapy can be utilised for orthodontic tooth movement by means of targeting specific microRNAs.

A recently introduced genome editing system known as clustered regularly interspaced short palindromic repeats sequence and CRISPR associated (Cas) gene mechanism (CRISPR/Cas9) has garnered wide attention in genome research and clinical application. CRISPR /Cas9 is a programmable protein that can edit, eliminate and turn on/off genes. Cas proteins act as DNA locator system that can be programmed by scientists to find whatever bit of DNA they decide to send it to. Once located, the Cas proteins can be enabled with the ability to cut out a particular gene, turn on or off a gene or replace a gene with something totally different. CRISPR/Cas9 works by utilizing Cas9 enzyme which act as a pair of DNA scissors for addition or removal of a gene, while another molecule known as guide RNA (gRNA) binds to DNA and guides Cas9 to edit the specific part of the genome. Until now, no CRISPR/Cas9 experiment has been involved in orthodontic tooth movement, although this technology has been implemented in mineralized tissue research (Farr *et al.*, 2018; Lambert *et al.*, 2016; Sakaguchi *et al.*, 2018; Subramaniam *et al.*, 2018). As a novel and controversial technology, the potentials of CRISPR/Cas9 is only beginning to be explored. Hence it is likely to take decades to develop and implement CRISPR/Cas9 for gene / genome editing in orthodontic treatment.

As orthodontic tooth movement is dependent on efficient remodelling of periodontal ligament and alveolar bone, it is correlated with inflammatory process. The compressive force during orthodontic tooth movement induces angiogenesis of periodontal ligament. Angiogenesis is the process of new blood vessel development, found in different situations such as embryonic tisssues, inflammatory processes, wound healing and repair, and tumourigenesis (Stuani *et al.*, 2021). Vascular endothelial growth factor (VEGF) is an angiogenesis inducer. As the primary mediator of angiogenesis, VEGF exerts a fundamental role in remodelling periodontal ligament and also in bone resorption and formation (Ribatti *et al.*, 2021). Studies on mice by (Ren *et al.*, 2004) demonstrated that preosteoclasts might be recruited to the periodontal ligament during tooth movement and hence these cells appear to be targets for acceleration of tooth movement. Applied forces which orthodontists apply, engender remodelling of both mineralized and non-mineralized paradental tissues as well as associated blood vessels and neural elements (Krishnan and Davidovitch, 2009). Using rat model of tooth movement, Yang *et al.*, (2018) (Yang *et al.*, 2018) demonstrated that the expression of VEGF may play an important role in early remodelling of periodontal tissues during orthodontic tooth movement. As VEGF leads to enhancement of osteoclasts recruitment, it is important for angiogenesis and particularly for tooth movement. Therefore, local administration of recombinant human vascular endothelial growth factor (rhVEGF) that has been found beneficial in increasing the amount of tooth movement (Kaku *et al.*, 2001; Kohno *et al.*, 2003) is another potential gene therapy modality in orthodontics.

GENE THERAPY IN REPAIR OF ROOT RESORPTION AND RETENTION STABILITY

Using viral envelope packaging and delivery system, Kanzaki *et al* (Kanzaki *et al*., 2004) performed the OPG gene transfer experiment to investigate the inhibition of orthodontic relapse in rats. The first molars in the rats were moved mesially for 3 weeks, followed by removal of springs to generate orthodontic relapse in the rats. After administering OPG gene therapy, rats were observed for 2 weeks. This study demonstrated that relapse was significantly inhibited 2 times compared to the mock and control groups. The bone mineral density as well as bone volume fraction of alveolar bone were significantly increased in the gene therapy group compared to the mock and control groups. The investigators suggested that local OPG gene therapy to periodontal tissues could inhibit relapse after orthodontic tooth movement via an inhibitory effect on osteoclastogenesis (Zhao *et al.*, 2012a). Kanzaki and colleagues (2006) (Kanzaki *et al.*, 2006) further investigated the effect of local OPG gene therapy on orthodontic root resorption with the same design of experiment by utilization of a microcomputed tomogram and histological analyses. The result showed no difference between root resorption at the beginning and the end of tooth movement in the OPG gene therapy group. However, they were able to conclude that repair of root resorption in the gene therapy group was higher than other control groups (Zhao *et al.*, 2012b).

GENE THERAPY FOR ORTHODONTIC PAIN ALLEVIATION

Gene therapy has proved itself helpful in targeting tissue-specific biological pathways associated with pain. Also, long-term sustained analgesia can be achieved, thereby eliminating the need for repeated dosages. The use of gene therapy for alleviation of pain is still limited to animal models. Pain relief could be achieved through delivering endogenous opioid genes into neurons (Tzabazis *et al.*, 2014). Gene therapy for the alleviation of cancer pain has been applied in a human clinical trial and acceptable outcomes were

achieved (Fink *et al.*, 2011). Different types of viral vectors have been developed for transducing genes of interest into target cells, including adenovirus (Watanabe *et al.*, 2014), lentivirus (Seo *et al.*, 2014), herpes simplex virus (Grinde, 2013) and adeno-associated virus (Fischer *et al.*, 2014). The rationale for using the herpes virus was attributed to its unique property of neurotropism, which enabled it to travel to the dorsal root ganglia (DRG) via nerve endings in the skin. In the DRG, it has been reported to code for an inhibitory neurotransmitter, an anti-inflammatory peptide or decrease the synthesis of an endogenous nociceptive molecule that results in alleviation of pain (Smith, 2012).

The transient receptor potential vanilloid 1 (TRPV1) gene has been widely recognised as a key component of both inflammatory and neuropathic pain in the sensory system (Gunthorpe and Chizh, 2009). RNA interference (RNAi) is a powerful tool to silence gene expression or translation by neutralising targeted mRNA molecules in mammalian cells and is widely used clinically (Simonato *et al.*, 2013). Small interfering RNAs (siRNAs) are also non-coding short duplex RNA molecules that exert gene silencing effects at the post-transcriptional level by targeting mRNAs. However, siRNAs are highly specific with only one mRNA target (Lam *et al.*, 2015). Since siRNA is efficient at interference, it has become more popular in the application of the targeted interference of gene expression and related gene functions that are involved in pain regulation (Leung and Whittaker, 2005; Röhl and Kurreck, 2006). The role of TRPV1 in modulating pain associated with tooth movement was examined by injecting a TRPV1 antagonist into the trigeminal ganglia of rats. The results showed that the expression levels of TRPV1 protein and mRNA were markedly high following tooth movement pain. The TRPV1 antagonist significantly reduced tooth movement pain. Therefore, TRPV1-based gene therapy has been suggested as a potential candidate treatment strategy for the relief of orthodontic pain (Guo *et al.*, 2019).

GENE THERAPY FOR GROWTH MODULATION

Scientific advances in the 21st century have helped to understand molecular factors that regulate condylar growth (Rabie and Hägg, 2002). The rapid development of recombinant DNA technology has led to the development of growth factor based approaches. Using the specific gene encoding the proteins, large quantities of the therapeutic proteins for treatment purposes can be synthesized (Reddi and Cunningham, 1991; Ripamonti and Reddi, 1994). Local administration of insulin-like growth factor (IGF-I) in the mandibular condyle of rats has been shown to induce actual bone formation (Ito *et al.*, 2004; Suzuki *et al.*, 2004a). Although these findings are promising, the possible applications of these growth factors are limited by their short biological half-life which requires repeated administrations and expensive dosages (Zhang *et al.*, 2002). Recent advances in molecular biology have led to fast progress in the development of gene therapy and the most promising novel approaches to maximally stimulate bone formation in animals as well as in humans (Chen *et al.*, 2002). Since Baum and O'Connel (Baum and O'Connel, 1995) first described the potential impact of gene therapy on dentistry, gene therapy has forged its own position as an innovative strategy to induce bone formation.

Rabie et al through their extensive studies on mandibular condyle, were able to construct a delivery vehicle where potential therapeutic genes could be delivered to the condyle (Dai and Rabie, 2008). As a potent regulator of neovascularization expressed during endochondral ossification of the condyle, VEGF has been shown to play an important role in mandibular condylar growth (Dai and Rabie, 2008; Leung *et al.*, 2004; Rabie and Hägg, 2002; Rabie *et al.*, 2002a; Rabie *et al.*, 2002b). The mandibular condylar chondrocytes express VEGF which incites neovascularization and marks the onset of endochondral ossification.

Some successful gain of function studies with recombinant VEGF proteins or gene therapy has yielded significant increases in vascularization and bone regeneration in a defects model (Street *et al.*, 2002; Tarkka *et al.*, 2003). With the use of recombinant adeno-associated virus and lentivirus, successful gene transfer to the TMJ has been reported in animal model (Dai and Rabie, 2008). Moreover, rhVEGF administration leads to enhanced blood vessel formation and ossification in bone defects (Leung *et al.*, 2004; Rabie *et al.*, 2002b).

In vivo gene therapy with adenovirus mediated VEGF was demonstrated to modify bone defect healing (Hiltunen *et al.*, 2003; Tarkka *et al.*, 2003). A better understanding of the genes responsible for mandibular growth and safe methods of gene delivery into tissues may become a possibility for the treatment of mandible-deficient malocclusions. Thus, VEGF has possible clinical applications for inducing bone formation. Moreover, Rabie et al (Rabie *et al*., 2007) successfully established recombinant adeno-associated virus (rAAV) mediated VEGF delivery system and identified transgene distribution in the condylar cartilage and significant increase in the expression of chondrogenic and osteogenic markers. Dai and Rabie provided further evidence that local rAAV mediated VEGF gene transfer enhances the size of mandibular condyle leading to mandibular condylar growth. It has provided a basis to regulate mandibular condylar growth for future clinical practice (Dai and Rabie, 2008).

Basic fibroblast growth factor (bFGF) is an important regulator of tissue growth (Franceschi, 2005). Previous studies have shown that low intensity pulsed ultrasound (LIPUS) has a stimulatory effect on bone growth (El-Bialy *et al.*, 2006; Kaur *et al.*, 2014;

Oyonarte *et al.*, 2009). Kaur and colleagues (Kaur *et al.*, 2014) undertook a pilot study to evaluate the possible synergestic effect of LIPUS (due to its stimulatory effect on bone growth) and local injection of nonviral bFGF plasmid DNA (pDNA) on mandibular growth in rats. Within the limitations of their pilot study, they concluded that the combination treatment of bFGF and LIPUS has selective effect on the mandibular condyle growth (Kaur *et al.*, 2014).

Limitations and challenges of Gene therapy

At present, the application of gene therapy in clinical practice is limited by its biosafety concerns (VandenDriessche *et al.*, 2003). The success expected from gene therapy depends on its delivery system. For the delivery of the gene, either viral or nonviral vectors may be used as carriers. Viral vectors provide efficient gene delivery to the targeted tissue cells and longer duration of gene expression. Nevertheless, use of viral vectors for transgenesis is still doubted to be hundred percent safe and free of adverse side effects. In some rare cases, immune response against the viral vectors may happen whereas in some other instances, integration of a gene at an unwanted location can trigger an oncogene leading to tumorigenesis. Due to safety concerns associated with viral vectors such as immunogenicity and oncogenicity (Hacein-Bey-Abina *et al.*, 2003) a nonviral vector is the preferred gene delivery approach. Several fundamental cell culture and animal experiments need to be undertaken to demonstrate the safety and efficacy of the treatment concept. This should be followed by clinical trials to ascertain and ensure the clinicians and patients about the efficacy of treatment. Even after successful delivery of a desired gene, the new gene needs to be activated and remain activated. As human cells have a habit of shutting down genes that are too active or exhibiting unusual behaviour, this poses a challenge causing gene therapy to be short lived. Yet another limitation is lack of knowledge about the orthodontic diseases at the molecular (DNA) level.

Despite these challenges and biosafety issues, some promising success stories of gene therapy are emerging in the field of dentistry. Ongoing researches in the gene therapy field provide an optimistic future for dentistry field especially for precision orthodontics. The application of gene therapy in orthodontics has just seen the beginning of an era of immense potential and possibility. Extensive researches and clinical trials need to be undertaken so as to ensure that gene therapy finally can be employed safely in humans.

FUTURE PERSPECTIVES

Knowledge of the genetics related to orthodontics is helping to understand the etiology of malocclusion, normal genetic variation between individuals and prevention of malocclusion to some degree. Advancements in understanding the genetic basis of craniofacial development and the gene variants associated with dentofacial deformities have resulted in the integration of genetics into orthodontics that will lead to significant advancements of orthodontic treatments. Great progress has taken place in the identification of the genes for craniofacial dysmorphologies including growth factors and transcription factors controlling morphogenesis and growth of craniofacial tissues, candidate genes for midfacial deformities, and genes affecting growth and treatment. Researches are ongoing on the specific effects of genetic variants in growth factors and cytokines as they might affect dental development, tooth movement and resorption, temporomandibular joint pain and assessment of skeletal maturation. The early concept of Class III malaocclusion was thought to be governed heavily by genetic factors. However, major advancements in research discovered that it is somewhat multifactorial involving the interaction of both genetic and epigenetic factors at some levels. Modern genomic studies on the genetic mechanisms of facial variations show signs of future success in helping to understand the etiology of malocclusion and variations of dentofacial morphology. This knowledge gives helpful insights on the limits of what orthodontic treatment could achieve, which in turn influences orthodontists in diagnosing the severity of Class III malocclusion, choosing the appropriate treatment timing, planning the proper treatment as well as preventing relapse from happening. Current emphasis which is on precision orthodontics, 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 on a patient by patient basis. Personalized orthodontics might be customized or precise orthodontic care can be implemented to an individual in whom the genomic evidence and clinical data can be utilized and the susceptibility of developing malocclusion(s) can be anticipated.

The impact of genetic factors on orthodontic treatment outcome is gaining greater importance. Gene therapy as a novel approach is worth pursuing in orthodontics as it is precise, personalizable and effective. More and more genome-wide association, whole genome and exome sequencing studies need to be undertaken to understand how the interaction between nature (genetic factors) and nurture (environmental factors including orthodontic treatment) together affect the treatment and thereby provide evidence base for the orthodontists. As none of the existing databases contains comprehensive dentofacial data for malocclusions, phenotype-genotype correlation studies of malocclusion are of crucial importance. The knowledge gained from such studies will aid in our understanding of the mechanisms responsible for human malocclusion and craniofacial anomalies. Orthodontists need to learn more about genetic and genomics of orthodontic related conditions, how to

interpret these genetic/genomic data and apply to practice including counselling the patient and family regarding the associated higher or lower risk for development of one or more conditions.

As very well stated by Hartsfield Jr (Hartsfield Jr *et al*., 2021), precision orthodontics is warranted in the following situtations i) when a patient's phenotype is driven or influenced by a specific etiology, ii) when a variable response to treatment is observed and/or iii) when variation in stability following orthodontic treatment with or without surgical correction is noted. Although testing of genetic factors for day to day orthodontic practice is not yet ready, promising results are emerging for utilization of genetic tests for monogenic traits such as primary failure of eruption and Class III malocclusion. Nevertheless, how much will precision orthodontics impact on the daily practice and into the future, still remains to be seen. In order to evaluate the impact that precision orthodontics will have on everyday orthodontic practice, exquisite research is warranted. For precision orthodontics to take a standpoint in standard care delivery, it is indeed necessary for orthodontics to evolve into a hybrid discipline utilising both mechanics as well as genomic components. Thus precision orthodontics can move towards better progression and optimise treatment by delivery of precise individualised care and thereby produce outstanding results.

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