Tooth agenesis is the failure of tooth bud development, causing definitive absence of the tooth. It is the most common dental anomaly, affecting up to one-quarter of the general population. The main cause is related to abnormal function of specific genes which play key roles during odontogenesis, particularly MSX1 and PAX9. MSX1 is a transcription factor highly expressed in the mesenchyme of developing tooth germs, whereas PAX9 is a transcription factor that shows a direct relationship with craniofacial development, particularly the formation of the palate and teeth. Despite the high frequency of tooth agenesis, there are as yet only a restricted number of mutations in MSX1 and PAX9 that have been associated with non-syndromic tooth agenesis. Thus, a deeper analysis of the gene networks underlying this anomaly is imperative. By means of a literature review based on Medline, PubMed, Lilacs, NCBI, and STRING, performed between 1991 and 2010 and focused on etiologically associated mutations, this work aimed to assess the latest advances in the genetic etiology of tooth agenesis and to offer an insight into how they can assist dental practice in the near future. A better knowledge of the genetic networks underlying tooth agenesis will lead to better treatment options and, perhaps, a tool for early diagnosis possibly related to DNA examination based on polymorphic variants. Such a test based on DNA analysis may be available to and accessible by clinicians, resulting in a more accurate diagnosis and allowing for a better approach to this anomaly.

Keywords: dental agenesis; molecular biology; MSX1 transcription factor; mutation; PAX9 transcription factor

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be used in the early diagnosis of the referred abnormality, which would allow patients to receive more effective treatment.

The aim of this review was to assess the latest advances in the genetic etiology of tooth agenesis and to offer an insight into how they can assist dental practice in the near future.

**Clinical Features of Tooth Agenesis**

Clinically, hypodontia (Fig. 2) presents in individuals congenitally missing from one to six teeth (except third molars), and it has been used to identify common and mild forms of agenesis (Arte 2001); oligodontia indicates that more than six teeth, except third molars, are lacking, and it is often applied to more severe cases (Stockton et al. 2000). Application of these terms should be carefully evaluated, since they may mislead clinicians regarding the severity of some cases, particularly those in which third molars are excluded. Anodontia constitutes an extreme case, the complete absence of teeth, and it is usually part of syndromes (Arte 2001).

Hypodontia is more prevalent in the permanent dentition, although deciduous dentition can also present the anomaly, at much lower rates. The most often absent teeth are third molars (Polder et al. 2004), followed by either upper lateral incisors or lower second premolars (Bredy et al. 1991; Arte 2001; Polder et al. 2004; Nieminen 2009). Both upper lateral incisors and lower second premolars account for 85% of all missing teeth (with third molar agenesis not considered) (Arte 2001).

Shimizu and Maeda (2009) reviewed the prevalence of congenital absence of teeth in the Japanese population. After having analyzed 11 reports involving 2200 affected individuals, they found the lower second premolar as the most frequently absent tooth, accounting for 27% of the total missing teeth. In a meta-analysis study, Polder et al. (2004) evaluated data for tooth agenesis in permanent dentition among Caucasian populations in North America, Australia, and Europe. They found that the majority of affected individuals (up to 83%) showed the absence of one or two permanent teeth and that unilateral agenesis of upper permanent lateral incisor is more prevalent than bilateral agenesis.

Since one of the most common forms of hypodontia is agenesis of upper permanent lateral incisors (ULIA), this has been specifically investigated. Recently, Pinho et al. reviewed the prevalence of...
(2010a) suggested that ULIA is a distinct kind of hypodontia. From a survey with 62 probands and first-degree relatives from Portugal, the results indicated that the probands’ relatives showing ULIA had a 15-fold greater risk of developing the same type of agenesis than the general population. Furthermore, these investigators found that ULIA almost never segregates with other forms of agenesis, which could indicate ULIA as a distinct clinical and genetic type of hypodontia.

Correlation between agenesis of a deciduous tooth and its permanent successor does exist, since agenesis of a given deciduous tooth is mostly followed by agenesis of the corresponding permanent tooth (Nieminen 2009). When it comes to families, the frequency of agenesis affecting one tooth class among relatives is significantly higher than that affecting different tooth classes (Arte 2001), supporting the genetic etiology of the anomaly.

Several dental anomalies have been associated with isolated tooth agenesis. Microdontia, impacted canines (Boeira Junior et al. 2000), and peg-shaped crowns can be easily identified in affected patients. Although at lower rates, ectopic eruption, transpositions, enamel hypoplasia, enlarged leeway space (space that remains during the transition from the deciduous to the permanent dentition), and retained deciduous teeth have also been shown to be linked. Furthermore, it has been suggested that dental developmental anomalies and tooth agenesis would result from different expressions (defects) of the same genes, mostly MSX1 and PAX9 (Ahmad et al. 1998; Nieminen 2009).

Possible influences of tooth agenesis on craniofacial form were investigated by Tavajohi-Kermani et al. (2002), who found little but significant correlation between agenesis and changes in cephalometric measurements, particularly regarding the maxilla. Significant decreases in maxillary jaw size were associated with tooth agenesis. These authors also concluded that missing upper teeth had a greater influence on craniofacial form than did missing lower teeth.

Etiology

The underlying genetic mechanism leading to tooth agenesis has lately been linked to some gene mutations, although some environmental factors—such as chemotherapy/radiotherapy, trauma in jaws, and maternal diabetes—have also been shown to be involved (Näsmann et al. 1997). Indeed, studies of families have shown a concordance in intra-familial phenotypes (van den Boogaard et al. 2000). For example, in most cases of twins, monozygotic show a rather more concordant phenotype than dizygotic twins (Vastardis 2000).

Interestingly, there seems to be an association between agenesis and cancer. Families segregating lip and/or palate cleft may show increased susceptibility to cancer, particularly colon cancer. Evidence has also shown that some genes, such as the AXIN2 (AXIS inhibition protein 2), may be concurrently correlated to tumor development and tooth agenesis (Menezes et al. 2009).

Roles of the MSX1 and PAX9 Genes

Studies of tooth development in mice have shown more than 200 genes to be involved in odontogenesis regulation, which is a highly complex phenomenon regulated at the molecular level. To date, literature has reported that genes such as MSX1 (muscle segment homeobox 1) and PAX9 (paired box 9), among others, play key roles in tooth development, showing sequential and reciprocal signaling processes instead of one-way pathways. That is, there are several ways for those processes to occur, thus preserving tooth development in most cases. For example, in mice, MSX1 and PAX9 genes form an auto-regulatory gene network where PAX9 activation is required for completion of tooth development (Nieminen 2007). Evidence from human population studies has also shown that there is a similar molecular mechanism regulating odontogenesis (Vieira et al. 2004).

MSX1 contains the homeobox (a specific sequence for interaction with DNA), and it has also been called the master regulatory gene, since it participates in the development of several organs. MSX1 is expressed in a temporally restricted manner, from the fourth gestational week until completion of root formation of all teeth (around 21 years). During embryogenesis, it also regulates the position of several organs other than the dentition, in addition to the regulation of patterning along the antero-posterior axis of human embryos. Theoretically, any mutation in such genes may cause cells to misread their position, forming organs in regions other than those where they should (Arte 2001).

MSX1 is located on chromosome 4 and has been found to be highly expressed in the mesenchyme of developing tooth germs (Jumlongras et al. 2001), particularly during the early stages (bud and cap).

PAX9 is a developmental control gene containing the paired domain, which is a sequence capable of specific interaction with DNA. Located on chromosome 14, it encodes for transcription factors that act in organogenesis regulation during early embryonic development. It is expressed in the mesenchyme in the maxillary and mandibular arches, showing a direct relationship with craniofacial development, especially in the formation of the palate and teeth. This gene also establishes the place and time of organ initiation or morphogenesis. Furthermore, it has been suggested that PAX9 acts in marking mesenchymal-specific sites where future teeth will form (Kim et al. 2006).

Mutations in these two genes are suggested to cause selective tooth agenesis: Defects in the MSX1 are particularly associated with second premolar and third molar agenesis, whereas PAX9 mutations are mostly associated with permanent molars. Concerning oligodontia phenotype, MSX1 is frequently associated with the absence of upper first bicuspids, whereas PAX9 is most frequently associated with the absence of the upper and lower second molars (Jumlongras et al. 2001). Nonetheless, there are cases
where mutations in these genes have not been found in patients with non-syndromic hypodontia, thus suggesting that other genes and/or a multigenic inheritance (involving genes in association) may also be considered as likely etiologies for this anomaly (Pinho et al. 2010b).

Interactions among Other Genes

Other genes potentially involved in tooth agenesis pathogenesis have been reported in the literature. In short, MSX1 and BMP4 (bone morphogenetic protein 4) form an auto-regulatory gene network in which PAX9 is included mainly at an early stage, although PAX9 activation is required for completion of later stages of tooth development (Peters and Balling 1999). Accordingly, any mutation may be able to unbalance the gene network, causing tooth buds to be arrested, which may lead to tooth agenesis. Wnt signaling has also been investigated, since it has been implicated in the regulation of embryonic patterning and morphogenesis of a large number of organs, including the development of human dentition. Indeed, a mutation in the Wnt-signaling regulator AXIN2 was identified in a Finnish family affected with oligodontia. Moreover, oligodontia would be a risk factor for the development of colorectal neoplasia in that family, suggesting a potential correlation between these two distinct phenotypes (Lammi et al. 2004).

TGFA is another gene claimed to be involved with tooth agenesis. Indeed, interaction between MSX1 and TGFA genes in individuals with oral clefts has been found. However, there is no consensus among authors concerning the interaction between MSX1 and TGFA. Some studies show no statistically significant evidence of interaction between these genes in human tooth agenesis (Vieira et al. 2004), whereas others indicate strong correlation (Jugessur et al. 2003) among individuals with clefts.

Mutations Associated with Tooth Agenesis and How This Knowledge Can Be Beneficial

The key role of molecular genetics regarding the identification of the genetic causes underlying tooth agenesis is being demonstrated. While several studies (Nieminen et al. 1995; Peters and Balling 1999; Scarel et al. 2000; Gerits et al. 2006; Swinnen et al. 2008) failed to detect mutations in MSX1 and PAX9, suggesting that other genes, among the more than 200 involved in tooth development, are probably implicated, indeed only a restricted number of mutations in MSX1 and PAX9 genes have been associated with hypodontia and/or oligodontia so far. Generally, these mutations cause loss of function in MSX1 and PAX9, thus leading to haploinsufficiency (reduced amount of functional protein). Accordingly, the amount of functional protein required to maintain tooth development is reduced, and abnormalities in odontogenesis may occur, including arrest of the tooth bud. The etiology of oligodontia has been linked to the haploinsufficiency of MSX1 and PAX9, whereas hypodontia has been more correlated with mutations in PAX9. The first mutation associating MSX1 and tooth agenesis was depicted in 1996 based on a family with autosomal-dominant agenesis of second premolars and third molars (Vastardis et al. 1996). The first mutation in PAX9 causally related to tooth agenesis was found in 2000 from a family segregating oligodontia lacking most permanent molars (Stockton et al. 2000).

Venous blood samples have been normally used to obtain DNA for subsequent genetic analysis; however, modern techniques for DNA isolation and purification have shown that buccal epithelial cells are a reliable source of DNA to search for mutations. Dentists are well-positioned to collect these buccal cells by simply scratching the inside of the patient’s cheek with a cytology brush. Theoretically, the easiest, fastest, most non-invasive and painless method for the collection of test materials from humans will lead to a larger amount of individuals agreeing to participate in studies for this purpose, since a significantly smaller number of individuals agree to donate when blood samples are required (Hansen et al. 2007).

Accordingly, it is assumed that novel mutations associated with tooth agenesis will be found in the coming years, substantially increasing our understanding regarding its genetic etiology.

MSX1, PAX9, Bioinformatics, and Dentistry

The investigation and sequencing of genomes around the world, as in the Human Genome Project, are providing massive amounts of data which require new technologies for analysis and interpretation. Also, new research fields are being created or enhanced for the further study of these information sources. Thus, bioinformatics (computational molecular biology) is a promising field that uses computers to handle biological information such as investigation of the genetic code, protein interactions, and clinical outcomes. Experimental knowledge coming from dozens of scientific journals around the world needs to be organized and correlated, whenever applicable. Since there are numerous proteins whose interactions remain to be identified, STRING version 8.2 software (Search Tool for the Retrieval of Interacting Genes/Proteins) can be a useful tool. MSX1 and PAX9 proteins form a genetic network where other proteins are also included. But how strong is the MSX1 and PAX9 relationship, and with what other proteins do they interact? MSX1 and PAX9 present an interaction scored 0.995 according to the STRING tool (Fig. 3). At the time of this analysis (http://string.embl.de/, accessed March, 2010), they correlated with each other more strongly than with others. Such information is possible from crossing multiple data as provided by specialized publications. Many other types of software are available through the World Wide Web, and the way information is being processed is constantly changing.

Regarding dentistry and other health sciences, it seems that the capability of achieving new levels for early diagnosis and approaches to treatment will involve further studies in molecular genetics as well as powerful tools to process all these new data. Nonetheless, the way clinicians will
deal with all this information in their offices is an imperative question that has already been raised (Wright and Hart 2002). How can this knowledge be effectively applied in treatment planning? Perhaps the answer relates to specific training in molecular genetics, given the new demands of clinical dentistry. Furthermore, clinicians will be given new tools for diagnosis and treatment evaluation to complement the current therapeutic approach, which will further personalize dental treatment.

**Current and Future Therapeutic Approaches**

Missing teeth compromise human health, both physically and emotionally, and usually require multidisciplinary treatment. Orthodontic space closure, deciduous tooth maintenance, implant therapy, and adhesive bridgework, among other prosthetic and esthetic dentistry resources, are the most common current approaches (Furquim et al. 1997). However, human health care in the 21st century demands treatment options which are more biologically compatible. At the same time, clinical dentistry is being reformed to support clinicians in this all-new horizon by training them for the new genomics tools. Molecular genetics and bioengineering play key roles in the development of new technologies, such as the use of BMP2 synthetic protein (laboratory production) to assist in the growth of alveolar bone for posterior implant therapy, which is already available for use. Another promising field is craniofacial genetics, research which is suggested to provide a better insight regarding the phenotypic correlation between facial morphology and tooth agenesis (Tavajohi-Kermani et al. 2002).

Although dentistry has achieved a high level of diagnostic and treatment accuracy, a biological replica of a missing tooth is the natural evolution of a therapeutic approach that is entirely compatible with the human organism. Regenerative dentistry is on its way, since the regeneration of tissues and organs has become feasible. Adult stem cells isolated from human dental tissues such as the dental pulp, dental follicle, and periodontal ligament theoretically match the purpose of differentiation into tooth-related cells that will produce dental tissues: that is, a bioengineered tooth. Nonetheless, there are still some obstacles to overcome, such as the correct number of stem cells and the growth factors to be combined, an accurate spatial arrangement, and how tooth size and morphology are managed. Still remaining unsolved is an accessible source of epithelial, tooth-related stem cells which will form enamel, since the only source known thus far is tooth germs from young children (Koussoulakou et al. 2009). Even if all of these issues had been addressed, the formation process of this “bio-tooth” until its completion would require a long-term course, which is not commercially feasible. However, cutting-edge technology, along with the growing knowledge base in the bioengineering field, will allow this aim to be accomplished.

There seems to have been an increasing trend toward agenesis during the 20th century. Since the available data are not sufficient to support this assumption (Mattheeuws et al. 2004), further research is necessary to unveil whether this trend was due to more accurate techniques and patient awareness, or whether humans are dealing with a real ten-
dency toward the increased frequency of tooth agenesis (Vastardis 2000; De Coster et al. 2009). In either case, a deeper analysis of the gene networks underlying tooth agenesis is critical. Such efforts will allow for better treatment options and, perhaps, an early diagnostic tool which would possibly lie on the DNA examination based on polymorphic variants (mutations). Once a great number of mutations are available, it will be possible to design such a DNA test to be performed, even in newborns and other individuals who lack complete dentition and are at risk of being affected. Thus, waiting for the manifestation of tooth agenesis will no longer be needed. This early diagnostic tool based on DNA analysis may be available to and accessible by clinicians, resulting in more accurate diagnosis and allowing for a better approach to tooth agenesis. However, a minimum case-based study of affected individuals is required for the establishment of such a diagnostic tool. Clinicians are able to contribute by referring affected families for investigative research, thus assisting in the development of new therapeutic resources.

Conclusion

The most common craniofacial anomaly, tooth agenesis, is still a challenge to be overcome. An etiology strongly conditioned by genetic factors, the small number of linked mutations found so far, the right time and locale for each involved gene to be expressed, and the complexity of the genetic networks regulating odontogenesis—these are some of the factors that need enlightenment. In contrast, the last decade brought a faster and more accurate method of processing and analyzing genetic data, increasing the knowledge regarding the etiology of tooth agenesis. Thus, training clinicians for the new genomic tools seems important, though, given the significant issues of data analysis, the expertise of geneticists is also required. Since one of the major obstacles to the modern investigation of disease is still the recruitment of a study population, any survey on the present issue, even involving only a few cases, may contribute. Likewise, clinicians from private offices and dentistry residency clinics are able to cooperate by referring families segregating tooth agenesis for genetic research. Such behaviors may assist in the identification of polymorphic variants and mutational ‘hotspots’ for tooth agenesis, since the genetic pathways underlying most cases are still unknown. Moreover, an early diagnosis based on DNA specificity may be performed by means of a comparative analysis between these genetic variants and DNA from unaffected individuals showing likelihood of a given individual presenting with agenesis.

Regardless of the restricted number of mutations associated with tooth agenesis to date, it is clear that some genes, such as MSX1 and PAX9, play key roles during the development of dentition. Thus, the key for the development of better resources for tooth agenesis diagnosis and treatment involves the clarification of processes regulating the initiation and morphogenesis of teeth, including gene defects such as mutations. Advances in technology and further studies evaluating gene expression in larger samples may be valuable for the development of an early diagnostic tool and a better approach to tooth agenesis.

Conflict of Interest

The authors have no conflict of interest.

References


