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FAMILIAL AGGREGATION OF CONGENITAL HYPODONTIA

by

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Abstract

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Prevalence rates of congenital hypodontia have been extensively characterized for various populations. In certain families, members are more likely to be affected with the condition than individuals from the general population, yet no specific genetic relationship has been established using genetic epidemiological means. To assess whether congenital hypodontia conforms to specific Mendelian patterns of inheritance, this study evaluated eighty-eight nuclear families from the Cleveland, Ohio metropolitan area via survey and radiographic follow-up. Data analysis included a calculation for correlation for each relationship. None were significant. The presence of a missing tooth in one family member did not imply that other members were, or were not, likely to have a missing tooth. Recurrence risk ratios for the sample populations studied were compared to the general population. For individuals missing second premolars, family members had a higher risk of missing teeth compared to the general population. For individuals missing maxillary lateral incisors, parents had a slightly higher risk of missing teeth compared to both siblings in these families and the general population. For individuals missing any tooth, family members had a slightly higher risk of missing teeth compared to the general

population. The results indicate that, while some risk was evident in certain samples studied, a clear pattern of inheritance was not detected. Congenital hypodontia appears to be a complex trait with many genetic variants. Molecular analysis of individual families with this trait might identify specific causes of hypodontia which were not detected by this epidemiologic approach.

DEDICATION

This work is dedicated to:

Our Lord, who makes all things possible,

my brothers, Jeff and Paul, who continue to amaze me with their creativity,

my parents, John and Barbara, who have sacrificed in every way possible for me,

my wife, Kim, who's tireless love and companionship knows no boundaries.

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TABLE OF CONTENTS

Page

ABSTRACT.....ii

DEDICATION.....iv

ACKNOWLEGDEMENTS.....v

LIST OF TABLES.....viii

INTRODUCTION.....1

REVIEW OF THE LITERATURE.....3

MATERIALS AND METHODS.....32

RESULTS.....37

DISCUSSION.....46

CONCLUSIONS.....54

APPENDICES.....55

REFERENCES.....63

LIST OF TABLES

Table Page

1. Distribution of Missing Teeth.....37

2. Correlation Values Unadjusted for Ascertainment.....39

3. Likelihood Ratio Tests Adjusted for Ascertainment.....41

4. Recurrence Risk Ratios.....43

INTRODUCTION

The last decade has seen a tremendous expansion of scientific knowledge in human genetics. Since the initiation of the Human Genome Project in 1990, our understanding of human genes and of the genetic contribution to disease has grown dramatically. The tools now exist to better define which segments of genetic information are significant in disease recognition and to better understand the pathogenesis of diseased biological processes. It is now documented that some variations in genes can contribute to a host of common conditions including congential hypodontia.

The genetic epidemiologic approach to understanding genetic causes of congenital hypodontia involves finding patterns of hypodontia and/or oligodontia in affected families. If specific patterns can be identified, investigations of specific gene sequences that are associated with these patterns may yield significant results. Several genetic causes may be uncovered, depending on the phenotypic presentation of congenital hypodontia in affected families.

Development and morphodifferentiation comprise a complex set of processes where any number of genetic abnormalities may compromise tooth formation. Recent studies have improved our ability to describe and understand tooth morphogenesis; these studies have yielded the identification of combinations of regulatory and structural genes required for tooth morphogenesis. These discoveries

of the normal developmental processes of odontogenesis paired with a better understanding of abnormal developmental processes may lead to new therapies for hypodontia. Hypodontia often complicates orthodontic treatment and can result in compromised esthetics and/or functional occlusion. In these instances, an excellent long-term result might be difficult to attain because typical treatment includes closing an unusually large space and a substitution of one tooth for another, or opening space for prosthetic replacement of the missing tooth.

Today's mechanical solutions may eventually be replaced by biologically based treatments. A congenitally missing tooth might be replaced with a laboratory "grown" tooth. The principles for the design and fabrication of tooth organs have been discovered. Presently, laboratories are showing promise with adult stem cell mesenchyme for application to tissue engineering. This will ultimately lead to bold frontiers in dentistry where dental replacements are like natural teeth.

LITERATURE REVIEW

Prevalence of Congenital Hypodontia

Several theories have been developed regarding the causes of congenital tooth absence.[1] One theory proposes that the absence of teeth is related to three morphological fields of the dentition. Typically, one tooth in each field is stable; usually the most mesial tooth (central incisor, first premolar, etc.). Flanking teeth to the distal become progressively less stable (lateral incisor, second premolar, etc.). Another theory proposes that the most posterior tooth of a tooth series would be most frequently missing. A third theory suggests that the variation in expression and penetrance of developing tooth germs is a function of the interaction between tooth germs during development. Here, agenesis develops where there is insufficient primordia for tooth germ initiation. Lateral incisors develop after central incisors and their initiation depends on the availability of local factors. If local factors are not sufficient, the tooth will not form. Peg lateral incisors develop in the presence of sufficient primordia, but a poor environment. An additional theory proposes that certain areas of embryonic fusion are more susceptible to epigenetic influences (agenesis). Finally, it has been proposed that tooth agenesis within a developmental field is most likely where innervation occurs last.

Early studies determined the prevalence of hypodontia in various sample populations to be between 2.06% and 10.1%, with most between 3% and 4%.[2] The

variation observed in these studies may be due to many factors. The two most likely are a small sample size and the different populations evaluated.

Several studies differ as to which permanent teeth are most likely to be absent. Hunstadbraten[2] determined that the third molar was the most likely tooth to be congenitally absent followed by the mandibular second premolar. The third and fourth most common missing teeth are maxillary second premolars and maxillary lateral incisors respectively. A more recent study by Arte et al.[3] in 2001, found that the lower second premolars were most likely found to be missing (47%), followed by upper second premolars (30%) and upper lateral incisors (17%) when third molars were not considered.

Other studies contradict the previously mentioned studies.[4,5] The most common missing tooth was the maxillary lateral incisor in a sample of over 11,000 college students, followed by the mandibular and maxillary second premolar.[4] Of individuals missing maxillary lateral incisors, nearly 52% were missing other teeth. Individuals missing second premolars showed a 69% chance of missing other teeth. Another study with a similar pattern found that the maxillary lateral incisor was most often missing followed by mandibular and maxillary second premolars respectively.[5]

In a descriptive study of over 5,200 patients, 231 were missing one or more teeth. Radiographs were used to verify the congenital absence of teeth. The mandibular second premolar was most often missing (57% of the cases) the maxillary lateral incisor was second (33%) and the maxillary second premolar was third (27%). Relatively few (16 of 231) were missing other teeth. Typically, individuals with

congenitally absent teeth were missing only a few teeth; 42.4% had one tooth missing, 38% had two teeth missing, and only 19.6% had three or more teeth missing.[6]

The prevalence of congenital hypodontia in a population other than Northern European produced different findings. The most common missing tooth was the mandibular incisor in 47% of a Japanese population.[7] The second-most common missing tooth was the mandibular second premolar, followed by the maxillary lateral incisor. An association between congenitally missing teeth and hypoplastic teeth was observed. Individuals were three-times more likely to have hypoplastic teeth when they presented with hypodontia.

The pattern of hypodontia differed for females (9.2%) and males (5.8%). These prevalence rates were duplicated when only maxillary lateral incisors were studied in other populations. Evaluation of over 5,000 college students revealed that females (1.3%) were affected to a greater, statistically significant degree than males (0.8%).[8]

A review paper by Graber,[9] in 1978, evaluated previous studies of hypodontia in non-syndromic individuals. He found a range from 1.6-9.6%, excluding third molar agenesis. Graber noted that several repeated studies had a serious error in data collection. They were performed without complete radiographic surveys to verify the tooth was missing. Without this, estimates of hypodontia tended to be inflated. Errors in sampling were also noted. The studies Graber evaluated did not identify how the participants were related to one another. Maxillary lateral incisors were the

most common tooth missing (40-45%). The mandibular second premolar was the second most common missing tooth. Also, most patterns were bilaterally symmetrical, except maxillary lateral incisors, where missing the left incisor was more common.[9]

These observations have helped in the development of two gene theories. The single gene theory proposes that crown size and shape and hypodontia are independent. In this senario, a single gene defect would present multiple pleiotropic manifestations. Here, a peg-shaped maxillary lateral incisor, is thought to be a microform of an oligodontic trait. The polygenic theory proposes that more than a single gene is responsible for phenotypic expression of congenital hypodontia. This could help explain the poor correlation between the many morphological variations and tooth absence, size, and shape.[9]

Determining the genetic basis for congenital hypodontia involves the evaluation of families of individuals previously identified with congenitally missing teeth. The members of these families have the trait in excess of individuals from the general population. A recent study found the prevalence in first degree relatives to be 39%, second degree relatives 36%, and the general population 5%.[3] Another study evaluated relatives of a group of children with missing teeth. These relatives had a prevalence rate for missing teeth of 33.8%, while the control group had a rate of 4.4%.[10] Furthermore, the relatives of probands missing 1-5 teeth had a prevalence rate of 30%, while the relatives of probands missing 6 or more teeth had a prevalence

rate of 47%.[10] As the severity of hypodontia increased in the probands, relatives were more likely to be affected.

In 1974, Suarez and Spence,[11] used previously published data to determine whether missing teeth in siblings and parents would match a single-locus or a polygenic model. The single-locus model has two quantitatively different alleles (A_1 and A_2) combined to form three possible genotypes (A_1A_1, A_1A_2, A_2A_2).[11] Individuals with hypodontia would lie beyond the liability threshold: if a recessive trait, only A_2A_2 would lie beyond the threshold; if a dominant trait, only A_1A_2 and A_2A_2 would lie beyond the threshold. They concluded that two thresholds, a moderate threshold and a severe threshold, would be necessary.

The polygenic model involves several loci as potential causes of congenital hypodontia. These loci and various environmental contributions would be additive in causing the phenotypic trait of hypodontia. It is assumed that the moderate threshold location (T_m) is between individuals with no missing teeth and individuals with one or more missing teeth and the severe threshold location (T_s) is between individuals with four and five missing teeth. Chi-square can be used to determine differences between expected and observed results from siblings and parents. Separate calculations for the single-locus and polygenic model found that the polygenic model best fit the data.[11]

The polygenetic mode of inheritance has subsequently been characterized as being an autosomal dominant trait with variable expressivity and incomplete penetrance. Brook[10] showed that the frequency of hypodontia among first degree

relatives equaled the square root of the frequency in the general population (control group). This supported multifactorial inheritance. A quasi-continuous distribution with the anomalous traits hypodontia and microdontia at one end of the bell-shaped curve, and traits of macrodontia and supernumerary teeth at the other end, was presented. In addition, quantitative differences were noted between males and females, a higher prevalence of supernumeraries and macrodontia in males and a higher prevalence of microdontia and hypodontia in females.[10]

Chosack et al.[12] attempted to validate the polygenic model of hypodontia using a sample of 305 probands and their parents and siblings. Among siblings and parents, the prevalence of hypodontia was significantly higher than for the general population. The prevalence of missing maxillary lateral incisors was over 10% compared to 2% from the general population. The prevalence of missing mandibular lateral incisors was over 9% compared to less than 1% in the general population. Offspring manifested clinical hypodontia more often when one parent presented with hypodontia. The authors suggested that hypodontia, in general, and lateral incisor hypodontia specifically, was a polygenic trait.

Another study evaluated 1,295 schoolchildren, both clinically and radiographically, for the prevalence of congenitally missing teeth.[13] Of the total number evaluated, 10.1% had congenitally missing teeth, not including third molars.[13] More mandibular teeth were found to be absent (52%) and more females had missing teeth (158) than males (108). The majority were missing a small number of teeth; seventy-five percent were missing two or fewer teeth. The most common missing

tooth was the mandibular second premolar (46%) followed by the maxillary second premolar (28%) and the maxillary lateral incisor (12%). These three teeth accounted for nearly 86% of all congenitally missing teeth, excluding third molars.

A study of 165 pairs of twins, 99 monozygotic and 66 dizygotic, found that nine pairs of monozygotic twins had missing teeth and five pairs of dizygotic twins had missing teeth.[14] Eight of the nine pairs of monozygotic twin were concordant in hypodontia, meaning that if one twin was missing a tooth so was the other. From the data, the empirical risk of hypodontia was calculated at 89%. Conversely, all five pairs of dizygotic twins showed discordance, meaning than none of the five pairs were both missing teeth. This revealed an empirical risk of zero. Hence, the risk of one dizygotic twin having hypodontia if the other twin had hypodontia was no different than if they were unrelated. There was no more of a genetic predisposition toward missing teeth than the general population.

Another study proposed that several genes were involved in hypodontia.[15] The type and number of teeth missing from family members were remarkably similar to the probands in 69% of the families studied. If the proband was missing both lateral incisors, 64 of 84 relatives were also missing both lateral incisors. If the proband was missing a tooth other than the maxillary lateral incisor, relatives were missing teeth other than lateral incisors in 26 of 32 cases. The authors concluded that some genotypes resulted in agenesis of maxillary lateral incisors, other genotypes caused agenesis of multiple teeth including the maxillary lateral incisors, and still other genotypes caused agenesis of teeth other than the maxillary lateral incisor.

Brook[16] studied dental anomalies in British schoolchildren with congenital hypodontia. He found that microdontia, supernumeraries, macrodontia, and impactions were in excess of a control population. These dental anomalies were more common in the permanent dentition than the deciduous dentition and affected girls more than boys. There was an association between hypodontia and macrodontia in these subjects. A higher occurrence of anomalies were found to exist in their relatives, suggesting a genetic mechanism. Additional anomalous conditions have been found associated with congenital hypodontia. One was the position of the maxillary canine in relation to missing lateral incisors in the permanent dentition. Peck et al.[17] published evidence supporting a genetic basis for palatal displacement of the maxillary canine. Their reasons included bilateral occurrence, sex differences, population differences, familial differences, and concurrence with other dental anomalies.

In a similar study, school children were evaluated for canine position and presence of lateral incisors. Forty-three percent presented with palatally positioned maxillary canines and missing or anomalous lateral incisors. This was significantly different from normally positioned maxillary canines and anomalous or congenitally absent maxillary lateral incisors 6% of the time.[18] The likelihood of maxillary canines being positioned palatally concurrent with missing or anomalous lateral incisors appears to be an environmental influence rather than a genetic one. Typically, the maxillary canine uses the slope of the lateral incisor root to erupt into a

normal position. When this root is inadequate, the canine will often miss the intended target.[18]

Pirinen et al.[19] evaluated a group of patients with palatally impacted canines, along with their first and second-degree relatives, for congenitally missing teeth. A review of the literature showed the palatally displaced canine was a common anomaly, with a prevalence between 1% and 2%. At least one congenitally missing permanent tooth was found in nearly 36% of the group with palatally displaced canines.[19] Another 9% had peg-shaped teeth. Hypodontia was observed in 20% of first-degree relatives and 19% of second-degree relatives. These prevalence rates lead Pirinen et al. to conclude these anomalies were genetically related.

Another dental anomaly associated with hypodontia is taurodontism. A multirooted tooth exhibiting a large pulp chamber and short root canals is described as a taurodontic tooth. Seow and Lai[20] investigated taurodontism and congenital hypodontia in a group of children with congenital hypodontia versus matched controls. In their analysis, the mandibular first molar was selected because of its clear visibility on a panoramic radiograph. Of the group of children with hypodontia, 35% exhibited taurodontism. Of the group of children without hypodontia, only 8% presented with taurodontism. In addition, they found, 56% of the children with taurodontism had multiple missing teeth; 30% were missing premolars, 9% were missing lateral incisors, and 4% were missing lateral incisors and premolars. Seow and Lai postulated that taurodontism resulted from an alteration in Hertwig's epithelial root sheath. Since it is a derivative of ectodermal origin, they proposed that

factors that might cause this condition could be similar to those causing a lack of tooth formation beyond bud stage.

In addition to taurodontism, another dental anomaly is crown size reduction. Garn and Lewis[21] recorded mesio-distal measurements of teeth from subjects with, and without, congenital hypodontia. Their results indicated that hypodontia was associated with crown-size reduction. Marked crown-size reduction was associated with several missing teeth. Individuals congenitally missing teeth typically presented with variant mesio-distal or occluso-gingival proportions of the remaining teeth. Anterior teeth were most commonly affected and posterior teeth less likely affected. Garn and Lewis proposed that typical crown dimensions were not applicable when performing Bolton tooth size discrepancies in individuals with hypodontia.

Sofaer et al.[22] studied the relationship between tooth number and size in the incisor region of the maxilla. The size of central incisors were analyzed in the presence of missing or peg-shaped lateral incisors from a sample of over 17,000 high school students. Peg-shaped lateral incisors were found to be associated with small central incisors. Unilaterally missing lateral incisors were associated with large central incisors. A central incisor adjacent to a missing lateral incisor site was asymmetrically larger than the adjacent central incisor.[22] The authors related this to an interaction between environment and genetics. Environment allowed for proper growth while genetics provided adequate primordia for initiation of tooth development. For example, a peg-shaped lateral incisor could result from a poor environment. This poor environment might impact the formation of the adjacent

central incisor. The environmental effect on the adjacent central incisor would tend to stimulate the tooth to be larger than normal. A missing premolar would have a similar effect.

The maxillary lateral incisor exhibited the largest variation in size in a study by Lysell and Myrberg.[23] The mesio-distal width of all permanent and deciduous teeth were measured and compared. The degree of tooth size variation was least in the permanent canines and first molars. These teeth appeared less susceptible to modifying environmental factors than the highly variable maxillary lateral incisor.

The quantification of genetic and environmental influences on mesio-distal tooth dimension were determined in a study by Townsend and Brown.[24] Measurements were performed on parents and progeny to determine heritability estimates of tooth size. The percentage contribution was found to be small for common environmental variance. Within-family environment accounted for 30% of the total phenotypic variance, and additive genetic variance accounted for 64% of the total phenotypic variability.[24]

Molecular Basis of Tooth Formation

Vertebrate organs are composed of epithelium and mesenchyme, which influence each other during organogenesis. Interactions between these tissues allow for initiation, morphogenesis, and cytodifferentiation. The process of odontogenesis is a specific type of organogenesis. The expression patterns of genes which encode transcription factors, cell surface molecules, and growth factors required for odontogenesis are implicit for formation of additional organ systems. Recent studies

demonstrate specific gene sequences and their products appear responsible for epithelial-mesenchymal interactions during odontogenesis.

Lumsden[26] evaluated initial pattern-forming events in odontogenesis at specific sites of initiation. Using recombination grafting procedures in rodent embryos, Nichols found that ectomesenchyme, which becomes the mandibular arch, originates from the midbrain and rostral hindbrain neural folds.[25] These cranial neural crest cells constitute the oral mesenchyme required for odontogenesis that are odontogenically uncommitted prior to migration. Differentiation appears to be specified by an interaction with epithelium at its destination. Hence, tooth formation appears to be dependent on a site-specific epithelium. This specificity, in conjunction with an uncommitted mesenchyme, suggests tooth development is a site-specific interaction of oral epithelium with competent mesenchyme.

At the stage where specific epithelial cells and competent mesenchyme collaborate, odontogenesis commences. First, initiation occurs as epithelial thickening forms the dental lamina. Subsequently, epithelium invaginates into mesenchymal tissues which proliferate and condense in the bud and early cap stages. Second, morphogenesis begins with epithelium convoluting into characteristic tooth shapes over condensing mesenchyme in the late cap and bell stages. Third, cellular differentiation occurs via an epithelial-mesenchymal interaction whereby epithelium forms enamel and mesenchyme forms dentin.[27]

There are several experimental approaches to studying odontogenesis including organ culture, cell lineage, and mouse mutation. These approaches have

elucidated roles of several complex processes during tooth formation. The role of epithelial-mesenchymal interactions begins early during initiation and continues through differentiation. Recombination experiments have shown that the odontogenic potential begins in the epithelium and switches to the mesenchyme sometime during the cap/bell stages. Subsequently, reciprocal signaling between the two tissues are required for late morphogenesis and differentiation to occur.

The role of ectodermal signals has also been established. The expression of one family of ectodermal signals, the bone morphogenic proteins (BMP's), show their potential as signals during early tooth development. Other signals from the ectoderm include fibroblast growth factor (FGF) 4 and Sonic Hedgehog (SHH) protein.[28] The muscle segment homeobox genes (Msx1 and 2) are involved at almost all sites of epithelial-mesenchymal tissue interaction during tooth development. They can be induced by epithelial signals, and are required for tooth formation.[28] Msx 1 mediates the transfer of BMP4 from epithelium to mesenchyme. This is believed to parallel the chronological shift in odontogenic potential from epithelium to mesenchyme.[28] At this stage of development, other mesenchymal signaling molecules and transcription factors are required for tooth induction. These include lymphoid enhancer factor 1 (Lef1), activin βA, Distal-less genes (Dlx1 and 2), and Pax9.[28] In addition, the extracellular matrix is integral in morphogenesis and cellular differentiation. Matrix proteins, which include; collagen types I, III, IV, laminin, several proteoglycans, and fibronectin, are expressed when the intact basement membrane regulates differentiation of mesenchymal cells into odontoblasts.[28]

Initiation begins with the dental lamina marking sites where signals from FGF's and BMP's will lead to determination of tooth regions. Determining the type of tooth involves differential activation of various transcription factors. These factors include; BMP4, Barx1, Lhx, Dlx, and Shh. Budding is the transition between initiation and morphogenesis. At this point, the inductive potential shifts from epithelium to mesenchyme and transient epithelial signaling centers are presumed to instruct the mesenchyme to activate certain transcription factors.[29] This active mesenchyme looks different under the microscope, often described as condensed. This condensing mesenchyme forms both the dental papilla between epithelial folds, and the dental follicle which surrounds the epithelium. This marks the cap stage where tooth shape begins under the direction of the enamel knot via the mesenchymal BMP4 signals.[29] The characteristic folding of the epithelium is regulated by FGF's.[29] Further morphogenesis is controlled via a network of signals from the epithelium. Largely, the same signaling cascades are used reiteratively throughout the remainder of tooth development.[29]

Some of these signaling cascades and their overwhelming complexities are revealed by Thesleff[30] in 2000. Several of the same factors are used reiteratively throughout development. Tooth formation begins in the epithelium via the Pitz2 gene. The protein from this gene has the capacity to produce BMP's, FGF's, and tumor necrosis factors (TNF) which initiate odontogenesis in the underlying mesenchyme.[30] This odontogenic mesenchyme via several genes (Lhx6, -7, Barx1, Msx1, -2, Pax9, Gli1, -2, -3, Dlx1, and -2) produce both BMP's and Activin

controling the growth and folding of the overlying epithelium.[30] With the shift of odontogenic potential back to the epithelium, the enamel knot has an integral role. At the time of enamel knot formation, p21, Msx2, and Lef1 genes produce BMP's, FGF's, Shh, and Wnt. Concurrently, dental papilla genes produce BMP's, FGF's, and Wnt to regulate the advancing development of cusps.[30]

The sequence of signals involved in oral epithelial-mesenchymal interactions are vital to odontogenesis. These signals include several proteins; TGFβ, FGF, EGF, hepatocyte growth factor (HGF), glial cell derived neurotrophic factor (GDNF), low and high-affinity neurotrophin receptor, midkine (MK), and SHH.[31] Other protein signaling families, BMP's and FGF's, have roles in initiation. Bone morphogenic proteins regulate Msx1, Msx2, and the early growth-response gene Egr1, during this time. FGF2 and FGF4 mimic the effect of the epithelium, and stimulate Msx1.[31] BMP's and FGF's are potential signals which transmit odontogenic potential from epithelium to mesenchyme early in odontogenesis.

Two of these signals are the transcription factors Msx1 and Lef1. When inactivated in transgenic mice, they lead to the arrest of tooth development at bud stage. Msx1 is triggered by epithelial signals, BMP's and FGF's, and is normally expressed in the mesenchyme after initiation. It is not related to incisor-premolar hypodontia.[31] Lef1 regulates an epithelial signal that is required for mesenchyme determination. The inactivation of these two genes affects the capacity of the mesenchyme to signal back to the epithelium which occurs soon the epithelium has initiated odontogenesis.[31] An important consequence of losing mesenchymal

signaling may be the inability of induction of the enamel knot. The enamel knot acts as a signaling center releasing signals FGF4, BMP2, 4, and 7, and Shh during late bud-early cap stages.[31] It may act as an organizing center that regulates tooth shape by altering size and position of cusps.

Whether the number or the type of teeth missing, there are several forms of hypodontia. These differences may be due to Eda and Msx gene abnormalities. Anodontia, particularly severe hypodontia, can be related to a syndrome known as anhidrotic ectodermal dysplasia where the gene Eda is non-functional.[32] This syndrome manifests with the lack of hair, sweat glands, and varying numbers of teeth. Mild or moderate hypodontia, involving molars, is associated with Msx abnormalities.[32] Msx mutation can produce a different phenotype than incisor-premolar hypodontia. In a study of five affected families with incisor-premolar hypodontia, Msx1 did not co-segregate with hypodontia.[32]

An interaction between epithelium and mesenchyme is central to odontogenesis. Mechanisms involved in this interaction have been studied for years using various models. Three mechanisms have been proposed: mediation by diffusable signals; mediation by the extracellular matrix; and, mediation by cell-to-cell contact. In the past decade, localization of growth factors in the inducing tissue has favored the acceptance of mediation by diffusion mechanism.

Two transcription factors involved in regulating odontogenesis are Msx1 and Pax9. Msx1 has previously been discussed. Pax9 expression in mesenchyme is controlled by signals from the ectoderm. It is required for tooth development beyond

the bud stage. Pax9 is expressed in mesenchyme and has been proposed as the marker for sites of tooth formation. It is induced and maintained by the diffusible protein signal FGF8 from the ectoderm.[33] This FGF-mediated induction of Pax9 has antagonists, BMP2 and BMP4 proteins. These BMP proteins can inhibit Pax9 in the early stages when it is regulated by the ectoderm, but not at later stages when ectoderm signals are no longer required to maintain Pax9 expression. Thus, tooth development is initiated in regions where the inducer (FGF) is present, the antagonist (BMP) is absent, and the mesenchyme is competent.[33]

Peters and Balling[34] discussed how mesenchymal Pax9 expression is induced only at those sites where FGF8/FGF9 are expressed in the overlying epithelium and where BMP4/2 doesn't interfere. While FGF and BMP have the opposite effect on expression of Pax9, they both stimulate expression of Msx1. Both Msx1 and Pax9 are essential for development beyond the bud stage. When the odontogenic potential switches from ectoderm to mesenchyme, the control hierarchy of Bmp and Pax9 switches, and Pax9 takes control.[34] The transition from bud to cap stage is characterized by differential cell proliferation in the inner enamel epithelium. Cells in the middle of the inner enamel epithelium do not replicate, forming the future enamel knot. Adjacent cells continue to proliferate and invaginate further. These enamel knot cells eventually undergo apoptosis. Prior to their programmed death they act as a signaling center, as previously mentioned, for several factors; Shh, BMP2, BMP4, BMP7, FGF4, and FGF9[34].

Stockton et al.,[35] in 2000, identified a frameshift mutation in the paired domain of Pax9 in a family with autosomal dominant oligodontia where most permanent molars were lacking. An insertion of guanine at nucleotide 219 was responsible for a frameshift mutation (addition of an extra G extends a series of 5 guanines to 6 causing premature termination of the protein). This mutation might have exerted an effect in one of several ways: loss of function due to absence of DNA-binding domain; haploinsufficiency secondary to mutant mRNA or protein instability; gain of function due to novel protein; or dominant-negative effect by interference with the product of normal Pax9 allele. These potential disruptions of normal Pax9 function could also be the cause of other gene sequence disruptions in other forms of oligodontia or hypodontia.

Another study evaluated a family with autosomal dominant tooth agenesis of all maxillary and mandibular second premolars and third molars revealed an Msx1 gene mutation.[36] Linkage analysis mapped the gene mutation to chromosome 4p16 between D4S432 and D4S1614. Upon analysis, a G→C transversion, resulting in the substitution of a positively charged arginine with a neutral proline at residue 31 of the Msx1 homeodomain, was discovered. The affect of the Arg31Pro missense mutation at other locations in the dental arch appears to be incomplete. The Msx1 gene appears to be non-critical at these sites, or other transcription factors have compensated for the Msx1 defect at these tooth positions.[36]

In another experiment, tissue recombination was performed to evaluate Msx1 and Msx2 expression.[37] Msx1 is expressed only in the mesenchyme. The

mesenchyme has two components, the dental papilla, which becomes the dentin and pulp, and the dental follicle, which becomes the cementum, periodontal ligament, and alveolar bone. Msx2 is expressed in the epithelium and the dental papilla of the mesenchyme. Mesenchymal expression of these Msx genes requires epithelium until the early bell stage. After this, Msx1 expression is not dependent on epithelial signals, while Msx2 is dependent. This suggests Msx1 in mesenchyme does not require a specific epithelial signal, while Msx2 requires interactions between dental epithelium and dental mesenchyme. Thus, Jowett et al.[37] postulate these two genes were involved in epithelial-mesenchymal interactions in the control of odontogenesis, but without positional specification.

Chen et al.[38] used Msx1 mutant mice to show the effects of reduced levels of BMP4, Lef1, and a syndecan protein. These signals, regulated by Msx1, are a diverse set of peptide growth factors required in various stages of tooth development. Their results showed that BMP4 is one of these signals. Msx1 is required early and often in tooth development and BMP4 is seen from the bud stage onward. The authors postulated that Msx1 is a mesenchymal gene with a capacity to regulate signals from the mesenchyme which act on the epithelium. It permits reciprocal inductive signaling between the two tissue layers, epithelium and mesenchyme, required for odontogenesis.

Kratochwil et al.[39] found that Lef1 expression is temporarily required in epithelium to induce a specific morphological event in mesenchyme using recombination techniques from normal and Lef1 deficient embryos. Lef1 is first

expressed in epithelium at initiation and later in condensing mesenchyme around the epithelial tooth bud. Finally, it is expressed during morphogenesis in both mesenchyme and the basal cell layer of epithelium. At this stage in odontogenesis, where Lef1 expression is prominent in the basal cell layer of epithelium, the formation of the dental papilla is presumably dependent on the expression of Lef1. Lef1 is also activated by Bmp4, suggesting its regulatory role in Bmp-mediated inductive tissue interactions.[39]

The homeobox family member, Hox8, was evaluated in the mouse embryo and compared to another family member, Hox7, in their expression patterns.[40] Hox8 is initially expressed in dental epithelium. During the bell stage, Hox8 switches to the differentiating mesenchymal odontoblasts of the dental papilla. Hox7 is expressed only in mesenchymal cells of the dental papilla and follicle. Expression of Hox7 and Hox8 were evaluated in prospective molar and incisor positions. In the first molar, Hox8 is first expressed in the mesenchyme at initiation. Then it is expressed in the buccal external enamel epithelium at bud stage. Finally, it is expressed in the enamel knot and lightly in the adjacent papilla mesenchyme at early cap stage. Expression is maximal in the enamel knot, septum, and external enamel epithelium in late cap stage. At early bell stage, Hox8 is expressed most prominently in the inner enamel epithelium where the future cusps form. By late bell stage, Hox8 expression shifts to the odontoblasts of the papilla mesenchyme. In the incisor, the same expression pattern exists, but in a wider, more uniform field. Hox8 has been suggested to be the earliest marker for sites of dental initiation.[40] It is the first molecular evidence for the

involvement of the enamel knot, septum, and navel in the shaping of the molar tooth. Involvement of Hox-8 with tooth shape is strengthened by its symmetrical expression in cuspless incisor teeth.

In generating mutant Dlx1 and Dlx2 gene transgenic mice, Thomas et al.[41] discovered mice homozygous for mutations of either Dlx1 or Dlx2 genes, showed normal development of teeth, while mice with homozygous mutations of both Dlx1 and Dlx2 were missing maxillary molars. These results indicated a redundant function of Dlx genes in the development of maxillary molars. The authors postulated that there were more functional redundant patterns in mandibular molar development than in maxillary molar development. The expression of additional Dlx genes in the mandibular molar mesenchyme would compensate for the loss of Dlx1 and Dlx2. In addition to separate programs for maxillary and mandibular molars, separate programs might also exist for incisor and molar morphogenetic pathways. Each program would require a certain set of mesenchymal genes. Initiation of molar tooth buds would occur when mesenchyme expressed the genes Dlx1, Dlx2, and Barx1 and incisor development would occur when mesenchyme expressed the genes Msx1 and Msx2.

Previous studies found FGF4 in the enamel knot during cap stage. Accordingly, its role in tooth morphogenesis and cuspal growth was speculated. However, Fgf4 knockout mice have been difficult to examine, and the effects on tooth development have not been explored. Tucker et al.[42] used a soluble receptor to block FGF from binding, which allowed enamel knot formation to proceed. Results

show a loss of Sonic hedgehog (Shh) expression. This indicated that enamel knot cells were lost or that Shh expression was dependent on FGF4, or both. Thus, Fgf4 may inhibit apoptosis in the cells near the enamel knot.

Kettunen and Thesleff[43] examined Fgf4, 8, and 9 using in situ hybridization of recombinant FGF proteins during tooth initiation and development. All three FGF proteins were confined to dental epithelial cells at all stages. These proteins acted as epithelial signals that allowed for epithelial-mesenchymal interaction during tooth development presumably by stimulating the expression of mesenchymal Msx1. FGF8 acted during tooth initiation, FGF4 acted during tooth development and shape formation, and FGF9 performed both functions. Before any morphological sign of tooth development, both FGF8 and FGF9 were found in the epithelium. Their expression continued into the beginning of the bud stage, and was later downregulated in late bud stage. Downregulation of FGF8 from the budding epithelium corresponds with the shift of odontogenic potential from epithelium to mesenchyme. FGF4 and FGF9 upregulated in the enamel knot in late bud stage and remained until cap stage. At early bell stage, both spread from the enamel knot to secondary enamel knots. They appeared to be involved with preventing premature apoptosis of the enamel knot cells. The authors proposed that FGF9 functions as a paracrine signal regulating odontoblast differentiation due to the intense expression in inner enamel epithelium until preodontoblast differentiation from the mesenchyme.[43]

Bone morphogenic proteins belong to the TGFβ family of signaling molecules. They have been implicated in several processes of odontogenesis. Aberg

et al.[44] evaluated the bone morphogenic proteins during odontogenesis. BMP's signal between epithelial and mesenchymal tissues during initiation, morphogenesis, and differentiation of odontoblasts, ameloblasts, and perhaps cementoblasts. The expression of BMP3, 5 and 6 are all different and restricted to one cell lineage while BMP2, 4, and 7 shift between lineages of both epithelial and mesenchymal origin. BMP3 is expressed only in dental mesenchymal cells of the dental papilla and is proposed to involve the formation of cementum. BMP5 is associated with cell differentiation and is expressed only in the dental epithelium, starting in the inner enamel epithelium and continuing in the secreting ameloblasts. BMP6 is found only in the dental mesenchyme. BMP2 and BMP7 spread from the primary enamel knot to the inner enamel epithelium showing an expression domain consistent with epithelial signals regulating the differentiation of the underlying mesenchymal cells into odontoblasts. BMP4 began in epithelium and moved to mesenchyme (corresponding with the odontogenic potential of tooth formation). This family may be part of the signaling network of the enamel knot that regulates patterning of tooth cusps. A redundancy in function, or formation of heterodimers between different Bmp's result in significant overlap in their expression patterns.

Vainio et al.[45] evaluated BMP4 as a soluble growth factor involved in tooth development. Beads soaked in BMP4 protein were added to dental mesenchyme, and mesenchymal changes were analyzed. BMP4 stimulated the transcription factors Egr1, Msx1, and Msx2. These are known epithelial inducers of mesenchyme. Thus, BMP4 is an inductive signal between epithelial and mesenchymal tissues.[45]

Another important function of BMP4 is the ability to induce the cyclin-dependent kinase inhibitor p21. This inhibitor is linked to a cessation of cell proliferation in the formation of the enamel knot region. The enamel knot performs a central function in the control of growth and patterning of the tooth cusps. It causes unequal growth of the enamel epithelium in forming tooth cusps by remaining non-proliferative while stimulating adjacent growth via FGF4. Three-dimensional reconstructions of in situ hybridizations have revealed BMP4 expression follows the expression of p21 in the enamel knot.[46] Hence, BMP4 is involved in the induction of the enamel knot due to it's inductive capacity of the inhibitor p21, and in the termination of the enamel knot via apoptosis. The inhibitor p21 is found maximally expressed at cap stage that closely correlates with the withdrawal of the enamel knot cells from the mitotic cycle. After a brief 24-hour period of activity, the enamel knot disappears with the upregulation of BMP4[46]. Jernvall et al. proposed that BMP4 overwhelms FGF4 to prevent premature apoptosis.

Studies have shown mutant genes Pax9 and Msx1 are associated with congenital hypodontia in isolated families. Arte et al.[47] examined epidermal growth factor (EGF), epidermal growth factor receptor (EGFR), and fibroblast growth factor 3 (FGF-3) in several cases of congenital hypodontia. All were implicated in mouse studies as important regulators of odontogenesis. Using linkage analysis to evaluate the number of recombination events, it was possible find the distance of a disease locus and a known marker. A lod score greater than 3 (odds ratio of 1000:1) is considered proof of linkage while a score less than –2 (odds ratio of 1:100) is

considered exclusion of linkage. The lod scores showed that there was no linkage of hypodontia with EGF, EGFR, FGF-3 genes. Mutations in these genes were not responsible for hypodontia in the families studied.[47]

Genetic Epidemiology

A common epidemiological measure of familial aggregation is the comparison of disease frequencies in relatives of affected subjects to the disease frequencies in the relatives of unaffected controls. Often, a descriptive study may have a design that will adequately evaluate linkage present in relatives of subjects previously determined to have some form of a trait in question. Both sib pair and regression coefficients between parents and offspring are ways to evaluate genetic causes to a trait. These are measures of heritability used to evaluate traits. In studies where a qualitative trait is analyzed, relative risk is calculated to determine the genetic component to familial aggregation.

Familial aggregation of a trait is initially examined from epidemiological data. This epidemiological evidence must show at least one of the following: (1) prevalence of the trait is higher among relatives of affected persons than rates observed in the general population, (2) a higher proportion of affected individuals have a relative with the trait than controls, (3) correlation values between related individuals are significantly higher than zero.[48] Traits that do cluster in families can do so for several reasons. Single or multiple genes can cause the trait to be inherited. Related individuals, like siblings that grow up in the same household, may be exposed to certain agents that may be implicated with the disease in question. Lastly,

cultural inheritance may increase susceptibility through lifestyle.[48] Several study designs exist to separate the relative importance of these factors on a trait in question.

Documention of familial aggregation for a trait or disease is followed by determining how much of this aggregation can be attributed to genetic causes. Genetic analysis focuses on correlations among relatives and partitions these correlations into components attributable to shared genes and shared environments. From these components heritability is calculated. Heritability is the ratio of genetic variance to the total phenotypic variance in the population.[49] There is heritability in the broad sense, which is the ratio of variance due to all genetic differences to total phenotypic variance, and in the narrow sense, which is the variance due to alleles at one or more loci to the total phenotypic variance. All measures of heritability must lie between zero and one. The lower limit occurs when all observed phenotypic variation is attributable to non-genetic factors, while the upper limit exists when no phenotypic variation is not due to genetic variation.[50]

As a general rule, greater environmental diversity will lower the estimate of heritability while a more homogeneous environment will give a higher estimate of heritability even though the genetic mechanism is the same in the two environments. As a result, one must be careful applying an estimated heritability obtained from one population to another for predictive purposes.[50]

The genetic component of variance used in estimating heritability is actually the sum of several components. These components are: additive genetic variance that is the effects of individual alleles on the trait, dominance variance that is the

interaction of alleles at the same locus, and epistatic variance that is the interaction between alleles at different loci.[51] Additive genetic variance represents variation in phenotype transmissible from parent to offspring and is the most observable correlation among relatives, being used to compute heritability in the narrow sense. Dominance variance only contributes to correlation between relatives who share a genotype identical by descent, which are full sibs and twins. Epistatic variance includes the effects of modifying loci and is very difficult to estimate. Components of environmental variance include variation due to environmental factors shared among relatives and factors unique to each individual. One of the more common methods for estimating heritability is the regression of offspring phenotypes from their parents. A big reason for this is the interest in resemblance between parent and offspring phenotypes. Greater precision is possible in estimation procedures when both parents can be measured and midparent values can be constructed. While correlation between single offspring and single parents are identical to the regression coefficient, this is not true with multiple offspring. Regression interpretation becomes much easier than correlation coefficients in these circumstances.[51]

An attractive alternative to parent-offspring regression in estimation procedures is the sib analysis. In sib analysis, the total phenotypic variance is partitioned into within-family and among-family components. Both can be interpreted in terms of covariance between relatives using analysis of variance statistics. Analysis of variance partitions total phenotypic variance into the sum of the within-family and among-family variances. Another relationship that proves

useful is covariance where the phenotypic covariance between members of the same group equals the variance among groups. Heritability can be roughly calculated for both half-sib and full-sib analysis from covariance calculations. Ideally, paternal half-sib designs are optimal because common maternal effects are eliminated. The potential for common environmental effects, the main drawback of any sib analysis, would be eliminated in this instance. Full-sib analyses are not optimal for the same reasons as maternal half-sib analyses. Dominance effects and common environmental effects will play a role in these analysis. However, supplementary full-sib analysis can be valuable because it would provide information on the significance of the variance associated with these previously mentioned effects.[51]

The phenotype of congenital hypodontia manifests as a qualitative trait whereby the tooth is either present or absent. The approach to measuring possible genetic factors in this instance is traditionally modeled where the phenotype in question, termed a liability, is the sole determinant of disease. Since liability controls the risk of disease, individuals with high liability values are at a high risk of disease. A risk function is assessed and, at some level on the liability scale, an individual becomes affected. They pass a liability threshold above (or below) which the disease is manifest.

As in studies of congenital hypodontia, attributes that are categorical (absent or present) on an observable scale, but believed to be continuous on an unobserved scale, are known as threshold characters. Individuals with phenotypic values above the threshold exhibit the trait, and those below it do not. Individuals with genotypic

values above the threshold are not at absolute risk because some have phenotypes below the threshold. Alternatively, some individuals with genotypic values below the threshold will have phenotypic values above it. Hence, the greater the environmental contribution to the variance of the trait, the more gradual the risk function on the genotype scale (genotype does not automatically result in phenotype).[51]

Studies performed to evaluate qualitative traits must have a narrow definition for the phenotypes in question and be highly repeatable. Both of these requirements are adequately met using the typical protocol of radiographic analysis of congenital hypodontia. Panoramic radiographs and periapical radiographs are excellent visual indicators for presence or absence of teeth, assuming that the subject is old enough to show proper calcification of permanent tooth buds. This usually occurs three-to-four years prior to eruption. Since the last permanent tooth to erupt, excluding third molars, usually occurs about eleven to twelve years of age, the earliest a study can reliably determine permanent tooth absence is for subjects seven to eight years of age. Absence of a tooth younger than this age would not necessarily mean that the tooth will not form, only that it has not yet formed.

MATERIALS AND METHODS

Study Population

Two hundred and eighty three subjects from the Case Western Reserve University, Department of Orthodontics, School of Dental Medicine and from two private practices in Berea, Ohio and Mayfield Heights, Ohio were selected as probands for the present study. Selection criteria for the probands were the presence of at least one radiographically verifiable congenitally absent permanent tooth other than third molars. Subjects under the age of eight years old were excluded from the study because the second premolars and maxillary canine teeth might not begin to show follicular development on radiographic exam until this age. Recruitment of these proband subjects followed from clinical exam and assessment of panoramic radiograph.

Survey Collection

A letter of invitation to participate in this study was mailed from one of the three orthodontic offices to all families with a self-addressed, stamped postcard to declare intent of participation. Sixty-two families mailed the postcard back to their orthodontist with the intent of not participating in the study. Thirty of the letters were returned from the post office with incorrect addresses. These families were also excluded from the study. One hundred and seven families who mailed the postcard back to their orthodontist with intent to participate received a packet from that

orthodontist or the Department of Orthodontics. These packets included an informed consent form, questionnaire, and instructions for each immediate family member. Each family could return the forms to their orthodontist's office, or similarly to the Department of Orthodontics by hand or mail. Immediate biological family members were asked to complete the survey answering biographical data including their name, gender, ethnicity, and specific relationship to the proband.

After several months, sixty-five families who had not responded were mailed a second letter with self-addressed, stamped postcards. In addition, forty- five of the one hundred and seven families had expressed interest in the study and were mailed appropriate forms, but had not returned them. For these families, additional packets were mailed with forms identical to the previous attempt. Follow-up calls were placed to answer questions. After several follow-up calls, eighteen families had not responded four months after the second mailings. These families were excluded from the study. Upon completion of data collection, eighty-three families who were mailed the letters of invitation never responded and were excluded from the study.

Eighty-nine families returned the completed informed consent forms and questionnaires. They were placed into one of two groups. Families who did not report any additional members with congenitally missing teeth were placed into one group. No follow-up was necessary for this group. The second group included families with at least one additional member with a self-reported congenitally missing tooth. These families were contacted to obtain their general practitioner's phone number. Each general practitioner was contacted to verify the congenital absence of

teeth via a previously administered clinical exam, dental history report, or radiographic exam. In four instances an adult family member did not have any dental records in the area, but knew which tooth or teeth they were missing. These individuals were included in the study.

Data Analysis

Data for the subjects in each family was entered into the spreadsheet program Microsoft® Excel® (Microsoft, Seattle, WA). Families were assigned identification numbers and their addresses and phone numbers were entered. Individuals were then assigned identification numbers; proband was 1, mother was 2, father was 3, siblings were 4-8. Gender was noted for the proband and siblings. Date of birth and ethnicity were entered for all family members. Teeth were recorded as missing with verification (m), present with verification (x), or present with self-report (u). The universal numbering system was used where the upper right central incisor was assigned the number 11, upper right lateral incisor assigned number 12 to upper right second molar assigned number 17. Each quadrant was scored similarly with the upper left quadrant numbered 21 through 27, the lower left quadrant numbered 31 through 37, and the lower right quadrant numbered 41 through 47.

At the completion of data collection, sixteen individuals who were under the age of twelve with no radiographic verification, two individuals with incomplete responses, and seven individuals who could not be contacted were recorded as unknown (k). In addition, the assumption was made that all self-reported teeth were correctly made. Thus, all teeth reported present were given an "x" value regardless of

radiographic confirmation. Finally, one proband and sibling were found to be monozygotic twins. By definition, monozygotic twins are genetically identical, so this family was excluded from the study.

General descriptive statistics, including prevalence of the trait in the Caucasian group and Overall groups, distribution by gender and age, as well as number and affected status of sib, brother, sister, brother-sister, and nuclear families were determined. Two phenotypic definitions were included; the number of missing teeth was used in the distribution statistics in the descriptive analysis and binary presence or absence of teeth was used in heritability and relative risk calculations.

Data analysis of this paper were obtained by using the program package SAGE [2002] (Statistical Analysis for Genetic Epidemiology, Statistical Solutions Ltd, Cork, Ireland) which included an estimation of heritability using calculation of three familial correlations, including spousal, parent-offspring, and sib-sib relationships, while allowing for ascertainment correction. Likelihood ratio tests were performed:

$$-2\ln \frac{L(R)}{L(F)} \sim \chi^2_{df}$$

where df = difference in the number of parameters, R = restricted model, F = full model, and R must be nested within the F model. As a model for the heritability calculation, an assumption of parent-offspring correlation equaling ½ genetic variance, sib-sib correlation equaling ½ genetic variance + ½ common environment variance + dominance variance, and spouse-spouse correlation equaling common environment variance was made.

Sibling recurrence risk ratio was calculated from the data, correcting for ascertainment bias, using the equation[52]:

$$\lambda_s = \frac{K_s}{K}$$

Recurrence risk for siblings (K_s) was calculated as the sum of affected siblings minus one and divided by the sum of the total siblings minus one.

Where the recurrence risk is estimated as:

$$K_s = \frac{\Sigma_f (a_f - 1)}{\Sigma_f (s_f - 1)}$$

Where a_f = number of affected sibs, and s_f =total number of sibs. Recurrence risk for parents was similarly calculated. The sum of affected minus one divided by the sum of total minus one. The prevalence (K) for the general population was estimated at 0.05 based on previously reported values in the literature.[2,4,5,6,9]

RESULTS

General Descriptive Statistics

A sample of eighty-eight families was analyzed for congenital hypodontia. The sample included 376 subjects which consisted of 88 probands and 288 relatives. A total of 127 (34%) of the 376 sample subjects were congenitally missing at least one tooth. A total of 39 (13%) of the 288 relatives were congenitally missing at least one tooth. Most individuals affected with congenital hypodontia were missing either one or two teeth. Including all individuals missing teeth, 42% were missing one tooth and 84% were missing up to two teeth. In addition, the number of missing teeth per person between probands and relatives was similar where 84% of probands and 85% of relatives were missing either one or two teeth. The distribution of the number of missing teeth per person is displayed in Table 1.

TABLE 1.--Distribution of Missing Teeth

	NUMBER OF TEETH MISSING						
	0	**1**	**2**	**3**	**4**	**5**	**6**
Proband	0	39	35	6	4	2	2
Relative	249	14	19	0	6	0	0
Total	249	53	54	6	10	2	2

Most probands were found to be missing second premolars and/or the maxillary lateral incisors. Thirty-two probands (36%) were missing either or both maxillary lateral incisors and forty-five probands (51%) were missing any of the second premolars. The thirty-two probands missing maxillary lateral incisors had 106 relatives, of which 8 (8%) were missing a maxillary lateral incisor and 11(10%) were missing any tooth except third molars that were not under investigation in the present study. The forty-five probands missing second premolars had 133 relatives, of which 18 (13%) were missing a second premolar and 27 (20%) were missing any tooth. More probands were missing second premolars than maxillary lateral incisors. The relatives of probands missing second premolars are more likely to be missing teeth than relatives of probands missing maxillary lateral incisors.

Familial Correlation

The sample was divided into five groups for the purpose of detecting correlations (Table 2). Sample 1 was the sample of all 88 families. Sample 2 includes the 45 families with both probands and relatives missing at least one second premolar. Sample 3 includes 45 families with the probands missing at least one second premolar and relatives missing any tooth. Sample 4 includes 32 families with both probands and relatives missing at least one maxillary lateral incisor. Finally, sample 5 includes 32 families with the probands missing at least one maxillary lateral incisor and relatives missing any tooth.

TABLE 2.--Correlation Values Unadjusted for Ascertainment

	Pairs	Affected	Total	Correlation
Sample 1	PO*	34	400	0.04
	Sibling	13	157	-0.34
	Sister	2	42	-0.47
	Brother	1	43	-0.48
	Brother-Sister	10	72	-0.15
Sample 2	PO	15	198	0.05
	Sibling	8	72	-0.30
	Sister	2	14	-0.27
	Brother	0	22	-0.62
	Brother-Sister	6	36	-0.08
Sample 3	PO	24	198	-0.01
	Sibling	10	72	-0.26
	Sister	2	14	-0.27
	Brother	1	22	-0.55
	Brother-Sister	7	36	-0.03
Sample 4	PO	7	166	0.02
	Sibling	1	79	-0.47
	Sister	0	28	-0.55
	Brother	0	20	-0.52
	Brother-Sister	1	31	-0.37
Sample 5	PO	9	166	0.03
	Sibling	3	79	-0.37
	Sister	0	28	-0.55
	Brother	0	20	-0.52
	Brother-Sister	3	31	-0.16

*PO denotes parent-offspring pair

Since only information from nuclear families was gathered, the familial relationships evaluated included: parent-offspring pairs, sib-sib pairs, sister pairs, brother pairs, and brother-sister pairs. Correlations were calculated for each relative pair type in each of the five samples. These values ranged between 0.05 and –0.62 as seen in Table 2. Correlation values may be as large as +1 or as small as –1. A positive value approaching 1 would show the two variables are positively related.

This means a relationship exists for the trait in question where the presence of a missing tooth in one subject in the pair implies the other subject in the pair is also likely to have a missing tooth. A negative value approaching 1 would show the two variables are negatively related. This means a relationship exists for the trait in question where the presence of a missing tooth in one subject in the pair implies the other subject is not likely to have a missing tooth. A correlation value approaching 0 shows the two variables are unrelated with regards to the phenotype. This was the case with the sample population studied here. Presence of a missing tooth in one subject did not imply the other subject in the pair was, or was not likely to have a missing tooth.

These correlation values were computed based on families ascertained through an affected proband. By definition, probands are individuals who brought the family to our attention. In this case, the probands are an extreme presentation of the phenotype in question. However, our ability to interpret familial correlations depends on having a random sample of families from the general population. Therefore, these same five samples were analyzed using likelihood ratio tests, which corrects for the ascertainment bias (Table 3).

TABLE 3.--Likelihood Ratio Tests Adjusted for Ascertainment

Model	Sample	Parameter Estimates					-2LN Likelihood	Chi-Square	p-value
		ρ_{FM}	ρ_{FO}	ρ_{MO}	ρ_{PO}	ρ_{SS}			
1	1	0.45	-0.02	-0.06	\	0.5	885.089		
2		0.45	\	\	-0.04	0.5	885.523	0.434	0.51
3		[0]	\	\	-0.03	[-.03]	887.948	2.425	0.33
1	2	-0.54	0.04	-0.05	\	0.5	372.018		
2		-0.54	\	\	-0.01	0.5	372.342	0.324	0.57
3		[0]	\	\	-0.05	[-.05]	374.872	2.530	0.28
1	3	0.26	-0.09	-0.09	\	0.5	486.533		
2		0.26	\	\	-0.09	0.5	486.534	0.001	0.97
3		[0]	\	\	-0.07	[-.07]	487.005	0.471	0.79
1	4	-0.18	-0.12	0.09	\	0.5	175.345		
2		-0.26	\	\	0.01	0.5	177.595	2.250	0.13
3		[0]	\	\	0.01	[.01]	178.252	0.657	0.72
1	5	-0.47	-0.16	0.17	\	0.5	327.300		
2		-0.53	\	\	0.04	0.5	329.880	2.580	0.11
3		[0]	\	\	0.02	[.02]	331.944	2.064	0.36

ρ_{FM} denotes spousal correlation
ρ_{FO} denotes father-offspring correlation
ρ_{MO} denotes mother-offspring correlation
ρ_{PO} denotes parent-offspring correlation
ρ_{SS} denotes sib-sib correlation
"\" denotes the parameter was not estimated
square brackets ([ ]) denotes the parameter was fixed to be equal to this value

Two hypotheses were tested. First, there was an evaluation of whether the correlation between father and offspring (ρ_{FO}) was equal to the correlation between mother and offspring (ρ_{MO}). Comparison between father and mother may show a sex-linked heritability of the phenotype of congenital hypodontia. Here, the null hypothesis stated the father-offspring and mother-offspring relationships were equal. Chi-square calculations for all five pedigrees resulted in non-significant p-values. The null hypothesis could not be rejected and a sex-linked cause to congenital hypodontia could not be established in the current sample.

Next tested was whether the correlation between parent and offspring (ρ_{PO}) was equal to the sib-sib correlation (ρ_{SS}), and whether the spousal correlation (ρ_{FM})

was equal to 0. The first evaluated differences between parent-offspring and sib-sib relationships in order to determine a component of genetic variance known as dominance variance.[50] Dominance variance represents interaction between alleles at a single locus which causes deviation of the affects of these alleles. Here, the parent-offspring relationship is equal to the additive genetic variance and the sib-sib correlation equals the additive genetic variance plus dominance variance. The null hypothesis stated the parent-offspring parameter estimates equaled sib-sib parameter estimates. We tested the marital correlation to determine if there is evidence for assortive mating. Significance here would allow some phenotypic difference to be attributed to common environment shared among family members living together. Chi-square calculations for all five pedigrees resulted in non-significant p-values. The null hypotheses could not be rejected. Dominance variance and/or environmental variance did not contribute to the affects of congenital hypodontia in our sample population.

The parent-offspring correlation values from the 5 sample groupings ranged between -0.09 and 0.04. The heritability, which is the portion of congenital hypodontia caused by genetic variation, ranged between 0.08 and 0.18. A phenotype with a substantial portion of variance due to genetic causes will yield a heritability value close to 1. However, the phenotype of congenital hypodontia had a heritability value close to 0. This means little to no genetic causes is suspected to contribute to congenital hypodontia.

Recurrence Risk Ratio

The recurrence risk ratio was calculated for siblings and parents for all five samples (Table 4). This represents the ratio of recurrence risk for a particular relative type to the prevalence rate for the dental phenotype in the general population. The first sample was analyzed using an average estimate from previously published reports of prevalence rates of congenitally missing teeth in the general population. This rate for absence of any tooth was 5%, or 0.05. The second and third samples were analyzed using a prevalence rate for second premolars calculated by the average of several studies of prevalence rates of congenital hypodontia. These studies showed that approximately 50% of all congenitally missing teeth were second premolars. Hence, the prevalence rate used to calculate recurrence risk ratios for these pedigrees were 0.025, which is one half of 0.05. Likewise, the fourth and fifth samples were analyzed using a prevalence rate for maxillary lateral incisors calculated by averaging the same studies previously mentioned. Approximately 40% of all congenitally missing teeth were maxillary lateral incisors. Hence, the prevalence rate used to calculate recurrence risk ratios for these samples was 0.02, which is 40% of 0.05.

TABLE 4.--Recurrence Risk Ratios

Sample	n	Prevalence	RR		RR Ratio	
			S	P	S	P
1	350	0.05	0.138	0.154	2.76	3.08
2	221	0.025	0.140	0.111	5.60	4.44
3	178	0.025	0.233	0.189	9.32	7.56
4	138	0.02	0.024	0.109	1.20	5.45
5	138	0.02	0.071	0.125	3.55	6.25

N denotes sample size
RR denotes recurrence risk
S denotes sibling
P denotes parent

Recurrence risk values are calculated in each respective sample by dividing the number of individuals affected with missing teeth into the total number of individuals. This is essentially the frequency of affected individuals in the sample population. The recurrence risk ratios are calculated by dividing the recurrence risk value (observed frequency) into the expected frequency in the general population, which is the prevalence rate listed in Table 4. These ratios represent the increased risk for a relative (either siblings or parents) above the risk for the general population. Samples 2 and 3 yielded slightly higher recurrence risk ratios compared to samples 4 and 5 which, in turn, yielded slightly higher recurrence risk ratios compared to sample 1. The risk for a relative of a proband who is missing any second premolar is slightly higher than for a relative of a proband missing any maxillary lateral incisor. The risk for a relative of a proband who is missing any maxillary lateral incisor is higher than for a relative of a proband missing any other tooth.

Most individuals (approximately 80-90%) who are affected with congenital hypodontia are missing either second premolars or maxillary lateral incisors.[2,4,5,6,9] Here, the risk that additional family members will be affected is greater than when an affected individual is missing other teeth. In addition, these family members would have a greater likelihood of presenting with congenital absence of any tooth versus the specific tooth their family member was missing. The recurrence risk ratios for these two types of teeth may, in actuality, be similar to all other teeth. However, with such low rates a much larger sample size would be necessary with an adequate number of individuals affected with congenital hypodontia of teeth other than second

DISCUSSION

In several previous studies, prevalence rates of congenital hypodontia exceeded what was expected for the general population.[2,4,5,6,9] Most dental professionals have sited such increased prevalence rates as evidence for a genetic cause of congenital hypodontia. However, there is a lack of systematic attempts to identify a recognizable pattern as a genetic basis of congenital hypodontia. Thus, the current study was undertaken to examine environmental, cultural, and/or genetic factors that contribute to the apparent familial clustering of this phenotype. In this study, all relationships in the nuclear family were evaluated in order to identify a heritable pattern to better explain the genetic basis for the phenotype of congenital hypodontia. No correlation was found between familial relationship and phenotype, suggesting that congenital hypodontia is a complex trait involving multiple genes that control odontogenesis. This makes the specific genes difficult to identify. The additive action of these genes might cause variations in the observed phenotypes, presenting as a normal distribution in the population. The present sample included a variety of phenotypes, which may help to explain the lack of correlation between family relationships and phenotype observed in this study. By including families with different phenotypes (mutations), when analyzed together, the results may be inconclusive in identifying one pattern of inheritance.

One example for a clear genetic inheritance pattern has not been established is the maxillary lateral incisor. Absence of this tooth has been commonly reported in published studies.[2,4,5,6,9] In the current study, the recurrence risk ratio for the absence of the maxillary lateral incisor was no higher than the risk in the general population. Thus, although it may be inherited as a complex trait, environmental influences may play a large role. If severe enough, an environmental disturbance could cause agenesis of the maxillary lateral incisor. However, a less catastrophic disturbance might lead, instead, to a microdontic peg-shaped maxillary lateral incisor.

The lack of correlation between family relationship and phenotype in this study suggests a weak genetic component in the etiology of tooth agenesis, and that it may be a complex trait. In such a trait, the simple correspondence between genotype and phenotype breaks down, and the segregation and independent assortment of alleles may not be observed.[53] Consequently, the pattern of congenital hypodontia observed in this study cannot be explained as a single-gene disorder with an autosomal recessive, autosomal dominant, or sex-linked mode of inheritance. Rather, as a complex trait, it suggests a polygenic mode of inheritance where there is a shared genetic contribution from a large, or moderate, number of independent genes, each with small effects that control odontogenesis.

Special challenges arise in the identification of specific genes that contribute to complex traits. These include locus heterogeneity, epistasis, incomplete penetrance, variable expressivity and pleiotropy.[54] For example, microdontia of maxillary lateral insicors may be an example of variable expressivity of genes

controlling odontogenesis. Furthermore, with incomplete penetrance, the gene could be masked by other modifying genes, bypassed at critical stages of development, or be muted by environmental factors.

Such complex interactions between multiple genes represents a logical extension of concepts born of various molecular studies. For example, it has been shown that metabolic pathways are not controlled by a single rate-limiting step, but that more than one step will have significant influence on the regulation of such pathways. Because the activity of multiple steps in these pathways may be influenced by gene mutations, individuals may differ at various steps in the pathway, causing additional genetic complexity.[55] These genes may have effects of different magnitudes, thereby further complicating the interpretation of family recurrence patterns.[53]

Currently, more than one hundred genes have been identified as having a role in odontogenesis. All are candidates for tooth agenesis.[56] Mutations of Msx1 and Pax9 have been discovered to cause hypodontia/oligodontia in humans. However, these genes probably cause only a few cases.

There are four mutations found involving Msx1, all of which involve second premolars and/or third molars. Three of the mutations have been shown to segregate in an autosomal dominant fashion. One is associated with individuals in a family with Witkop tooth-and-nail sydrome.[56] There are three known mutations involving Pax9 that cause agenesis of maxillary and mandibular molars. All modify

downstream transcriptional factors halting odontogenesis at the bud stage. Like Msx1 mutations, these mutations have been discovered in one family each.[56]

Several other gene mutations, including Dlx1, Dlx2, Fgf8, Smad2, and certain activin receptors cause different types of tooth agenesis in mice, provide evidence that different types of teeth may have independent developmental mechanisms, and different genetic factors may be involved in the agenesis of specific types of teeth.[56] These genes, acting alone or in combination with other genes, could lead to specific phenotypic patterns of which tooth types are missing.

Given that congenital hypodontia is a complex trait with environmental and other factors affecting phenotypic expression, it is a challenge to offer some estimate of risk, even in the absence of identifiable genes or defined mechanism of inheritance. This can be calculated using recurrence risk ratios. In this study, smaller recurrence risk ratios for congenitally missing teeth were associated with the sample that included all subjects, regardless of missing tooth. Samples that included only subjects missing maxillary lateral incisors or second premolars had slightly higher recurrence risk ratios. Published prevalence rates of congenital hypodontia show that central incisors, canines, first premolars, and first molars are rarely missing.[5,6,9] Therefore, to include subjects missing these teeth in an analysis constructing risks for family members decreases the recurrence risk ratio for teeth that are most often absent. Consequently, removal of subjects with such rarely missing teeth from certain samples produced slightly larger risk values associated with subjects missing second premolars and/or maxillary lateral incisors.

The sibling recurrence risk involving the maxillary lateral incisor was very low, suggesting that this tooth may have a distinct set of factors with a different pattern of inheritance. Another explanation is that the small sample size contributed to its lack of representation in the target population. This could also account for the differences observed when comparing sample 3 to samples 2, 4, and 5.

Similar recurrence risk patterns were found in previous studies of cleft lip and/or cleft palate. Familial recurrence patterns fit well with both oligogenic models with as few as four loci as well as they fit with polygenic models with as many as twenty loci.[57] As an explanation for these disparate models of inheritance, some have proposed that analytic problems with samples could be the result of genetic heterogeneity, wherein, some families present with a monogenic mechanism and others with a polygenic mechanism. Thus, just as in congenital hypodontia, multiple genes control formation of the lip and palate. By including families with defects in any one of these multiple genes, when analyzed together, the results may be inconclusive in defining a single mode of inheritance. Thus, the presence of several genetic forms of congenital hypodontia in different pedigrees could also obscure the overall pattern of transmission if all pedigrees are pooled, as in this study.

Limitations

This survey-based study was subject to several limitations, including errors in self-report, a recall bias, and a small sample size. First, this study relied on subjects reporting historical information correctly, thus allowing for errors in self-reported information. Relying on casual family histories obtained from an interview has

serious limitations that can lead to erroneous interpretation.[50] In an effort to overcome this potential error, trained dental professionals validated a reported absence of teeth with radiographs whenever possible. However, because subjects not missing teeth were not radiographically verified, the reliability of self-reported data remains a potential source of error.

Self-reported data could also have led to underreporting of congenital hypodontia. For example, the population studied included individuals at a minimum of twelve years of age. It is possible that individuals at this age may still have had deciduous teeth, and not have known their succedaneous teeth were missing.

Similarly, relying on self-reported data introduced a potential for recall bias. For example, some parents could not remember if they were affected with congenital hypodontia. These individuals may not have had recent dental care or access to any radiographic information that would verify congenital absence of teeth versus absence of teeth due to caries or periodontal disease.

One problem with calculating variances is that they are assumed to be independent. This means that genetic factors and environmental factors have no relationship with each other. However, in human observational studies, including this one, it is impossible to force complete independence between genotypes and environmental factors influencing the trait.

Furthermore, this study was also limited by small sample size. Once the sample population was broken down for analysis of subjects missing second premolars and maxillary lateral incisors, small sample size may have contributed to

increased standard error. If sample size could be increased, standard error would decrease, thus allowing correlation values to gain statistical significance. However, assuming the sample was representative of a population affected with congenital hypodontia, familial correlation values would remain as reported even with increasing sample size.

Future Studies

A promising area of investigation involves the identification of the genetic cause of syndromes that present with tooth agenesis. This could be a tool for selecting candidate genes for tooth agenesis.[56] Two such reports have identified two chromosomal abnormalities associated with tooth agenesis. An individual with Klippel-Feil anomaly (characterized by congenital vertebral fusion of the cervical spine, clinically short neck, limitation of head and neck movements, low-set posterior hairline, and various defects of other organ systems). The investigators mapped the condition to an inversion of chromosome 2 (p12.q34).[58] They concluded that the inversion may involve genes associated with tooth formation since Klippel-Feil anomaly had not previously involved tooth absence nor had this chromosomal abnormality ever been reported with this condition. Similarly, genetic evaluation of a child with ectodermal dysplasia and congenital absence of permanent mandibular incisors produced a previously unknown translocation of chromosomes 1 and 6 (p22.1:p22.1), suggesting additional genes associated with tooth agenesis.[59] The authors suggest this chromosomal rearrangement might have disrupted one or more genes important in ectodermal development and odontogenesis.

Investigators should continue to search for the identify of specific genes associated with distinct individual or familial phenotypes of hypodontia. A recent study of an extended family in Pakistan with congenital hypodontia identified a new chromosome location for this condition after excluding the involvement of previously candidate genes in hypodontia. The genome-wide search with markers found on chromosome 16 q12.1 was homozygous only in individuals with congenital hypodontia.[60]

CONCLUSIONS

In this study, all relationships in the nuclear family were evaluated in order to identify a heritable pattern to better explain the genetic basis for the phenotype of congenital hypodontia. Heritability estimates ranged between 0.08 and 0.18. In addition, no correlation was found between familial relationship and phenotype, suggesting that congenital hypodontia is a complex trait involving multiple genes. Given that congenital hypodontia is a complex trait, with environmental and other factors affecting phenotypic expression, it remains necessary to offer some estimate of risk, using recurrence risk ratios, even in the absence of identifiable genes or a defined mechanism of inheritance. Samples where the proband was missing a second premolar yielded slightly higher recurrence risk ratios (4.4–9.3) compared to samples where the proband was missing a maxillary lateral incisor (3.6–6.3) which, in turn, yielded slightly higher recurrence risk ratios compared to the sample which included all probands (2.8–3.1). The only exception was the sibling recurrence risk ratio where both proband and sibling were missing maxillary lateral incisors which had a low recurrence risk ratio (1.2). These ratios represent the increased risk for a relative (either siblings or parents) above the risk for the general population.

Alternate mechanisms seem to exist in congenital hypodontia between lateral incisors, second premolars, and other types of teeth as evidenced from the different recurrence risk ratios. Discovery of these mechanisms may require evaluation of congenital hypodontia on a family-by-family basis.

APPENDIX 1

IRB Approval

Case Western Reserve University
Institutional Review Board
NOTICE OF APPROVAL

Responsible Investigator: Manish Valiathan
Department: DEN -Dental Clinic Ortho
IRB Protocol #20010605
Title: Genetic Analysis of Congenital Hypoplasia

Approval Date: Friday, July 19, 2002
Continuing Review Deadline: Friday, July 04, 2003
Expiration Date: Friday, July 18, 2003

The Institutional Review Board (IRB) has APPROVED the above continuing protocol through the full review process.

It has been determined that this study involves minimal risk, and that the following vulnerable populations will be involved: minors.

As an investigator of human subjects, your responsibilities include the following:

1. Report all adverse events and unanticipated problems involving human subjects to the Office of Research Administration (ORA) within three (3) days of your knowledge of the occurrence.

2. Provide the ORA with a complete Continuing Review form (available at the CWRU IRB Web Pages, or from the ORA) by the continuing review deadline noted above, and when the study is terminated.

3. Discontinue all work pertaining to this protocol if a continuing review approval is not finalized by the expiration date noted above.

4. Submit all proposed changes to the protocol to the ORA, and receive approval from the IRB, before implementation of the change.

5. Keep all research data and consent documents in your possession for at least three (3) years after the completion of the research activity.

APPENDIX 2

Consent Form

Assent and Parental Permission
Genetic Analysis of Congenital Hypodontia

You are being asked to participate in a research study dealing with a possible genetic cause for missing teeth. You were selected as a possible participant because you or a family member has been identified with missing teeth.
Researchers at Case Western Reserve University are conducting this study.

Background Information:
The purpose of the study is to determine the reasons for missing teeth in family members. The absence of teeth, affects the position and size of the rest of your teeth, your bite, overall oral health and often, facial and dental appearance.

Procedures:

If you consent to participate in this study, we would ask you to do the following things:

- Allow us to count your teeth and check if any teeth may be missing. Time needed: 1 minute. No instruments will be used.
- Complete a simple and short questionnaire. The questions will gather information about the age, race, teeth that may be missing, medical conditions, and your dental history. The time required will be within 3-4 minutes.

The total time involved will be between approximately 5 minutes.

Future studies may involve you providing us with a blood sample (3ml/kg which is usually about 2-3 teaspoons) from your arm or brush a cotton swab against your cheek. Time required 1 minute.

Risks and Benefits to being in the study:

There are no known risks involved with answering a short questionnaire. If family members do have missing teeth we would require verification via radiograph. We will be evaluating existing radiographs from your dentist or orthodontist, so there is no risk of radiation exposure involved. Your only benefit will be your contribution to the understanding of a complex and common condition.

Confidentiality:

Access to research records will normally be limited to the researchers. However, the University's Institutional Review Board (IRB) and other regulatory agencies, may review

the research records to ensure that the rights of human subjects are being adequately protected. The information gathered via the clinical assessment and questionnaire will be kept private. In any sort of report we might publish, we will not include any information possible that will make it possible to identify a participant. Research records will be kept in a locked room and only authorized researchers will have access to the records.

Please check one of the following

I give permission for medical record and demographic information to be stored indefinitely

I would like my medical record and demographic information destroyed after the stated project is completed

Voluntary nature of the study:

Your participation is voluntary, and your refusal to participate will not affect your current or future relation with the University. If you decide to participate, you are free to withdraw at any time without penalty or loss of benefits to which you are otherwise entitled.

Contacts and Questions:

The researchers conducting this study are Dr. Manish Valiathan and Dr. Christopher Spoonhower. You may ask any questions you may have now. If you have questions later, you may contact them at (216) 368-0673 or (216) 368-4649.

If you would like to talk to someone other than the researcher's about (1) concerns regarding the study, (2) research participant rights, (3) research related injuries, or (4) other human subject issues, please contact Case Western Reserve University's Office of Research Administration at (216) 368-4510 or write:

Case Western Reserve University

Office of Research Administration

4 Adelbert Hall

10900 Euclid Avenue

Cleveland, OH 44106-7015

You will be given a copy of this form for your records.

Statement of consent:

I have read the above information. I have received answers to the questions I have asked. I consent to participate in the study.

Signature of the participant: Date

Signature of the Parent or Guardian Date

Signature of the person obtaining consent Date

APPENDIX 3

Questionnaire

Family Questionnaire to filled out by all Biological relatives

Family Identification Number: To be filled in by the investigator
Subject Number: To be filled in by the investigator

First Name: Last Name: Gender (circle): M F
Date of birth:
Address
Telephone

Ethnic Background /Race (you may circle more than one if necessary):

White African American/Black

Hispanic Asian

American Indian or Alaska Native

Native Hawaiian or other Pacific Islander

Other

**Please circle your relationship with _____:
Mother Father Brother Sister Other (specify)_____

1. Do you have any permanent teeth that are missing? (Circle) Yes No

2. If yes, why? (Circle) Cavities Gum disease Trauma

Orthodontics Never formed

3. Does anyone else in your family, unavailable to fill out this form, have teeth that never formed?

Yes No

4. If you answered Yes to the above question, is their relationship to you on your mothers or fathers side? How are they related to you? Please be specific.

5. Scientific journals have shown that there may been an association between teeth that never form and certain conditions of the hands and feet. Are there any unusual conditions relating to your hands or feet?

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