Primary failure of eruption and $PTH1R$: The importance of a genetic diagnosis for orthodontic treatment planning

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Introduction: Primary failure of eruption (PFE) is characterized by nonsyndromic eruption failure of permanent teeth in the absence of mechanical obstruction. Recent studies support that this dental phenotype is inherited and that mutations in $PTH1R$ genes explain several familial cases of PFE. The objective of our study was to investigate how genetic analysis can be used with clinical diagnostic information for improved orthodontic management of PFE.

Methods: We evaluated a family ($n = 12$) that segregated an autosomal dominant form of PFE with 5 affected and 7 unaffected persons. Nine available family members (5 male, 4 female) were enrolled and subsequently characterized clinically and genetically.

Results: In this family, PFE segregated with a novel mutation in the $PTH1R$ gene. A heterozygous c.1353-1 G $\rightarrow$ A sequence alteration caused a putative splice-site mutation and skipping of exon 15 that segregated with the PFE phenotype in all affected family members.

Conclusions: A $PTH1R$ mutation is strongly associated with failure of orthodontically assisted eruption or tooth movement and should therefore alert clinicians to treat PFE and ankylosed teeth with similar caution—ie, avoid orthodontic treatment with a continuous archwire. (Am J Orthod Dentofacial Orthop 2010;137:160.e1-160.e7)

Primary failure of eruption (PFE), originally described by Proffit and Vig, is characterized by nonsyndromic eruption failure of permanent teeth in the absence of mechanical obstruction. The hallmark features of this condition are (1) infraocclusion of affected teeth, (2) increasing significant posterior open-bite malocclusion accompanying normal vertical facial growth, and (3) inability to move affected teeth orthodontically.

Many studies have noted the heritable basis of this dental phenotype, and recently mutations in parathyroid hormone receptor 1 ($PTH1R$) have been identified in several familial cases of PFE. A detailed clinical analysis showed several types of nonsyndromic PFE, including type I and type II, with both types primarily affecting the posterior segments, either unilaterally or bilaterally. Type II is further characterized by greater eruption potential of the most distal tooth affected with PFE. It is not clear whether specific phenotypes (clinical variations described as type I vs type II) are associated with distinct genetic mutations or whether the clinical variation represents the broad spectrum associated with, for instance, $PTH1R$. It is known that, despite clinical severity or type, PFE does not respond to orthodontic force. Previous studies in our laboratory ruled out $AMELX$, $RUNX2$, $POSTN$, and $AMBN$ as candidate genes for PFE in families and individuals. However, recent advances in gene discovery, particularly with PFE, will most likely provide a novel diagnostic tool to aid in the clinical management of orthodontic patients when failure of eruption is suspected.

Eruption failure can be a part of a syndrome or occur as an isolated condition, such as PFE. In either case, it is critically important to distinguish between eruption failure due to local or mechanical causes (cysts, interference of adjacent tooth, lateral pressure from the tongue, or secondary to a syndrome such as cleidocranial dysplasia) and failure of the eruption mechanism completely. The orthodontic management of mechanical failure of eruption (MFE) is quite different from that of PFE. In the case of syndrome involvement,
the orthodontic diagnosis triage should include whether the syndrome results in MFE, including but not limited to osteopetrotic bone, fibrous gingival tissue, or impacted teeth, or whether it is due to delayed eruption. The genetic alteration of at least 1 gene, PTH1R, is evidence that there is also a biologic distinction between PFE and other eruption disorders such as MFE.9 Hence, once MFE is ruled out, genetic analysis of PTH1R should be a critical part of the orthodontic diagnostic regimen.

PTH1R resides on the small arm of chromosome 3 (3p22-21.1) and encodes a G-coupled protein receptor for both parathyroid hormone (PTH) and parathyroid hormone-related protein (PTHrP). A key function of PTH is regulation of calcium metabolism, whereas PTHrP plays a major role in skeletal development during early bone growth by regulating chondrocyte proliferation and differentiation. Recessive mutations in PTH1R have been identified in people with Blomstrand chondrodysplasia (BOCD; OMIM: 215045), a genetic disorder characterized by advanced endochondral bone maturation, increased bone density, and short stature.10 Those affected with BOCD also had abnormalities in breast development and several impacted teeth. In BOCD, autosomal recessive mutations have led to mutational inactivation of PTH receptors.10 Other conditions associated with mutations in PTH1R include Jansen metaphyseal chondrodysplasia (OMIM: 156400), enchondromatosis, and Eiken syndrome (OMIM:600002), all of which include cartilage or skeletal dysplasia.11-13 Despite the many disorders associated with mutations in PTH1R, the recent report of PTH1R mutations associated with PFE makes this a high-priority candidate gene for confirming diagnosis of a nonsyndromic PFE phenotype.9 The objective of our study was therefore to investigate how genetic analysis can be used with clinical diagnostic information for improved orthodontic management of PFE.

MATERIAL AND METHODS

Enrollment for this study was based on PFE in at least 2 family members. All subjects (adults and minors) consented to participate (including a release for dental records). Approval for this study was granted by the Biomedical Institutional Review Board at the University of North Carolina at Chapel Hill. Through detailed interviews, a pedigree (Fig 1) was extended in 1 family. This family consisted of 12 people from 11 to 72 years of age. Five male and 4 female members were available for clinical and molecular testing. A comprehensive clinical evaluation was performed to determine (1) a positive diagnosis of PFE with no mechanical or secondary barrier and also based on at least 1 unerupted tooth after unsuccessful orthodontic treatment for the proband; (2) the skeletal relationship including vertical (overbite or open bite) and sagittal (Angle Class I, Class II, or Class III molar relationship) based on clinical examinations and cephalometric radiographs; and (3) anomalies in growth or stature either absent or present. The clinical evaluation included clinical photographs; panoramic and cephalometric radiographs were obtained for participating family members.

Molecular analysis was performed after extraction and purification of DNA from buccal cells (PureGene kit, Gentra Systems, Minneapolis, Minn) or saliva (Oragene, DNA Genotek, Toronto, Ontario, Canada). We amplified and sequenced all coding exons of PTH1R (exons 3-16) for the 9 subjects using primer sets as described previously.9 To include splice junctions in our analysis, primer sets were designed to delineate regions that included a minimum of 25 bases of intron sequence in addition to the exon sequence. Amplification was performed by using Accuprime polymerase chain reaction buffer and enzyme mix (Life Technologies/Invitrogen, Bethesda, Md) under the following conditions: 10 minutes at 95°C activation/premelt, followed by 35 cycles of 30 seconds at 94°C melt, 30 seconds at 60°C anneal, and 3 minutes of 72°C extension. The polymerase chain reaction products were purified by using ExoSaplt (USB, Cleveland, Ohio) and sequenced at the University of North Carolina at Chapel Hill’s Genome Analysis Core facility.
Sequences were compared to wild type \textit{PTH1R} (accession NM_000316.2) from GenBank release GRCh37 by using the BLAST algorithm.\textsuperscript{14} For our study, previously reported single nucleotide polymorphisms and synonymous substitutions in the coding region were ignored.

RESULTS

Pedigree analysis by visual inspection (Fig 1) suggested an autosomal dominant inheritance with variable expressivity of PFE. For all examined persons, there was a complete absence of systemic disorders, and stature was within normal limits but varied as expected according to population norms. Two affected family members were classified as PFE type I (subjects III:3 and II:2); 2 were type II (subjects III:2 and III:4). In the family, the severity varied greatly. For example, the pretreatment records of subject III:4 showed a much less severe manifestation of PFE with only unilateral involvement (Fig 2, A-C). Subjects III:4 and III:2 (Fig 3) had previously been diagnosed with ankylosis rather than PFE, since the more severe classic appearance of PFE was lacking (only 2 or 3 affected teeth in 1 quadrant). This is in contrast to their full sibling, subject III:3 (Fig 4), whose pretreatment photos showed a severe bilateral open bite that included the canines through the second molars. Subject II:2 (Fig 5, A-C) was similar in severity to her son (subject III:3). In this family, an identical genetic diagnosis (see next section) was an equalizing element; although the clinical diagnoses were disparate between affected members, the resultant orthodontic failure was the same.

Fig 2. A-C, Type II PFE was observed in pretreatment photos of subject III:4, with a Class I molar and canine relationship. D-F, This mild presentation of a unilateral open bite (indicated by the arrow) was initially mistaken for isolated ankylosis, which significantly worsened with continuous archwire treatment. The resultant posterior lateral open bite could be corrected only with single-tooth osteotomies to reposition the teeth occlusally.

Fig 3. A-C, Pretreatment records of subject III:2 show type II PFE with slightly more eruption potential of the distal-most affected tooth on the right side. Similar to his sister (subject III:4), he has a unilateral pattern of PFE with a Class I relationship on the unaffected side (left).
Clinical and cephalometric evaluation of vertical and sagittal relationships showed that affected persons were rarely Class II skeletal or dental and more likely Class III. Cosegregation of Class III malocclusion and PFE traits was observed. Specifically, 2 subjects affected with PFE were also diagnosed as Class III skeletal; subject I:2 was not affected with PFE but had a Class III malocclusion. In the 2 subjects with PFE and Class III, orthognathic surgery was required to correct the Class III sagittal relationship. Furthermore, these persons with a Class III skeletal relationship had a more severe posterior lateral open bite. Analysis of the clinical pretreatment and posttreatment records also showed that, despite the clinical severity of the PFE phenotype, treatment with a continuous archwire resulted in the same outcome: failure to erupt. The response to treatment was often intrusion of the adjacent teeth (Fig 2, F), creating a more severe malocclusion than before orthodontic treatment.

Direct sequencing of PTH1R from the 9 participants (5 affected, 4 unaffected) showed the cosegregation of a novel mutation with the PFE phenotype. Mutational analysis of PTH1R showed a heterozygous alteration, c.1353-1 G > A, which resulted in a putative splice-site mutation and skipping of exon 15 (Fig 6). This mutation, which is predicted to cause loss of function of the PTH1R protein, segregated in an autosomal dominant fashion with the phenotype. Absence of the alteration in subjects I:1 and I:2 ruled out an autosomal recessive mode of inheritance. The c.1353-1 G > A mutation appeared in subject II:2 (her sibling, II:3, was unavailable for testing but was reported to have a similar eruption disorder) and 3 of her children, subjects III:2, III:3, and III:4. Sequence data from the unaffected subjects (I:1, I:2, and III:5) showed no alterations of PTH1R. Functional studies are currently underway to specifically determine the consequence of this sequence alteration in the PTH1R gene.

**DISCUSSION**

Our pedigree and clinical analysis findings further confirm that PFE is an inherited disorder, and that the inheritance can be autosomal dominant with variable expressivity. We ruled out autosomal recessive inheritance in this family, since we found 1 of 2 scenarios: (1) the mutation occurred as a new, spontaneous mutation in a founder, or (2) when 2 generations were affected, only 1 parent carried the PTH1R mutation. Moreover, autosomal recessive mutations of the PTH1R gene have been reported in the literature with severe forms of dwarfism; this form is often lethal and results in death shortly after birth. It has been proposed that, in the case of BOCD, autosomal recessive mutations of PTH1R most likely result in a complete lack of functional PTH receptors. Hence, the skeletal abnormalities seen in BOCD are explained by a lack of functional PTH receptors; conversely, autosomal dominant mutations in PFE result in reduced gene dosage with some functional protein remaining.
One characteristic of BOCD is mandibular hypoplasia, presumably caused by condylar cartilage hypoplasia in affected persons. Since 2 subjects with PFE also had Class III skeletal patterns, the Class III skeletal patterns could have been the result of growth disturbances in the cartilaginous nasal capsule, leading to maxillary hypoplasia rather than exuberant mandibular growth.

Hence, patients with PFE might allow an opportunity to study the genetic and epigenetic factors associated with localized intramembranous bone growth and an attempt to relate those findings to more generalized endochondral skeletal growth disorders.

The finding that both mild and severe cases carried the same mutation in 1 family challenged previous
diagnoses of ankylosis for isolated unerupted teeth. In this family, 2 affected members had been diagnosed with ankylosis, determined by bone sounding. Because our evaluation found that all family members had PFE, it shows a need to establish better diagnostic tools to distinguish between ankylosis and PFE. Alternatively, perhaps these 2 conditions belong to the same biologic spectrum and are effectively the same disorder. Future studies that explore the molecular and cellular events of ankylosis vs PFE will help to elucidate the pathways that contribute to normal eruption. When either MFE or delayed eruption is ruled out, the orthodontist would be wise to use a genetic analysis to improve the orthodontic management of PFE patients.

It is still unclear why highly variable clinical expressivity is observed in PFE, with some persons affected bilaterally and others affected unilaterally in the same family. Likewise, there is no apparent explanation for why the posterior dentition is preferentially affected. We speculated that similar to other patterning genes that are involved in tooth development (eg, MSX1, DLX2, PAX9), the PTH1R gene product acts in a temporally and spatially specific manner, affecting the posterior vs anterior segments of the alveolus and corresponding teeth.

The discovery of a gene that is responsible for PFE does not eliminate the likelihood that additional genes, yet to be discovered, are also responsible for this condition. Similar to tooth agenesis and other craniofacial disorders, many genes in critical pathways interact with PTH1R. Although the recent report of autosomal dominant mutations in PTH1R makes this gene an ideal candidate gene for analysis of an eruption failure phenotype, future research should address these questions: what is the specific role of PTH1R in the spectrum of eruption failure phenotypes, and are additional genes responsible for familial eruption failure?

CONCLUSIONS

Although molecular studies in mice have significantly advanced our understanding of the cellular and molecular pathways involved in tooth eruption, its specific genetic and cellular determinants and their relationships to clinical disorders are still poorly understood. By taking advantage of the genetic knowledge by the identification of PTH1R, we can begin to understand these determinants and avoid unnecessary procedures such as futile attempts to extrude teeth orthodontically, thereby sparing PFE patients excessive costs and protracted treatment times. Additional genotype and phenotype studies of PFE will also aid in determining whether affected persons (carrying mutations in PTH1R or other yet unidentified genes) might respond to orthodontic forces. To date, there are only a few anecdotal cases of successful extrusion of teeth affected with PFE. We hope that, with the identification of additional genes involved in abnormal tooth eruption, we will someday elucidate the genetic basis of several critical biologic aspects that underlie all clinical orthodontics: (1) normal tooth eruption, (2) normal dentoalveolar growth and development, (3) tissue behavior in normal physiologic tooth drift, (4) tissue reaction to orthodontic tooth movement, and (5) variability in response to orthodontic treatment. The ultimate goal of genetic research in orthodontics is to shift the emphasis in diagnosis and treatment planning from a wholly phenotypic or clinical perspective to greater consideration of a patient’s genotype.

Since we now have no mecanotherapeutic means for modifying dentoalveolar growth in PFE, any attempt at early orthodontic intervention for these patients is futile. Once growth is complete, several multidisciplinary treatment strategies can partially solve the severe posterior open-bite malocclusions that are characteristic of this disorder. Single-tooth or multiple-tooth osteotomies or selective extractions followed by implants can often lead to a functioning occlusion. Thus, the advantage of making an early diagnosis of PFE is that it allows a clinician the peace of mind to merely observe and document the unfavorable growth changes that will inevitably take place. This treatment-planning recommendation is meant to be in sharp contrast to an ex post facto diagnosis of PFE after unsuccessful orthodontic extrusion of the teeth. The disadvantage of a therapeutic diagnosis (early orthodontic intervention particularly with a continuous archwire) is that it can actually make the situation worse. Thus, the best treatment for an accurately established early diagnosis of PFE is initially no treatment but reserving the multidisciplinary options for affected patients for a later time after the completion of growth.

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REFERENCES


