

Absence of Association Between Transforming Growth Factor- β 1 Promoter Polymorphisms and Hypodontia

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Abstract: Hypodontia, the congenital absence of one or a few teeth, is one of the most common alterations of the human dentition. The most common permanent missing teeth are the third molars, second premolars, and maxillary lateral incisors. Although hypodontia does not represent a serious public health problem, it may cause masticatory and speech dysfunctions and esthetic problems. Transforming growth factor- β 1 (TGF- β 1) is believed to play an important role in tooth development. Its gene is expressed at bud, cap, and bell stages of odontogenesis. Genetic polymorphisms in the TGF- β 1 gene promoter were shown to interfere with the transcriptional activity of this gene. To further investigate the role of the TGF- β 1 gene in human hypodontia, we analyzed the frequencies of the -509 polymorphism (C-T) alleles and -800 polymorphism (G-A) alleles and genotypes in the TGF- β 1 gene promoter in 51 Caucasian subjects with hypodontia and 48 control individuals. Our data suggest that these TGF- β 1 promoter polymorphisms are not associated with hypodontia. (*Angle Orthod* 2004;74:665-671.)

Key Words: TGF- β 1; Hypodontia; Tooth agenesis

INTRODUCTION

Hypodontia, the congenital absence of one or a few teeth, is one of the most common alterations of the human dentition. Hypodontia can occur as a familial (autosomal dominant, recessive, or X linked) or isolated sporadic trait.¹ The most common permanent teeth missing are the third molars (20%), second premolars (3.4%), and maxillary lateral incisors (2.2%).² Although hypodontia does not represent a serious public health problem, it may cause masticatory and speech dysfunctions and esthetic problems. In the past few years, several growth and transcription factors were shown to be expressed in developing teeth.^{3,4} The direct participation of several genes in tooth development was evidenced by the lack of teeth in mutant knockout mice models.⁵ Autosomal dominant forms of hypodontia were shown to be caused by mutations in the MSX1 and PAX9 genes in human families.⁶⁻¹¹ However, the origin of the isolated spo-

radic agenesis, the most common form of hypodontia in humans, is still unknown.

Although the exact molecular mechanisms involved in the tissue interactions that regulate tooth development are largely unknown, there is evidence that extracellular matrix (ECM) molecules and growth factors as well as their receptors may play a central role.^{12,13} Transforming growth factor- β 1 (TGF- β 1) is a signaling molecule that participates in the cascade of signaling events during early tooth development. Its gene is expressed in both epithelia and mesenchyme during bud, cap, and bell stages of odontogenesis.¹² At bud and cap stages, the dental mesenchyme is rapidly proliferating, whereas the dental epithelium intensely expresses TGF- β 1 ribonucleic acid (RNA), and the mesenchyme itself also expresses TGF- β 1 although at lower levels. Thus, the local expression of TGF- β 1 in the dental epithelium may regulate cell proliferation in the underlying dental mesenchyme and contribute to the determination of tooth morphology.^{12,14} Another possible function of TGF- β 1 during tooth morphogenesis is regulation of ECM deposition. TGF- β 1 is known to promote the synthesis of ECM, to modify cell surface matrix receptors, and to prevent degradation of ECM.^{15,16}

Gene polymorphisms are mechanisms by which individuals may exhibit variations within the range of what is considered biologically normal. Gene polymorphisms were also shown to be associated with disease susceptibility. Most polymorphisms are single nucleotide exchanges that occur with high frequency in the human genome and may

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TABLE 1. Genotypes and Profile of Permanent Dentition in Affected Individuals^a

C-509T	G-800A	Upper		8R	7R	6R	5R	4R	3R	2R	1R	1L	2L	3L	4L	5L	6L	7L	8L
		Lower																	
CT	GG	1	*																*
CT	AG	2	*				*												*
CC	GG	3	*																*
CC	GG	4	*																*
CT	AG	5	*																*
CT	GG	6	*																*
TT	GG	7	*				*												*
CC	AG	8	*																*
CT	GG	9	*																*
CC	GG	10	*							*			*						*
CT	GG	11	*							*	*								*
CT	GG	12	*																*
TT	GG	13	*							*						*			*
CC	GG	14	*																*
CT	GG	15	*																*
CT	AG	16	*																*
CT	GG	17	*																*
CT	GG	18	*																*
CT	GG	19	*																*
CT	GG	20	*																*
CT	GG	21	*																*
CC	GG	22	*																*
CT	GG	23	*																*
CT	GG	24	*																*
CT	GG	25	*																*
CT	GG	26	*																*
CC	GG	27	*																*
CC	AG	28	*					*							*				*
CC	AG	29										*	*						
CT	GG	30								*			*						
CT	AG	31													*	*			
CC	GG	32					*									*			*
TT	AG	33					*							*					*

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TABLE 1. Continued

C-509T	G-800A	Upper Lower	8R	7R	6R	5R	4R	3R	2R	1R	1L	2L	3L	4L	5L	6L	7L	8L
CC	GG	34																
CT	GG	35							*						*			
CT	GG	36													*			
TT	GG	37					*											
CC	GG	38							*						*			
CT	GG	39							*						*			
CT	GG	40				*									*			
CT	AG	41							*						*			
CT	GG	42					*											
CC	GG	42						*						*				
CC	GG	44							*						*			
CC	GG	45							*						*			
CT	GG	46							*	*	*	*	*	*	*			
CT	GG	47							*						*			
CT	GG	48							*						*			
TT	GG	49							*						*			
CC	GG	50							*						*			
CC	GG	51							*						*			

^a The first two columns represent the genotypes of C-509T and G-800A polymorphic sites. The numbers in the vertical rows represent the position of the tooth (1 central incisors; 2 lateral incisors; 3 canines; 4 and 5 first and second premolars; 6, 7 and 8 first, second and third molars, respectively). L indicates left quadrant; R, right quadrant; *, missing teeth.

affect the function of genes.¹⁷ Recent evidence indicates that there is a substantial genetic component in the control of total and active TGF-β concentration in the circulation. In particular, two polymorphisms identified in the promoter region of the TGF-β1 gene—C-509T and G-800A—were associated significantly with the plasma concentration of TGF-β1.^{18,19} Polymorphisms in the promoter region of TGF-β1 gene have been associated with asthma severity,²⁰ adult periodontitis,²¹ renal parenchymal scarring,²² and Alzheimer disease.²³ The aim of the present study was to test whether the C-509T and G-800A single nucleotide polymorphisms of the TGF-β1 gene are associated with human hypodontia.

MATERIAL AND METHODS

Subject selection and sampling

Fifty-one unrelated individuals with hypodontia and without signs of other disorders (Table 1) and 48 healthy

control individuals (without hypodontia) were interviewed and the data documented. The congenital absence of teeth was confirmed by X-ray analysis. All subjects were of Caucasian origin and demonstrated no other dental anomalies.

The sampling of epithelial buccal cells was performed as described previously.²⁴ Briefly, 99 individuals undertook a one-minute mouthwash containing five mL of 3% glucose. After the mouthwash, a sterile wood spatula was used to scrape the oral mucosa. The tip of the spatula was then shaken into the retained mouthwash solution. Buccal epithelial cells were pelleted by centrifugation at 2000 rpm for 10 minutes. The supernatant was discarded and the cell pellet resuspended in 500 μL of extraction buffer (10 mM Tris-HCl [pH 7.8], five mM ethylenediaminetetraacetic acid [EDTA], 0.5% sodium dodecyl sulfate [SDS]). The samples were then frozen at -20°C until used for Deoxyribonucleic acid (DNA) extraction. After thawing, the samples were incubated overnight with 100 ng/mL proteinase K (Sigma, St. Louis, Mo) at 37°C with agitation. DNA was then pu-

rified by sequential phenol-chloroform extraction and salt-ethanol precipitation. DNA was dissolved in 70 μ L of TE buffer (10 mM Tris [pH 7.8], one mM EDTA). The concentration was estimated by measurements of OD 260.

Polymerase chain reaction and restriction endonuclease digestion

Polymorphism at position -509. The oligonucleotides 5' TTTTGCCATGTGCCAGTAG 3' and 5' CACCAGAGAAAGAGGACCAG 3' were used as primers. Polymerase chain reactions (PCR) were carried out with 500 ng genomic DNA in a total volume of 50 μ L, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, one μ M of each primer, 200 μ M each deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP), and deoxythymidine triphosphate (dTTP), 2.5 mM MgCl₂, and 2.5 units *Taq* DNA polymerase (Amersham Pharmacia Biotech, Uppsala, Sweden). The reaction was incubated for three minutes at 95°C, followed by 35 cycles of one minute at 95°C, one minute at 58°C, and one minute at 72°C, and a final extension at 72°C for five minutes.

Polymorphism at position -800. The oligonucleotides 5' CCCGGCTCCATTTCCAGGTG 3' and 5' TGCTCTTGA CCACTGTGCCA 3' were used as primers. PCR were carried out with 500 ng genomic DNA in a total volume of 50 μ L, containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, one μ M of each primer, 200 μ M each dATP, dCTP, dGTP, and dTTP, 2.5 mM MgCl₂, and 2.5 units *Taq* DNA polymerase (Amersham Pharmacia Biotech). The reaction was incubated for three minutes at 95°C, followed by 35 cycles of one minute at 95°C, one minute at 62°C, and one minute at 72°C, and a final extension at 72°C for five minutes.

The PCR products were then digested with 1.5 units of the *Eco8II* and *MaeIII* restriction enzymes for the analysis of the C-509T and G-800A promoter polymorphisms, respectively. The digest was mixed with 5 μ L of loading buffer and electrophoresed on a 7.5% vertical nondenaturing polyacrylamide gel. The DNA bands were evidenced by the rapid silver staining method.²⁵

Statistical analysis

The significance of the differences in observed frequencies of polymorphism in both groups was assessed by Chi-squared-test (χ^2). Differences were considered significant when $P < .05$. To calculate putative haplotypes and to verify Hardy-Weinberg expectations and linkage disequilibrium, the computer program package ARLEQUIN, version 2.0 was used.²⁶ The Clump software was used to assess differences in haplotype frequencies between control and test groups. This program is designed for use in genetic case-control studies where multiple alleles are being considered and the observed frequencies of some alleles are rare.²⁷

TABLE 2. Distribution of the TGF- β_1 Allele and Genotype in the Control and Test Group^a

SNP ^a	Control Group		Test Group		P Value
	n	%	n	%	
C-509T					
Allele					
C	61	63.5	63	61.8	.91 (chi-squared)
T	35	36.5	39	38.2	
Genotype					
C/C	20	41.6	17	33.3	.41 (Fisher exact test)
T/T	7	16.6	5	9.8	
C/T	21	43.8	29	56.9	
G-800A					
Allele					
G	87	90.6	93	91.2	.91 (chi-squared)
A	9	9.4	9	8.8	
Genotype					
G/G	39	81.3	42	82.4	1 (Fisher exact test)
A/A	0	0	0	0	
G/A	9	18.7	9	17.6	

^a TGF- β_1 indicates transforming growth factor- β_1 ; SNP, single nucleotide polymorphism.

RESULTS

In C-509T position, the *Eco8II* enzyme cleaves the PCR products into two fragments of 551 and 189 bp, when the polymorphic site contains allele C (but not T), whereas, in G-800A position, the *MaeIII* digests the G allele creating two fragments of 325 and 63 bp.

The statistical analysis did not show significant differences in the alleles and genotypes of both promoter polymorphisms of TGF- β_1 gene (C-509T and G-800A) between the two sample groups. In the C-509T polymorphism, the frequency of T allele was 36.4% in the control group and 38.2% in the test group (hypodontia, $P = .91$). The C-T genotype frequency was 43.8% in the control group and 56.9% in the test group ($P = .41$). Regarding the G-800A polymorphism, the allele G was observed with a very similar frequency in both groups (90.6% and 91.2%, respectively; $P = .91$). The genotype G-A was observed in 18.8% of the control group, whereas this same genotype was found in 17.6% of the test group ($P = 1$). The frequencies of different alleles and genotype of the TGF- β_1 gene are shown in Table 2. Comparisons were made with the affected individuals separated into two subgroups including third molar ($n = 28$) and incisor agenesis ($n = 18$), respectively. No significant differences in allele and genotype frequencies were observed when these two groups were confronted or when they were compared with control individuals (not shown). The genotype distribution for the two polymorphisms studied was consistent with the assumption of Hardy-Weinberg equilibrium in both groups. No linkage disequilibrium has been detected. The distri-

TABLE 3. Frequency of the Haplotype of the TGF- β ₁ Gene in the Control and Test Group^a

Haplotype G-800A/ C-509T	Control Group		Test Group		P Value
	n	%	n	%	
Allele					
GC	52	54.2	55	53.9	.92 (clump)
GT	35	36.5	38	37.3	
AC	9	9.4	8	7.8	
AT	0	0	1	1.0	
Genotype					
GC/GT	18	37.5	24	47.1	.55 (clump)
AC/GT	3	6.25	5	9.8	
GC/GC	14	29.2	14	27.5	
AC/GC	6	12.5	3	5.9	
AT/GT	0	0	1	1.9	
GT/GT	7	14.6	4	7.8	

^a TGF- β ₁ indicates transforming growth factor- β ₁.

bution of the haplotypes arranged as alleles and genotypes were similar between control and test groups ($P = .92$ and $P = .55$, respectively), and the most frequent haplotype in both groups was CG (Table 3). The phenotypic and genotypic characterization of the affected individuals is shown in Table 1.

DISCUSSION

TGF- β ₁ is a pleiotropic cytokine that plays a role in inflammation, tissue repair, as well as in embryonic growth and development.²⁸ The C-to-T polymorphism at -509 position of the human TGF- β ₁ promoter creates two different alleles located in a region that is thought to be a negative regulatory area of TGF- β ₁ gene.¹⁷ This polymorphism creates an YY1 activator consensus sequence.²⁹ The G-800A polymorphism is located in a region having enhancerlike activity (-1132 to -732), which is thought to overcome the negative regulatory region.³⁰ Although the role of TGF- β ₁ in tooth development has not been extensively studied, the expression of its messenger RNA (mRNA) detected by in situ hybridization experiments indicates that this molecule plays a role during the diverse phases of odontogenesis.¹² During the bud and cap stages of mouse molar odontogenesis, TGF- β ₁ is expressed in dental epithelium and mesenchyme indicating that this factor plays a role in the growth of tooth primordia. Histologic analysis of odontogenesis in TGF- β ₁ knockout mice showed that the morphogenesis, cytodifferentiation, and histogenesis were unaffected.³¹ Although no mRNA for was detected by in situ hybridization, TGF- β ₁ immunoreactive material was found in the tissues of knockout mice. D'Souza and Litz suggested that maternally-derived TGF- β ₁ could be responsible for the rescue of developmental events. Unlike the mouse, the development of most human permanent teeth occurs months or years after birth.

A major issue is to understand how genetic variations

affect development and are translated into changes in morphology. It is well established that morphological changes that occur among and within species are ultimately determined by genetic interference in the developmental process because small changes during development can produce large changes in adult morphology.^{32,33} Experimental studies have linked the spatial and temporal patterns of expression of regulatory genes during development and variations in morphology between species.^{32,34-36} Experimental and theoretical approaches have stressed the importance of *cis*-regulatory DNA sequences that control the transcription of genes as the basic modular unit that determines the positional information in developing structures.^{37,38} Mutations within individual modules can alter gene transcription in a tissue-specific manner, allowing a mutation to exert its effect on a few morphogenetic fields.³⁵

Teeth are becoming a major model in the novel field of evolutionary developmental biology, which links the molecular strategies that control organ development and morphological evolution.^{5,39-42} The complexity and dynamics of tooth organogenesis can be exemplified by observing the changes in dental patterning that occurred during the course of vertebrate evolution. Changes in the number of teeth tend to occur in the reverse order of how the teeth are formed during development, which also characterizes the general pattern of tooth loss observed during the evolution of placental mammals.⁴³ This process could be associated with changes in the regulation of tooth budding morphogenesis. Similar to hypodontia, most evolutionary changes in tooth number resulted from the loss of one or two elements, in most cases the last member of a tooth family. Polymorphic genetic loci have been directly associated with morphological variation in nonvertebrates, and there is to our knowledge no example of genetic polymorphism that causes morphological variation in vertebrates.⁴⁴ In this regard, the study of genetic polymorphisms in tooth agenesis is a specially suited model for understanding the association of gene polymorphisms and changes in morphology. Teeth are serially homologous structures, and the effects of gene variations on the development of these structures can be quantified easily.^{42,45} Individuals with distinct polymorphic alleles may exhibit subtle and specific phenotypic variations in dental patterning. In this sense, association studies between gene polymorphisms and hypodontia as well as other mild malformations that reflect qualitative defects of embryogenesis⁴⁶ may help in understanding the molecular mechanisms that account for the subtle phenotypic variations that characterize distinct human populations and ethnic groups.

Our data indicate that the TGF- β ₁ promoter polymorphisms are not associated with hypodontia. The lack of association in our results could be a consequence of the sample size. However, the very high P values found in our analysis are indication that there is indeed a lack of correlation. It is also possible that changes in the transcriptional

activity in the TGF- β 1 promoter caused by the polymorphisms studied are compensated by other factors in the cascade of events that regulate tooth development.⁴ In addition, other polymorphisms present in the coding sequence of the TGF- β 1 gene may have a larger effect on the development of tooth germ.⁴⁷

CONCLUSION

The results presented in the present study indicate that the -509 (C-T) and -800 (G-A) TGF- β 1 promoter polymorphisms are not associated with hypodontia.

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REFERENCES

- Vastardis H. The genetics of human tooth agenesis: new discoveries for understanding dental anomalies. *Am J Orthod Dentofacial Orthop.* 2000;117:650-656.
- Simons AL, Stritzel F, Stamatou J. Anomalies associated with hypodontia of the permanent lateral incisors and second premolars. *J Clin Pediatr Dent.* 1993;17:109-111.
- Thesleff I. Two genes for missing teeth. *Nat Genet.* 1996;13:379-380.
- Jernvall J, Thesleff I. Reiterative signaling and patterning during mammalian tooth morphogenesis. *Mech Dev.* 2000;92:19-29.
- Line SRP. Molecular morphogenetic fields in the development of human dentition. *J Theor Biol.* 2001;211:67-75.
- Vastardis H, Karimbux N, Guthua SW, Seidman JG, Seidman CE. A human MSX1 homeodomain missense mutation causes selective tooth agenesis. *Nat Genet.* 1996;13:417-421.
- van den Boogaard M-JH, Dorland M, Beemer FA, van Amstel HKP. MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. *Nat Genet.* 2000;24:342-343.
- Stockton DW, Das P, Goldenberg M, D'Souza RN, Patel PI. Mutation of PAX9 is associated with oligodontia. *Nat Genet.* 2000;24:18-19.
- Jumlongrass D, Bei M, Stimson JM, et al. A nonsense mutation in MSX1 causes Witkop syndrome. *Am J Hum Genet.* 2001;69:67-74.
- Nieminen P, Arte S, Tanner D, Paulin L, Alaluusua S, Thesleff I, Pirinen S. Identification of a nonsense mutation in the PAX9 gene in molar oligodontia. *Eur J Hum Genet.* 2001;9:743-746.
- Frasier-Bowers SA, Guo DC, Cavender A, Xue L, Evans B, King T, Milewicz D, D'Souza RN. A novel mutation in human PAX9 causes molar oligodontia. *J Dent Res.* 2002;81:129-133.
- Vahtokari S, Vainio S, Thesleff I. Associations between transforming growth factor- β 1 RNA expression and epithelial-mesenchymal interactions during tooth morphogenesis. *Development.* 1991;113:985-994.
- Shimo T, Wu C, Billings PC, Piddington R, Rosenbloom J, Pacifici M, Koyama E. Expression, gene regulation and roles of Fisp12/CTGF in developing tooth germs. *Dev Dyn.* 2002;224:267-278.
- Makela TP, Alitalo R, Paulsson Y, Westermarck B, Heldin CH, Alitalo K. Regulation of platelet-derived growth factor gene expression by transforming growth factor beta and phorbol ester in human leukemia cell lines. *Mol Cell Biol.* 1987;7:3656-3662.
- Rasmussen S, Rapraeger A. Altered structure of the hybrid cell surface proteoglycan of mammary epithelial cells in response to transforming factor-beta. *J Cell Biol.* 1988;107:1959-1967.
- Rizzino A. Transforming growth factor-beta: multiple effects on cell differentiation and extracellular matrices. *Dev Biol.* 1988;130:411-422.
- Hobbs K, Negri J, Klinnert M, Rosenwasser LJ, Borish L. Interleukin-10 and transforming growth factor-beta promoter polymorphisms in allergies and asthma. *Am J Respir Crit Care Med.* 1998;158:1958-1962.
- Grainger DJ, Metcalfe JC. Transforming growth factor-beta and cardiovascular protection. In: Rubanyi GM, Dzau VJ, eds. *The Endothelium in Clinical Practice. Source and Target of Novel Therapies.* 1st ed. New York: Marcel Dekker Inc; 1997:203-243.
- Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD. Genetic control of the circulating concentration of transforming growth factor type beta1. *Hum Mol Genet.* 1999;8:93-97.
- Pulleyn LJ, Newton R, Adcock IM, Barnes PJ. TGF-beta 1 association with asthma severity. *Hum Genet.* 2001;109:623-627.
- Souza AP, Trevilatto PC, Scarel-Caminaga RM, de Brito RB Jr, Line SRP. Analysis of the TGF- β 1 promoter polymorphism (C-509T) in patients with chronic periodontitis. *J Clin Periodontol.* 2003;30:519-523.
- Cotton SA, Gbadegesin RA, Williams S, Brenchley PE, Webb NJ. Role of TGF- β 1 in renal parenchymal scarring following childhood renal tract infection. *Kidney Int.* 2002;61:61-67.
- Luedeckung EK, DeKosky ST, Mehdi H, Ganguli M, Kamboh MI. Analysis of polymorphisms in the transforming growth factor-beta 1 gene and Alzheimer's disease. *Hum Genet.* 2000;106:565-569.
- Trevilatto PC, Line SRP. Use of buccal epithelial cells for PCR amplification of large DNA fragments. *J Forensic Odontostomatol.* 2000;18:6-9.
- Sanguineti CJ, Dias Neto E, Simpson SJ. Rapid silver staining and recovery of PCR products separated on polyacrylamide gels. *Biotechniques.* 1994;17:914-921.
- Schneider S, Roessler D, Excoffier L. *ARLEQUIN, Version 2000: A Software for Population Genetic Data Analysis.* University of Geneva, Switzerland: Genetics and Biometry Laboratory; 2000.
- Sham PC, Curtis D. Monte Carlo tests for associations between disease and alleles at highly polymorphic loci. *Ann Hum Genet.* 1995;59:97-105.
- Clark DA, Coker R. Transforming growth factor beta (TGF-beta). *Int J Biochem Cell Biol.* 1998;30:293-298.
- Kim S-J, Glick A, Sporn MB, Roberts AB. Characterization of the promoter region of the TGF- β 1 gene. *J Biol Chem.* 1989;264:402-408.
- Shrivastava A, Calame K. An analysis of genes regulated by multi-functional transcriptional regulatory Yin-Yang-1. *Nucleic Acids Res.* 1994;24:5151-5155.
- D'Souza RN, Litz M. Analysis of tooth development in mice bearing a TGF-beta 1 null mutation. *Connect Tissue Res.* 1995;32:41-46.
- Kim J, Kerr JQ, Min GS. Molecular heterochrony in the early development of *Drosophila*. *Proc Natl Acad Sci USA.* 2000;97:212-216.
- Raff RA. Evo-devo. The evolution of a new discipline. *Nat Rev Genet.* 2000;1:74-79.
- Warren R, Nagy L, Selegue J, Gates J, Carrol S. Evolution of homeotic gene regulation and function in flies and butterflies. *Nature.* 1994;372:458-461.
- Stern DL. Perspective: evolutionary developmental biology and the problem of variation. *Evolution.* 2000;54:1079-1091.
- Ronshaugen M, McGinnis N, Inglis D, Chou D, Zhao J, McGinnis W. Structure and expression patterns of *Drosophila* TULP

- and TUSP, members of the tubby-like gene family. *Mech Dev.* 2002;117:209–215.
37. Kopp A, Duncan I, Carrol SB. Genetic control and evolution of sexually dimorphic characters in *Drosophila*. *Nature.* 2000;408:553–559.
 38. Edelman GM, Gally JA. Degeneracy and complexity in biological systems. *Proc Natl Acad Sci USA.* 2001;98:13763–13768.
 39. Neubuser A, Peters H, Balling R, Martin GR. Antagonistic interactions between FGF and BMP signaling pathways: a mechanism for positioning the sites of tooth formation. *Cell.* 1997;90:247–255.
 40. Jernvall J. Linking development with generation of novelty in mammalian teeth. *Proc Natl Acad Sci USA.* 2000;97:2641–2645.
 41. Polly PD. Development and evolution occlude: evolution of development in mammalian teeth. *Proc Natl Acad Sci USA.* 2000;97:14019–14021.
 42. Line SRP. Variation of tooth number in mammalian dentition: connecting genetics, development and evolution. *Evol Dev.* 2003;5:295–304.
 43. Peyer B. *Comparative Odontology.* Chicago, Ill: The University of Chicago Press; 1968.
 44. Zimmerman E, Palsson A, Gibson G. Quantitative trace loci affecting components of wing shape in *Drosophila melanogaster*. *Genetics.* 2000;155:671–683.
 45. Line SRP. Molecular strategies in the evolution of mammalian dental patterning. *Evol Ecol.* 2001;15:73–79.
 46. Opitz JM. Heterogeneity and minor anomalies. *Am J Med Genet.* 1999;91:254–255.
 47. Langdahl BL, Cartens M, Stenkjaer L, Ericksen EF. Polymorphisms in the transforming growth factor beta 1 gene and osteoporosis. *Bone.* 2003;32:297–310.