Genetic Linkage of Hereditary Gingival Fibromatosis to Chromosome 2p21

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Summary

Gingival fibromatosis is characterized by a slowly progressive benign enlargement of the oral gingival tissues. The condition results in the teeth being partially or totally engulfed by keratinized gingiva, causing aesthetic and functional problems. Both genetic and pharmacologically induced forms of gingival fibromatosis are known. The most common genetic form, hereditary gingival fibromatosis (HGF), is usually transmitted as an autosomal dominant trait, although sporadic cases are common and autosomal recessive inheritance has been reported. The genetic basis of gingival fibromatosis is unknown. We identified an extended family ($n = 32$) segregating an autosomal dominant form of isolated gingival fibromatosis. Using a genomewide search strategy, we identified genetic linkage ($Z_{max} = 5.05$, $\theta = .00$) for the HGF phenotype to polymorphic markers in the genetic region of chromosome 2p21 bounded by the loci D2S1788 and D2S441. This is the first report of linkage for isolated HGF, and the findings have implications for identification of the underlying genetic basis of gingival fibromatosis.

Introduction

Hereditary gingival fibromatosis (HGF) is characterized by a slowly progressive, benign enlargement of the keratinized oral gingival tissues. Gingival tissues surrounding both the maxillary and the mandibular dentition may be affected. As a result, the teeth become buried, to varying degrees, beneath the redundant hyperplastic tissues, which results in both aesthetic and functional problems (Laband et al. 1964; Cuestas-Carnero and Bornancini 1988). Although HGF most commonly presents as an isolated clinical finding, it is also known to occur as part of a number of syndromes (Gorlin et al. 1976). The relationship between isolated hereditary and syndromic presentations of gingival fibromatosis is unclear.

In addition to Mendelian and syndromic forms, gingival fibromatosis is also known to be induced by certain classes of pharmacologics, including phenytoin, calcium-channel blockers, and cyclosporin (Hassell and Hefti 1991). These drugs are used in the treatment of seizure disorders (phenytoin), hypertension and angina (calcium-channel blockers), and organ transplants and autoimmune diseases (cyclosporin). Although these drugs differ in their primary target tissues, they share similarities with respect to their pharmacological mechanisms of action at the cellular level, and they may act similarly on a common secondary target tissue, such as gingival connective tissue (Dongari et al. 1993). The reported incidences of significant gingival fibromatosis occurring with these drugs are 13%–15% (phenytoin), 10%–15% (calcium-channel blockers; e.g., nifedipine), 30% (cyclosporin), and 40% (combined cyclosporin and nifedipine, used to treat transplant patients). This differential potential to develop gingival fibromatosis after exposure to these pharmacologics may have a genetic basis (Pernu et al. 1994) that determines whether an individual is a "responder" (develops gingival overgrowth) or not. Genetic polymorphisms may account for the differential response to pharmacotopic agents associated with drug-induced gingival fibromatosis. Polymorphisms have been identified, at both genetic and phenotypic levels, in a variety of xenobiotic metabolizing enzymes that predispose certain individuals to physiologic effects of particular pharmacologic agents (Daly et al. 1993). Although genetic factors appear to play a significant role in many types of gingival fibromatosis, the underlying genes responsible for these disorders are unknown.

Identification of the genetic basis of HGF may provide a beginning to understand genetic and pharmacologically induced forms of gingival fibromatosis. The purpose of the present investigation was to identify genetic linkage, as a first step toward identification of the gene

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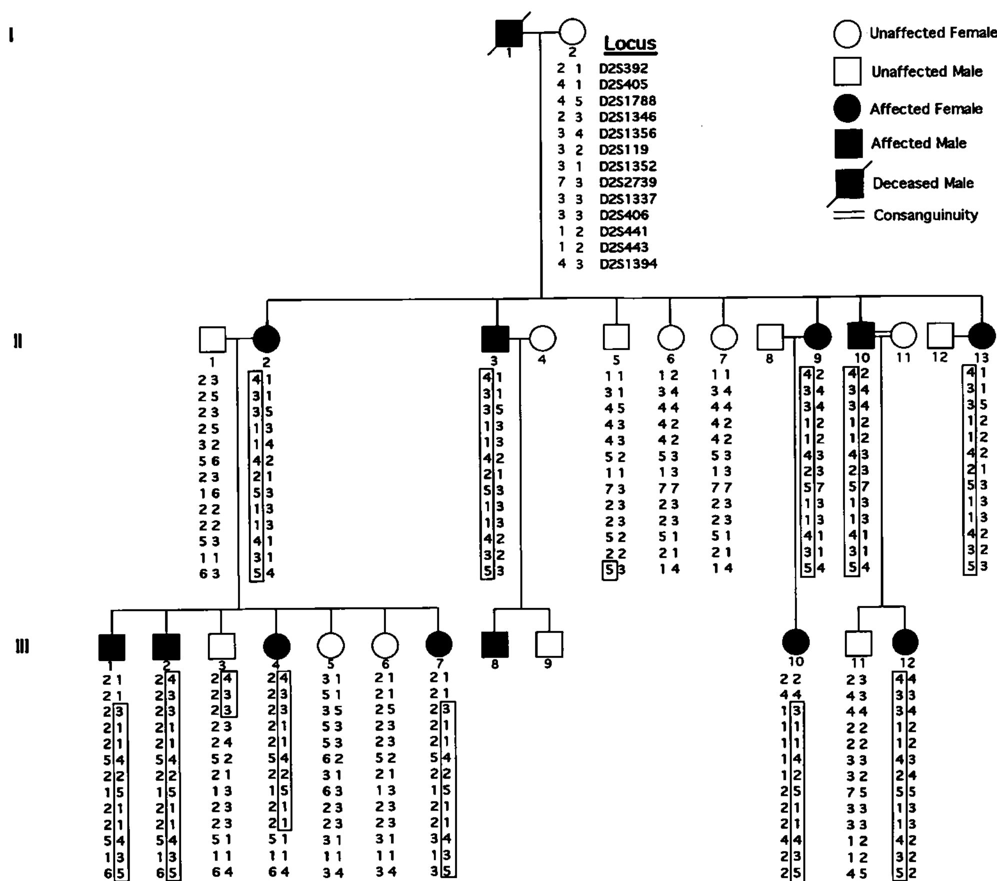

Figure 1 Pedigree of family segregating the HGF phenotype. Genetic haplotypes are presented for all individuals genotyped. Boxes surround alleles inherited from individual I-1.

defect(s) responsible for HGF. We have identified an extended family segregating a highly penetrant, autosomal dominant form of isolated HGF ($n = 32$, 12 affected). Using a genomewide search strategy, we obtained conclusive evidence for linkage of an HGF locus to markers on chromosome 2p21. This is the first report of linkage for isolated HGF, and it has implications for identification of the underlying genetic basis.

Material and Methods

Ascertainment of Family Members

A three-generation Brazilian family segregating for HGF was identified when the proband presented to the University of Taubaté dental clinic, seeking treatment for her enlarged gingiva. Figure 1 shows the family pedigree. Informed consent was provided by all study participants prior to their inclusion in the study. All individuals received an oral/ dental examination and, on the basis of presence or absence of gingival enlargement, were classified as either affected or unaffected. To be classified as affected, an individual had to have keratinized gingival tissues covering at least one-third of the clinical crowns of a minimum of five teeth. All individuals were asked about exposure to prescription and nonprescription medications in general and about exposure to specific pharmacologics associated with gingival overgrowth, including phenytoin, cyclosporin, and calcium-channel blockers.

DNA-Marker Analysis

Peripheral venous blood (7.5 ml) was obtained by standard venipuncture. Genomic DNA was extracted by means of the QIAamp blood kit (QIAMP). We performed an initial genomewide scan by use of the Weber version 6A low-density markers (Research Genetics), using standard techniques for PCR amplification with radioactively labeled γ-[^{32}P] primers, according to the manufacturer's protocol, with a PCR 9600 thermocycler (Applied Biosystems) (Weissenbach et al. 1992). After identification of a linkage relationship, individuals were genotyped for a high-density array of DNA markers that

Table 1

LOD Scores at Standard Values of θ and at the Maximum Likelihood Estimate (Z_{max}) of θ_{max}, Equal Values of θ in Both Sexes, for Markers from Chromosome 2p

Marker[a]	Location (cM)[b]	n[c]	Heterozygosity	LOD Score at θ= .00	.01	.05	.1	.2	.3	.4	Z_{max}	θ_{max}
D2S392	57	4	.68	$-\infty$	−2.94	−.39	.47	.96	.85	.43	.97	.22
D2S405	58	5	.80	$-\infty$	−5.09	−2.40	−1.33	-.42	−.05	.06	.01	.41
D2S1788	64	5	.89	$-\infty$	2.97	3.37	3.28	2.72	1.92	.94	3.37	.05
D2S1346	68	5	.93	5.05	4.97	4.65	4.23	3.32	2.29	1.11	5.05	.00
D2S1348	68	5	.93	5.05	4.97	4.65	4.23	3.32	2.29	1.11	5.05	.00
D2S119	75	6	.80	5.05	4.97	4.65	4.23	3.32	2.29	1.11	5.05	.00
D2S1352	82	3	.75	5.01	4.94	4.62	4.20	3.30	2.27	1.11	5.01	.00
D2S2739	...[d]	6	.95	5.04	4.96	4.64	4.23	3.31	2.28	1.11	5.04	.00
D2S406	91	3	.82	5.02	4.94	4.62	4.20	3.30	2.27	1.11	5.03	.00
D2S1337	91	3	.65	5.02	4.94	4.62	4.20	3.30	2.27	1.11	5.02	.00
D2S441	101	5	.75	$-\infty$	3.04	3.43	3.33	2.77	1.96	.96	3.44	.56
D2S443	103	6	.81	$-\infty$	2.97	3.37	3.28	2.72	1.92	.94	3.38	.57
D2S1394	108	3	.81	$-\infty$	3.04	3.43	3.33	2.77	1.96	.96	3.44	.56

[a] Marker loci showing no recombination with the HGF phenotype are underlined.
[b] From telomeric end of 2p.
[c] Total number of different alleles identified in the pedigree set.
[d] Most likely location between D2S1346 and D2S441.

spanned the genetic interval from D2S392 to D2S1394 (table 1). These markers were selected from the Cooperative Human Linkage Center Chromosome 2 version v8 c7 integrated marker map (http://www.chlc.org) and the Whitehead Institute STS-based Map of the Human Genome (http://www-genome.wi.mit.edu). After PCR amplification, individual samples were separated on a 6% PAGE 7M urea gel (30 W, 1,500 V). An M13 sequencing ladder (Sequenase kit, USB) was loaded onto each gel, to permit sizing of individual alleles. After electrophoresis, gels were wrapped in cellophane, exposed in a phosphorimaging cassette for 15 min, and scanned (Molecular Dynamics). Alleles were scored, and genotype data were entered into the pedigree file of the LINKAGE computer package (Lathrop and Lalouel 1984).

Linkage Analysis

Autosomal dominant inheritance with complete penetrance was assumed, and LOD scores were generated. The affected allele frequency was taken as .0001. Marker allele frequencies were assumed to be uniformly distributed. Calculations using marker allele frequencies reported in available databases were also performed, but these changes had minimal effects on the LOD scores generated. The number of alleles at each marker locus is shown in table 1. Two-point linkage analysis was performed by use of the MLINK program version 5.1 from the LINKAGE computer program (Lathrop and Lalouel 1984). At any given locus, results for the pedigree were used to generate a final LOD score for each marker tested. Precise values for maximum LOD scores (Z_{max}) were calculated with the ILINK program from the same computer package. Multipoint analyses were performed by FASTLINK (Cottingham et al. 1993; Schaffer et al. 1994). A multipoint map was constructed from several runs with overlapping sets of marker loci. Haplotype analysis was used for error elimination during the linkage scan and for determination of the critical linkage segment. Haplotype construction was performed using the CRIMAP program with the CHROMPIC option (Lander and Green 1987).

Cytogenetic Studies

To evaluate the possibility of chromosomal changes, cytogenetic studies were performed on 72-h phytohemagglutinin-stimulated peripheral blood cultures from two affected individuals (III-4 and III-7) and from one unaffected individual (III-6). Routine cultures were established in RPMI 1640 by means of standard laboratory techniques. Chromosome preparations were obtained by use of a modified ethidium bromide procedure (Ikeuchi 1984). Standard International System for Cytogenetic Nomenclature was followed, with high-resolution (650 band level) analysis performed on 30 trypsin-Giemsa–banded metaphases.

Results

Clinical Findings

Of the 32 family members identified, 29 received an oral examination (fig. 1). Twelve of these individuals, with enlarged keratinized gingival tissues covering at least one-third of the clinical crown of five or more teeth, were classified as affected. In affected individuals, all teeth were generally affected, although the severity of

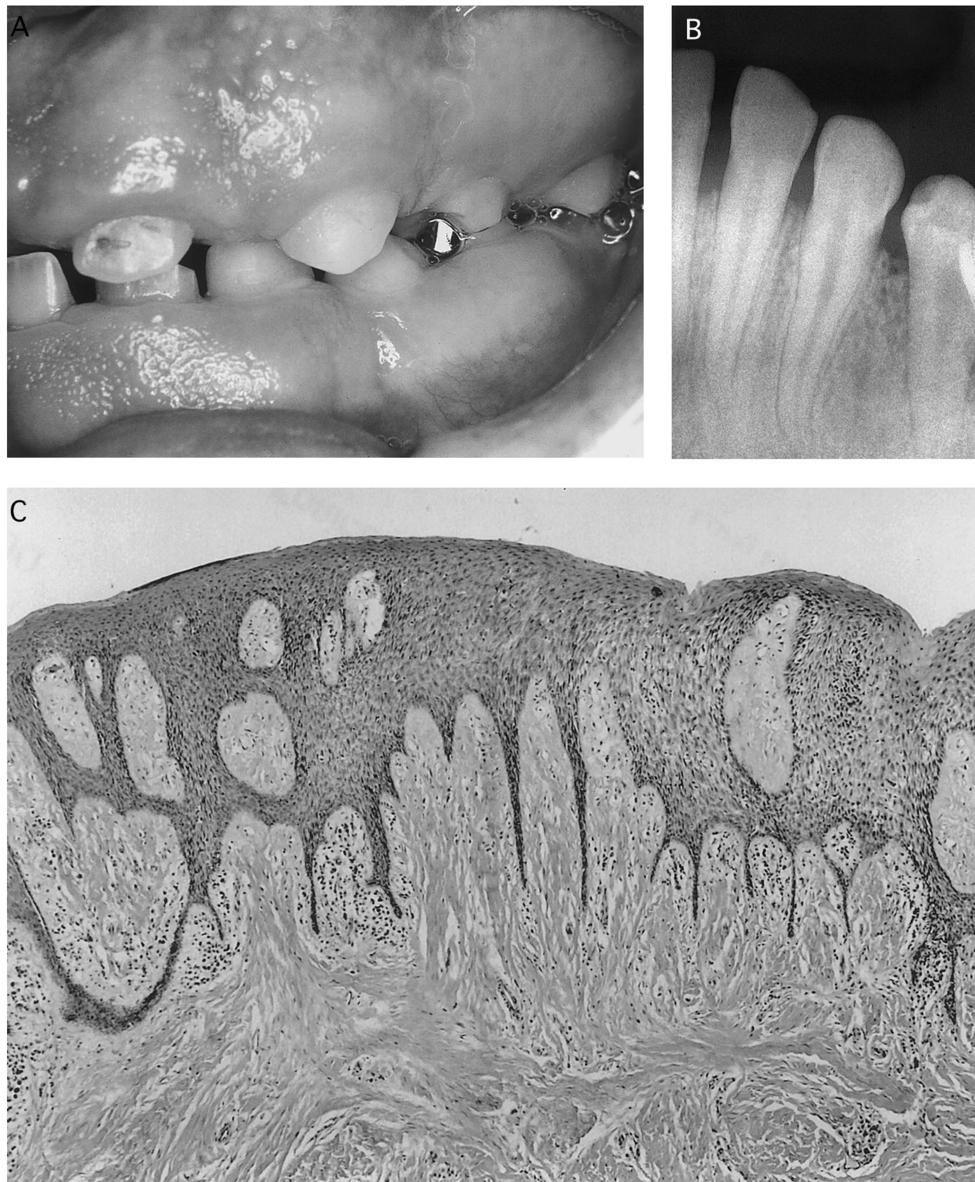

Figure 2 *A,* Clinical photograph (individual III-4) showing gingival fibromatosis. *B,* Periapical radiograph (individual III-4) showing normal level of alveolar bone. *C,* Histological section showing extension of epithelial rete pegs into underlying corneum (hematoxylin and eosin stain, original magnification × 100).

gingival enlargement varied considerably, from gingival enlargement covering one-third of clinical crowns of teeth to enlargement fully engulfing the crowns (fig. 2*A*). Periapical radiographs showed that there was no increase in alveolar bone surrounding involved teeth, consistent with the clinical impression of increased gingival soft tissues (fig. 2*B*). The proband (individual III-4) was treated surgically to remove the excess gingival tissues, and a recurrence of gingival overgrowth occurred. Histological evaluations of surgical resection tissue were consistent with gingival fibromatosis, and no evidence of an osseous component was seen (fig. 2*C*). No family members reported taking any class of pharmocologics (phenytoin, calcium-channel blockers, and cyclosporin) implicated in drug-induced gingival overgrowth. All family members were systemically healthy and were of normal intelligence. No family members reported a positive history of hearing loss, epilepsy, or hypertrichosis, findings associated with syndromic forms of gingival fibromatosis. Gingival fibromatosis was present in all generations, male-to-male transmission of the condition was observed, and there were approximately equal propor-

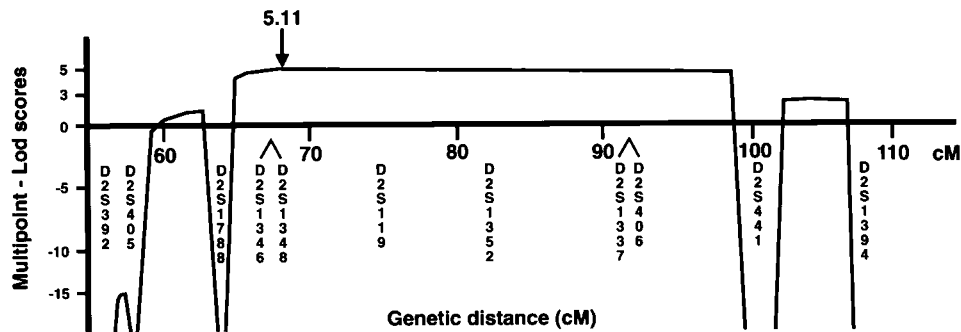

Figure 3 Multipoint LOD-score calculations between the HGF phenotype and markers D2S392, D2S405, D2S1788, D2S1346, D2S1348, D2S119, D2S1352, D2S1337, D2S406, D2S441, and D2S1394. The multipoint LOD score is represented on the x axis. The positions of the DNA marker loci tested are located on the y axis, together with the genetic location coordinate (in centimorgans) according to the Généthon map; the zero coordinate is at the short-arm telomere (Gyapay et al. 1994). Marker D2S2739 has not been included in the multipoint analysis because its precise genetic location was not known.

tions of affected males (.55) and females (.54). The paternal grandfather was deceased, but his family dentist reported that the grandfather had been affected by gingival fibromatosis. Segregation of gingival fibromatosis in this family was consistent with autosomal dominant transmission with high penetrance. Clinical findings for individuals classified as affected were consistent with isolated HGF (MIM 135300).

Two-Point Linkage Analysis

Using a genomewide search strategy, we genotyped all individuals for a low-density STRP locus–screening panel (Weber Version 6A). Two-point LOD scores supportive of linkage were obtained for only two markers, D2S1788 (Z_{max} = 3.37, recombination fraction (θ) of .05) and D2S441 (Z_{max} = 3.43, θ = .05). All individuals were then genotyped with markers from a high-density map of additional closely spaced markers that spanned the genetic interval between D2S392 and D2S1394. Two-point LOD scores for markers spanning the candidate interval are summarized in table 1.

A Z_{max} of 5.05 was obtained for D2S1346, D2S1348, and D2S119 at θ = .00. Results of the linkage analysis for the other genome-scan markers (outside of the chromosome 2p region) were not supportive of linkage with HGF in this family. Although the absolute value of two-point LOD scores calculated for HGF with these genetic markers varied, ~70% of the markers were able to significantly reject the linkage hypothesis in the vicinity of the marker tested (i.e., two-point LOD scores <-2.0 for values of θ ranging from .00 to .10). For those markers that did not reject linkage at the level of statistical significance, several were uninformative or only partially informative, none were supportive of linkage, and no LOD scores >1.0 were obtained for any marker tested, at any specified value of θ. Because of the report of gingival fibromatosis associated with recombinant 8 syndrome, we also tested for a linkage relationship between HGF and 18 genetic markers spanning chromosome 8. Results of two-point LOD score calculations provided statistical evidence against linkage for HGF with the genetic markers tested (LOD score $\leqslant -2.0$).

Haplotype and Multipoint Linkage Analysis

To define the smallest interval containing the HGF locus, we analyzed individuals for recombination events, by haplotype reconstruction. The cosegregating segment in which recombination was not detected was flanked by the markers D2S1788 and D2S441. Multipoint linkage analysis using 11 STRP markers yielded a maximum multipoint LOD score of 5.11 at D2S1346 and D2S1348. Using a criterion of LOD −1.0 to determine the 95% confidence interval (Conneally et al. 1985), we determined that the HGF locus lies between the genetic interval flanked by D2S441 and D2S1788 (fig. 3).

Possible Suppression of Meiotic Recombination in the HGF 2p21 Candidate Interval

The genetic interval in the candidate region is ~37 cM. The amount of recombination observed in this interval appears to be less than expected. We can evaluate this by calculating the probability of observing no recombinants in the interval for sex-average genetic maps. This is calculated by use of the equation $P = (1-\theta)^n$, where θ is the distance in which no recombination is observed and n is the number of equivalent meioses, calculated with the EQUIV program from the Linkage Utility Package (J. Ott, personal communication).

For the sex-averaged map, the distance between markers unseparated by recombination is 37 cM, corresponding to θ = .31. The calculated P value is therefore .002,

which suggests that absence of recombination is not merely due to chance. The male and female genetic map distances in this region of chromosome 2p21 are not equivalent. The male genetic map distance from D2S1788 to D2S441 is ~18.9 cM, and the female genetic map distance is 58.9 cM (Chromosome 2 Version 4.0 Recmin extended Weber Version 6 screening set sex-specific maps [http://www.chlc.org]). Even when we consider the differing lengths of the male and female genetic maps, we can calculate the probability of observing no recombinants, using the equation $P_{mf} = (1 - \theta_m)^{n_m}(1 - \theta_f)^{n_f}$, where θ_m is the male distance, θ_f is the female distance, n_m is the number of male meioses, and n_f is the number of female meioses. Using this equation and the appropriate distances (male genetic distance [18.9 cM] corresponds to $\theta = .18$, female genetic distance [58.9 cM] corresponds to $\theta = .41$) and meioses, we determined that the probability of observing no recombinants is .003, consistent with the sex-average calculation. One possible explanation for this phenomenon is a chromosomal rearrangement that reduces the efficiency of chromatid pairing in meiosis, resulting in a decrease in meiotic recombination.

Cytogenetics

Cytogenetic analysis on stimulated peripheral blood revealed normal karyotypes (46,XX) in all cells of the three individuals examined (III-4, III-6, and III-7). Extended examination of the 2p21 chromosomal region did not identify any chromosomal rearrangements that could account for the suppression of recombination observed across the interval of interest.

Discussion

Results of the present study have identified a major gene locus for HGF on chromosome 2p21. Results of the genomewide scan did not demonstrate support for linkage at any other site, and no LOD score >1.0 was found outside the chromosome 2p21 region. Because of the association of gingival fibromatosis with recombinant chromosome 8 syndrome, we genotyped 18 STRP markers spanning chromosome 8. Results of linkage analysis with these markers excluded an HGF locus on chromosome 8 (LOD scores <−2.0). Although the results for this family are quite conclusive, it will be necessary to evaluate this linkage relationship in additional families to determine whether this region represents a common HGF linkage. The genetic region segregating with the HGF phenotype is ~37 cM. The amount of genetic recombination observed across this region is less than expected ($P = .003$). The reason for the apparent suppression of meiotic recombination in the HGF candidate interval observed in this family is unknown. Results of high-resolution chromosomal banding studies performed on two affected and one unaffected family members did not indicate the presence of any cytogenetic rearrangement that would account for meiotic suppression. It is difficult to imagine how any factor that suppresses meiotic recombination could be causal for gingival fibromatosis. However, genetic linkage studies of other families demonstrate meiotic recombination across the interval flanked by D2S1788 and D2S441, indicating that the suppression of recombination in this interval may be unique to this family. Whether this is related to HGF in this family is unknown.

In the current family, HGF appears to segregate as a highly penetrant autosomal dominant trait. Inheritance patterns for isolated gingival fibromatosis are most consistent with autosomal dominant inheritance with incomplete penetrance and variable clinical expression (Jorgenson and Cocker 1974; Raeste et al. 1978), but autosomal recessive transmission has also been reported, and the condition is often reported to be sporadic. Whether the high frequency of sporadic cases reflects a high new-mutation rate, incomplete penetrance, or variable clinical expression and underdiagnosis of mild cases is unclear. Unfortunately, there are no biologic or molecular markers to aid in the diagnosis of gingival fibromatosis, and, as a result, diagnosis of affected individuals can be problematic, particularly in cases of mild expression. The availability of genetically linked DNA markers will, for the first time, provide the means for studying HGF.

Histological evaluation of HGF tissues show a fairly nonspecific hyperplasia of fibrous tissue of the corneum. The affected gingival tissues are composed mainly of dense connective tissue that is rich in collagen fibrils but contains only a few fibroblasts. The overlying epithelium is typically normal but may be slightly hyperplastic with rete pegs extending deeply into the corneum (fig. 2*C*). Although osseous involvement has been reported in HGF, it is apparently uncommon (Fritz 1970). There was no evidence of an osseous component of gingival enlargement in the present family. Osseous involvement was assessed by histological evaluation of surgical resection tissues and by assessment of standard periapical radiographs (fig. 2*B*). Gingival fibromatosis typically appears to consist of increased soft tissue, primarily epithelium and corneum. Although a number of potential biological mechanisms have been proposed for HGF, the genetic basis for gingival fibromatosis is currently unknown. Fibromatosis tissues have been reported to contain fibroblasts that have low growth activity but are active in the production of greater amounts of collagen and other extracellular substances (e.g., glycosaminoglycans) compared with normal fibroblasts (Shirasuna et al. 1989). Preliminary studies of gingival tissues from the proband of the family studied in the present report sug-

gest that altered collagen cross-linking may be etiologically important. For assessment of collagen cross-linking, gingival resection tissues were pulverized in liquid nitrogen and reduced with NaB^3H_4, hydrolyzed with 6N HCl, and subjected to amino acid and cross-link analyses. Results revealed a 30% increase in dehydro-dihydroxlysinonorleucine cross-linking in fibromatous gingiva compared with control gingiva (Pallos et al. 1997). Increased collagen cross-linking may increase resistance to degradation and may contribute to collagen accumulation. These preliminary results suggest that altered posttranslational modifications of collagen may be etiologically important in HGF.

In addition to Mendelian autosomal transmission, chromosomal anomalies have been identified in syndromic forms of gingival fibromatosis (Gorlin et al. 1976). The most common syndromic forms of gingival fibromatosis occur with hypertrichosis, epilepsy, and mental retardation. Other syndromic forms of gingival fibromatosis include sensorineural hearing loss; juvenile hyaline fibromatosis; corneal dystrophy (Rutherford syndrome); ear, nose, bone, and nail defects (Laband syndrome); microphthalmia, athetosis, and hypopigmentation (Cross syndrome); cherubism, hypertrichosis, mental and somatic retardation, and epilepsy (Ramon syndrome); and growth-hormone deficiency (Gorlin et al. 1976). Recently, Fryns (1996) reported a partial duplication of chromosome 2p13-p21 in an individual with gingival fibromatosis and mental retardation. The region of chromosome 2p13-p21 duplication reported by Fryns contains the HGF candidate region identified in the present study. These observations raise the possibility that a common gene locus on chromosome 2p may be involved in at least some isolated Mendelian and syndromic forms of HGF. It is possible that isolated gingival fibromatosis may result from a single gene mutation, whereas syndromic forms may result from alterations of multiple genes and/or from a gene-dosage affect. The generality of the chromosome 2p21 locus for Mendelian or syndromic forms of gingival fibromatosis is unknown. Syndromic gingival fibromatosis with sensorineural deafness has been reported (OMIM 135550), and it is possibly significant that a gene for deafness (DFNB9) has recently been identified in the 2p22 region (Hartsfield et al. 1985; Chaib et al. 1996). Although it is unknown whether Mendelian forms of gingival fibromatosis are genetically heterogeneous, the association of gingival fibromatosis with recombinant chromosome 8 syndrome suggests that there are genetically distinct forms of syndromic gingival fibromatosis (Sujansky et al. 1993).

In addition to Mendelian and syndromic forms of gingival fibromatosis, pharmacologically associated forms are also known. Gingival enlargement may occur secondary to exposure to certain classes of pharmacologics, including phenytoin, cyclosporin, and calcium-channel blockers. Whether these conditions share any etiologic commonality is not known. Not everyone exposed to these pharmacologics develops gingival fibromatosis, and it is possible that allelomorphic polymorphisms at one or more different genetic loci may account for this differential response to one or more of these pharmacologic agents. A common mechanism of action of the pharmacological agents associated with gingival fibromatosis involves cellular regulation of calcium. The genetic interval containing the HGF locus identified in the present study includes at least two genes important in calcium regulation, calmodulin (CALM2) and sodium-calcium exchanger 1 (NCX1). Calmodulin is the archetype of the family of calcium-modulated proteins of which >20 members have been identified. Calmodulin functions in growth and the cell cycle as well as in signal transduction and in synthesis and release of neurotransmitters. Calmodulin is the delta subunit of phosphorylase kinase, which has three other types of subunits. Although only one form of calmodulin has been found in humans, three distinct human cDNAs have been isolated that encode the identical polypeptide. The existence of three expressible genes for calmodulin may indicate that one is a housekeeping gene and that the additional copies are differentially regulated to modulate calmodulin function (OMIM 114180). The amino acid sequence of the sodium-calcium–exchanger gene NCX1 does not resemble any previously described protein. In the heart, the exchanger may play a key role in digitalis action. The exchanger is the dominant mechanism in returning the cardiac myocyte to its resting state after excitation. The NCX1 gene is located just inside the chromosome 2p21 interval of interest, between D2S1348 and D2S119. Both NCX1 and CALM2 lie within the chromosome 2p21 interval that cosegregates with HGF and, as such, must be considered for a possible role in HGF. However, mutational studies have not yet been performed, and the roles of NXC1 and CALM2, if any, in gingival fibromatosis are unknown. It is possible that naturally occurring polymorphisms in these genes are important in pharmacologically induced forms of gingival overgrowth, whereas mutations in one of these genes may be important in HGF.

The biologic basis for gingival fibromatosis is unknown, but this first report of linkage for isolated HGF may provide the basis for strategies to identify the responsible gene. Identification of linkage for isolated HGF will also permit evaluation of the generality of HGF linkage to chromosome 2p, thereby testing support for allelic versus nonallelic heterogeneity for isolated gingival fibromatosis. Elucidation of the gene mutation responsible for HGF will permit investigation of possible common etiologic factors between different genetic and pharmacologically induced conditions manifesting gingival fibromatosis.

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References

Chaib H, Place C, Salem N, Chardenoux S, Vincent C, Weissenbach J, El-Zir E, et al (1996) A gene responsible for a sensorineural nonsyndromic recessive deafness maps to chromosome 2p22-23. Hum Mol Genet 5:155–158

Conneally PM, Edwards JH, Kidd KK, Lalouel JM, Morton NE, Ott J, White R (1985) Report of the committee on methods of linkage analysis and reporting. Cytogenet Cell Genet 40:356–359

Cottingham RW Jr, Idury RM, Schaffer AA (1993) Faster sequential genetic linkage computations. Am J Hum Genet 53: 252–264

Cuestas-Carnero R, Bornancini CA (1988) Hereditary generalized gingival fibromatosis associated with hypertrichosis: report of five cases in one family. J Oral Maxillofac Surg 46:415–420

Daly AK, Cholerton S, Gregory W, Idle JR (1993) Metabolic polymorphisms. Pharmacol Ther 57:129–160

Dongari A, McConnell HT, Langlais RP (1993) Drug-induced gingival overgrowth. Oral Surg Oral Med Oral Pathol 76: 543–548

Fritz ME (1970) Idiopathic gingival fibromatosis with extensive osseous involvement in a 12-year-old boy. Oral Surg Oral Med Oral Pathol 30:755–758

Fryns JP (1996) Gingival fibromatosis and partial duplication of the short arm of chromosome 2 (dup(2)(p13→p21)). Ann Genet 39:54–55

Gorlin RJ, Pinborg JJ, Cohen MM Jr (1976) Syndromes of the head and neck, 2d ed. McGraw-Hill, New York, pp 329–336

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Mare S, et al (1994) The 1993–94 Généthon human genetic linkage map. Nat Genet 7:246–339

Hartsfield JK Jr, Bixler D, Hazen RH (1985) Gingival fibromatosis with sensorineural hearing loss: an autosomal dominant trait. Am J Med Genet 22:623–627

Hassell TM, Hefti AF (1991) Drug-induced gingival overgrowth: old problem, new problem. Crit Rev Oral Biol Med 2:103–137

Ikeuchi T (1984) Inhibitory effect of ethidium bromide on mitotic chromosome condensation and its application to high-resolution banding. Cytogenet Cell Genet 38:56–61

Jorgenson RJ, Cocker ME (1974) Variation in the inheritance and expression of gingival fibromatosis. J Periodontol 45: 472–477

Laband PF, Habib G, Humphreys GS (1964) Hereditary gingival fibromatosis. Oral Surg Oral Med Oral Pathol 17: 339–351

Lander ES, Green P (1987) Construction of multilocus genetic maps in humans. Proc Natl Acad Sci USA 84:2363–2367

Lathrop GM, Lalouel, JM (1984) Fast calculations of LOD scores and genetic risks on small computers. Am J Hum Genet 36:460–465

Pallos D, Hart TC, Cortelli JR, Yamauchi M (1997) Altered post-translational modification of collagen in hereditary gingival fibromatosis. J Dent Res 76:32,147

Pernu HE, Knuuttila MLE, Huttunen KRH, Tiilikainen ASK (1994) Drug-induced gingival overgrowth and class II major histocompatibility antigens. Transplantation 57:1811–1823

Raeste AM, Collan Y, Kilpinen E (1978) Hereditary fibrous hyperplasia of the gingiva with varying penetrance and expressivity. Scand J Dent Res 86:357–365

Schaffer AA, Gupta SK, Shriam K, Cottingham RW Jr (1994) Avoiding recomputation in genetic linkage analysis. Hum Hered 44:225–237

Shirasuna K, Okura M, Watatani K, Hayashido Y, Saka M, Matsuya T (1988) Abnormal cellular property of fibroblasts from congenital gingival fibromatosis. J Oral Pathol 17: 381–385

Sujansky E, Smith ACM, Prescott KE, Freehauf CL, Clericuzio C, Robinson A (1993) Natural history of the recombinant (8) syndrome. Am J Med Genet 47:512–525

Weissenbach J, Gyapay G, Dib C, Vignal A, Morissette J, Millasseau P, Vayseix G, et al (1992) A second-generation linkage map of the human genome. Nature 359:794–801