Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2009; 36: 627–633 doi: 10.1111/j.1600-051X.2009.01438.x

Further evidence of genetic heterogeneity segregating with hereditary gingival fibromatosis

Xiaoqian Ye[1,*], Lisong Shi[2,*], Wei Yin[1,*], Liuyan Meng[1], Qing Kenneth Wang[2,3,4] and Zhuan Bian[1]

[1]Key Laboratory for Oral Biomedical Engineering of Ministry of Education, Hospital and School of Stomatology, Wuhan University, Wuhan, China; [2]Key Laboratory of Molecular Biophysics of Ministry of Education, Center for Human Genome Research, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, China; [3]Department of Molecular Cardiology, Center for Cardiovascular Genetics, Lerner Research Institute, Cleveland Clinic, and [4]Department of Molecular Medicine, Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland, OH, USA

Ye X, Shi L, Yin W, Meng L, Wang QK, Bian Z. Further evidence of genetic heterogeneity segregating with hereditary gingival fibromatosis. J Clin Periodontol 2009; 36: 627–633. doi: 10.1111/j.1600-051X.2009.01438.x.

Abstract

Aim: To clinically characterize and map the disease-associated locus in a five-generation Chinese family with autosomal dominant early-onset hereditary gingival fibromatosis (HGF).

Material and Methods: A complete oral examination was conducted. Genomic DNA samples were obtained from 14 individuals. Short tandem repeats markers, which encompass four previously known loci related to HGF, were genotyped. Two-point log of the odds (LOD) scores were calculated using MLINK program of the LINKAGE software, multipoint and non-parametric linkage (NPL) analysis were performed using the GENEHUNTER software.

Results: Clinical evaluation and histological examination of this family suggested typical features of HGF. The onset age was early in the generations, ranging between 1 and 2 years. None of the tested markers showed cosegregation among affected individuals. Genotyping data from four putative regions yielded significant negative two-point LOD scores (< -2.0) at $\theta = 0$. The maximum multipoint LOD scores and NPL analysis revealed exclusion of these loci as well.

Conclusions: Exclusion of linkage in this family to any of the known HGF loci proved the existence of a novel locus for autosomal dominant HGF and showed that this rare disorder is far more heterogeneous than previously expected.

Key words: genotyping; hereditary gingival fibromatosis (HGF); heterogeneity; linkage analysis; LOD score

Accepted for publication 10 May 2009

Hereditary gingival fibromatosis (HGF, OMIM 135300), also known as elephantiasis gingiva or congenital macrogingiva, develops as a slowly progressive, benign, localized or generalized enlargement of keratinized gingiva (Gorlin et al. 1990). Unlike inflammatory gingival enlargement, HGF appears normal in colour, and feels firm and nodular on palpation (Coletta & Graner 2006, DeAngelo et al. 2007). The overgrowth tissues may partially or totally cover the dental crowns, causing diastemas, and may delay or impede tooth eruption, pseudopocketing and aggressive periodontitis due to poor oral hygiene (Casavecchia et al. 2004). In severe cases, it may lead to crowding oral cavity, mastication and speech impediments, or even lip closure difficulties (Häkkinen & Csiszar 2007). The onset age of HGF usually coincides with the eruption of permanent teeth; however, it may present at birth occasionally (Anderson et al. 1969). HGF occurs as either an isolated disease or combined with some rare syndromes or chromosome disorders. Syndromic forms of HGF have been reported in the Zimmerman–Laband syndrome (Bakaeen & Scully 1991), juvenile hyaline fibromatosis (Dowling et al. 2003), the Klippel–Trenaunay–Weber syndrome (Hallett et al. 1995), Prune-belly syndrome (Harrison et al. 1998), von Recklinghausen neurofibromatosis (Bekisz et al. 2000), Costello syndrome (Hennekam 2003), sensorineural hearing loss (Kasaboğlu et al. 2004), hearing loss with supernumerary teeth (Wynne et al. 1995), cherubism (Yalçin et al. 1999), growth hormone deficiency (Oikarinen et al. 1990), amelogenesis imperfecta (Martelli-Júnior et al. 2008), etc. More-

Conflict of interest and source of funding

The authors declare that they have no conflicts of interests.

This study was supported by the National Natural Science Foundation of China (30500562), the Chenguang Plan for Distinguished Youth of Wuhan, China (200850731374), the Foundation for the author of National Excellent Doctoral Dissertation (2007B69) and the Program for New Century Excellent Talents in University, Ministry of Education, China.

*Contributed equally to this work.

over, medications such as cyclosporine A, phenytoin, nifedipine and other calcium channel blockers are the most common causes of gingival overgrowth. These drug-induced gingival enlargements produce a phenocopy of HGF (Seymour et al. 1996).

HGF is genetically heterogeneous and can be inherited as an autosomal dominant (Hart et al. 1998, Xiao et al. 2000), autosomal recessive (Goldblatt & Singer 1992, Singer et al. 1993) and sporadic forms of inheritance. To date, four loci have been mapped: one maternally inherited locus (GINGF4 on chromosome 11p) and three other autosomal dominant loci (GINGF and GINGF3 on chromosome 2p, GINGF2 on chromosome 5q). No recessive locus has yet been identified.

The first HGF locus, GINGF, was localized to chromosome 2p21–p22, between D2S1788 and D2S441, by genetic mapping in a large autosomal dominant Brazilian pedigree. Further analysis in additional family members confirmed linkage to this interval (Shashi et al. 1999), and later the GINGF gene was identified when patients were found to have a heterozygous frameshift mutation c.3248–3249insC in the *Son of Sevenless-1* gene (*SOS1*), which encodes a RAS-specific guanine nucleotide exchange factor (Hart et al. 2002, Jang et al. 2007). The second HGF locus, GINGF2, was mapped to chromosome 5q13–q22 (Xiao et al. 2001). The third locus, GINGF3, was identified on chromosome 2p22.3–p23.3 in a large Chinese family segregating HGF in an autosomal dominant fashion (Ye et al. 2005). The fourth locus, GINGF4, was maternally inherited on chromosome 11p15 in two Chinese pedigrees (Zhu et al. 2007). Studies involving patients with various chromosome aberrations have also suggested additional loci on other regions, including 2p13–p16 (Fryns 1996, Shashi et al. 1999), 7q8 (Morey & Higgins 1990), 14q22–q32 (Yen et al. 1989, Rivera et al. 1992) and chromosome 8 (Sujansky et al. 1993).

Despite intensive pathological and biochemical research (Meng et al. 2007, 2008, Sobral et al. 2007, Kather et al. 2008), the aetiology of HGF remains unknown. In the current study, we test and exclude linkage to the four known HGF loci in a new pedigree originating from China. These data provide evidence for a novel autosomal dominant locus and imply the existence of at least five genes responsible for this phenotype.

Material and Methods

Pedigree and sample collection

A five-generation family with HGF was ascertained from a remote village in Henan province, China (Fig. 1). All family members received careful oral examinations by two experienced dentists. Information from the deceased parents was obtained by interview with either their siblings or from their medical records. Diagnosis was based on the previously described criteria (Hart et al. 1998): (1) individuals were considered to be affected if they demonstrated keratinized gingival fibrous overgrowth covering at least one-third of the dental crowns of a minimum of five teeth; (2) individuals should not be exposed to drugs including phenytoin, cyclosporin A, nifedipine and other calcium-channel blockers; and (3) individuals should have no systemic abnormalities of hypertrichosis, epilepsy, progressive hearing loss or mental retardation. After appropriate written informed consent was obtained from adults and the parents of children, venous blood samples were collected from all available family members. Gingival tissues were obtained by gingivectomy from the proband (individual V:1) at the Hospital of Stomatology, Wuhan University, China. Gingival specimens were fixed in formaldehyde and embedded in paraffin. Sections of 5 mm were stained with haematoxylin and eosin (H&E), and viewed by light microscopy. This study complied with the Declaration of Helsinki Principles and was approved by the Hospital and School of Stomatology, Wuhan University Committee on Ethics in Research.

Genotyping

Genomic DNA was extracted from peripheral blood according to standard procedures using the QIAmp blood kit (Qiagen, Hilden, Germany). The DNA was quantified by spectrophotometry, and diluted to a concentration of 50 ng/μl for polymerase chain reaction (PCR) amplification. All available 14 family members were genotyped. We performed a first round of search using only 12 short tandem repeats polymorphism markers covering four previously reported candidate regions: 2p21–22, 2p22.3–p23.3, 5q13–22 and 11p15. PCRs were carried out in a 25 μl volume with 50 ng of genomic DNA, 20 pmol of each primer (a fluorescent and an unlabelled primer in each pair), 200 mM each dNTP, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin and 1 U *Taq* DNA polymerase (Takara, Dalian, China). After the initial denaturation step at 95°C for 5 min., the samples were processed through 31 cycles of 95°C for 30 s, 50–63°C for 30 s and 72°C for 30 s. A final extension step was performed at 72°C for 10 min. Following amplification, PCR products were electrophoresed on an ABI PRISM 3100 Genetic Analyzer (Applied Biosytems, Foster, CA, USA). Genotyping data were collected using the GeneMapper v 3.0 software. The map order and intermarker distances were based on the National

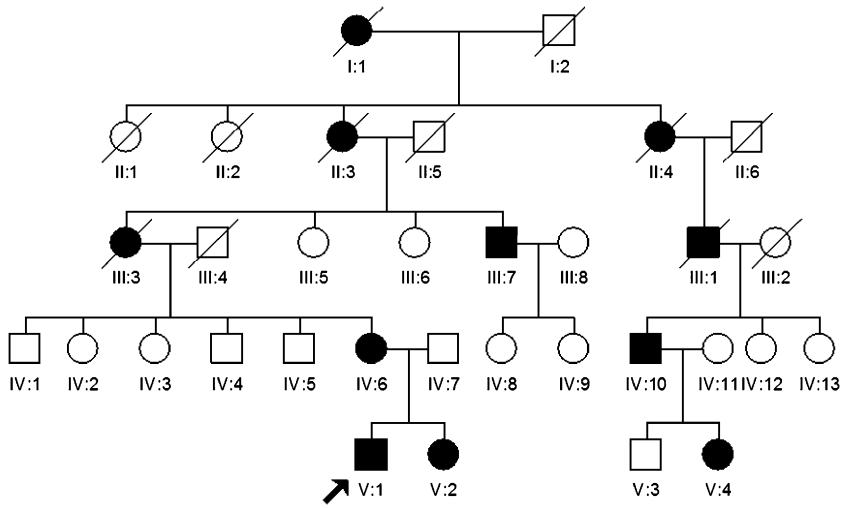

Fig. 1. The pedigree structure of the Chinese family with hereditary gingival fibromatosis is shown. Affected individuals are shown as filled squares (males) or circles (females), and normal individuals are indicated by open symbols. Deceased individuals are marked with slashes. The proband is indicated by an arrow.

Center for Biotechnology Information (NCBI) build 36.3. To confirm the exclusive mapping of these regions, additional 10 high-density markers were selected from the Marshfield Comprehensive human genetic maps (http://research.marshfieldclinic.org/).

Linkage analysis

Inheritance of disease in the family was modelled as an autosomal dominant, two-allele system with 100% penetrance, 0.1% phenocopy rate. The affected allele frequency was set as 0.0001 in compliance with other published studies. Meiotic recombination frequencies were considered to be equal for males and females. Because we have only a few founders available from this family, we could not estimate the marker–allele frequencies from the family data. Marker–allele frequencies were thus determined to be uniformly distributed. Two-point linkage analysis was performed by means of the MLINK program within LINKAGE software package v 5.0. For multipoint log of the odds (LOD) scores as well as non-parametric (NPL) analysis, we used GENEHUNTER software v 2.1 on the entire pedigree. An LOD or an NPL score over 3 was considered to indicate significant support of linkage and that between 2 and 3 was considered as a suggestive linkage. An LOD or an NPL score below -2 was considered to indicate exclusion of linkage.

Results

Clinical findings

Of the large family investigated, 14 individuals received oral examinations and six (three males, three females) were found to be affected by enlarged keratinized gingival tissues, with clear evidence of both complete penetrance and variable expression (Fig. 1). Gingival enlargement was the cardinal symptom emerging in infancy (average onset age between 1 and 2 years old), with the majority occurring during the first year after birth. The exception was individual III: seven, whose onset occurred at about age 3. The proband (V:1) was 9 years old at our initial visit. His medical history was remarkable in that he developed gingival enlargement at the age of about 8 months. The intraoral examination revealed severe gingival fibromatosis with few permanent first molars and maxillary incisors, which were partially erupted. The pink, firm and dense overgrown gingiva tissue could be seen in both arches, preventing adequate lip closure (Fig. 2a). There were no signs of gingivitis. The primary teeth had no mobility. Panoramic radiography revealed the presence of all permanent teeth; however, the physiologic root resorptions of primary teeth were delayed (Fig. 2b). The patient had been biting and chewing on the gingiva. He was treated surgically to remove the excess gingival tissues. Specimens stained with H&E revealed a well-structured squamous epithelium, but the rete ridges were significantly elongated apparently (Fig. 2c). These histological findings are consistent with HGF and similar to those reported in other families. The mother (IV:6, 33 years) and 10-month-old sister (V:2) were also affected by signs of HGF. Examination of the mother showed that her anterior removable denture was broken and the gingiva had covered the vestibule of the mouth. The 10-month-old girl also showed gingival enlargement, especially affecting the maxillary posterior and tuberosities. Clinical prognose for other patients (III:7, IV:10, V:4) were favourable. They had been gingival overgrowth-free after surgical treatment at the age of 16–25 years. The patients did not have any other systemic findings, such as hypertrichosis, hearing deficit or mental retardation.

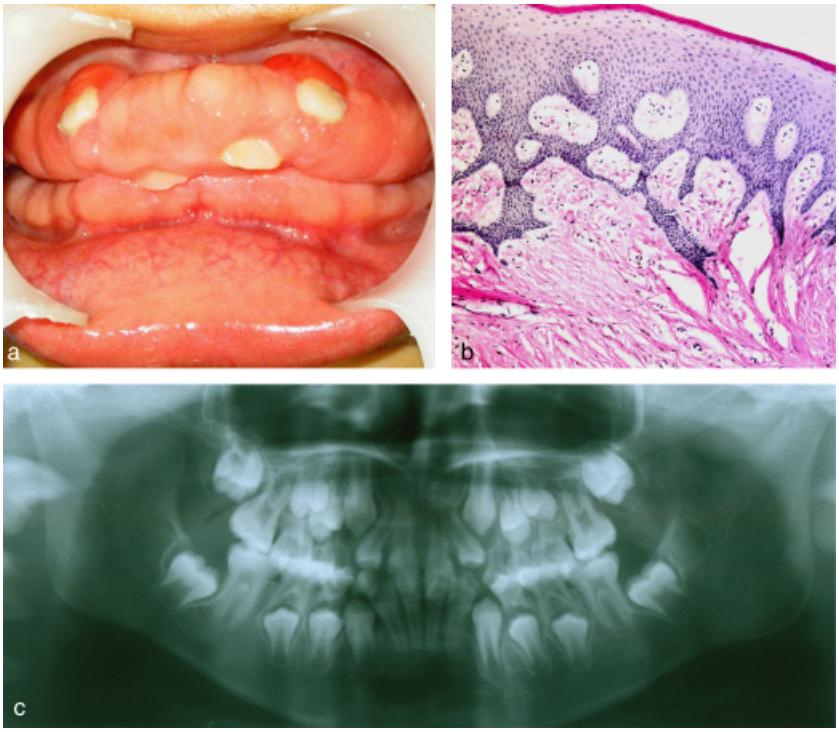

Fig. 2. Clinical characteristics of the proband in the Chinese family with hereditary gingival fibromatosis. (a) Gingival overgrowth in the anterior facial area. Severe gingival enlargement covering almost two-thirds of the clinical crowns. (b) The panoramic radiography revealed the presence of all permanent teeth, but, prolonged retention and delayed root resorption of the primary teeth. (c) Histologic morphology from gingival tissue of the proband showing the increase in the amount of connective tissue, densely arranged collagen and significant extension of the epithelial rete ridges (haematoxylin–eosin stain, magnification $\times 10$).

Exclusion of the HGF loci

HGF occurring in this family was inherited through an autosomal dominant inheritance pattern, with male-to-male transmission. Analysis therefore focused on the four known HGF loci with the following markers (from telomere to centromere): D2S162, D2S305, D2S2259, D2S2368; D5S407, D5S647, D5S424, D5S641, D5S428, D5S644, D5S433, D5S2027, D5S471; D11S4046, D11S1338, D11S902 and D11S904. For convincing exclusive mapping of chromosome 2, all individuals were genotyped with a subset of high-density markers (D2S168, D2S165, D2S367, D2S391 and D2S337) that covered the candidate interval. Using a fully penetrant dominant model, two-point linkage analysis yielded strong negative

Table 1. Two-point log of the odds (LOD) scores between the disease and candidate loci with a penetrance rate of 100% and a phenocopy rate of 0.1%

Loci (chromosome band)	Markers	Genetic distance (cM)*	LOD score (θ =)						
			0.0	0.01	0.05	0.1	0.2	0.3	0.4
GINGF (2p21–22)	D2S162	20.03	− 4.61	− 2.04	− 1.19	− 0.77	− 0.36	− 0.16	− 0.06
	D2S168	27.06	− 4.57	− 1.58	− 0.91	− 0.63	− 0.33	− 0.15	− 0.04
	D2S305	38.87	− 3.96	− 1.60	− 0.86	− 0.53	− 0.23	− 0.10	− 0.04
	D2S165	47.43	− 3.07	− 0.33	0.19	0.31	0.31	0.22	0.09
	D2S367	54.96	− 4.52	− 1.79	− 0.85	− 0.46	− 0.15	− 0.06	− 0.03
GINGF3 (2p22.3–p23.3)	D2S2259	64.29	− 5.31	− 2.49	− 1.37	− 0.80	− 0.29	− 0.09	− 0.03
	D2S391	70.31	− 0.22	− 0.18	− 0.08	− 0.03	0.01	0.01	0.01
	D2S337	80.69	− 9.00	− 4.46	− 2.76	− 1.91	− 1.02	− 0.53	− 0.21
	D2S2368	85.48	− 9.57	− 3.29	− 1.77	− 1.09	− 0.46	− 0.17	− 0.04
	D5S407	64.67	− 3.95	− 0.98	− 0.34	− 0.11	0.05	0.08	0.05
	D5S647	74.07	− 5.81	− 1.43	− 0.70	− 0.40	− 0.15	− 0.05	− 0.01
	D5S424	81.95	− 0.61	− 0.53	− 0.32	− 0.18	− 0.07	− 0.03	− 0.01
GINGF2 (5q13–22)	D5S641	92.38	0.20	0.21	0.22	0.19	0.11	0.05	0.01
	D5S428	95.40	− 3.42	− 0.69	− 0.1	0.07	0.14	0.11	0.06
	D5S644	104.76	0.63	0.61	0.53	0.44	0.29	0.16	0.06
	D5S433	111.97	− 4.87	− 2.15	− 1.13	− 0.65	− 0.23	− 0.07	− 0.02
	D5S2027	119.50	− 4.14	− 1.34	− 0.65	− 0.36	− 0.10	− 0.00	0.02
	D5S471	129.83	− 3.57	− 0.91	− 0.27	− 0.05	0.07	0.07	0.03
	D11S4046	2.79	− 3.20	− 1.48	− 0.77	− 0.47	− 0.19	− 0.07	− 0.01
GINGF4 (11p15)	D11S1338	12.92	− 4.22	− 0.66	− 0.07	0.11	0.15	0.09	0.03
	D11S902	21.47	− 4.33	− 0.97	− 0.35	− 0.14	− 0.02	0.00	0.00
	D11S904	33.57	0.25	0.24	0.20	0.15	0.08	0.03	0.01

*Genetic distance starts from the short arm telomere of each chromosome.

LOD scores, which are summarized in Table 1. Multipoint and non-parametric linkage (NPL) analyses were also carried out and the results are shown in Fig. 3a–c. Negative multipoint and NPL LOD scores were obtained, excluding these loci as a cause in this family. To determine the recombination events among the family members, haplotypes were constructed and exclusion evidences of segregation with the above loci were confirmed (data not shown). Our data thus suggest that a highly genetic heterogeneity and a novel locus contribute to this early-onset form of HGF.

Discussion

HGF is a clinically and genetically heterogeneous disorder (Takagi et al. 1991, Hart et al. 2000). It has been defined as a slowly evolving condition, but the early manifestations and subsequent clinical course are still unclear. We described a Chinese family with autosomal dominant inheritance. Histological examination of the HGF specimens showed dense connective tissues, which are mainly composed of accumulation of excess collagen, relatively few fibroblasts and blood vessels. The overlying epithelium is normal but is slightly hyperplastic in some areas, with elongated rete pegs extending into the corneum (Fig. 2c). These findings are similar to those reported for the HGF family linked to chromosome 2p21 (Hart et al. 1998, 2000). The major clinical characteristics, disease history and histological examination support the diagnosis of HGF, and the symptoms seemed to vary between members, in keeping with the broad heterogeneity. Affected individuals of different ages in the family allowed investigation of the natural history of manifestation. HGF in this family appeared in early childhood (starting at around 1 or 2 years old), worsened until puberty and stabilized in adult age as a slowly progressive disturbance. Age at onset and the evolving course of HGF from infancy to puberty were the main differential clinical features compared with previously described pedigrees. Interestingly, we noticed that another Chinese family manifested with early-onset autosomal dominant HGF was mapped on a novel locus, GINGF2 (5q13–q22) (Xiao et al. 2001). The clinical signs described in this family fit well with the classic HGF and displayed histological outcomes relatively identical to the description seen in the family linking to GINGF2 and other HGF loci. Focusing on the same ethnic origin, it is probable that the two families with early-onset forms of HGF have genetic and environmental homogeneity. This makes it reasonable to suppose that the additional gene(s) involved in could be structurally and/or functionally related. A genetic study is needed to clarify the classification.

To date, it is difficult to distinguish between the clinical phenotypes seen at all dominant HGF loci without molecular genetic testing. We tested a total of four known candidate genomic regions on chromosomes 2p21–p22, 2p22.3–p23.3, 5q13–q22 and 11p15 in the family by linkage and haplotype analysis. Nearly all markers, in particular D2S367 and D2S391 on chromosome 2, D5S647 at chromosome 5, D11S1338 and D11S902 on chromosome 11, yielded low LOD scores. These markers were among the best markers in original genome scan reports. NPL and multipoint LOD scores at these loci did not yield increased linkage. Because statistically significant negative LOD scores excluded linkage to these chromosome regions, several conditions were considered: (1) HGF is easily misdiagnosed for its varied clinical manifestations. Hence, we rechecked all the clinical data of the patients in this family. All individuals affected were confirmed according to standard diagnostic criteria. They had isolated HGF without association with other systemic conditions. (2)

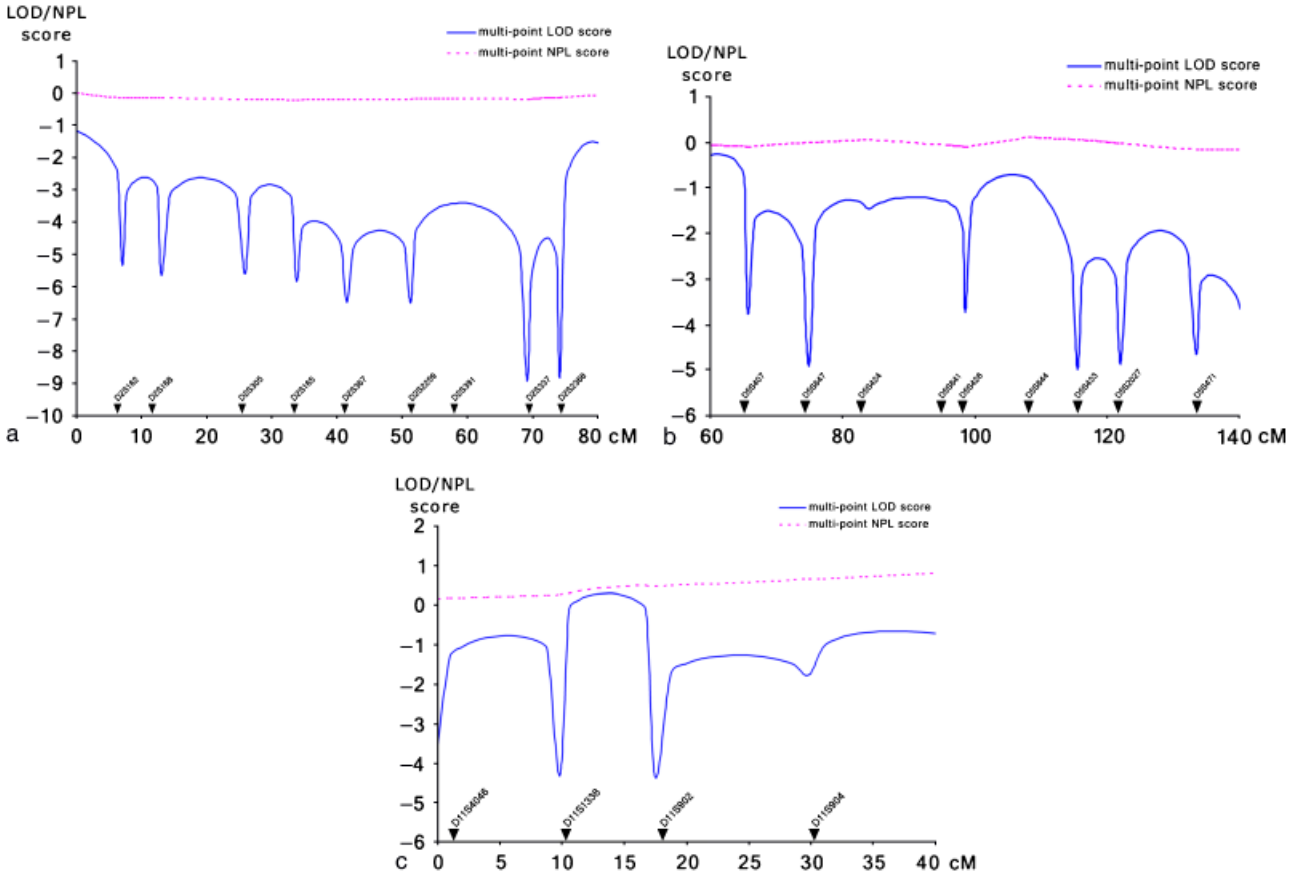

Fig. 3. Multipoint linkage analysis and non-parametric linkage analysis (NPL) for the Chinese family with hereditary gingival fibromatosis. The *X*-axis represents the genetic distance between markers, and the *Y*-axis represents the log of the odds (LOD) and NPL scores. Arrows indicate the genetic marker positions on relevant chromosomes. An LOD or an NPL score over 3 was considered to indicate significant support of linkage and that between 2 and 3 was considered to indicate suggestive linkage. An LOD or an NPL score below –2 was considered to indicate exclusion of linkage. (a) Multipoint LOD (blue solid line) and NPL (cerise-dashed line) distribution curve around nine short tandem repeats (STR)-markers covering GINGF and GINGF3 loci on chromosome 2. (b) Multipoint LOD (blue solid line) and NPL (cerise-dashed line) distribution curve around nine STR-markers covering the GINGF2 locus on chromosome 5. (c) Multipoint LOD (blue solid line) and NPL (cerise-dashed line) distribution curve around four STR markers covering the HGF locus on chromosome 11.

Parametric linkage analysis could be highly sensitive to misspecification of the linkage model. A non-parametric analysis was performed subsequently and the results were in accordance with the parametric analysis. (3) The unremarkable LOD scores and NPL scores obtained in this family may be a reection of a small family size and uninformative markers, and so another five markers were added. The subsequent haplotype analysis excluded the HGF locus from the region of chromosomes 2, 5 and 11 as well (data not shown). The absence of linkage to the known loci associated with HGF together confirms the genetic heterogeneity and implied the presence of a different locus yet to be identified. The increasing heterogeneity observed in HGF was also evident from recent *SOS1* mutation screens. We have previously excluded the coding regions of the *SOS1* gene in three families (Ye et al. 2005). Although the GINGF locus has historically been reported as the major autosomal dominant locus for HGF (Hart et al. 1998, Shashi et al. 1999, Xiao et al. 2000), mutations in *SOS1* have been shown to account for only one HGF pedigree, suggesting that other unidentified genes may play a significant role in this condition.

In conclusion, the current findings demonstrated further genetic locus heterogeneity for HGF and suggest that the phenotype in this family is caused by another, yet unmapped, gene. Based on this study and our earlier results, the *SOS1* gene and the GINGF locus are not as important in the aetiology of HGF as originally expected. This high heterogeneity of HGF would also explain at least in part the paucity of limited success so far in isolating causative genes. Considering the informative power of the recruited family, a genome-wide scan approach will likely result in the mapping of causative gene, thus facilitating the identification of genetic background of this disorder and other fibrotic processes.

Acknowledgements

The authors are indebted to all the family members for their assistance in this study. The authors would like to thank Dr. Changbo Huang from Hospital of Stomatology of Xiangfan City, China, for cooperation in recruiting patients for this study.

References

Anderson, J., Cunliffe, W. J., Roberts, D. F. & Close, H. (1969) Hereditary gingival

fibromatosis. *British Medical Journal* **3**, 218–219.

Bakaeen, G. & Scully, C. (1991) Hereditary gingival fibromatosis in a family with the Zimmermann–Laband syndrome. *Journal of Oral Pathology Medicine* **20**, 457–459.

Bekisz, O., Darimont, F. & Rompen, E. H. (2000) Diffuse but unilateral gingival enlargement associated with von Recklinghausen neurofibromatosis: a case report. *Journal of Clinical Periodontology* **27**, 361–365.

Casavecchia, P., Uzel, M. I., Kantarci, A., Hasturk, H., Dibart, S., Hart, T. C., Trackman, P. C. & Van Dyke, T. E. (2004) Hereditary gingival fibromatosis associated with generalized aggressive periodontitis: a case report. *Journal of Periodontology* **75**, 770–778.

Coletta, R. D. & Graner, E. (2006) Hereditary gingival fibromatosis: a systematic review. *Journal of Periodontology* **77**, 753–764.

DeAngelo, S., Murphy, J., Claman, L., Kalmar, J. & Leblebicioglu, B. (2007) Hereditary gingival fibromatosis – a review. *Compendium of Continuing Education in Dentistry* **28**, 138–143.

Dowling, O., Difeo, A., Ramirez, M. C., Tukel, T., Narla, G., Bonafe, L., Kayserili, H., Yuksel-Apak, M., Paller, A. S., Norton, K., Teebi, A. S., Grum-Tokars, V., Martin, G. S., Davis, G. E., Glucksman, M. J. & Martignetti, J. A. (2003) Mutations in capillary morphogenesis gene-2 result in the allelic disorders juvenile hyaline fibromatosis and infantile systemic hyalinosis. *American Journal of Human Genetics* **73**, 957–966.

Fryns, J. P. (1996) Gingival Wbromatosis and partial duplication of the short arm of chromosome 2 (dup(2) (p13–>p21)). *Annals Genetics* **39**, 54–55.

Goldblatt, J. & Singer, S. L. (1992) Autosomal recessive gingival fibromatosis with distinctive facies. *Clinical Genetics* **42**, 306–308.

Gorlin, R., Cohen, M. M. & Levis, L. S. (1990) *Syndromes of the Head and Neck*, 3rd edition. New York: Oxford University Press.

Häkkinen, L. & Csiszar, A. (2007) Hereditary gingival fibromatosis: characteristics and novel putative pathogenic mechanisms. *Journal of Dental Research* **86**, 25–34.

Hallett, K. B., Bankier, A., Chow, C. W., Bateman, J. & Hall, R. K. (1995) Gingival fibromatosis and Klippel–Trenaunay–Weber syndrome. Case report. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics* **79**, 578–582.

Harrison, M., Odell, E. W., Agrawal, M., Saravanamuttu, R. & Longhurst, P. (1998) Gingival fibromatosis with prune-belly syndrome. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics* **86**, 304–307.

Hart, T. C., Pallos, D., Bowden, D. W., Bolyard, J., Pettenati, M. J. & Cortelli, J. R. (1998) Genetic linkage of hereditary gingival fibromatosis to chromosome 2p21. *American Journal of Human Genetics* **62**, 876–883.

Hart, T. C., Pallos, D., Bozzo, L., Almeida, O. P., Marazita, M. L., O'Connell, J. R. & Cortelli, J. R. (2000) Evidence of genetic heterogeneity. *Journal of Dental Research* **79**, 1758–1764.

Hart, T. C., Zhang, Y., Gorry, M. C., Hart, P. S., Cooper, M., Marazita, M. L., Marks, J. M., Cortelli, J. R. & Pallos, D. (2002) A mutation in the SOS1 gene causes hereditary gingival fibromatosis type 1. *American Journal of Human Genetic* **70**, 943–954.

Hennekam, R. C. (2003) Costello syndrome: an overview. *American Journal of Medical Genetics C Seminar Medical Genetics* **117**, 42–48.

Jang, S. I., Lee, E. J., Hart, P. S., Ramaswami, M., Pallos, D. & Hart, T. C. (2007) Germline gain of function with SOS1 mutation in hereditary gingival fibromatosis. *Journal of Biological Chemistry* **13**, 20245–20255.

Kasaboğlu, O., Tümer, C. & Balci, S. (2004) Hereditary gingival fibromatosis and sensorineural hearing loss in a 42-year-old man with Jones syndrome. *Genetic Counseling* **15**, 213–218.

Kather, J., Salgado, M. A., Salgado, U. F., Cortelli, J. R. & Pallos, D. (2008) Clinical and histomorphometric characteristics of three different families with hereditary gingival fibromatosis. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics* **105**, 348–352.

Martelli-Júnior, H., Bonan, P. R., Dos Santos, L. A., Santos, S. M., Cavalcanti, M. G. & Coletta, R. D. (2008) Case reports of a new syndrome associating gingival fibromatosis and dental abnormalities in a consanguineous family. *Journal of Periodontology* **79**, 1287–1296.

Meng, L., Huang, M., Ye, X., Fan, M. & Bian, Z. (2007) Increased expression of collagen prolyl 4-hydroxylases in Chinese patients with hereditary gingival fibromatosis. *Archive of Oral Biology* **52**, 1209–1214.

Meng, L., Ye, X., Fan, M., Xiong, X., Von den Hoff, J. W. & Bian, Z. (2008) Keratinocytes modify fibroblast metabolism in hereditary gingival fibromatosis. *Archive of Oral Biology* **53**, 1050–1057.

Morey, M. A. & Higgins, R. R. (1990) Ectroamelia syndrome associated with an interstitial deletion of 7q. *American Journal of Medical Genetics* **35**, 95–99.

Oikarinen, K., Salo, T., Käär, M. L., Lahtela, P. & Altonen, M. (1990) Hereditary gingival fibromatosis associated with growth hormone deficiency. *British Journal of Oral Maxillofacial Surgery* **28**, 335–339.

Rivera, H., Ramirez-Duenas, M. L., Figuera, L. E., Gonzalez-Montes, R. M. & Vasquez, A. I. (1992) Opposite imbalances of distal 14q in two unrelated patients. *Annals Genetics* **35**, 97–100.

Seymour, R. A., Thomason, J. M. & Ellis, J. S. (1996) The pathogenesis of drug-induced gingival overgrowth. *Journal of Clinical Periodontology* **23**, 163–175.

Shashi, V., Pallos, D., Pettenati, M. J., Cortelli, J. R., Fryns, J. P., von Kap-Herr, C. & Hart, T. C. (1999) Genetic heterogeneity of gingival fibromatosis on chromosome 2p. *Journal of Medical Genetics* **36**, 683–686.

Singer, S. L., Goldblatt, J., Hallam, L. A. & Winters, J. C. (1993) Hereditary gingival fibromatosis with a recessive mode of inheritance. Case reports. *Australia Dental Journal* **38**, 427–432.

Sobral, L. M., Montan, P. F., Martelli-Junior, H., Graner, E. & Coletta, R. D. (2007) Opposite effects of TGF-beta1 and IFN-gamma on transdifferentiation of myofibroblast in human gingival cell cultures. *J Clin Periodontol* **34**, 397–406.

Sujansky, E., Smith, A. C., Prescott, K. E., Freehauf, C. L., Clericuzio, C. & Robinson, A. (1993) Natural history of the recombinant (eight) syndrome. *American Journal of Medical Genetics* **47**, 512–525.

Takagi, M., Yamamoto, H., Mega, H., Hsieh, K. J., Shioda, S. & Enomoto, S. (1991) Heterogeneity in the gingival fibromatoses. *Cancer* **68**, 2202–2212.

Wynne, S. E., Aldred, M. J. & Bartold, P. M. (1995) Hereditary gingival fibromatosis associated with hearing loss and supernumerary teeth – a new syndrome. *Journal of Periodontology* **66**, 75–79.

Xiao, S., Bu, L., Zhu, L., Zheng, G., Yang, M., Qian, M., Hu, L., Liu, J., Zhao, G. & Kong, X. (2001) A new locus for hereditary gingival fibromatosis (GINGF2) maps to 5q13–q22. *Genomics* **74**, 180–185.

Xiao, S., Wang, X., Qu, B., Yang, M., Liu, G., Bu, L., Wang, Y., Zhu, L., Lei, H., Hu, L., Zhang, X., Liu, J., Zhao, G. & Kong, X. (2000) Refinement of the locus for autosomal dominant hereditary gingival fibromatosis (GINGF) to a 3.8-cM region on 2p21. *Genomics* **68**, 247–252.

Yalçin, S., Yalçin, F., Soydinç, M., Palandüz, S. & Günhan, O. (1999) Gingival fibromatosis combined with cherubism and psychomotor retardation: a rare syndromes. *Journal of Periodontology* **70**, 201–204.

Ye, X., Shi, L., Cheng, Y., Peng, Q., Huang, S., Liu, J., Huang, M., Peng, B. & Bian, Z. (2005) A novel locus for autosomal dominant hereditary gingival fibromatosis, GINGF3, maps to chromosome 2p22.3–p23.3. *Clinical Genetics* **68**, 239–244.

Yen, F. S., Podruch, P. E. & Weisskopf, B. (1989) A terminal deletion (14)(q31.1) in a child with microcephaly, narrow palate, gingival hypertrophy, protuberant ears, and mild mental retardation. *Journal of Medical Genetics* **26**, 130–133.

Zhu, Y., Zhang, W., Huo, Z., Zhang, Y., Xia, Y., Li, B., Kong, X. & Hu, L. (2007) A novel locus for maternally inherited human gingival fibromatosis at chromosome 11p15. *Human Genetics* **121**, 113–123.

Address:
Zhuan Bian
Key Laboratory for Oral biomedical Engineering of Ministry of Education
Hospital and School of Stomatology
Wuhan University
Luoyu Road 237
Wuhan, 430079
China
E-mail: kqyywjtx@public.wh.hb.cn

Clinical Relevance

Scientific rationale for the study: HGF shows remarkable heterogeneity, with at least four loci implicated. A Chinese family presenting with early-onset HGF was investigated and genotyped to prove or exclude linkage of all known loci.

Principal findings: No linkage evidence was found in two-point, multipoint as well as non-parametric linkage analysis to the tested loci.

Practical implications: The results further confirmed the wide heterogeneity of HGF. Further evidence of genetic heterogeneity segregating with HGF.