

Refinement of the *GINGF3* locus for hereditary gingival fibromatosis

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Abstract Hereditary gingival fibromatosis (HGF) is a rare, clinically variable disorder characterized by slowly progressive fibrous overgrowth of the gingiva. Four gene loci have been mapped for autosomal dominant non-syndromic HGF (adHGF). The molecular basis of adHGF remains largely unknown, with only a single *SOS1* gene mutation identified so far at the gingival fibromatosis 1 (*GINGF1*) locus in one family. We identified an adHGF family with ten affected individuals in whom onset of gingival fibromatosis concurred with the eruption of the primary teeth. In order to identify the molecular basis in this family, we tested for linkage of the disease to known adHGF loci. A maximal multipoint logarithm of the odds score of 3.91 was obtained with marker *D2S390* ($\theta=0$) at the *GINGF3* locus on chromosome 2p23.3–p22.3, and linkage to other known loci was excluded. Sequencing two candidate genes, *ALK* and *C2orf18*, and a single nucleotide polymorphisms array analysis did not reveal a mutation or copy number variation in a patient from the family. We refined the *GINGF3* locus to a 6.56-cM, 8.27-Mb region containing 112 known and hypothetical genes, and our data and a

search of the literature suggest that *GINGF3* is a major adHGF locus.

Keywords Nodular · Alveolar · Signaling · *ALK* gene · *C2orf18* · Major gene · Primary teeth · Gingiva · SNP · Array · Copy number analysis

Introduction

Gingival fibromatosis (gingival hyperplasia) may result from systemic medication with calcium-channel blockers, cyclosporin, dilantin, and phenytoin, or it may be hereditary. In severe cases, the gingival enlargement may cover the crowns of teeth and cause severe functional and esthetic concerns. Hereditary gingival fibromatosis (HGF) is a rare condition that can occur as an isolated disease or as part of a number of syndromes or chromosomal abnormalities [1, 2]. The onset of gingival overgrowth generally coincides with the eruption of the permanent incisors, infrequently with the eruption of the primary dentition, and it rarely presents already at birth. Among the non-syndromic HGFs, autosomal recessive [3] and autosomal dominant inheritance have been described [2]. Linkage studies have localized loci for autosomal dominant non-syndromic forms of gingival fibromatosis (adHGF) to chromosomes 2p21–p22 (*GINGF1*, OMIM 135300) [4, 5], 2p22.3–p23.3 (*GINGF3*, OMIM 609955) [6], and 5q13–q22 (*GINGF2*, OMIM 605544) [7]. Delineation of chromosome 2p13–p21 duplication in a patient with HGF in association with mental retardation and facial dysmorphism suggested another HGF locus on chromosome 2p13–p21 [8]. Recently, an apparently maternally inherited form of gingival fibromatosis was mapped to chromosome 11p15 in two unrelated Chinese families [9]. The molecular basis of HGF

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remains largely unknown. One mutation in the *SOS1* (son of sevenless one) gene (OMIM 182530) has been identified at the *GINGF1* locus in a single adHGF family. Diagnosis of HGF is based on medical history and clinical examination, since there are currently no specific immunohistochemical markers available.

Materials and methods

We ascertained a German four-generation family with autosomal dominant transmission of HGF without evidence for parental imprinting through a proband. Fifteen family members were examined and nine (five male and four female) were classified as affected (Fig. 1, individuals 12170, 3, 11284, 13230, 11602, 12163, 13232, 11603, and 11606) based on the following criteria: enlarged gingiva covering at least one third of clinical dental crowns of five or more teeth and lack of exposure of affected members to any inducible drugs; individuals were otherwise healthy. The deceased male founder of this family (Fig. 1, individual 1) was reported as having been affected by HGF. Five spouses of family members were also examined, and samples were taken for molecular investigation (individuals 12021, 11378, 13231, 11253, and 11760 in Fig. 1). No samples were available from individuals 1–6 shown in Fig. 1, and their genotypes were inferred and haplotypes were re-constructed (see below).

Informed consent was obtained from all participants following genetic counseling. DNA was extracted from peripheral blood samples, from paraffin-embedded tissue sections, and from buccal smears, and RNA was extracted from cultured fibroblasts using an automated extractor according to the manufacturer's protocols (GenoM 48, Qiagen, Vienna, Austria). For linkage analysis, highly polymorphic microsatellite markers were selected from 2p21–p23.3 and from 5q13–q22 (<http://www.gdb.org/>). Locus order and sex-averaged inter-marker distances were taken from the Marshfield map (<http://research.marshfieldclinic.org/genetics/>). Marker alleles were detected by electrophoresing the PCR products on 6% polyacrylamide gels followed by silver staining. We used the Allegro program [10] to compute haplotypes as well as two-point and multipoint logarithm of the odds (LOD) scores, under the assumption of autosomal dominant inheritance with 100% penetrance, and with the disease-allele frequency set at 0.001, and equal marker allele frequencies. A whole-genome analysis of copy number variation was conducted in one patient (12170, Fig. 1) using the Affymetrix Genome-Wide Human SNP 6.0 array according to the specifications of the manufacturer. Oligonucleotide primer sequences and conditions to sequence the *ALK* and *C2orf18* genes in complementary DNA (cDNA) are available on request.

Results

Remarkably, the onset of gingival fibromatosis consistently concurred with the eruption of the primary teeth in all affected members of this family. Gingival fibromatosis developed slowly progressive and variably consisted of localized or generalized enlargement of keratinized gingiva and consisted of dense fibrous tissue that feels firm and nodular on palpation (Fig. 2a–d). Gingival excess tissue resulted in periodontal problems. Difficulties in daily oral hygiene and long-term smoking were recorded in patients with most severe findings (Fig. 2c, d). Histological evaluation of specimens from different individuals of the family showed hyperplasia of fibrous tissue characterized by squamous epithelium with elongated rete ridges overlying dramatically increased cell poor fibrous tissue (Fig. 3). A pseudoepitheliomatous hyperplasia of the squamous epithelium with formation of papillae was seen in severely affected individuals.

Haplotype and LOD score analyses excluded *GINGF1* (Figs. 1 and 4 and Table 1) and *GINGF2* (data not shown) in this adHGF family. Linkage of the disease to *GINGF3* was demonstrated with a maximal two-point LOD score of 3.45 with marker *D2S171* ($\theta=0$; Table 1) and a maximal multipoint LOD score of 3.91 with marker *D2S390* ($\theta=0$; Fig. 4). Recombinations defined markers *D2S220* and *D2S352* as boundaries of the linked interval (Fig. 1). The candidate region defined in the presented family overlaps with the 11.42-cM, 13.04-Mb *GINGF3* interval flanked by marker loci *D2S2221* and *D2S1788*, originally defined in one Chinese family [6]. According to the current NCBI draft sequence of the human genome, build 36.3, the original and the refined *GINGF3* candidate region contain 131 and 112 known and hypothetical genes. Prioritization of candidate genes for mutation analysis considers expression in gingival or connective tissue and a putative function of the gene product in cell cycle control or extracellular matrix composition. Three interactive, web-based implementations, SUSPECTS (<http://www.genetics.med.ed.ac.uk/suspects/>), GeneWanderer (<http://compbio.charite.de/genewanderer/GeneWanderer>), and GeneDistiller (<http://www.genedistiller.org>) [11] were queried in addition to a manual approach to identify the disease gene based on expression and functional data contained in the NCBI database (<http://www.ncbi.nlm.nih.gov/sites/gene>). However, sequencing two candidate genes, *ALK*, encoding a receptor protein–tyrosine kinase and *C2orf18* encoding an adenine nucleotide translocase 2 binding protein in cDNA from fibroblasts did not reveal a mutation in a patient from the presented family. A whole-genome analysis of copy number variation in the same patient detected neither aberrations at the *GINGF3* locus nor elsewhere.

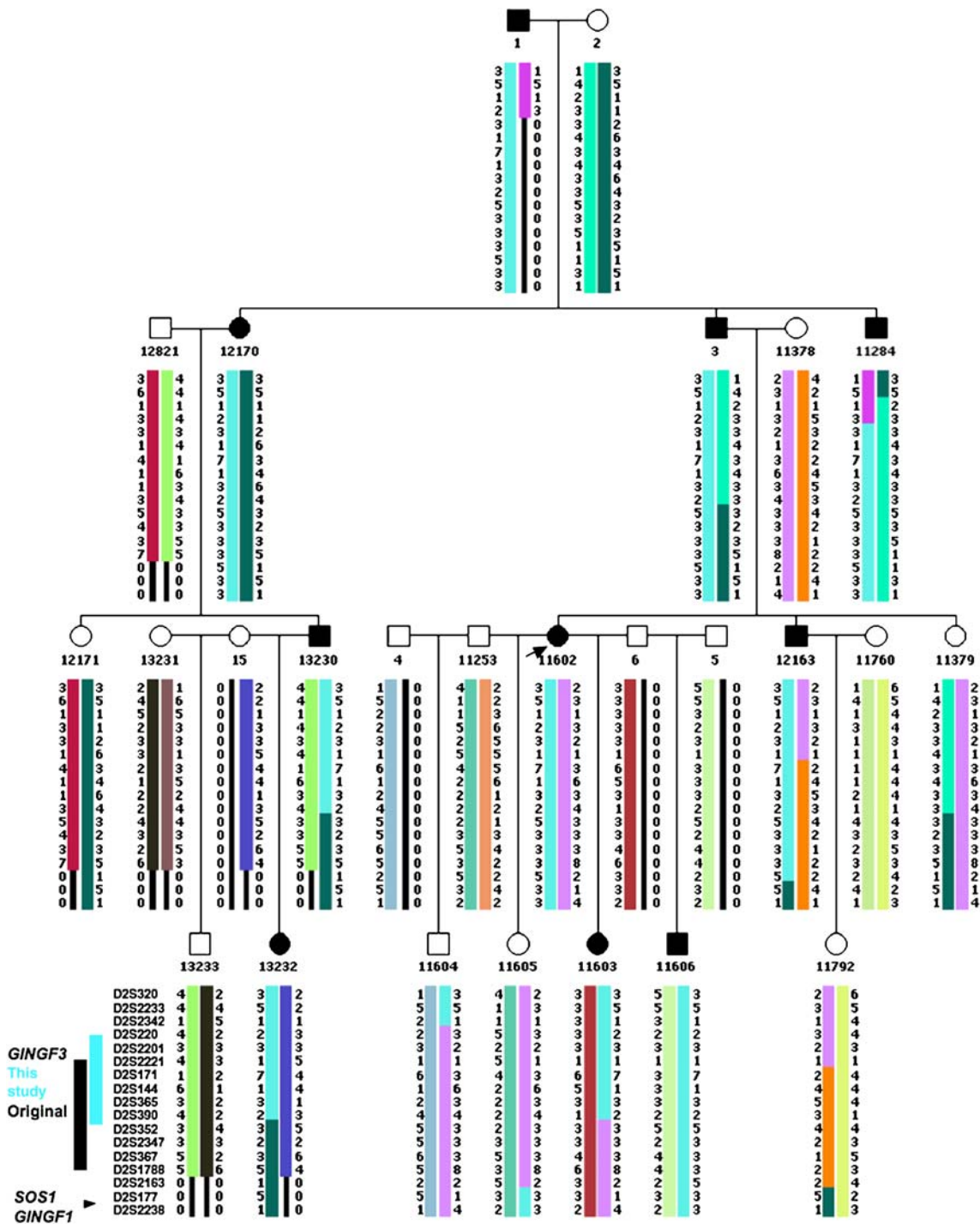


Fig. 1 Pedigree and haplotypes of the HGF family under study. The proband, indicated by an *arrow*, has two affected and two unaffected children with four different partners. Haplotype analysis excluded the SOS1 gene at the GINGF1 locus as the disease gene in this family and

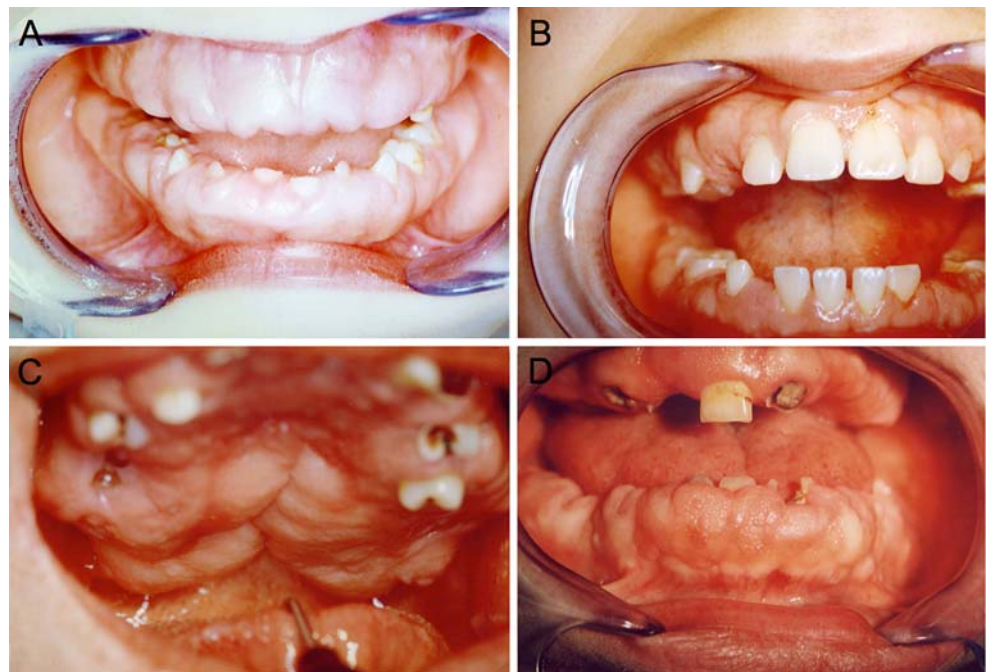
narrowed the original 11.42-cM, 13.04-Mb GINGF3 locus, flanked by marker loci *D2S2221* and *D2S1788*, down to the region between markers *D2S220* and *D2S352*

Discussion

The candidate region defined in the presented family overlaps with the *GINGF3* interval originally defined in one Chinese family [6]. Under the hypothesis that this locus

contains a single HGF gene, we refined it to a 6.56-cM, 8.28-Mb interval flanked by maker loci *D2S2221* and *D2S352* (Figs. 1, 4) and confirm the presence of an additional HGF gene on chromosome 2p distinct from the GINGF1 locus. The data of at least five published smaller

Fig. 2 Gingival overgrowth in **a** a 6-year-old female and **b** 13-year-old male showing symmetrical gingival hyperplasia. **c** Severe involvement in a male at age 36 years and **d** a female proband aged 37 years, 7 years after last surgical intervention



adHGF families are compatible with linkage to this locus [5, 6]. These previous reports and our data suggest that *GINGF3* is a major adHGF locus.

Previous studies on HGF suggest a function of the *GINGF3* gene product in cell cycle control, and more specifically, the gene product might directly or indirectly interact with *SOS1*. A heterozygous *SOS1* single base insertion leading to a C-terminally truncated protein, leading to sustained activation of RAS/MAPK signaling

in fibroblasts, was identified in the form of HGF [12, 13]. Mutations in another component of the RAS/MAPK pathway, *HRAS*, can cause Costello syndrome, a malformation-dysmorphism syndrome with gingival hypertrophy as a feature [14]. Most recently, dysregulation of the human mitogen-activated protein kinase kinase 6 (MAP2K6) was implicated in the pathogenesis of a syndromic form of HGF, congenital generalized hypertrichosis terminalis [15]. Therefore, genes from the candi-

Fig. 3 Histopathological findings in two individuals from the presented family. **a** Squamous epithelium with elongated rete ridges overlaying dramatically increased cell poor fibrous tissue (original magnification, $\times 100$, H&E stain). **b** Pseudoepitheliomatous hyperplasia of the squamous epithelium with formation of papillae in individuals with marked inflammatory infiltrates (original magnification, $\times 100$, H&E stain)

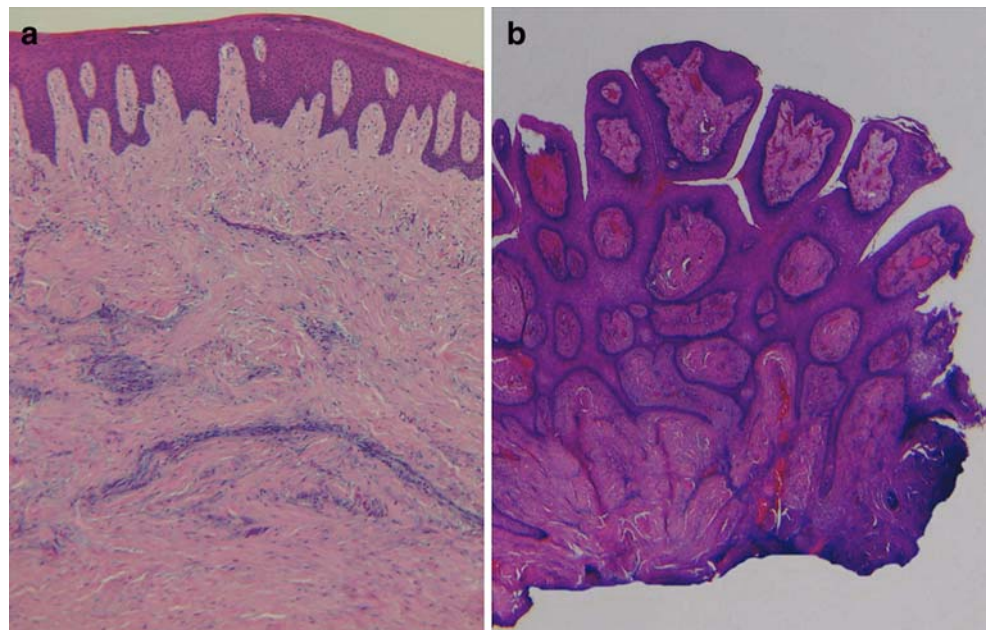
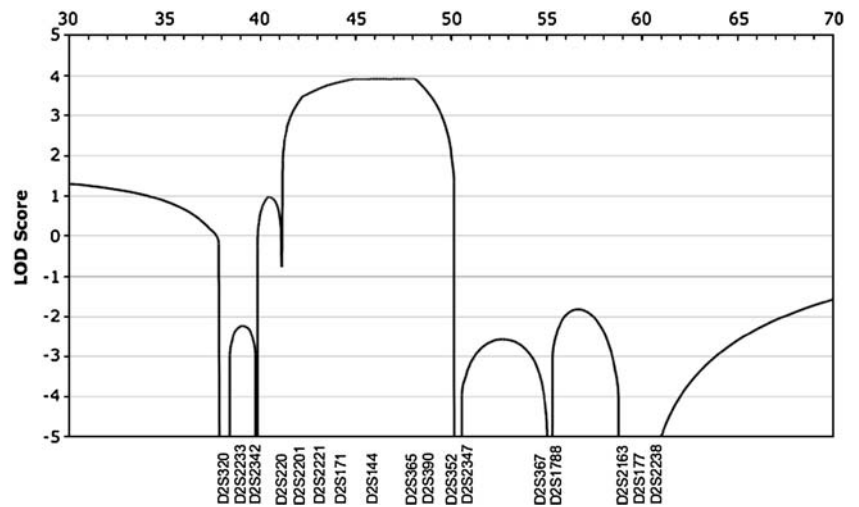


Fig. 4 Multipoint LOD score. The order of analyzed markers on chromosome 2p is shown on the X-axis, and distances are given in centiMorgan. The Y-axis refers to the LOD score



date region encoding for components of the RAS/MAPK pathway will be given priority regarding sequence analysis to identify the mutation causing HGF in the presented family. For example, *RAB10* from the interval encodes a member of the RAS (see HRAS; OMIM 190020) superfamily of small GTPases. RAB proteins localize to exocytic and endocytic compartments and regulate intracellular vesicle trafficking; however, the particular function of RAB10 is unknown. Most genes from the candidate region are incompletely characterized such as *GPNI*, which

encodes a guanosine triphosphatase enzyme that may play a role in DNA repair and may function in activation of transcription, and *AGBL5*, a putative ATP/GTP binding protein. *CIB4*, encoding calcium and integrin binding family member 4, may represent a further HGF candidate gene considering that calcium channel blockers can induce gingival hyperplasia. Candidates further include *PPP1CB*, encoding one of the three catalytic subunits of protein phosphatase 1, which is known to be involved in the regulation of cell division, glycogen metabolism, muscle

Table 1 Two-point linkage analysis between the HGF locus and chromosome 2p markers

| Marker | Genetic Distance (cM) | LOD score at θ | | | | | | | | Z_{\max} | θ_{\max} |
|----------------------|-----------------------|-----------------------|-------|-------|-------|-------|-------|-------|-------|------------|-----------------|
| | | 0.00 | 0.01 | 0.05 | 0.10 | 0.20 | 0.30 | 0.40 | | | |
| D2S320 | 38.33 | $-\infty$ | 1.04 | 1.53 | 1.58 | 1.44 | 1.22 | 1.01 | 1.58 | 0.09 | |
| D2S2233 | 39.93 | $-\infty$ | 0.78 | 1.29 | 1.36 | 1.24 | 1.06 | 0.87 | 1.36 | 0.10 | |
| D2S2342 | 40.47 | 0.95 | 0.93 | 0.84 | 0.74 | 0.56 | 0.41 | 0.29 | 0.95 | 0 | |
| D2S220 | 42.65 | -0.69 | 1.26 | 1.76 | 1.82 | 1.67 | 1.44 | 1.22 | 1.82 | 0.09 | |
| D2S2201 ^a | 41.57 | 1.83 | 1.79 | 1.64 | 1.45 | 1.13 | 0.87 | 0.66 | 1.83 | 0 | |
| D2S2221 | 44.09 | 2.19 | 2.15 | 1.99 | 1.80 | 1.46 | 1.16 | 0.92 | 2.19 | 0 | |
| D2S171 | 45.30 | 3.45 | 3.39 | 3.16 | 2.89 | 2.40 | 1.97 | 1.60 | 3.45 | 0 | |
| D2S144 | 45.30 | 3.42 | 3.36 | 3.13 | 2.86 | 2.37 | 1.95 | 1.59 | 3.42 | 0 | |
| D2S365 | 47.97 | 0.95 | 0.93 | 0.85 | 0.75 | 0.58 | 0.44 | 0.33 | 0.95 | 0 | |
| D2S390 | 48.50 | 3.31 | 3.25 | 3.03 | 2.77 | 2.30 | 1.89 | 1.54 | 3.31 | 0 | |
| D2S352 | 50.65 | $-\infty$ | -1.51 | -0.29 | 0.11 | 0.33 | 0.35 | 0.32 | 0.36 | 0.27 | |
| D2S2347 | 50.65 | $-\infty$ | -0.34 | 0.24 | 0.40 | 0.45 | 0.42 | 0.37 | 0.45 | 0.19 | |
| D2S367 | 54.96 | 0.95 | 0.93 | 0.88 | 0.82 | 0.69 | 0.59 | 0.49 | 0.95 | 0 | |
| D2S1788 | 55.51 | $-\infty$ | -0.92 | 0.27 | 0.62 | 0.76 | 0.71 | 0.61 | 0.76 | 0.20 | |
| D2S2163 | 59.36 | $-\infty$ | -0.61 | -0.02 | 0.15 | 0.23 | 0.22 | 0.19 | 0.23 | 0.23 | |
| D2S177 | 59.36 | $-\infty$ | -4.01 | -2.05 | -1.32 | -0.71 | -0.43 | -0.28 | -0.28 | 0.40 | |
| D2S2238 | 60.45 | $-\infty$ | -2.49 | -1.20 | -0.73 | -0.36 | -0.21 | -0.13 | -0.13 | 0.40 | |

^a The physical map of chromosome 2 (NCBI draft sequence of the human genome, build 36.3) indicates that D2S2201 localizes centromeric to D2S220, contrasting with the Marshfield genetic map of chromosome 2.

contractility, protein synthesis, and HIV-1 viral transcription, and the protein encoded by *FKBP1B*, a member of the immunophilin protein family, which play a role in immunoregulation and basic cellular processes involving protein folding and trafficking. *FKBP1B* is a cis-trans prolyl isomerase that binds the immunosuppressant FK506 (Tacrolimus), which can induce gingival overgrowth as a side-effect in treated patients [16, 17].

There are clinical and histological differences in the presented family as compared with *SOS1*-related gingival fibromatosis, which might reflect the different molecular causes of the disease. As frequently seen in HGF, an overall cell poor increase in gingival tissue with scarce blood vessels and islets with dense fibroblasts comparable to the findings in the Chinese family originally defining the *GINGF3* locus [6] was noted in our patients contrasting with *SOS1*-related gingival fibromatosis, which features a higher increase in the number of fibroblasts compared with the increase in extracellular matrix [18]. The family presented here is also remarkable in that all patients consistently had an early onset of the disease, at the time of eruption of the primary teeth, also similar to the original Chinese *GINGF3* family [6], together arguing for a clinically recognizable subtype of adHGF linked to *GINGF3*.

Identification of the genetic mutations involved in HGF would provide novel aids for disease diagnosis, uncover targets for novel treatment modalities, and improve our understanding of the molecular mechanisms underlying HGF and other fibrotic processes.

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Competing interest statement The authors declare that they have no competing financial interests.

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