

## CORRESPONDENCE

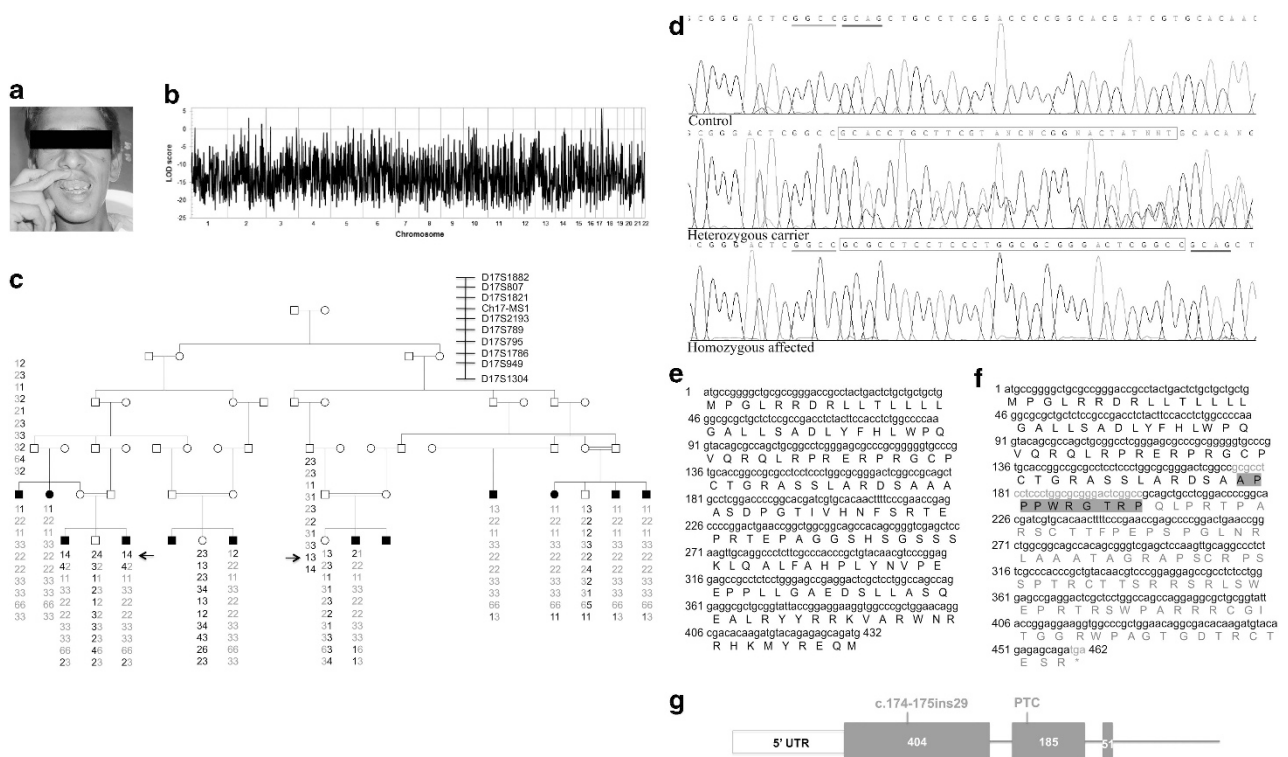
# Autosomal recessive gingival hyperplasia and dental anomalies caused by a 29-base pair duplication in the *FAM20A* gene

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Amelogenesis imperfecta (AI; MIM 104530) is a heterogeneous collection of disorders encompassing inherited congenital defects of dental enamel formation, causing

variably small, discolored, pitted, grooved, or fragile primary or permanent teeth. At least 14 syndromic and non-syndromic AI subtypes have been described, including

autosomal dominant and recessive, and X-linked recessive. Gene mutations that underlie both the non-syndromic forms and the tricho-dento-osseous (MIM



**Figure 1** Homozygous 29-bp duplication in the *FAM20A* gene, in a Pakistani family with gingival hyperplasia and dental anomalies. **(a)** Clinical phenotype of an affected individual reveals teeth abnormalities and gingival hyperplasia. **(b)** Autozygosity mapping using the low-density Affymetrix 10K genotyping array. A maximum LOD score of 5.63 was obtained for an 11-Mb region on chromosome 17. **(c)** Pedigree and haplotype analysis. Microsatellite markers spanning the 11-Mb region of homozygosity were used to genotype 16 family members and allowed narrowing of the linkage interval to 3.69 Mb on chromosome 17. Arrows indicate the key recombination events between markers D17S807 and D17S1821, and D17S1786 and D17S949. **(d)** Sanger sequencing identifies a homozygous 29-bp duplication in the first exon of the *FAM20A* gene, c.174-175ins29, in affected individuals. Representative electropherograms for control, heterozygous carrier and homozygous affected individuals are shown (red box shows inserted nucleotides, 5' and 3' nucleotides flanking the insertion are underlined in blue and green, respectively). **(e** and **f**) Translation of wild-type **(e)** and mutant **(f)** *FAM20A* transcript. For simplicity, only part of the wild-type protein sequence is shown in **e**. If translated, the mutant *FAM20A* protein would contain 10 additional amino acids (highlighted in red) followed by 85 foreign amino acids (in blue) and a PTC in exon 2. Owing to its nature, c.174-175ins29 is likely to result in activation of the Nonsense mediated mRNA decay (NMD) pathway and degradation of the mutant transcript prior to translation. **(g)** Schematic diagram of exons 1–3 of the *FAM20A* gene showing the c.174-175ins29 duplication and PTC in exon 2, located 257 bp downstream of the mutation site and 1164 bp upstream of the wild-type termination codon within the mRNA sequence. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

190320) and Jalili syndromes (MIM 217080) have recently been described.<sup>1</sup>

We studied a large consanguineous Pakistani family in which all members had severe gingival hyperplasia and dental abnormalities, including delayed tooth eruption or failure of tooth development (Figure 1a). On examination, the adults had primarily gingival disease, while children had prominent dental abnormalities with minimal, possibly secondary, gingival hyperplasia. Many family members could not chew hard food, because their gingiva covered molar and pre-molar teeth. None of the family members had any renal abnormalities.

After obtaining informed consent, we undertook a genome-wide scan on 16 family members using the Affymetrix 10K SNP. Haplotypes, or clusters of SNPs that tend to be inherited together, were inferred from the data by Genespring GT (Agilent Software). Initial analysis included genome-wide autozygosity mapping to identify regions identical by descent that were shared among affected individuals, and parametric linkage analysis assuming a recessive mode of inheritance with 100% penetrance and a disease allele frequency of 0.001. We identified an 11-Mb region of excess homozygosity shared among affected individuals on chromosome 17 (autozygosity logarithm of the odds (LOD) = 6.53). Microsatellite markers spanning this region were then used to genotype all family members. Key recombination events were detected between markers D17S807 and D17S1821, and D17S1786 and D17S949 (Figure 1b), which allowed the linkage interval to be narrowed to 3.69 Mb. This region contains 34 genes.

Homozygous mutations in the gene *FAM20A* have recently been shown to cause a phenotype similar to that observed in our family.<sup>2,3</sup> As *FAM20A* was included in our linkage interval, we used a candidate gene approach; we PCR amplified and directly sequenced all 11 exons of *FAM20A* (ABI Prism 310 Automated Sequencer). We identified a homozygous 29-bp duplication in exon 1, c.174-175ins29, which causes a frameshift and a premature termination codon (PTC) in exon 2, 257-bp downstream of the mutation site (Figures 1d-g). The mutation occurs between the first and second nucleotides of codon 59 (A, arginine), resulting in the potential insertion of 10

amino acids (APPPWRGTRP) followed by 85 foreign amino acids (Figures 1e and f). However, given the location of the PTC, this mutation likely activates nonsense mRNA-mediated decay, an mRNA surveillance pathway that promotes degradation of mRNAs containing PTCs, preventing the synthesis of truncated or potentially harmful proteins. Consequently, the levels of *FAM20A* protein in the homozygous affected individuals are expected to be markedly reduced or absent. The c.174-175ins29 duplication, which was excluded from 100 unrelated control Pakistani individuals by direct sequencing, completely co-segregated with the disease phenotype. This is the first reported duplication in the *FAM20A* gene.

*FAM20A* belongs to a family of secreted proteins (*FAM20A*, *FAM20B*, *FAM20C*), which regulates differentiation of hematopoietic cells and bone development.<sup>4,5</sup> Within the mouse incisor tooth, *FAM20A* mRNA and protein are expressed in the secretory and maturation stages of the ameloblasts, the suprabasal layers of the gingival epithelium, and the odontoblasts, a localization pattern consistent with the phenotype of AI and gingival hyperplasia.<sup>2</sup> Vogel *et al.*<sup>6</sup> studied knock-out mice for *FAM20A* and *FAM20C*, and identified that mice which were *FAM20A* (-/-) and *FAM20C* (-/-) had severe AI. Additionally, *FAM20A* (-/-) mice had disseminated intrapulmonary and intra-arterial calcifications, raising the possibility of systemic disease in patients with *FAM20A* mutations.

Although further mechanistic studies are required to investigate the molecular function of *FAM20A*, published data support its crucial role for enamel development and gingival homeostasis. We have identified the first duplication in *FAM20A* and the fifth independent mutation associated with gingival hyperplasia and dental anomalies.

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**Author Contributions:** RMC and MK carried out the molecular genetic studies, sequence alignments and preparation of the manuscript. LR prepared the manuscript. MW performed physical examination and clinical diagnosis of the patients. YS and LP performed the linkage studies. LP performed the statistical analysis. AMC provided oversight and conceptual guidance to the project, input into the functional significance of candidate genes, supervision of laboratory personnel, management of collaborations, preparation of the manuscript and all reporting requirements for granting agencies.

Rita M Cabral<sup>1,4</sup>, Mazen Kurban<sup>1,4</sup>,  
Lisa Rothman<sup>1</sup>, Muhammad Wajid<sup>1</sup>,  
Yutaka Shimomura<sup>1</sup>, Lynn Petukhova<sup>1,2</sup> and  
Angela M Christiano<sup>1,3</sup>

<sup>1</sup>Department of Dermatology, Columbia University, New York, NY, USA;

<sup>2</sup>Department of Epidemiology, Columbia University, New York, NY, USA;

<sup>3</sup>Department of Genetics and Development, Columbia University, New York, NY, USA and

<sup>4</sup>These authors contributed equally to this work.,

E-mail: amc65@columbia.edu

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