论文题目：遗传性牙龈纤维瘤病和牙本质生成不全-II 型致病基因的定位与克隆

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摘　要

遗传性牙龈纤维瘤病（Hereditary gingival fibromatosis，HGF）是一种较罕见口腔遗传性疾病，有孤立和综合征两种发病形式。其主要的遗传方式为常染色体显性遗传。临床特征为上下颌牙龈或全口牙龈呈慢性、进行性、弥漫性增生肥大，严重影响美观和口腔功能。最近，该病的致病基因被定位于 2p21 区 11cM 的候选区域。为了进一步方便克隆 HGF 的致病基因，我们收集了国内 5 个 HGF 家系。利用 2p21 区域更精细的 STRP 标记，将候选区域定位于 D2S2144 和 D2S2163 两个位点之间，与 Hart 报道的候选区域有 2.8Mb 的重叠区。NCX1 和 CALM2 过去一直被认为是 2 个重要的疾病候选基因，现已被反射杂种制图的结果排除在我们定位的候选区域之外。我们的 SSCP 突变筛选结果显示，该候选区域另 4 个重要的候选基因 CYP1B1、PRKR、PRKCN、FEZ2 以及应用 Genscan、Genfinder 和 GRAIL 预测出的功能与 HGF 相关的 NCX1 样的基因都与 HGF 无因果关系。

此外，在收集的 5 个家系中，睢宁家系的所有患者都是在一周岁以内患病，而其它家系都在 2 岁以后才开始发病，因此我们称之为早发性牙龈纤维瘤病。该家系的致病基因不与 2p21 的 STR 标记连锁，全基因组扫描和连锁分析将该家系的 HGF 致病基因定位于 5q13-q22 的 D5S1404 和 D5S1462 两位点之间。首次运用家系连锁分析的方法为 HGF 表型异质性提供了分子遗传学基础。

牙本质生成不全是一种常染色体显性遗传性疾病，其病因为牙本质生成和矿化紊乱。最近，该病的致病基因的候选区域已从 D4S2691-D4S2692 6.6cM 的范围缩小到 GATA62A11-D4S1563 2.0Mb。我们利用国内 5 个 DGI-II 大家系，将致病基因的候选区域进一步缩小至 800Kb。突变筛选试验的结果显示，在淮阴家系中，DSPP 基因第三内含子剪接位点的供位 GT 突变为 AT，在转录过程中可能导致 DSPP 基因第三外显子的缺失；在南京家系中，DSPP 基因第一外显子的最后一位密码子 CCA 颠换为 ACA(P17T)；在徐州家系中，DSPP 基因第二外显子的第一位密码子 GTT 转换为 TTT(V18F)。其它基因都没有与 DGI-II 呈因果关系的突变，只是发现了一些编码区的单核苷酸态（cSNP）。但我们不能排除这些基因的内含子和以及一些调节区可能存在的突变。800kb 的区域相对较小、易于操作，因此我们构建了覆盖候选区域的 BAC contigs，同时也构建了候选区域高覆盖率的 BAC 测序亚克隆库，为建立候选区域的转录图谱及测序提供了基础。

关键词：　遗传性牙龈纤维瘤病　牙本质生成不全-II 型　定位候选克隆

全基因组扫描　连锁分析

Mapping and cloning of Hereditary Gingival Fibromatosis and Dentinogenesis imperfecta type II

Hereditary Gingival Fibromatosis (HGF) is an oral inheritable disease characterized by a slowly progressive enlargement of the gingival tissues surrounding both the maxillary and the mandibular dentition which results in both aesthetic and functional problems. Recently, an autosomal dominant gingival fibromatosis locus was mapped to an 11cM interval bounded by D2S1788 and D2S2298. In order to refine the previously mapped region and facility the identification of the underlying genes responsible for the disorder, we collected five hereditary gingival fibromatosis families which were typed by use of polymorphic markers on 2p21. In the four families, the gingival fibronmatosis locus was located to an approximately 8.7cM region on 2p21which overlaps by 2.8Mb with previously mapped interval.

High-resolution radiation hybrid mapping showed that two important genes, CALM2 and NCX1 which previously mapped to HGF candidate interval were outside of the HGF critical region. SSCP analysis and sequencing of coding region of candidate genes CYP1B1, PRKR, PRKCN, PEZ2 and the other relevant gene (NCX-like) which was predicted by GENSCAN, GENFINDER and GRAIL failed to reveal any disease-specific mutations in affected individuals and normal controls, suggesting that mutations in these genes may not play a causative role in the pathogenesis of disorder.

All affected individuals in the fifth family (SN pedigree) began their gingival enlargement within one year old. Other HGF families took onset after two years old. So we called the SN pedigree as " early-onset type" HGF. The SN pedigree HGF locus did not cosegregate with the ATR markers on 2p21. Using a genomewide search strategy and linkage analysis, we identified a new genetic linkage(Zmax=4.81 θ=0.00) at a position of 111.97cM between D5S1462 and D5S1721 for the HGF phenotype to polymorphic markers in the genetic region of chromosome 5q13-q22. Haplotype reconstruction established the centromeric boundary to D5S1491, and the telomeric boundary to D5S1453 assuming complete penetrance and no phenocopy.

Dentinogenesis imperfecta type II is an autosomal dominant disorder of dentin formation and mineralization. Recently, the critical region has been narrowed from the 6.6cM D4S2691-D4S2692 interval to the 2.0Mb GATA2A11-D4S1563 interval at human chromosome 4q21. In the current investigation, five extensive Chinese DGI-II pedigrees were collected. Linkage analysis with STRP at 4q21 region has further refined the candidate region to a 800Kb interval. The results of the mutation-screening assay with PCR-SSCP and sequence of DSPP gene have demonstrated that a G to A transition was detected in the donor splice site (GT) of intron 3 in HY pedigree. In NJ family all affected members carry a CCA to ACA transversion at condon 17(P17T). A G to T tansversation in the first nucleotide of exon 2 was identified in affected individuals of XZ family. Other genes in critical region have been excluded from a causative role in the pathogenesis of DGI-II. These results however do not exclude mutations in other families or

occurred in non –coding and regulational regions of these genes, the 800Kb critical region is an easily manipulated fragment. Therefore, the BAC contigs spanning the critical interval were created and construction of BAC sequencing subclone library has been finished. The work will prove to be a central recource in the creation of a transcription map and the sequence of the region and will aid in the ultimate cloning of the DGI-II locus in other DGI-II of the region and will aid in the ultimate cloning if the DGI-II locus in other DGI-II families,

Key Words: Hereditary gingival fibromatosis, Dentinogenesis imperfecta type II,

Positional cloning, Genomwide search, Linkage analysis