that the *HLA-A* locus was indeed a good proxy for variability within the *HLA-B–HLA-DQ* segment. In addition, 3 of the 47 *five-locus* IBS haplotypes were shown to be distinguishable by typing for loci within the *HLA-B–HLA-DQ* segment. Another 2 of the 47 IBS haplotypes were split by typing for *HLA-G* (telomeric to *HLA-A*) and for *LMP7, TAP1, LMP2,* and *HLA-DPB1* (centromeric to *HLA-DQ*). The newly refined haplotype data continue to show decreased HLA haplotype matching between spouses, providing, in the context of no significant decrease in allele matching at *HLA-A, HLA-B*, or *HLA-DR,* support for the argument that it is the haplospecific configuration of alleles at all (or at least many) of the loci in the MHC region, not allelic differences at individual HLA loci, that is important.

LOWELL R. WEITKAMP[1] AND CAROLE OBER[2]
[1]*Department of Psychiatry and Division of Genetics, University of Rochester Medical Center, Rochester, NY; and* [2]*Center for Medical Genetics, Department of Obstetrics and Gynecology, The University of Chicago, Chicago*

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Address for correspondence and reprints: Dr. Lowell R. Weitkamp, Division of Genetics, Box 641, University of Rochester Medical Center, 601 Elmwood Avenue, Rochester, NY 14642. E-mail: lweitk@medicine.rochester.edu

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A Locus for Autosomal Recessive Hypodontia with Associated Dental Anomalies Maps to Chromosome 16q12.1

To the Editor:

Hypodontia is characterized by the congenital absence of one or more teeth and may include deciduous and/or permanent dentition. The permanent teeth most frequently missing are second premolars, maxillary lateral incisors, and third premolars (Schulze 1970). Congenitally missing deciduous teeth are uncommon, but, when occurring, usually involve the maxillary lateral incisors, mandibular lateral incisors, and mandibular cuspids (Grahnen and Granath 1961). Segregation analyses in many family studies have suggested that hypodontia is a single-gene defect, often transmitted as an autosomal dominant trait with incomplete penetrance and variable expressivity (Burzynski and Escobar 1983; Svinhufud et al. 1988). In this study, we report a consanguineous kindred from Pakistan, with hypodontia associated with various dental anomalies, transmitted as an autosomal recessive trait. Clinical investigation of the affected family members demonstrated hypodontia associated with dental anomalies such as malformation, enamel hypoplasia, and failure of eruption of teeth, leading prematurely to the edentulous state. To localize the gene responsible for the disease, we performed genomewide screening with a panel of 386 markers spaced at 10-cM intervals. We demonstrated linkage to a 10-cM region on chromosome 16q12.1, with a maximum two-point LOD score of 5.76 for marker D16S3140.

Tooth development begins as an epithelial bud, which undergoes complex morphogenesis and is regulated by interactions between the epithelial and mesenchymal tissue layers. The first step in tooth development is the migration of mesenchymal cells from the neural crest into the tooth-forming zone of the vertebrate jaw, known as the "maxillary and mandibular arches." When the mesenchymal cells arrive, they induce the overlying dental epithelium to thicken, forming the dental lamina. The epithelium subsequently invaginates into the mesenchyme to form the dental organ, around which the mesenchyme then proliferates and condenses into the dental papilla. Together, the dental organ and the dental papilla form the tooth germ (also known as the "tooth organ"; Thesleff 1996; Lewin 1997). Secreted signaling molecules that transmit the sequential and reciprocal inductive interactions between the epithelium and mesenchyme have been identified, and transcription factors that are needed for the signaling cascades (Thesleff 1996), such as the homeobox genes (MSX) 1 and 2, the epidermal growth factor (EGF) and its receptor (EGFR), and transforming growth factor (TGF) B1 (Vainio et al. 1993), are now known. Recent studies (Vaahtokari et al. 1996*a,* 1996*b*) have demonstrated that the enamel knot, the transient cluster of epithelial cells in the center of the developing tooth germ, may represent a major signaling center regulating tooth-shape development by expressing growth factors such as bone morphogenetic proteins (BMP) 2, 4, and 7, sonic hedgehog, and fibroblast growth factor 4. Yet, despite a large body of scientific investigation, only one mutation in a single gene, MSX1, has been identified, in one family with an autosomal dominant form of hypodontia (Vastardis et al. 1996).

To search for the gene defect in hypodontia, we stud-

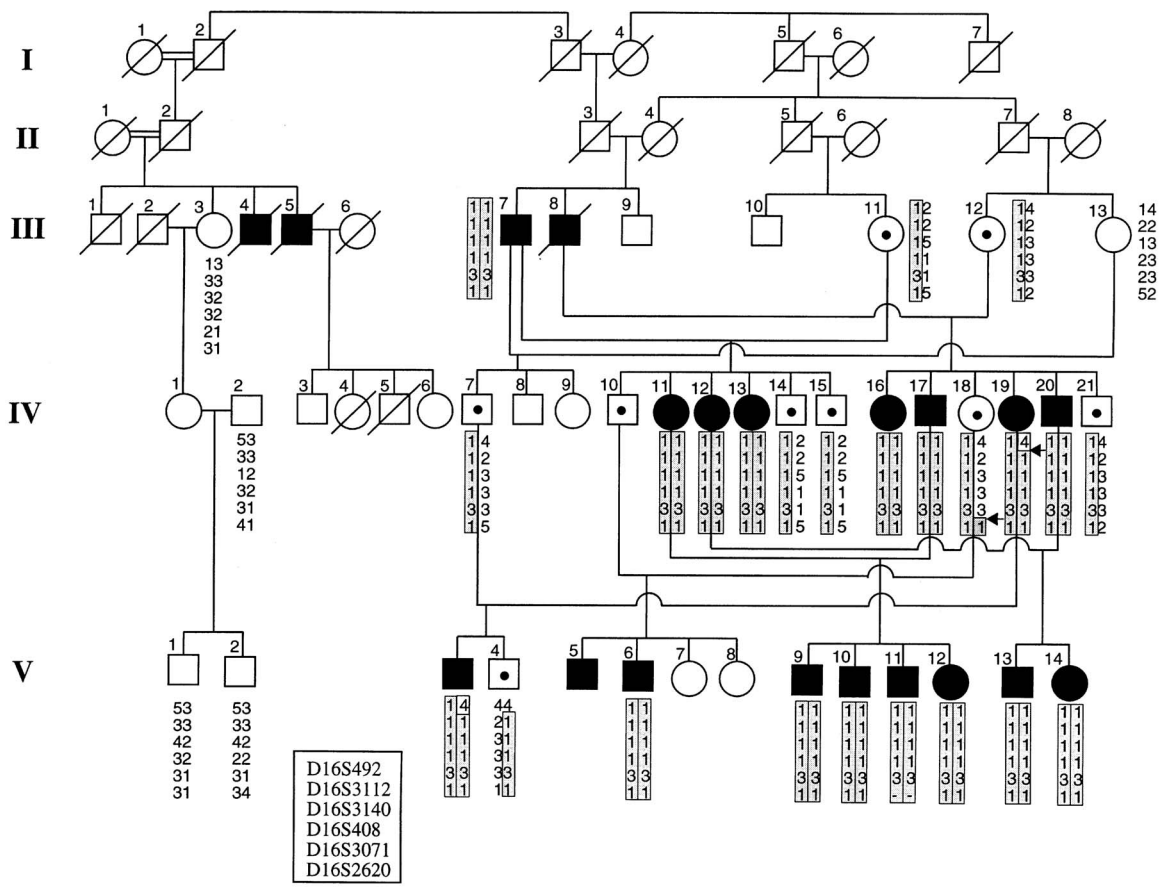

Figure 1 Pedigree showing disease-associated haplotypes. Blackened circles and squares represent affected females and males, respectively, and symbols with a central black dot represent obligate heterozygotes. The disease-associated haplotype is highlighted by a grey-shaded box beneath each symbol. Recombination events are indicated by an arrow adjacent to the haplotype, for individuals IV-18 and IV-19. The marker order is indicated in the inset (*bottom right*).

ied a single highly consanguineous family with an autosomal recessive form of hypodontia and other dental anomalies (fig. 1). The family described in this report resides in Sind, Pakistan. The family members rarely marry outside the family, and consequently consanguineous unions are common. The pedigree was drawn by interviewing the elders of the family. Information was checked and rechecked by interviewing different persons. Living affected and unaffected subjects were clinically examined at Abbassi Shaheed Hospital, Karachi, Pakistan, and blood samples were collected, with informed consent, at the local hospital. Clinical status was based on dental history, intraoral examination, and panoramic radiographs. Dental examinations of the affected subjects in our family demonstrated a range of dental anomalies involving several maxillary and mandibular teeth. The main clinical problems encountered were delays in the formation and eruption of the teeth (fig. 2). The skin, hair, nails, and sweat glands were normal, and no evidence for an ectodermal dysplasia was observed. The pedigree (fig. 1) provided convincing evidence for an autosomal recessive mode of inheritance, and consanguineous loops accounted for all the affected persons being homozygous for the mutant allele. To elucidate the gene defect in the family, we initially searched for linkage by using polymorphic markers within several candidate genes—including MSX1 (4p16), MSX2 (5q34–35), EGF (4q25–27), and EGFR (7p12–14)—previously reported to be involved in early tooth morphogenesis (Partanen et al. 1985; Kronmiller et al. 1991; Mackenzie et al. 1991, 1992). When linkage to these candidate genes was excluded, we undertook a genomewide search.

An initial genomewide screen with microsatellite markers evenly spaced at 10-cM intervals was conducted by use of the DNA from three of the affected individuals

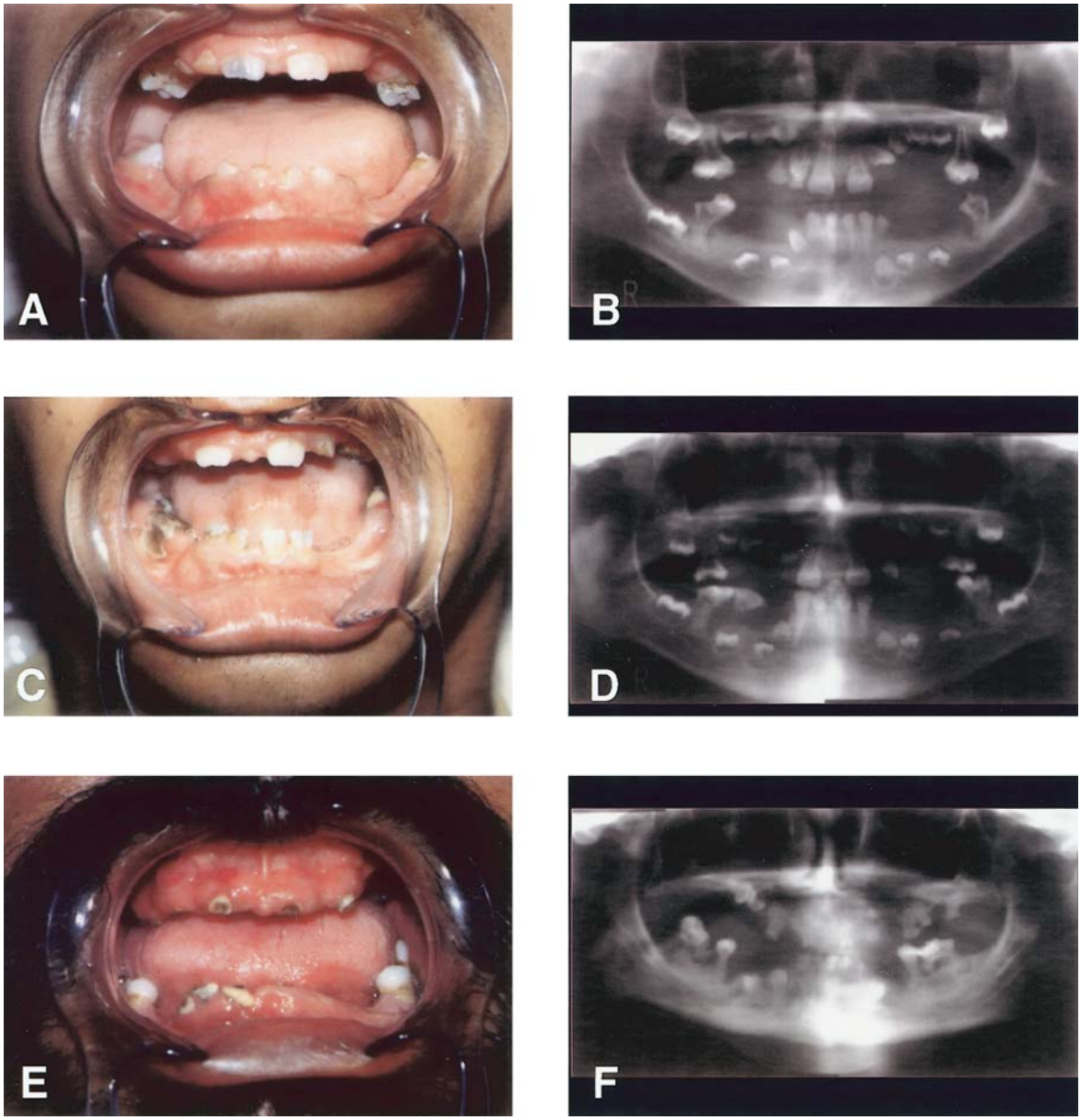

Figure 2 *A,* Subject V-9, a 15-year-old male, showing mixed dentition, with the first permanent molars, maxillary central incisors, and mandibular right cuspid erupted. In addition, the maxillary right lateral incisor and canine and the mandibular incisors and left canine from primary dentition were present. Maxillary central incisors had erupted only halfway and were widely spaced. The mucosa and other oral structures appeared normal in color and texture. *B,* Panoramic radiograph showing a number of unerupted teeth, including the maxillary lateral incisors, cuspids, bicuspids, and second molars and the mandibular left cuspid and bicuspids, right bicuspids, and second molars. All teeth, representing various stages of development, were malpositioned and malformed, showing only crown formation. Maxillary and mandibular last molars and left second molars were completely missing. Of the teeth that had erupted, the maxillary central incisors had open apices with wide pulp chambers and a loss of distinction between the chamber and the canal. The erupted maxillary and mandibular permanent molars had tapering cone-shaped fused roots curved toward the apices, and their pulp chambers and canals were wide, merging together, with loss of detail. *C,* Subject V-10, a 14-year-old male, showing the presence of maxillary central incisors and first molars and of mandibular incisors and first molars. The only deciduous tooth present was the maxillary right canine. *D,* Panoramic radiograph showing the presence of a number of unerupted teeth, including the maxillary lateral incisors, cuspids, bicuspids, and second molars and the mandibular cuspids, bicuspids, and second molars. The widely spaced maxillary central incisors had wide pulp chambers and canals with open apices. The pulp canals of the mandibular incisors were distorted, with complete loss of detail. All permanent first molars had fused roots and open apices and showed little distinction between the pulp chamber and the canal. *E,* Upper jaw of subject IV-17, a 38-year-old male, containing only six teeth, including the central incisors, canines, right second bicuspid, and right first molar with roots visible above the mucosal level. The lower jaw showed only nine erupted teeth, including the central incisors, right lateral incisor, right cuspid, both second bicuspids, and first molars. *F,* Panoramic radiograph showing a few malformed unerupted teeth, which might have corresponded to the maxillary left bicuspids, mandibular right and left canines, and left first bicuspid.

Table 1

LOD Scores for Linkage of the Hypodontia Locus to Chromosome 16q12.1 Markers

	LOD Score at Recombination Fraction of						
Marker	.00	.01	.05	.1	.2	.3	.4
D16S492	∞	3.31	3.61	3.4	2.65	1.77	.86
D16S3112	5.44	5.34	4.91	4.36	3.23	2.06	.9
D16S3140	5.76	5.65	5.21	4.65	3.49	2.31	1.12
D16S408	4.07	3.99	3.67	3.27	2.44	1.58	.73
D16S3071	2.64	2.58	2.35	2.06	1.47	.91	.4
D16S2620	∞	3.53	3.8	3.54	2.7	1.72	.76

(III-7, IV-12, and IV-17). The initial genomewide search involving the three affected individuals was carried out at Research Genetics, Inc. In the course of screening 386 markers, 17 genomic regions were found to be homozygous in all three affected subjects and were tested further in the rest of the affected and unaffected members of the family. A marker on chromosome 16q12.1 (D16S3112) was found to be homozygous in only the affected individuals. Further analysis with markers from this region resulted in the identification of homozygosity, in affected individuals, for markers D16S3112 and D16S3140. By use of the FASTLINK 3.0P package (Schaffer 1996), which enables retention of all inbreeding loops in the family, a two-point LOD score of 5.76 at .00 recombination was obtained for marker D16S3140 (table 1). Autosomal recessive inheritance with complete penetrance was assumed, using a disease-allele frequency of .0001. The order of the markers was derived from on-line genetic mapping data at the Genome Medical Center, University of Wisconsin (http://www.marshmed.org/genetics/). The LOD score remains significant over a range of allele-frequency estimates. Recombination events observed in individuals IV-19 and IV-18, with markers D16S492 and D16S2620, respectively, placed the disease locus in a 10-cM interval between these two markers.

Physical mapping data for this region include a single linked YAC contig, WI-16.4, and radiation hybrid mapping data have placed a number of expressed sequence tags (ESTs) in the region between D16S492 and D16S2620. Currently, there are no potential candidate genes for the disease; however, two ESTs show strong DNA homology to the drosophila *son of sevenless* gene and the *Saccharomyces cerevisiae* gene sequences for the general negative regulator of transcription subunit 1. Discovery of the first gene directly implicated in the pathogenesis of inherited autosomal recessive hypodontia associated with various forms of dental anomalies could generate a new direction of scientific investigation into the tooth bud as a model for genetic control of morphogenesis and development, particularly of epithelial-mesenchymal signaling interactions.

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Wasim Ahmad,[1] Valeria Brancolini,[4] Muhammad Faiyaz ul Haque,[5] HaMut Lam,[1] Sayed ul Haque,[5] Mahmud Haider,[6] Aijaz Maimon,[5] Vincent M. Aita,[2] Jason Owen,[7] Donna Brown,[7] David J. Zegarelli,[3] Mahmud Ahmad,[5] Jurg Ott,[4] and Angela M. Christiano[1]

[1]*Department of Dermatology,* [2]*Department of Genetics and Development, and* [3]*School of Dental and Oral Surgery, Columbia University, and* [4]*Laboratory for Statistical Genetics, The Rockefeller University, New York;* [5]*Department of Biological Sciences, Quaid-i-Azam University, Islamabad, Pakistan;* [6]*Abbassi Shaheed Hospital, Karachi, Pakistan; and* [7]*Research Genetics, Inc., Huntsville, AL*

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Address for correspondence and reprints: Dr. Angela M. Christiano, Department of Dermatology, Columbia University, College of Physicians and Surgeons, 630 West 168th Street VC15-1526, New York, NY, 10032. E-mail: amc65@columbia.edu

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The African Origin of the Common Mutation in African American Patients with Glycogen-Storage Disease Type II

To the Editor:

Conventional historiographical research provides abundant evidence of the African roots of African American populations, but, because of the absence of complete documentary records—for example, the point of embarkation of a particular slave vessel does not necessarily indicate who was actually on that vessel, and slave cargoes tended to be composed of mixed populations—it remains a frustrating task to identify exactly who was transported to the Americas (Curtin 1969; Parish 1989; Thornton 1992). The presence of a genetic marker in an African American population, however, might furnish a verifiable link, for the individuals who carry the trait, to a specific tribe or even to a point of origin.

In the autosomal recessive disorder glycogen-storage disease type II (GSD II [MIM 232300]), a deficiency of acid maltase (GAA; acid α-glucosidase) leads to the pathological accumulation of glycogen in lysosomes. In its most severe form, progressive cardiomyopathy causes cardiorespiratory failure and death within the 1st year of life (Pompe syndrome). Among the mutations identified (see Lin and Shieh 1995; Raben et al. 1995; Tsunoda et al. 1996; Adams et al. 1997; reviewed by Reuser et al. 1995; Hirschhorn and Huie 1997), three that lead to the total loss of enzyme activity occur frequently in particular ethnic groups: deletion of exon 18 in Caucasians (Boerkoel et al. 1992; Huie et al. 1994*a;* Van der Kraan et al. 1994; Kroos et al. 1995), deletion of T525 in northern Europeans (Hermans et al. 1994; Kroos et al. 1995), and D645E in Chinese patients from Taiwan (Shieh et al. 1994; Lin and Shieh 1996). The chance to study several affected infants of African parents has permitted us to identify a common African mutation, to confirm our previous suggestion that the mutation is also common in African Americans (Adams et al. 1997), and, thereby, to explore the molecular roots of GSD II in African Americans.

We initially studied a 3-mo-old infant (patient 1), from the Ivory Coast, of healthy, nonconsanguineous parents; the mother is Mandingo, and the father is Guéré (table 1). The patient is a compound heterozygote harboring a previously described C2560T transition in exon 18 (Hermans et al. 1993*a;* Adams et al. 1997) and a novel T2846A transversion in exon 20. An unusual feature of the novel exon 20 mutation (V949D) is its localization at the carboxy terminus of the 952 amino acid precursor, the tail that is removed during processing into mature forms (Wisselaar et al. 1993). Expression studies showed that the mutation results in complete inactivation of the enzyme: catalytic activity of the mutant protein in transfected COS cells did not exceed the background levels—778, 30, and 26 nmol 4-4-methylumbelliferone/h/mg cell protein for the wild-type, mutant, and mock-transfected cells, respectively. The mature protein was not detected by western analysis, thereby adequately explaining the absence of enzyme activity in this allele (data not shown). Apparently, the mutation results in a degradation of the precursor molecules prior to processing and maturation.

The paternally inherited nonsense mutation in exon 18 (R854X), which resides on a silent allele, had been previously described in a compound-heterozygous adult African American patient (cell line GM 01935) (Hermans et al. 1993*a*) and in a half–African American (African American father and Caucasian mother) child (patient 6) with the juvenile form of the disease (table 1). The data thus strongly pointed to an African origin of the R854X mutation in the African Americans and prompted us to look for more patients of a similar background. Two other infants born of western African parents were available for study. The R854X mutation was present on one allele of a 2-mo-old infant (patient 2) of healthy, nonconsanguineous parents who were of Hausa origin. One parent was from the province of Katsina,