Letters to the Editor

Am. J. Hum. Genet. 62:985–986, 1998

HLA and Mate Choice

To the Editor:

The papers on HLA and mate choice, by Hedrick and Black (1997), who studied South Amerindian tribes, and by Ober et al. (1997), who studied the relatively closed and partially inbred Hutterite populations, as well as the invited editorial by Beauchamp and Yamazaki (1997), point out the conflicting evidence for this potential relationship and some of the possible reasons for it. I would like to suggest an alternative approach.

My colleagues and I have shown that there is a relationship between recurrent spontaneous abortion and genes linked to HLA-DR and between unexplained infertility and genes linked to HLA-DQ (Gill 1992; Jin et al. 1995). It is important to note that the data showed that genes *linked* to the genes encoding HLA antigens—and not the HLA genes themselves—are involved in these associations between HLA and reproductive defects. The same conclusion has been proposed for the association between HLA and susceptibility to rheumatoid arthritis (Dizier et al. 1993), between HLA-DR and HLA-DQ and insulin-dependent diabetes mellitus (Clerget-Darpoux et al. 1991; Dizier et al. 1994), and between HLA and multiple sclerosis (Francis et al. 1991).

I propose that the potential association between HLA and mate selection may reside in the HLA-B–DR-DQ region and that this association should be explored in detail. Inclusion of the HLA-A locus in the analysis can obscure this potential effect considerably (Ho et al. 1994). Hedrick and Black (1997) typed only for HLA-A and HLA-B, and Ober et al. (1997) did not give the details of the genetic structure of the haplotypes in the various mating combinations. The latter group, however, has published on the relationship between HLA-DR and fertilization or implantation in the same Hutterite populations (Ober et al. 1992; Ober 1995); thus, it seems reasonable that, if mate selection has an association with genes in the HLA complex, these genes may reside in the HLA-B–DR-DQ region.

If a relationship between the major histocompatibility complex (MHC) and mate selection is borne out in humans, it may reflect an evolutionary reproductive drive to avoid homozygosity for MHC-linked recessive reproductive genetic defects (Gill 1997*a*, 1997*b*). It is interesting to speculate that this type of evolutionary drive may also be the basis for the near-universal human taboo against incest.

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Reply to Gill

To the Editor:

The "alternative approach" to our analysis (Ober et al. 1997) proffered by Gill has two parts: (1) "genes linked to the genes encoding HLA antigens, and not the HLA antigens themselves, are involved in these associations"; and (2) "the potential association between HLA and mate choice may reside in the HLA-B-DR-DQ region." In reply, we cite the first sentence of the final paragraph of our paper: "In summary, these data are consistent with the conclusion that genes in the HLA region may influence mate choice in humans" (Ober et al. 1997, p. 503).

We see no conflict between the data showing decreased HLA haplotype matching between Hutterite mates (Ober et al. 1997) and the absence of decreased allele matching at the *HLA-A* or *HLA-B* loci in South Amerindians (Hedrick and Black 1997). Indeed, among the same 411 Hutterite couples studied for HLA-haplotype matching, there was no significant evidence favoring decreased allele matching at the *HLA-A, HLA-B,* or *HLA-DR* loci, as shown in table 1. (The nonsignificant decrease in the number of observed allele matches at each of the three loci is consistent, in a population with a limited repertoire of haplotypes, with the significant HLA-haplotype effect on mating structure reported.) That any single HLA locus or other locus in the HLA region would, per se, be critical to mate choice seems unlikely, since this would represent a very narrow evolutionary strategy for avoidance of homozygosity; rather, we suggest that it is the haplospecific configurations of alleles—that is, haplotypes—that is essential to the decreased between-mate haplotype matching that we reported.

Table 1

Expected and Observed Numbers of Couples Matching for an HLA Allele

Locus	No. Expected[a]/No. Observed	χ^2_4	*P*
HLA-A	207.0/198	.789	.37
HLA-B	151.8/143	.809	.37
HLA-DR	184.5/178	.416	.52

[a] Calculated by method 1 of Ober et al. (1997).

We cannot determine, on the basis of the Hutterite data, whether the *HLA-B–HLA-DR* segment or the *HLA-DQ* segment may be more important than adjacent major-histocompatibility-complex (MHC) regions in this respect, but we do disagree that exclusion of the *HLA-A* locus from the haplotype-matching analysis—even if only the *HLA-B–HLA-DQ* region were relevant to mate choice—would have increased the statistical significance of our findings. The Hutterite genealogy was founded by 62 progenitors ~12 generations ago. Given a 1% recombination frequency between *HLA-A* and *HLA-B,* it is likely that haplotypes identical by state (IBS) for *HLA-B, HLA-DR,* and *HLA-DQ* alleles but with different *HLA-A* alleles are not identical by descent (IBD). Among the 48 ancestral five-locus (*HLA-A, HLA-C, HLA-B, HLA-DR,* and *HLA-DQ*) haplotypes, there were 35 unique three-locus (*HLA-B, HLA-DR,* and *HLA-DQ*) haplotypes (see Kostyu et al. 1993, table 3). *HLA-B–HLA-DQ* haplotypes that were IBS but not IBD might differ—and, in our judgment, are likely to differ—at unknown loci within the *HLA-B–HLA-DQ* segment. Thus, we concluded that, in the Hutterite genealogy, *HLA-A* allelic variability might serve as a proxy for allelic variability in other parts of the haplotype, including but not limited to the *HLA-B–HLA-DQ* segment.

To address this question, we have since typed the 48 serologically defined ancestral five-locus haplotypes by molecular methods and for 11 additional MHC loci, including four loci—*TNFa, BF, C4A,* and *C4B*—located between *HLA-B* and *HLA-DR* (authors' unpublished data). On the basis of these new results, the number of unique ancestral five-locus haplotypes was revised from 48 to 47, but the number of unique three-locus haplotypes remained unchanged, at 35. Therefore, there were 12 three-locus haplotypes that were IBS but that, on the basis of *HLA-A* allelic differences, probably were not IBD with other identical three-locus haplotypes. Ten of these 12 three-locus IBS haplotypes have now been shown to have different alleles at loci within the *HLA-B–HLA-DR* segment, thereby verifying the supposition

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.

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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-

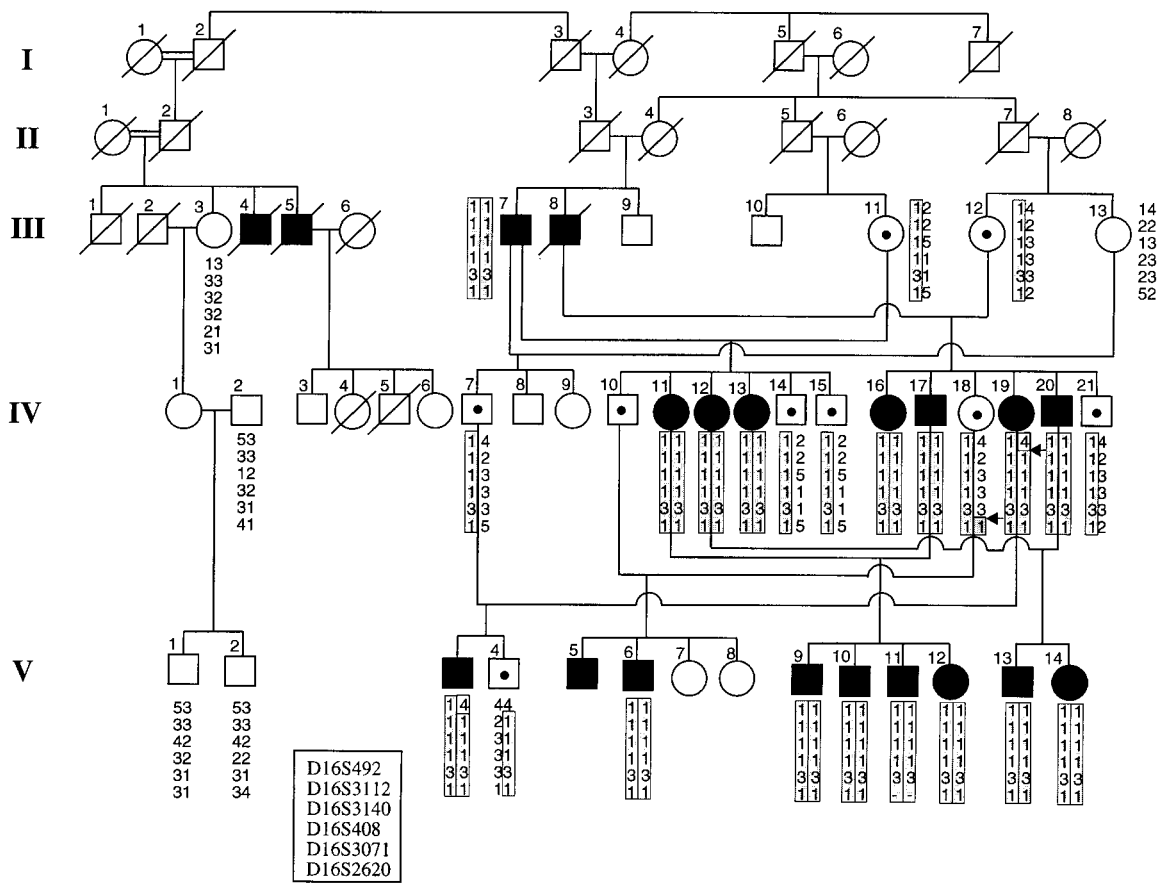

Figure 1 Pedigree showing disease-associated haplotypes. Blackened circles and squares represent affected females and males, respectively, and symbols with a central black dot represent obligate heterozygotes. The disease-associated haplotype is highlighted by a grey-shaded box beneath each symbol. Recombination 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

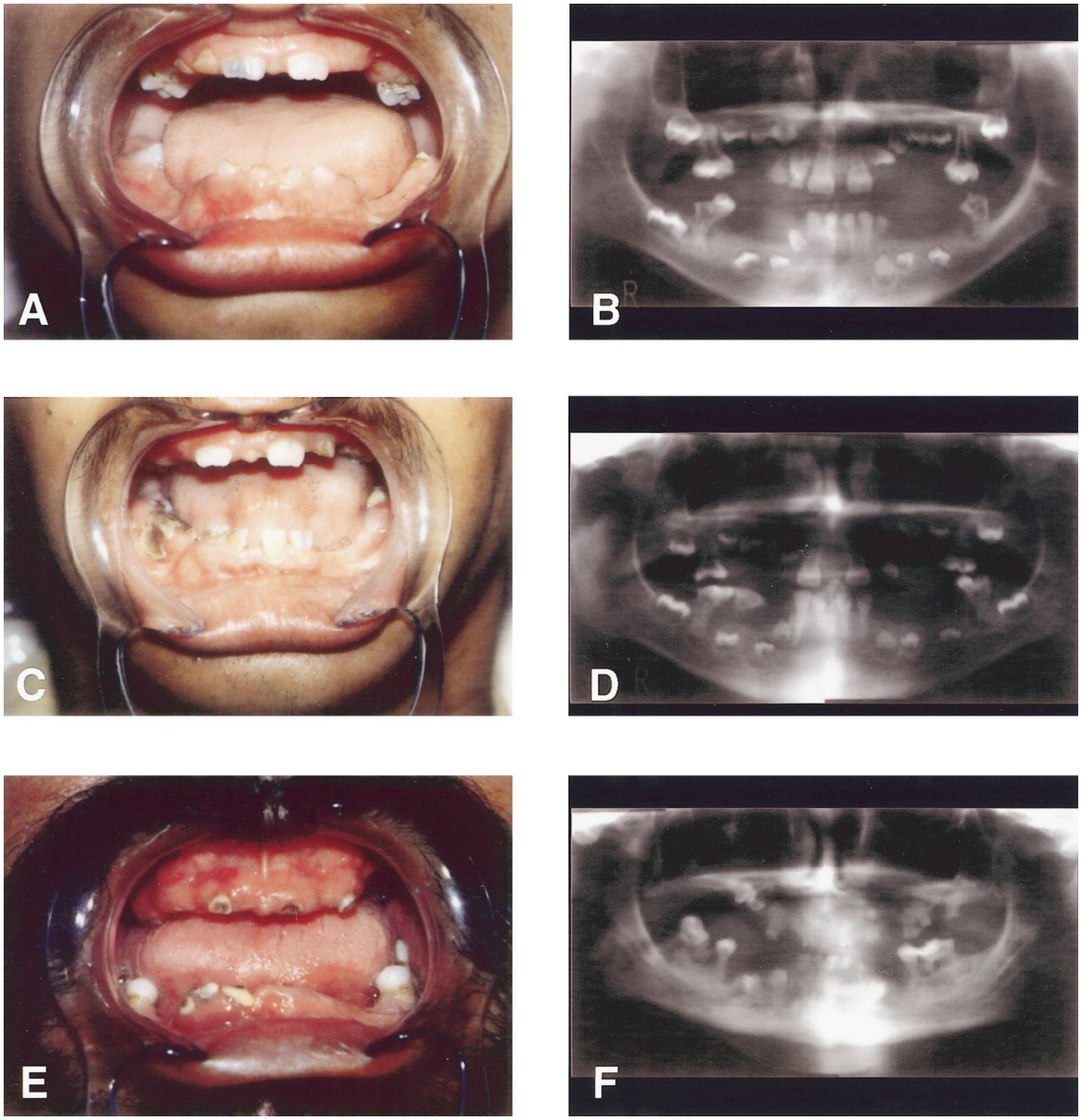

Figure 2 *A,* Subject V-9, a 15-year-old male, showing mixed dentition, with the first permanent molars, maxillary central incisors, and mandibular right cuspid erupted. In addition, the maxillary right lateral incisor and canine and the mandibular incisors and left canine from primary dentition were 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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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,

Table 1

Presence of R854X or Other Mutations on African or African American Chromosomes

Group and Patient	Mutation Status of	
	Chromosome 1	Chromosome 2
African:		
Patient 1 (Ivory Coast)	R854X	V949D
Patient 2 (Nigeria)	R854X	Negative[a]
Patient 3 (Ghana)	R854X	R854X
Patient 4 (Ovambo-Namibia)	R854X	R854X
Patient 5 (Zulu-South Africa)	Negative	Negative
African American:[b]		
Patient 6 (mixed)	R854X	IVS6 −22 t→g
Patient 7	R854X	Negative
Patient 8	R854X	Negative
Patient 9 (C2123)	R854X	R854X
Patient 10 (C1992)	Negative	Negative
Patient 11 (C9752)	Negative	Negative
GM 00248	R854X	R854X
GM 00338	R854X	R854X
GM 01935	R854X	D654E
GM 03329	Negative	M519V
GM 04912	Negative	Negative
GM 12932	Negative	Negative

Note.—This study was carried out under protocols approved by the institutional review boards of the National Institute of Arthritis and Musculoskeletal and Skin Diseases and of the institutions where the samples were obtained. Details of the primers, PCR conditions, and identification of mutations are available on request.

[a] R854X not present.

[b] Patient 6 has been reported in detail elsewhere (Adams et al. 1997). Patient 7 was the 6-wk-old daughter of healthy, nonconsanguineous African American parents; patient 8 was a 6-mo-old African American male referred to New York University Medical Center by Dr. H. Mussel; patients 9–11 (C2123, C1992, and C9752) were referred to the New York State Institute for Basic Research and New York University Medical Center. The cell lines designated "GM" were obtained from the National Institutes of Health Mutant Repository, Coriell Cell Repositories (Camden, NJ) and were derived from African American patients with infantile-onset GSD II (GM 00248, GM 12932, GM 03329, GM 00338, and GM 04912) (Martiniuk et al. 1986; Zhong et al. 1991) or adult-onset GSD II (GM 01935). Additional clinical information is present in the Coriell catalogue. Mutations and additional molecular information have been reported for GM 03329 (Huie et al. 1994*b*) and GM 01935 (Martiniuk et al. 1991; Hermans et al. 1993*a*). The diagnosis of severe deficiency of GAA was demonstrated in each of these patients by enzymatic assay of fibroblasts and/or muscle.

and the other was from the northeastern Borno province (parental DNAs were not available); the patient's male sibling had died at age 5 mo, of unknown causes. Both alleles of a 4-mo-old Ghanaian infant (patient 3) of healthy, nonconsanguineous parents, who were from the Twi subgroup of the Ashanti tribe and were living in Accra, bore R854X. We also sought the mutation in infants of different backgrounds who were from southern Africa and who had been reported by van der Ploeg et al. (1989). Both alleles of an Ovambo infant (patient 4) from Namibia carried R854X, and a Zulu infant (patient 5) was negative. (R854X was also absent in the two infants from southern Africa who were of uncertain ethnic background.) R854X was present, therefore, in four of five African infants of known ethnic background, on 6 of 10 chromosomes.

Among the 12 African American patients studied (patients 6–11 and six GM cell lines), 3 are homozygous, and 4 are heterozygous, for R854X (table 1). Therefore, 10 of 23 African American chromosomes (the mother of patient 6 is Caucasian) carry the R854X mutation, resulting in an allele frequency of .43 among African Americans with GSD II.

The other mutation described in an African American (Hermans et al. 1993*a*) and also identified in Chinese patients—that is, D645E—was not found in any of our cases. In African American patients (Hermans et al. 1993*b*) but not Chinese patients (C-Y Lin, personal communication), the mutation is associated with two polymorphic sites in exons 17 and 19 (I816 and I927) found in several healthy unrelated African Americans, suggesting that the Chinese and African American D645E mutations occurred independently. By contrast, all eight polymorphic sites (596G, 668A, 921T, 1203A, 1374T, 2154C, 2338A, and 2553A) residing on the R854X mutated African American allele in our juvenile case (Adams et al. 1997) are identical to those found in the infants from the Ivory Coast, Ghana, and Namibia, a finding that suggests a common haplotype and a common origin of the R854X mutation.

The R854X mutation was not found in 7 infantile- and 34 adult-onset Caucasian patients with GSD II. This represents 48 chromosomes at risk for a "null" mutation: 2 from each infant and only 1 from each adult, since adult patients have some enzyme activity. These results clearly document that R854X is not frequent in all populations. However, the mutation was found in homozygosity in an infantile patient from consanguineous Pakistani parents and was found in heterozygosity in a Mexican American patient and in a French patient. The R854X mutation is a C→T transition at a CG dinucleotide and therefore is at a site susceptible to recurrent mutations. Further investigation of haplotypes could reveal whether these are independently recurring events.

Of the 12 million African captives, <600,000 ever reached North American shores. The slave trade to North America experienced two particularly strong periods: one just before the American Revolution of 1776, and the second at the turn of the 19th century, just before the passage, in 1808, of a law prohibiting the importation of African slaves (Rawley 1985). The first Africans to be enslaved in the 16th century were peoples from the western Atlantic region. Traders then worked their way incrementally along the coast, focusing next on Guinea Coast peoples, and, at the height of the late-18th

Figure 1 Geographic location of western African populations described in the text.

century trade, peoples of the Guinea Coast, often of Ashanti origin, were most common in slave cargoes. The trade eventually reached Angola, around 1800 (Curtin 1969).

The four African ethnic groups currently known to carry R854X (fig. 1) have a history of long-standing interaction. The Hausa, although they claim northern Nigeria as their homeland, are widely dispersed throughout western Africa. Their commercial activities, initiated as early as 1100, brought them into direct and prolonged contact with the western Atlantic region, where they encountered the Guéré of the Ivory Coast. The Guéré, in turn, were a subgroup of the Kru who, because of their boating skills, were used as deckhands on European trading vessels sailing in Africa's coastal waters. The Hausa also had strong contacts with the Ashanti nation, in what is today the nation of Ghana, both through trade connections and through residential enclaves. The Ovambo of Namibia, although they were beyond the reach of the Hausa trade network, are a Bantu-speaking group descended from a population originally located on the Bauchi plateau of northern Nigeria, adjacent to the Hausa homelands. Segments of this Nigerian group, who would eventually become the Ovambo, undertook a gradual southward migration that brought them to their current location in Angola and northern Namibia, sometime during the period of 800–900 (Turnbull 1977; Curtin and Bohannon 1988). Thus, although it is probable that the closest African ancestors of the African American patients were culturally Ashanti and were brought to North America from the Guinea Coast, their genetic makeup strongly suggests that they have Hausa roots. It is reasonable to speculate that the mutation occurred some time before the southward migration of the Ovambo away from the territory of the Hausas, possibly in their common ancestral population from north-central Africa.

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Linkage Thresholds for Two-stage Genome Scans

To the Editor:

Two-stage genome scans are a common design in linkage studies. In the first stage, genotypes are generated for a standard set of ~300 microsatellite markers, and linkage analysis is carried out. In the second stage, any interesting "regions" (or "hits") that were found during the first stage are followed with a much denser map of markers to extract all available linkage information.

How should the statistical significance of the final results be determined? We have argued elsewhere that thresholds computed for a dense map of markers covering the entire genome should be applied (Lander and Kruglyak 1995). The logic behind this recommendation is simple. False-positive peaks that would have been detected with a dense whole-genome map almost always come up as hits in the initial stage of a two-stage scan. The reason for this is that the marker density for the initial stage is specifically chosen to detect peaks of moderate size (whether real or false positive). Since these hits are then followed up with a dense map, the final results are virtually the same as if a dense whole-genome map had been used at the outset.

Recently, Sawcer et al. (1997) challenged this recommendation. They carried out simulations to determine the significance of results obtained in a genome

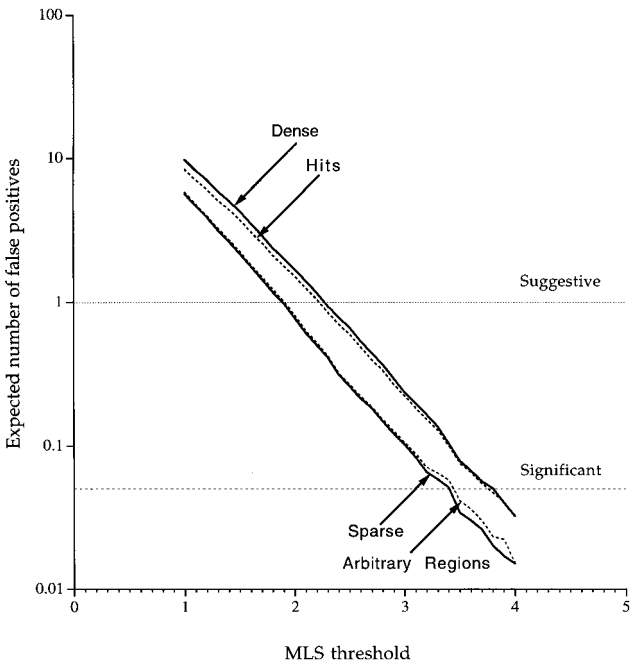

Figure 1 The expected number of false positives at or above a given MLS threshold for the four scenarios described in the text, plotted on a log scale.

scan for multiple sclerosis (MS), and they concluded that "the practice of adding markers around provisional linkage hits in a genome screen has relatively little effect on the false-positive rate" (Sawcer et al. 1997, p. 227). The goal of this letter is to point out the fallacy in the study of Sawcer et al. (1997) and to demonstrate unequivocally that increasing map density around linkage hits has a strong effect on the false-positive rate, making it essentially the same as that expected with a dense whole-genome map.

In the MS genome scan, Sawcer et al. (1996) identified provisional hits in the initial screen and then followed them up by increasing the marker density in the surrounding regions "to increase the information extraction in those areas showing possible linkage" (Sawcer et al. 1997, p. 224). Sawcer et al. (1996, 1997) carried out simulations (with marker genotypes generated under the hypothesis of no linkage) to evaluate the significance of the genome scan findings. However, the simulations had a serious flaw. Instead of using increased marker density around the hits occurring in each simulated genome scan (which would accurately model the actual follow-up strategy), they used increased marker density around the hits obtained in the actual MS genome scan, despite the fact that these regions did not correspond to hits in most simulated genome scans. Thus, the simulations modeled a strategy of increasing map density in arbitrary locations rather than around provisional hits. Not surprisingly, Sawcer et al. (1997) observed that the locations of hits in simulated genome scans showed little correlation with the regions of higher map density. The simulations did not accurately model the experiment and, as a consequence, considerably underestimated the false-positive rate.

To illustrate this effect, we carried out simulations modeling the different approaches. We generated 1,000 replicates of a genome scan of 100 sib pairs, under the null hypothesis of no linkage, with each replicate containing genotype data on 23 chromosomes of length 150 cM each. Markers were assumed to have four equally frequent alleles (heterozygosity 0.75). For each replicate, we examined four scenarios of marker density: sparse, dense, follow-up of hits (FH), and follow-up of arbitrary regions (FA). In the sparse scenario, markers were spaced every 10 cM. In the dense scenario, markers were spaced every 1 cM. In the FH scenario, markers were spaced every 10 cM, as in the sparse scenario, except that regions in which the score from the sparse map exceeded a given threshold were saturated with markers, at 1-cM density, in a 10-cM window around each peak. This scenario was intended to model an actual two-stage study. Finally, in the FA scenario, markers were spaced every 10 cM, as in the sparse scenario, except that the marker density was increased to 1 cM in arbitrarily selected 10-cM regions, with the number of such regions constrained to equal the number of regions followed up in the FH scenario. This scenario was intended to model the incorrect simulation approach of Sawcer et al. (1997). Linkage analysis was carried out by using MAPMAKER/SIBS (Kruglyak and Lander 1995) to compute the maximum LOD score (MLS) statistic of Holmans (1993). The threshold for follow-up was an MLS of 1.0. On average, 5.7 such regions were followed up per genome scan; higher or lower thresholds for follow-up did not change the results substantially.

The results are shown in figure 1, which plots the expected number of false positives in a genome scan for the four scenarios. As expected, the false-positive rate for follow-up of arbitrary regions closely follows that for the sparse map, while the rate for follow-up of hits closely follows that for the dense map. The respective thresholds for suggestive linkage (one expected false positive per genome scan) are ~1.9 for the sparse and FA scenarios and ~2.3 for the dense and FH scenarios. The thresholds for significant linkage (one expected false positive per 20 genome scans) are ~3.4 for the sparse and FA scenarios and ~3.8 for the dense and FH scenarios. The use of simulations with follow-up of arbitrary regions underestimates the true false-positive rate by a factor of ~2. The thresholds for follow-up of hits are not very different from the theoretical dense-map values

of 2.6 for suggestive and 4.0 for significant linkage (Lander and Kruglyak 1995).

We therefore conclude that the original recommendation to use dense-map thresholds to assess the significance of results from two-stage genome scans is appropriate. Of course, it may still be desirable to carry out simulations, to take into account specific features of a particular study and to avoid relying on asymptotic assumptions. In this case, such simulations must accurately model the methodology of the study, including follow-up of interesting regions. Otherwise, the false-positive rate can be underestimated considerably.

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Response to Kruglyak

To the Editor:

We accept that our simulations (Sawcer et al. 1997) did not accurately model our experiment in the important respect that extra markers were added after an interim analysis. However, this was not the only respect in which our genome screen differed from the situation that generated the theoretical results discussed in the original paper of Lander and Kruglyak (1995). Kruglyak's recent simulations still differ from our genome screen in important respects: his simulations used uniform markers spaced on a uniform map and involved a genome with chromosomes of a uniform length and families with a uniform pedigree structure. Our simulation study was as much aimed at assessing the importance of these features of real studies. To do this required massive computational effort, and it was simply not feasible for us to include the two-stage aspects at the same time. We should also point out that, in common with most "two-stage" studies, our second-stage study did not simply add extra markers—it added extra families as well. In such circumstances, it is not clear whether the simulations of Kruglyak are quite as relevant, since it will surely no longer be inevitable that the effect of increasing the marker density will almost always be to increase the maximum LOD score (MLS). It would be interesting to see the results of a simulation exercise that also considered the introduction of further families at the second stage. Intuitively, we might expect them to be intermediate between our results and those of Kruglyak.

This aspect of our genome study was one factor that led us to neglect the addition of extra markers. A second was that our general experience has not confirmed the assertion that the effect of adding extra markers is almost always to increase the MLS. We often find the reverse—the initial peaks disappear when additional markers are added! However, we also find Kruglyak's results persuasive. It is well recognized that genotyping and mapping errors tend to reduce the observed MLS (Ott 1991), and it is possible that these account for the discrepancy between our perceptions and the simulation results, since neither set of simulations considered these.

To summarize, we accept that our simulation study oversimplified the conditions of our genome screen in one important respect. But the real study had a number of further difficult aspects, and Kruglyak's simulations also neglect these aspects. We must concede, however, that perhaps we overstated the generality of some of our conclusions. Finally, we would make a plea that this debate should not be concerned solely with the "significance" of a single high MLS. In many cases, it may well be of considerable interest that substantially raised MLSs were found in a number of regions, even though none of them is singly convincing. This is another aspect of the interpretation of genome screens that may be valuably addressed by simulation.

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