sequence of expansion and their relationship to the methylation event are likely to provide new insight into the molecular mechanism leading to the manifestations of fragile X syndrome.

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Acknowledgments

We thank Dr. J. L. Mandel for providing the probes StB12.3, StB12.XX, and F33.

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0002-9297/96/5801-0027$02.00

Am. J. Hum. Genet. 58:239–241, 1996

Exclusion of Linkage between Cleft Lip With or Without Cleft Palate and Markers on Chromosomes 4 and 6

To the Editor:

Nonsyndromic cleft lip with or without associated cleft palate (CLP) is a common craniofacial defect, occurring in ~1/1,000 live births. While the defect generally oc-

curs sporadically, multiplex families have been reported. Segregation analyses have demonstrated that, in some families, CLP is inherited as an autosomal dominant/codominant disorder with low penetrance (Hecht et al. 1991). Several clefting loci have been proposed on multiple chromosomes, including 6p24, 4q, and 19q13.1. Association studies and linkage studies suggested a locus that mapped to 6p24 (Eiberg et al. 1987; Mehra and Verma 1991). We were unable to confirm this in a linkage study of 12 multigenerational families (Hecht et al. 1993). A subsequent linkage study by Carinci et al. (1995), however, found evidence for linkage to this region in 14 of 21 clefting families. Additionally, Davies et al. (1995) studied the chromosomes of three individuals with cleft lip and palate, all of whom had a rearrangement involving 6p24. Their investigation supported a locus at 6p24. Carinci et al. (1995) reported that the most likely position for a clefting locus was at D6S89, which is centromeric to EDN1. This is in contrast to the findings of Davies et al. (1995), who suggested a placement telomeric to EDN1. F13A, which had been implicated in the initial association studies, is telomeric to EDN1. Thus, the region between F13A and D6S89 encompasses the regions proposed by both Davies et al. and Carinci et al. A second clefting locus, at 4q, was proposed by Beiraghi et al. (1994), who studied a single multigenerational family by linkage analysis. Their data suggested a locus near D4S175 and D4S192.

In a recent study of 39 multigenerational clefting families, we demonstrated linkage to 19q13.1 in 17 of the families (Stein et al. 1995). To determine if the loci at 6p24 and 4q were important in those families that did not map to 19q13.1, we typed our families for markers mapping to these regions and compared the results from the families linked to 19q with the results from the 19q-unlinked families. DNA was available from 33 of the 39 families. Eighteen of the 33 families had a probability of $<.5$ of linkage to 19q13.1, on the basis of HOMOG analysis. The families were typed for the PCR markers F13A, D6S89, D6S105, D4S175, and D4S192 by our standard laboratory protocols (Stein et al. 1995). Two-point LOD scores between CLP and the markers were calculated by the MLINK program of the LINKAGE package (Lathrop et al. 1984). An autosomal dominant mode of inheritance was assumed with a penetrance of .24 in females and .32 in males and with a phenocopy rate of .001 (Hecht et al. 1991). Frequencies of alleles for markers were calculated by use of a panel of 30 unrelated and unaffected individuals. Evidence for heterogeneity was evaluated by HOMOG (Ott 1985).

Although some of the families yielded small positive LOD scores to markers on chromosomes 6p and/or 4q, overall the LOD scores were negative and provided evidence for exclusion (table 1). This was true for both the 19q-unlinked families and the 19q-linked families. More important, there was no evidence for heterogeneity in either group or when the groups were combined. This was true even when the 19q-unlinked group was limited to those families that had a probability $<.3$ of mapping to 19q (11 families). These results do not rule out the possibility that loci at either 4q or 6p play a role in the etiology of CLP in other families, as is suggested by

Table 1

Two-Point LOD Scores between CLP and Markers on Chromosomes 4 and 6

	Two-Point LOD Score at Recombination Fraction of						
Marker and 19q Status	0	.001	.01	.05	.15	.2	.3
D4S192:							
Linked	−7.355	−7.087	−5.603	−3.241	−2.079	−1.436	−1.014
Unlinked	−9.119	−8.636	−6.041	−2.459	−1.007	−.343	−.008
D4S175:							
Linked	−11.157	−9.438	−6.035	−2.595	−1.189	−.497	−.105
Unlinked	−13.503	−12.642	−8.858	−3.861	−1.639	−.541	−.064
F13A:							
Linked	−3.498	−3.322	−2.411	−1.123	−.591	−.359	−.251
Unlinked	−11.981	−10.946	−7.905	−4.364	−2.639	−1.663	−1.029
D6S105:							
Linked	−5.152	−4.968	−4.019	−2.348	−1.421	−.921	−.616
Unlinked	−14.622	−12.941	−5.933	−8.657	−4.831	−2.856	−1.688
D6S89:							
Linked	−10.029	−8.678	−6.194	−3.194	−1.88	−1.228	−.835
Unlinked	−17.591	−16.076	−11.617	−6.396	−3.982	−2.671	−1.842

the other studies. They do, however, provide further evidence for the complex nature of the etiology of CLP.

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Acknowledgments

This work was supported by NIH grant NIDR5R29 DE09954-03 to J.T.H. and by NIH grant DE09164 to D.L.G.

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0002-9297/96/5801-0028$02.00

Am. J. Hum. Genet. 58:241–243, 1996

Multiple Mutations in a Specific Gene in a Small Geographic Area: A Common Phenomenon?

To the Editor:

We read with interest the article from Allamand et al. (1995), which demonstrates in a genetic isolate the presence of at least six different haplotypes in the limb-girdle muscular dystrophy type 2A chromosome. Several hypotheses were proposed by the authors to explain this finding, but, after the identification of calpain, the gene involved in the disorder, multiple mutations were proved to be at the origin of this observation (Richard et al. 1995). The authors proposed that both the presence of multiple distinct calpain mutations within the Reunion Island pedigrees and the relatively low frequency of the disease in the isolate may be explained by a digenic inheritance of the disorder. Their hypothesis postulates that, although calpain mutations may be frequent in all populations, the disease manifestations are controlled by another frequently mutated nuclear or mitochondrial gene in the Reunion isolate.

We would like to propose an alternative model to explain the "Reunion paradox": a relatively recent occurrence of several mutations in the calpain gene in the island of La Reunion. The inhabitants of this island formed a genetic isolate founded by relatively few individuals; therefore it is expected that most of the individuals living today in the Reunion isolate will have some common ancestors. In addition, in most of the genome there will be differences from one individual to another, because of recombinations that have occurred since the founding of the isolate. In such a population, the occurrence of multiple mutations in a single gene will lead, a few generations later, to several observations. All the affected individuals will show linkage for the same genomic region, since the same gene is involved; however, the haplotypes on which the mutations occur will be different. All the carriers will have a common ancestor, as will almost all the individuals in the isolate, because the island was settled by few individuals. The disease will appear to be relatively rare, since the mutations will be present only in the descendants of the individuals in which the first mutations occurred and will not be present in all the descendants of the common ancestor. This hypothesis therefore may explain the "paradox" observed by Beckman and collaborators; however, it implies a very high mutation rate, a phenomenon that was indeed observed, in a small region of the Galilee in Israel, for two other loci (Bach et al. 1993; Heinisch et al. 1995).

Two lysosomal storage diseases—namely, Hurler syndrome and metachromatic leukodystrophy (MLD)—