Letters to the Editor

Am. J. Hum. Genet. 46:1214–1215, 1990

Linkage Disequilibrium between Two Restriction Sites in the COL2A1 Gene

To the Editor:

RFLPs in the COL2A1 gene, localized on chromosome 12q13, have been found useful in analyzing linkage relationships in various disorders (Francomano et al. 1987; Anderson et al. 1990). Haplotyping of these polymorphisms is often useful to increase the informativeness of families under study. Significant linkage disequilibrium, however, makes haplotyping less valuable. We decided to examine the three common RFLPs in the COL2A1 gene to determine whether these sites were in linkage disequilibrium. The *Hin*dIII polymorphism is between exons 20 and 30, with a rare allele frequency of .41; the *Pvu*II polymorphic site is about 500 bp 3′ to exon 30, with a rare allele frequency of .47; the *Hin*fI polymorphism is within the first few exons of the gene and has allelic frequencies of .42, .35, and .23. Two COL2A1 probes were used in the present study. pXK2.1, a 2.1-kb fragment of HCOLIIC, detected the *Hin*dIII and *Pvu*II polymorphisms (Nunez et al. 1985; Sykes et al. 1985; R. C. Schwartz and P. Tsipouras, unpublished data). The *Hin*fI polymorphism was detected with pEB1.6, a 1.6-kb fragment of HCOLF (Strom 1988; Anderson et al. 1990). Gene frequencies and haplotype frequencies were calculated using maximum-likelihood methods. Over 80 informative chromosomes were analyzed. The most significant disequilibrium was detected between the most physically close RFLPs, *Hin*dIII and *Pvu*II, which are estimated to be about 3.5-kb apart. The maximum values of linkage disequilibrium and the percentage degree of disequilibrium between each pair of RFLPs are presented in table 1.

0002-9297/90/4606-0023$02.00

Table 1

Maximum Linkage Disequilibrium Values in the COL2A1 RFLPs and Loss in PIC Values That Is Due to Association

	Theoretical Maximum Disequilibrium	Observed Disequilibrium as Percentage of Maximum Disequilibrium	Level of Significance	PIC Loss That Is Due to Association
*Pvu*II-*Hin*dIII	.219	73%	1%	.150
*Pvu*II-*Hin*fI	.122	41%	2%	.024
*Hin*dIII-*Hin*fI	.136	1.5%	NS	.067

NOTE.—NS = not significant.

We have also shown the PIC reduction that is due to the association. We conclude that, because of significant linkage disequilibrium, the genotyping of both the *Hin*dIII and *Pvu*II polymorphisms for a linkage study is less useful than what one would predict on the basis of gene frequencies.

ROBIN C. SCHWARTZ,
MANSOOR SARFARAZI,
AND PETROS TSIPOURAS

Department of Pediatrics
University of Connecticut Health Center
Farmington

References

Anderson IJ, Goldberg RB, Marion RW, Upholt WB, Tsipouras P (1990) Spondyloepiphyseal dysplasia congenita: genetic linkage to type II collagen (COL2A1). Am J Hum Genet 46:896–901

Francomano CA, Liberfarb RM, Hirose R, Maumanee IH, Streeten EA, Meyers DA, Pyeritz RE (1987) The Stickler syndrome is closely linked to COL2A1, the structural gene for type II collagen. Genomics 1:293–296

Nunez AM, Francomano C, Young MF, Martin G, Yamada Y (1985) Isolation and partial characterization of genomic clones coding for a human pro-alpha 1(II) collagen chain and demonstration of restriction fragment length polymorphisms at the 3′ end of the gene. Biochemistry 24:6343–6348

Strom CM (1988) A three allele restriction fragment length polymorphism within the human COL2A1 gene. Nucleic Acids Res 16:9077

Sykes B, Smith R, Vipond S, Paterson C, Cheah KSE, Solomon E (1985) Exclusion of the alpha-1 (II) collagen gene as a mutant locus in type IA osteogenesis imperfecta. J Med Genet 22:187–191

Am. J. Hum. Genet. 46:1215–1216, 1990

Codon 129 Changes in the Prion Protein Gene in Caucasians

To the Editor:

A number of mutations in the prion protein (PrP) gene in patients with Creutzfeldt-Jacob disease (CJD) or Gerstmann Sträussler syndrome (GSS) have been reported (Doh-ura et al. 1989; Goldgaber et al. 1989; Hsiao et al. 1989; Owen et al. 1989). Recently Goldfarb et al. (1989) reported a "double-allele mutation" in the PrP gene in two of three patients with Kuru, in three of three patients with familial CJD from unrelated families, and in two of two patients with iatrogenic CJD caused by treatment with contaminated pituitary-derived human growth hormone. The mutation, a methionine(M)-to-valine(V) substitution, results from an ATG-to-GTG change in codon 129 of the Prp gene. This change abolishes an *Nsp*I restriction site and creates a *Mae*II restriction site and formed the basis for the screening of samples for this mutation.

Goldfarb et al. failed to detect the double-allele mutation (V/V) in normal controls or in cases of sporadic CJD but did detect the presence of a single-allele mutation (M/V) in codon 129 in three of 24 control subjects and in three of 15 cases of sporadic CJD. They suggested that the consistent detection of the double-allele mutation in the initial seven patients studied who had related spongiform disorders indicated that this particular mutation may serve as a genetic background for some cases of Kuru and CJD.

In view of the potential importance of this finding, we have studied the incidence of codon 129 changes in 36 unrelated Caucasian control subjects. Changes in codon 129 were detected with allele-specific oligonucleotide probes by using the method of Saiki et al. (1986), modified to include the use of tetramethylammonium chloride in the wash procedures as described by Wood et al. (1985). Two oligonucleotides (15 mers) spanning codon 129 were synthesized, with one corresponding to the sequence of the M^{129} allele and the other to the sequence of the V^{129} allele, i.e., 5′-CGGCTACATGCTGGG-3′ and 5′-CGGCTACGTGCTGGG-3′, respectively. The open reading frame of the PrP gene was amplified using the polymerase chain reaction as described elsewhere (Collinge et al. 1989), and slot blots were prepared on Hybond-N-nylon membranes (Amersham, England). After being end-labeled with ^{32}P-ATP, the oligonucleotides were sequentially hybridized to the blots and autoradiographs were prepared. Unlike Goldfarb et al., in our control population we found the double-allele (V/V) change in five subjects and a single-allele (M/V) change in 13 subjects, whereas the remaining 18 subjects had no change in codon 129 (M/M). Examples are presented in figure 1.

Thus the change in codon 129 is not a mutation but represents a common polymorphism in the PrP gene. It is difficult to explain the discrepancy between the results of the present study and those of Goldfarb et al., but it is noteworthy that Doh-ura et al. (1989), who did not detect a double-allele (V/V) change in codon 129 in 33 controls (comprising 16 Japanese, 11 Thai, and 8 Nepali), stated that Caucasians have a higher