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Mapping Genes by Drift-Generated Linkage Disequilibrium

To the Editor:

In human populations that have remained of small and constant size, high levels of linkage disequilibrium (LD) are generated by genetic drift (Slatkin 1994; Laan and Pääbo 1997). Theoretical considerations suggest that such LD can be used to identify chromosomal regions involved in diseases or other traits, by "drift mapping" (Terwilliger et al. 1998). This concept relies on the assumption that when "cases" and "controls" are compared within a population in which extensive LD exists, disequilibrium will be observed between the trait and marker loci close to the gene(s) that contributes to the trait. Furthermore, genetic differentiation between the cases and controls will be observed in genomic regions contributing to the trait, whereas no differentiation will be seen in other parts of the genome. Computer simulations indicate that, under reasonable assumptions with regard to population size, population age, and marker heterozygosity (Terwilliger et al. 1998), it might be possible to map genes by use of this approach.

To empirically evaluate this idea, we have studied polymorphic loci in and around the gene that encodes the renin-binding protein (RnBP), a component of the renin-angiotensin system involved in the regulation of blood pressure. The RnBP gene is located on Xq28 and contains a point mutation, T61C, that occurs with a frequency of .18 in Germans (Knöll et al. 1997). We scored this polymorphism in males from the Saami and the Finns, two populations that differ radically in their demographic history. Whereas the Saami have not expanded during historical times and show no indication of expansion in tests based on DNA sequence variability (von Haeseler et al. 1996), the Finns are thought to have expanded drastically during the past few thousand years, on the basis of both epidemiological (Peltonen et al. 1995) and genetic evidence (Sajantila et al. 1996). The frequencies of the C allele were found to be .21 and .19 in the Saami and the Finns, respectively. The fact that the C allele occurs at appreciable frequencies in three European populations indicates that it is older than these populations. It is therefore a useful model of alleles involved in complex traits, since such alleles are expected to be both frequent in the population and of old age.

Four microsatellites located ~1.0–7.8 cM from the RnBP gene (fig. 1), as well as the T61C polymorphism, were typed in 53 Saami and 80 Finns. In addition, 10 microsatellite loci on Xp22 and Xq13, which had numbers of alleles comparable to the numbers of those around the RnBP gene, were typed in the same individuals (Laan and Pääbo 1997; authors' unpublished data), to assess the extent to which loci situated far from the RnBP gene might yield spurious associations with the T61C polymorphism. When the RnBP polymorphism and the microsatellite loci were analyzed for allelic as-

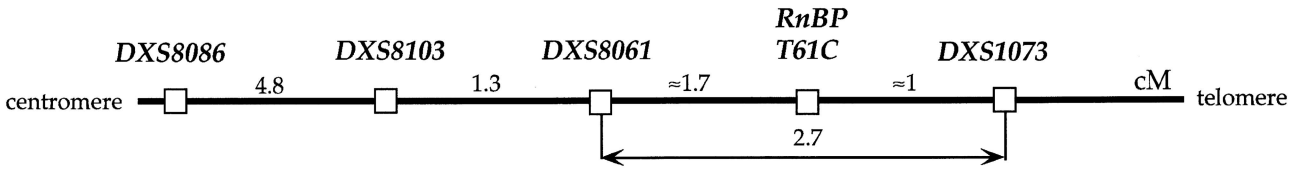

Figure 1 Genetic map (Nelson et al. 1995; Dib et al. 1996; Esposito et al. 1997; Nagaraja et al. 1997) of studied microsatellite loci around the RnBP gene.

sociation (table 1), DXS8061, located ~1.7 cM from the RnBP gene, showed LD in the Saami, at a significance level that would allow for gene mapping ($P = .00002$). All other P values, including that for DXS8061 in the Finns, were $\geqslant .003$. To evaluate the false-positive rate for the observed data, a randomization analysis was performed, in which the microsatellite haplotypes across the three regions on the X chromosome were kept together while the RnBP C/T alleles were randomly shuffled between the different haplotypes. The LD statistic was calculated for each microsatellite locus, and the most significant marker from each replicate was retained. After 10,000,000 randomizations, the significance of the association between DXS8061 remained significant at the .001 level in the Saami, whereas no association in the Finns was significant at the .05 level. It is noteworthy that, in contrast to DXS8061, DXS1073, located ~1.0 cM from the RnBP gene, showed no LD in the Saami. This result can be attributed to the fact that DXS1073 displays a single allele in the Saami carrying the C allele; it also underscores the fact that, because the extent of LD between closely linked loci is highly stochastic in constant populations (Weir 1996; Terwilliger et al. 1998), drift mapping is unlikely to allow fine-scale localization of genes.

When F_{ST} (Weir 1996) was used as a measure of genetic differentiation between the C and T chromosomes (table 1), differentiation ($P = .00000$) was seen for DXS8061 in the Saami. Furthermore, it was tested whether haplotypes consisting of four microsatellite loci would still show significant differentiation between the C and T alleles. This was the case, at a high level of significance ($P = .00000$), for the Saami but not for the Finns ($P = .02760$). Furthermore, no differentiation was detected in either the Saami or the Finns, for any of the four-locus haplotypes on Xp22 and Xq13 (data not shown).

It is noteworthy that the C allele is associated with different alleles (and haplotypes) of flanking microsatellites in the Saami and the Finns (data not shown). Thus, the ancestral haplotype on which the T61C mutation originally occurred has been lost in at least one population—and, possibly, in both. As predicted elsewhere (Terwilliger et al. 1998), this does not affect the efficiency of "drift mapping," whereas "shared segment" analyses of the combined data set would fail to show positive evidence of a gene in this region.

In conclusion, the Saami exhibit P values, both for LD between a marker locus and the T61C polymorphism and for population differentiation between the C and T chromosomes around the RnBP locus, of <.0001; this would allow ~10,000 tests to be performed without an unreasonably high background of false positives. By contrast, no comparable signals are seen in the Finns. Thus, these results show that, in principle, LD generated by drift in a small and constant population can be used to localize a gene, whereas it is difficult, if not impossible, in a population that has expanded. A further potential advantage of populations such as the Saami is that they may retain much of their statistical power in the presence of allelic heterogeneity (Terwilliger et al. 1998). Although several issues not addressed here complicate real mapping projects, we are hopeful that chromosomal regions involved in complex traits could be identified by an approach based on LD in populations such as the Saami.

Table 1

LD between T61C and Microsatellites and Genetic Differentiation between C and T Chromosomes

Region and Marker	LD (P)		F_{ST} (P)	
	Saami	Finns	Saami	Finns
Xq28:				
DXS1073	.09244	.00878	.169 (.04870)	.144 (.00540)
DXS8061	.00002	.03446	.443 (.00000)	.010 (.28567)
DXS8103	.13335	.32284	.089 (.06949)	.010 (.26387)
DXS8086	.40300	.72854	.053 (.19008)	−.022 (.78802)
Haplotype	N/A	N/A	.213 (.00000)	.034 (.02760)
Xq13:				
DXS983	.68754	.55828	−.010 (.42876)	−.032 (.94841)
DXS1225	.28491	.25807	.019 (.27327)	−.014 (.65133)
DXS8082	.13440	.43646	.039 (.17868)	.005 (.32887)
DXS8037	.26383	.18007	−.013 (.43976)	.019 (.17988)
DXS995	.05786	.21628	.205 (.03950)	.033 (.12239)
Xp22:				
DXS7105	.54625	.79768	−.009 (.42616)	−.016 (.62774)
DXS7163	.00316	.49793	.139 (.01850)	−.014 (.56234)
DXS1052	.69146	.39611	−.038 (.60054)	.015 (.22038)
DXS1229	1.00000	.02095	−.062 (.99990)	.113 (.01220)
DXS999	.82214	.00660	−.022 (.60774)	.050 (.05139)

Note.—P values were computed by use of ARLEQUIN software (Schneider et al. 1997).

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Evidence for a Common Ethnic Origin of Cystic Fibrosis Mutation 3120+1G→A in Diverse Populations

To the Editor:

Cystic fibrosis (CF) is a common recessive disorder in Caucasians, but little is known about its incidence in other populations (Welsh et al. 1995). In a recent study, however, Macek et al. (1997*b*) described a subset of specific CF transmembrane-conductance regulator (CFTR) gene mutations in African American CF patients. One splicing mutation, 3120+1G→A in intron 16, was particularly frequent and accounted for approximately half the "African" CF chromosomes in the group that Macek et al. studied (Macek et al. 1997*b*). This mutation also has been identified in four native African CF patients, on 5/8 chromosomes (Carles et al. 1996). Furthermore, it has been demonstrated that 3120+1G→A is a predominant CF mutation in the Eastern Oasis population of Saudi Arabia (El-Harith et al. 1997). Finally, three Greek CF families have been reported to harbor this mutation (Tzetis et al. 1997). These observations indicate that CF mutation 3120+1G→A is present in diverse populations from different continents.

To examine whether the 3120+1G→A mutation has a common origin in all these populations or whether its widespread distribution is the result of recurrent mutational events, we analyzed DNA samples obtained from 17 unrelated CF patients in four different populations and from 8 unrelated African CF carriers (fig. 1). In the first cohort, six CF patients were of African American descent, three CF patients originated from Saudi-Arabia, three CF patients were of Greek origin, and five CF patients were native Africans (four families came from South Africa, and one family came from Cameroon). In the second cohort, eight native African individuals who had been identified as mutation carriers in a population-based screening in South Africa (C. Padoa and M. Ramsay, unpublished data) were included here as a confirmatory group. The presence of the 3120+1G→A mutation in these different ethnic groups was confirmed by direct sequencing. We have typed six intra- and six extragenic RF40LP markers that had been useful in previous studies to characterize the origins of numerous other CFTR mutations (Estivill et al. 1987; Dörk et al. 1992, 1994; Ramsay et al. 1993; Sereth et al. 1993; Cuppens et al. 1994; Morral et al. 1996). In addition, we investigated the three highly informative intragenic CFTR microsatellites that are located in intron 8 (IVS8CA) and intron 17b (IVS17bTA and IVS17bCA) of the CFTR gene (Zielenski et al. 1991; Morral and Estivill 1992; Morral et al. 1993).

A common extended 3120+1G→A–associated haplotype could be derived in each of the four study populations (table 1). The phasing of haplotypes was based either on homozygosity or on the analysis of parental samples in all African and Arab CF families, as well as in two African American and two Greek CF families. In the remaining single CF patients, other haplotypes for the 3120+1G→A allele than those deduced in table 1 would be formally possible. Three of the four single African American patients, however, were compound