

A Global Perspective on Genetic Variation at the *ADH* Genes Reveals Unusual Patterns of Linkage Disequilibrium and Diversity

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Variants of different Class I alcohol dehydrogenase (*ADH*) genes have been shown to be associated with an effect that is protective against alcoholism. Previous work from our laboratory has shown that the two sites showing the association are in linkage disequilibrium and has identified the *ADH1B* Arg47His site as causative, with the *ADH1C* Ile349Val site showing association only because of the disequilibrium. Here, we describe an initial study of the nature of linkage disequilibrium and genetic variation, in population samples from different regions of the world, in a larger segment of the *ADH* cluster (including the three Class I *ADH* genes and *ADH7*). Linkage disequilibrium across ~40 kb of the Class I *ADH* cluster is moderate to strong in all population samples that we studied. We observed nominally significant pairwise linkage disequilibrium, in some populations, between the *ADH7* site and some Class I *ADH* sites, at moderate values and at a molecular distance as great as 100 kb. Our data indicate (1) that most *ADH*-alcoholism association studies have failed to consider many sites in the *ADH* cluster that may harbor etiologically significant alleles and (2) that the relevance of the various *ADH* sites will be population dependent. Some individual sites in the Class I *ADH* cluster show F_{st} values that are among the highest seen among several dozen unlinked sites that were studied in the same subset of populations. The high F_{st} values can be attributed to the discrepant frequencies of specific alleles in eastern Asia relative to those in other regions of the world. These alleles are part of a single haplotype that exists at high (>65%) frequency only in the eastern-Asian samples. It seems unlikely that this haplotype, which is rare or unobserved in other populations, reached such high frequency because of random genetic drift alone.

Introduction

The seven known alcohol dehydrogenase (*ADH*) genes encode enzymes that catalyze the conversion of alcohols to aldehydes. All seven genes exist in a cluster extending ~380 kb on the long arm of chromosome 4 (i.e., 4q21-23 [GenBank accession numbers AP002026, AP002027, AP002028, and AC097530]). The Class I *ADH* genes (*ADH1A* [MIM 103700], *ADH1B* [MIM 103720], and *ADH1C* [MIM 103730]) exist in a tighter cluster of ~77 kb, flanked upstream by *ADH7* (MIM 600086) and downstream by *ADH6* (MIM 103735), *ADH4* (MIM

103740), and *ADH5* (MIM 103710), in that order (fig. 1). Although the greatest similarity seen is among the Class I genes, all seven *ADH* enzymes are very similar in amino acid sequence and structure but differ in preferred substrates (Edenberg 2000). Two of the three Class I genes are known to have alleles that produce enzymes that catalyze the oxidation of ethanol at different rates (Edenberg and Bosron 1997).

A note on nomenclature is warranted. Allelic nomenclature based on the protein differences is not adequate for analyses of individual polymorphic sites in the genomic sequence of the gene cluster. For example, at the protein level, the allelic series for *ADH1B* (previously called “*ADH2*”) is generated by variation at two different sites at the genomic level: the *ADH1B**1 allele is composed of 47Arg and 369Arg, the *ADH1B**2 allele is composed of 47His and 369Arg, and the *ADH1B**3 allele is composed of 47Arg and 369Cys. We have not seen the “double variant” (composed of 47His and 369Cys),

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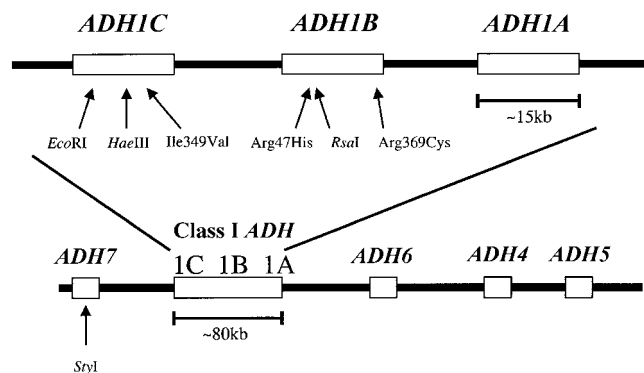


Figure 1 Relative map of SNP sites studied. A detailed map of the Class I cluster is expanded above the map of the gene cluster as a whole. In the map of the whole gene cluster, the names of the Class I ADH genes are abbreviated to “1A,” “1B,” and “1C,” for “ADH1A,” “ADH1B,” and “ADH1C,” respectively.

but we assume that it could exist. For our present purposes, we consider the sites separately.

The functional variants in the corresponding metabolic enzymes make the Class I ADH genes obvious candidates for risk of developing alcoholism. Alleles at two ADH genes that encode enzymes with higher V_{\max} values—namely, *ADH1B**47His (previously called “ADH2*2”), at the Arg47His (exon 3) SNP, and *ADH1C**349Ile (previously called “ADH3*1”), at the Ile349Val (exon 8) SNP—have consistently been found at significantly lower frequencies in alcoholic individuals than in non-alcoholic controls in eastern-Asian samples (Thomasson et al. 1991; Chen et al. 1996; Shen et al. 1997; Tanaka et al. 1997; Osier et al. 1999; Li et al. 2001). Also, in a genomewide linkage study in families mostly of European ancestry, the Collaborative Studies on Genetics of Alcoholism (COGA) group (Reich et al. 1998) found evidence that supports the genetic linkage between alcoholism and the region of chromosome 4 that includes the ADH genes. Long et al. (1998) also found evidence, in a sample of an Amerindian population, that supports the genetic linkage between alcohol dependence and a nearby region on chromosome 4.

The two functional variants are ~21 kb apart and are in strong linkage disequilibrium. Earlier, we demonstrated, through analysis of haplotype frequencies, that the association with alcoholism is caused by the allelic variation at the *ADH1B* Arg47His site and that the decreased frequency of the *ADH1C**349Ile allele in samples of Taiwanese Chinese alcoholic individuals, relative to the frequency in control subjects, is most likely due to linkage disequilibrium with the *ADH1B**47His allele (Osier et al. 1999). Similar haplotype-based analyses will be important for future work that seeks to resolve the role that polymorphisms at the various ADH

genes play in protection against alcoholism. Such haplotype analyses must be preceded by the discovery and characterization of new polymorphisms.

The frequencies of ADH alleles are distinctly different in various populations. *ADH1B**47His is present at frequencies >0.33 in eastern-Asian populations and <0.25 in populations from other regions (Goedde et al. 1992; Thomasson et al. 1994; Neumark et al. 1998). The low frequency, outside eastern Asia, of the commonly studied *ADH1B**47His allele makes it an unlikely contributor, in those regions, to protection against alcoholism, but other polymorphisms in the same or other ADH genes may be important. For example, the *ADH1C**349Ile allele (previously called “ADH3*2”) is found in Europeans (e.g., see Edman and Maret 1992; Poupon et al. 1992), and an expressed variant in exon 9 of *ADH1B* (Cys for Arg at codon 369; previously called “ADH2*3”) is found in African American samples (Bosron et al. 1979; 1983; Carr et al. 1989).

Linkage disequilibrium in the ADH gene family has also been shown to exist for other sites. Murray et al. (1987) found evidence of linkage disequilibrium between *StuI* and *XbaI* polymorphic sites in *ADH1C* in a sample of European Americans. Edman and Maret (1992) examined linkage disequilibrium, in samples of Swedes and mixed European Americans, between sites in *ADH1B*, *ADH1C*, *ADH4*, and *ADH5* by use of the measure Δ . They found significant linkage disequilibrium between sites in the two Class I genes and between *ADH4* and *ADH5*. They did not, however, find significant evidence of linkage disequilibrium between the Class I sites and either the *ADH4* site or the *ADH5* site, possibly because of the small size of the samples that they analyzed. Through analysis of Swedish nuclear families, Edman and Maret (1992) estimated haplotype frequencies and found that 10 of 16 possible haplotypes were present in a sample of 33 chromosomes.

To increase our understanding of both genetic diversity and the nature and extent of linkage disequilibrium in the ADH cluster, we have examined a large number of sites and populations. We have genotyped individuals from 40 populations for seven SNPs—including the two commonly studied coding polymorphisms, another previously described coding polymorphism *ADH1B* Arg369Cys (Carr et al. 1989), a novel *ADH1C* *HaeIII* (exon 5) site silent SNP, the *ADH1B* *RsaI* (intron 3) site SNP (Smith 1986; Osier et al. 1999), a novel *ADH1C* *EcoRI* (intron 2) site SNP, and a novel *ADH7* *StyI* (intron 5) site SNP. All sites were chosen because they can be typed by PCR and digestion with inexpensive, readily available, restriction enzymes. We have estimated the haplotype frequencies for all six Class I ADH sites, and we have examined linkage disequilibrium among the sets of five and six Class I ADH sites and across the segment

between the *ADH7* (intron 5) SNP and all six Class I ADH sites. Patterns of linkage disequilibrium are strong within the Class I cluster, but linkage disequilibrium between the *ADH7* site and the Class I sites varies among populations. This suggests that studies of ADH and alcoholism should evaluate the effect linkage disequilibrium has in the larger ADH gene family on both a site-by-site and population-by-population basis.

Material and Methods

Samples

The populations studied at Yale are those previously studied for the *ADH1B RsaI* (intron 3) site (Osier et al. 1999) plus samples of several new populations, noted briefly here. Additional cell lines from Druze and Ethiopian Jews were obtained from the National Laboratory for the Genetics of Israeli Populations (Tel Aviv University, Israel). The Nigerian samples were collected by Prof. Friday Okonofua, Dr. Frank Oronsaye, and Dr. Adekunle Odunsi: most of the Yoruba samples were from urban health care workers from Benin City; the Hausa samples were from Zaria, in north-central Nigeria; and the Ibo samples were from Enugu, in eastern Nigeria. The Kachari sample was collected by Dr. Ranjan Deka from the Assam province, in India. The Irish sample primarily consists of individuals from Roscommon County, in Ireland, and was collected by Prof. Kenneth Kendler and his colleagues in Ireland. The African American sample consists of cell lines that were purchased from the National Institute of General Medical Sciences (NIGMS) Human Genetic Cell Repository (Coriell Institute for Medical Research). The African American cell lines are among those listed by the NIGMS as being from the African American panel and, as such, may represent a broad cross section of African Americans. The samples of southeastern Bantu-speakers and !Kung San were provided by H.S. Both samples consist of unrelated individuals; the southeastern Bantu-speakers are urban South Africans with ancestry involving various native groups, primarily groups that speak different languages from the Bantu family. The Northern Moroccan, Central Moroccan, Saharan, Catalan, and Basque samples were collected by J.B. and D.C. (Comas et al. 2000; Bosch et al. 2001).

All individuals in all population samples were normal, apparently healthy volunteers, and no diagnoses of alcoholism or related disorders were performed, except in the Taiwanese Han, Ami, and Atayal, as described by Osier et al. (1999). These samples were enriched, relative to population frequencies, for individuals with alcohol dependence. All samples were collected with both the approval of the appropriate institutional review boards and informed consent from the participants.

The majority of the samples were typed in the labo-

ratory at Yale. The !Kung San and southeastern-Bantu-speaker samples were typed in the laboratory of H.S. by M.O. while a visitor at the South African Institute for Medical Research (Johannesburg). The Northern Moroccan, Central Moroccan, Saharan, Catalan, and Basque samples were typed in Barcelona by D.C.

Sequencing, Polymorphism Detection, and Ancestral-State Determination

Sequencing of the clone pADH73 (Smith 1986) and PCR products of genomic DNA was performed by cycle sequencing with ABI PRISM Dye Terminator Cycle Sequencing Core Kits on an ABI 373S Automatic DNA Sequencer. The new polymorphisms—*ADH1C EcoRI*, *ADH1C HaeIII*, and *ADH7 StyI*—were ascertained in different ways, as described in the dbSNP records. Following the logic of Iyengar et al. (1998), we determined ancestral states for all ADH sites by direct sequencing of PCR products from genomic DNA from nonhuman primates. The PCR products were generated using the same primers as those in the protocols given for human typing with genomic DNA from at least one chimpanzee (Cheetah, Dodo, and/or Colin), at least one gorilla (M'kubwa, Oko, Machi, and/or Abe), and at least one orangutan (Puti, Tupa, and/or CP81).

Typing Protocols

The *ADH1B RsaI* (intron 3) site was typed as described elsewhere (Osier et al. 1999). The *ADH1B Arg47His* and *ADH1C Ile349Val* polymorphisms were typed as described elsewhere (Osier et al. 1999) for the Taiwanese Chinese, Ami, Atayal, and Maya samples. All other typings, including the *ADH1B Arg47His* and *ADH1C Ile349Val* polymorphisms for other populations, were performed as PCR-RFLPs. Details of the individual typing protocols are given in table 1.

Statistical Analyses

Genotype and allele frequencies for the separate polymorphisms were calculated by direct counting under the assumption that each was a biallelic codominant system; binomial SEs were calculated separately, for each allele-frequency estimate, as $\sqrt{pq/2N}$. F_{st} values were calculated by the program DISTANCE (cf. Kidd and Cavalli-Sforza 1974), which also calculates Wright's F_{st} as $\sigma_p^2/(\bar{p}\bar{q})$ (Wright 1969). Maximum-likelihood estimates of haplotype frequencies were calculated by HAPLO (Hawley and Kidd 1995) from the individual multisite phenotypes of individuals in each population. HAPLO output includes a jackknife estimate of SEs for the frequency estimates. Overall and pairwise measures of linkage disequilibrium were evaluated using the ξ coefficient by the HAPLO program (Zhao et al. 1999; Kidd et al. 2000). Linkage disequilibrium measured by D' was calculated

Table 1**PCR Reagents and Conditions**

POLYMORPHIC SITE (LOCATION)	FORWARD AND REVERSE PRIMERS	CYCLING CONDITIONS ^a [NO. OF CYCLES]	ENZYME ^b	FRAGMENT SIZE(S) (bp)	
				Site Absent	Site Present
<i>ADH7 StyI</i> (intron 5)	A7IN5DW2 (5'-TAT TAA ATT ATT GCT TAA TAA CTG G-3'), A7IN5UP1 (5'-TTC CTG TGT CTC TTA CAG TG-3')	95°C (15 s), 54°C (15 s), 72°C (60 s) [40]	<i>StyI</i>	477	263 + 214
<i>ADH1C:</i>					
<i>EcoRI</i> (intron 2) ^c	A3EX2DW (5'-TTG CAC CTC CTA AGG CTC-3'), A3EcoUP2 (5'-TCT AAT GCA AAT TGA TTG TGA AC-3')	95°C (15 s), 51°C (15 s), 72°C (75 s) [40]	<i>EcoRI</i>	323	242 + 81
<i>HaeIII</i> (exon 5)	A3EX5FOR2 (5'-TGA GTT TGC ACA TTA GTT ATG G-3'), A3EX5REV1 (5'-TGC TCT CAG TTC TTT CTG GG-3')	95°C (30 s), 56°C (30 s), 72°C (60 s) [35]	<i>HaeIII</i>	435	193 + 242
Ile349Val ^c	A3FXNFOR1 (5'-TTG TTT ATC TGT GAT TTT TTT TGT-3'), A3FXNREV3 (5'-CGT TAC TGT AGA ATA CAA AGC-3')	95°C (15 s), 51°C (15 s), 72°C (75 s) [40]	<i>SspI</i>	378	274 + 104
<i>ADH1B:</i>					
Arg47His	A2FXNFOR (5'-ATT CTA AAT TGT TTA ATT CAA GAA G-3'), A2FXNREV (5'-ACT AAC ACA GAA TTA CTG GAC-3')	95°C (30 s), 56°C (30 s), 72°C (60 s) [35]	<i>MspI</i>	685	443 + 242
Arg369Cys ^c	HE39 ^d (5'-TGG ACT TCA CAA CAA GCA TGT-3'), HE40 ^d (5'-TTG ATA ACA TCT CTG AAG AGC TGA-3')	95°C (15 s), 58°C (15 s), 72°C (60 s) [40]	<i>AluNI</i>	201	130 + 71

NOTE.—All PCR was performed using 100 ng genomic template, 100 ng each primer, 200 μ M dNTP, 2.0 mM MgCl₂, 50 mM KCl, 10 mM Tris HCl (pH 8.4), and 0.5 U AmpliTaq DNA polymerase (Perkin Elmer) in a total volume of 25 μ l.

^a All cycling protocols were performed on a Perkin Elmer 9600, with an initial hold at 95°C, for 5 min, and a final hold at 72°C, for 10 min.

^b PCR products were digested with 5 U of the appropriate restriction enzyme by use of the buffer that was recommended by the manufacturer.

^c Dimethyl sulfoxide was added to a final concentration of 5% by volume.

^d Xu et al. (1988).

by the LINKD program (Kidd et al. 2000), which calculates D' as described by Lewontin (1964).

Results

Site Descriptions

The three polymorphisms that result in amino acid changes (*ADH1C* Ile349Val [exon 8], *ADH1B* Arg47His [exon 3], and *ADH1B* Arg369Cys [exon 9]) have been documented by Smith (1986) and Carr et al. (1989), and the *ADH1B* *RsaI* (intron 3) site by Smith (1986) and Osier et al. (1999). The molecular spacing of the sites on chromosome 4, as well as the Allele Frequency Database (ALFRED) site unique identification numbers (UIDs) and dbSNP submitted-SNP accession numbers (assay IDs), are listed in table 2. Ancestral states for the Class I ADH sites were determined, thereby allowing the ancestral haplotype to be inferred, and are listed in table 3.

Individual-Site Frequencies

All individual-site allele-frequency data and the five- and six-site haplotype frequency estimates are in ALFRED (see Osier et al. 2001) (table 2). Future updates to the frequency data will be placed in the database. Individual-site frequencies are presented graphically in figure 2. The mean number of typed chromosomes per population, $2N$, across all sites is 111.8; the sample size of each population is given in table 4.

F_{st} Values

For some sites, allele frequencies in eastern-Asian populations seem markedly different from those in non-eastern-Asian populations. To examine the magnitude of these differences, we calculated F_{st} values for a set of 33 populations that includes the 8 eastern-Asian populations and compared them to F_{st} values that were calculated for a subset of 25 non-eastern-Asian populations (table 5). For a comparison with other loci, we calculated F_{st} values, in the same populations, for a total of 86 single sites that were not linked to the *ADH* cluster (Pakstis et al. 2002). For the full set of 33 populations, these 86 sites had a mean \pm SD F_{st} value of 0.15 ± 0.08 . The F_{st} value for the *ADH1B* Arg47His polymorphism ($F_{st} = 0.48$) is the highest, for the full set of 33 populations, that we have seen among the 86 single sites that were not linked to the *ADH* cluster; the value for this polymorphism is followed closely by that for the *ADH1B* *RsaI* polymorphism ($F_{st} = 0.41$). The *ADH1B* *RsaI* and *ADH1B* Arg47His polymorphisms, respectively, are 3.3 and 4.3 SDs from the mean F_{st} value. All F_{st} values for the ADH sites decrease when the eastern-Asian populations are removed from the calculation, but the change is most extreme for the *ADH1B* Arg47His and *ADH1B* *RsaI* (intron 3) polymorphisms. When the eastern-Asian populations are removed from the F_{st} calculations, the 86 sites had a mean \pm SD F_{st} value of 0.14 ± 0.07 . The *ADH1B* Arg47His site is still 2.8 SDs from the mean F_{st} value, but the *ADH1B* *RsaI* site is at the mean. The higher heterozygosity for the *ADH1B* Arg47His site in southwestern-Asian populations rela-

Table 2
SNPs in the *ADH* Cluster

POLYMORPHIC SITE (LOCATION)	DISTANCE FROM <i>ADH1C</i> <i>EcoRI</i> SITE ^a (kb)	UNIQUE IDENTIFIER		REFERENCE
		ALFRED UID ^b	dbSNP Assay ID ^c	
<i>ADH7</i> <i>StyI</i> (intron 5)	–71.7	SI000231G	ss2978365	Present study
<i>ADH1C</i> :				
<i>EcoRI</i> (intron 2)	.0	SI000226K	ss2978361	Present study
<i>HaeIII</i> (exon 5)	2.7	SI000227L	ss2978362	Present study
Ile349Val (exon 8)	8.0	SI000228M	ss2978363	Smith (1986)
<i>ADH1B</i> :				
Arg47His (exon 3)	29.5	SI000229N	ss2978360	Smith (1986)
<i>RsaI</i> (intron 3)	30.4	SI000002C	ss2978359	Smith (1986)
Arg369Cys (exon 9)	39.8	SI000230F	ss2978364	Carr et al. (1989)
Class I SNPs:				
Five-site haplotype	NA	SI000659U	NA	Present study
Six-site haplotype	NA	SI000259C	NA	Present study

NOTE.—NA = not applicable.

^a Negative value indicates distance upstream in the cluster; positive values indicate distance downstream from the *ADH1C* *EcoRI* SNP.

^b “UIDs” are unique identifiers in the ALFRED database for locating allele frequencies and typing protocols.

^c “Assay IDs” are unique identifiers in the dbSNP database for definition of the molecular position of each SNP.

tive to other populations probably accounts for the high F_{st} value even when the eastern-Asian populations are removed. The difference between F_{st} values with and without the sample data from the eastern-Asian populations that we report does not appear to be due to the informativeness of these two sites, since the expected “global” heterozygosity, H_t (mean of the estimated heterozygosity for all populations) is comparable for the *ADH1C* exon 5 *HaeIII* and *ADH1C* Ile349Val sites when the eastern-Asian populations are included (for *ADH1C* *HaeIII* [exon 5], $F_{st} = 0.323$; for *ADH1C* Ile349Val, $F_{st} = 0.317$; for *ADH1B* Arg47His, $F_{st} = 0.166$; and for *ADH1B* *RsaI* [intron 3], $F_{st} = 0.256$).

Haplotype Frequencies

Table 4 lists all estimated frequencies of six-site Class I haplotypes that were observed at a frequency of at least 5% in some population that we have studied. The “Residual” column is the total frequency, in the specific population, of all haplotypes that never exceeded a frequency, in any population studied, of 5%. In all but five populations, the haplotypes listed account for >95% of all chromosomes in the sample. Most of the eastern-Asian samples (San Francisco Han, Taiwanese Han, Hakka, Japanese, Ami, and Atayal) have high frequencies (>0.60) of the 221221 haplotype. This haplotype consists of the *ADH1C* *EcoRI* site-present allele, the *ADH1C* *HaeIII* (exon 5) site-present allele, the *ADH1C**349Ile allele (high-activity enzyme), the *ADH1B**47His allele (high-activity enzyme), and the *ADH1B* *RsaI* site-present allele. This haplotype was not observed in most African or in any Amerindian populations that we studied and was observed at low frequencies in some European/Mid-

dle Eastern samples, the Nasioi, the Micronesians, and the Yakut.

The haplotype composed of the ancestral alleles at all Class I sites—that is, 212111, the ancestral haplotype—is infrequent to absent in Africa, except in the !Kung San sample, in which it is estimated as having a frequency of 10.6%. This haplotype is much more frequent in some European (e.g., Finns, with a frequency of 0.31) and Native American (e.g., Mexican Pima, with a frequency of 0.49) samples. It is also present in most eastern-Asian populations.

Linkage Disequilibrium

When the calculation is possible, pairwise linkage disequilibrium measured by D' is generally strong and significant within the Class I *ADH* cluster. There are sporadic combinations of sites in some populations for which pairwise linkage disequilibrium is not significant or strong, but this can almost always be accounted for by small sample size or low heterozygosity for one or both sites.

The ξ coefficient measures overall nonrandomness of alleles on chromosomes (i.e., of alleles constituting haplotypes), for a series of polymorphic sites. The ξ coefficient is based on a comparison of the likelihood based on estimated haplotype frequencies (in table 4) with the distribution of likelihoods under the assumption of no linkage disequilibrium (Zhao et al. 1999). Values of the ξ coefficient for the Class I *ADH* sites are given in table 6. For the Class I *ADH* sites, we have examined five sites—specifically, the *ADH1C* *EcoRI*, *ADH1C* *HaeIII* (exon 5), *ADH1C* Ile349Val, *ADH1B* Arg47His, and *ADH1B* *RsaI* polymorphic sites, spanning 39.8 kb—

Table 3**Sequences and Ancestral States**

POLYMORPHIC SITE (LOCATION)	SEQUENCE ^a				CONSENSUS ANCESTRAL STATE (SYMBOL)
	Human	Chimpanzee	Gorilla	Orangutan	
<i>ADH7 StyI</i> (intron 5)	<u>G/C</u> CATGG	<u>C</u> CATGG	<u>C</u> CATGG	Unknown	Site present (2)
<i>ADH1C</i> :					
<i>EcoRI</i> (intron 2)	GAAT <u>T/G</u> C	GAAT <u>T</u> C	GAAT <u>T</u> C	<u>AAAC T</u> C	Site present ^b (2)
<i>HaeIII</i> (exon 5)	<u>A/G</u> GCC	<u>A</u> GCC	<u>A</u> GCC	<u>A</u> GCC	Site absent (1)
Ile349Val	AAT <u>A/G</u> TT	AAT <u>G</u> TT	AAT <u>G</u> TT	AAT <u>G</u> TT	349Val (2)
<i>ADH1B</i> :					
Arg47His	C <u>A/G</u> CACAGATG	C <u>G</u> CACAGATG	C <u>G</u> CACAGATG	C <u>G</u> CACAGATG	47Arg (1)
<i>RsaI</i> (intron 3)	G <u>T/C</u> AC	G <u>C</u> AC	G <u>C</u> AC	G <u>C</u> AC	Site absent (1)
Arg369Cys	CAGTATC <u>C/T</u> G	CAGTATC <u>C</u> G	CAGTATC <u>C</u> G	CAGTATC <u>C</u> G	369Arg (1)

NOTE.—SNP are indicated in boldface, and the sequence differences are italicized and underlined.

^a Oriented in the 5'→3' direction in the gene cluster.

^b With the exception of orangutan, in which two different base changes result in the restriction site being absent.

that are variable in a wide range of global population samples. We have calculated separate ξ values for the above five Class I ADH sites and the “African-specific” *ADH1B* Arg369Cys polymorphism in the African populations.

Pairwise linkage disequilibrium between the *ADH7 StyI* site and each of the five Class I sites was examined for all 41 samples (table 7). The permutation test was significant at $P \leq .01$ for 10.2% of all tests and at $P \leq .05$ for 24.4% of all tests, suggesting that most of the D' values with P values .05 are likely to be meaningful, even though many (197) tests were performed. However, the individual significance levels are probably not reliable above the $P \leq .01$ level. Significant ($P \leq .030$) and moderate-to-strong D' values between the *ADH1B* Arg369Cys and *ADH7 StyI* sites (~112 kb) are observed in the !Kung San ($D' = -1.00$), Biaka ($D' = -0.88$), Yoruba ($D' = -0.54$), and Ibo ($D' = -0.75$) samples, but values were not significant for the other sub-Saharan African samples for any other combination of Class I sites with the *ADH7 StyI* site (excepting the Ethiopians in northeastern Africa). Interestingly, in the European samples, where several of the Class I sites were initially identified (*ADH1C EcoRI* and *HaeIII*) or have high individual-site heterozygosities (*ADH1B RsaI*; *ADH1C* Ile349Val) linkage disequilibrium with the *ADH7 StyI* site was generally not significant. The few exceptions are one Class I site (*ADH1C EcoRI*) in the Finns ($D' = 0.38$; $P \leq .05$) and Irish ($D' < 0.51$; $P \leq .05$), one site in the Catalans (*ADH1B* Arg47His [$D' = 1.00$; $P \leq .01$]), and one site in the Basque (*ADH1B* Arg369Cys [$D' = 1.00$; $P \leq .01$]). In the Japanese sample, there was significant ($P \leq .05$) evidence of strong linkage disequilibrium ($|D'| > 0.80$) between the *ADH7 StyI* site and four of five polymorphic Class I sites (*ADH1C EcoRI*, *ADH1C HaeIII*, *ADH1B* Arg47His, and *ADH1B RsaI*). There was not significant evidence of linkage disequilibrium between the *ADH7*

StyI site and any Class I site for the other eastern-Asian samples, except for a few specific pairwise combinations for the San Francisco Chinese (*ADH1B* Arg47His), Hakka (*ADH1C* Ile349Val), Atayal (*ADH1B RsaI*), and Cambodians (*ADH1C* Ile349Val). In the South American Amerindian samples, there was significant evidence of strong linkage disequilibrium between the *ADH7* and *ADH1C* sites except for one pairwise combination (Suri, *ADH1C EcoRI*). Otherwise, the sporadic occurrences of significant P values for linkage disequilibrium showed no geographic pattern.

Using a segment test for evidence of linkage disequilibrium between the six Class I ADH sites considered as a group and the *ADH7* site should clarify linkage disequilibrium between the *ADH7 StyI* site and the Class I cluster as a whole by effectively using the Class I haplotypes. This integrates into one test the information from all five polymorphic sites (six sites in the African populations). At the .01 level of significance, we observe (table 6) evidence of linkage disequilibrium in only seven populations from different geographic regions. These are primarily populations that have (or had) undergone a population bottleneck (e.g., Ethiopian Jews, Samaritans, Micronesians, Ticuna, and Karitiana), but, for other populations (Central Moroccans and Cheyenne), that simple explanation for the significant linkage disequilibrium does not seem likely. In all populations, ξ values for the specified segment are moderate or weak ($0 < |\xi| < 0.64$).

Discussion

Global Patterns of Allele-Frequency Variation

As shown in figure 2, most of the SNPs that we studied were segregating in all major geographic regions of the world, and allele frequencies for the individual sites tend to be similar within each geographic region. The most

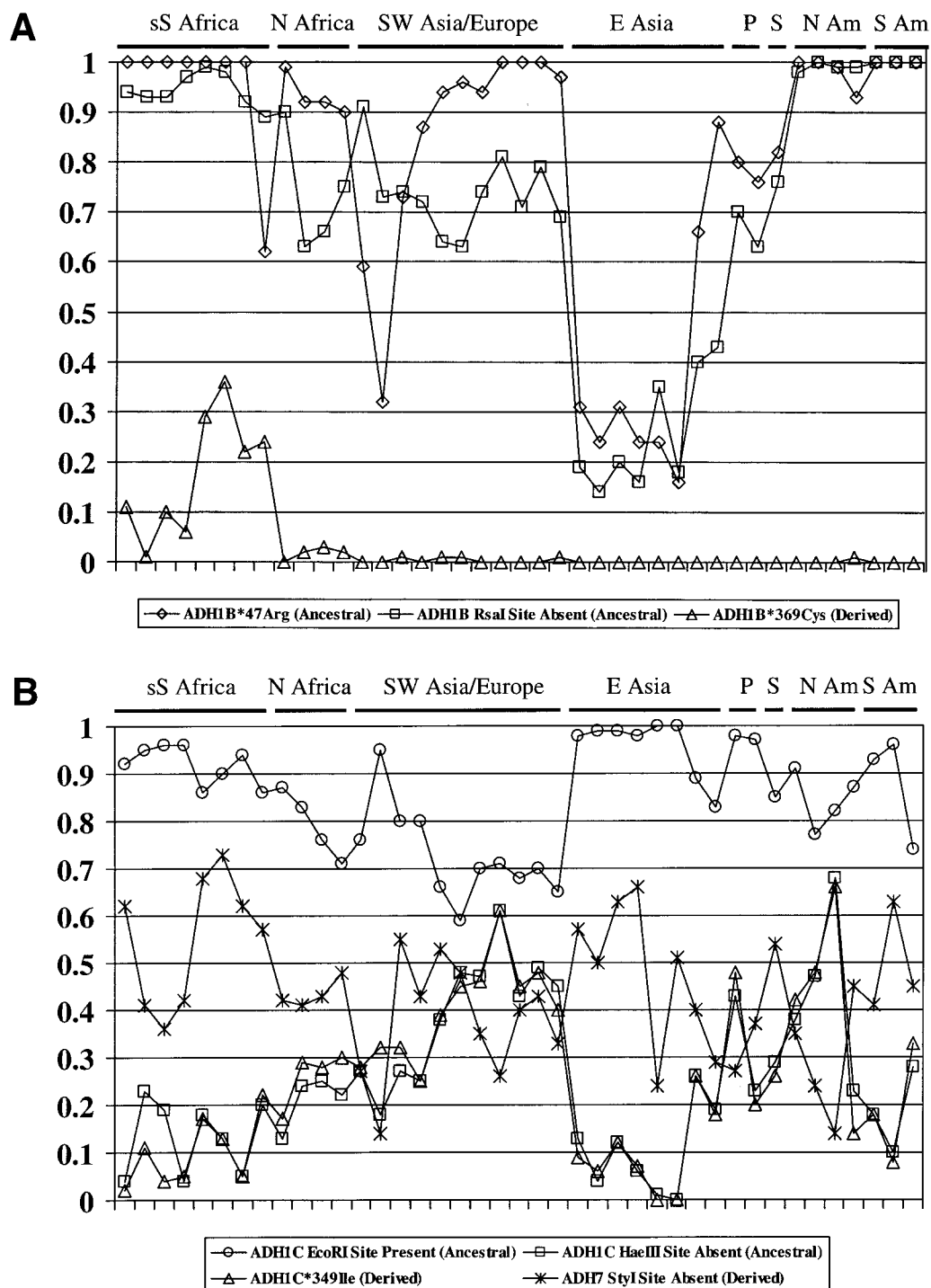


Figure 2 *ADH* SNP allele frequencies. In each panel, the populations are in the same order, from left to right (as in tables 4, 6, and 7 and from top to bottom). Geographic regions are indicated, across the top of each panel, by the abbreviations “sS Africa,” for “sub-Saharan Africa”; “N Africa,” for “northern Africa”; “SW Asia,” for “southwestern Asia”; “E Asia,” for “eastern Asia”; “P,” for “Pacific” (Micronesians and Nasioi); “S,” for “Siberia” (Yakut); “N Am,” for “North America”; and “S Am,” for “South America.” The allele graphed for each SNP is indicated. *A*, Allele frequencies at the three *ADH1B* SNPs. *B*, Allele frequencies at the three *ADH1C* SNPs and the *ADH7* SNP.

Table 4

Class I *ADH* Six-Site Haplotype Frequencies

POPULATION	2N	E(Het) ^a	RESIDUAL ^b	ESTIMATED FREQUENCY OF HAPLOTYPE ^c														
				1	1	1	1	2	2	2	2	2	2	2	2	2	2	
				1	1 ^d	1	1	1 ^d	1 ^{d,e}	1	1 ^f	1 ^d	2 ^{d,g}	1 ^d	1 ^{d,f}	1 ^{d,f}	1	1 ^f
Sub-Saharan African:																		
Southeastern Bantu-speakers	96	407	15	14	28	0	42	0	0	0	0	761	92	49	0	0	0	0
!Kung San	82	524	4	23	0	0	26	86	106	3	0	672	12	68	0	0	0	0
Biaka	134	497	48	0	24	0	0	108	0	0	0	694	59	67	0	0	0	0
Mbuti	74	230	0	0	27	13	0	0	0	0	0	875	57	14	0	0	14	0
Yoruba	152	612	0	0	147	13	0	0	16	0	0	528	294	0	0	0	0	0
Ibo	94	616	0	0	96	0	0	0	31	0	0	501	350	21	0	0	0	0
Hausa	78	543	0	0	51	0	13	0	0	0	0	630	225	81	0	0	0	0
African Americans	176	684	47	0	136	3	0	12	28	0	0	503	198	67	0	6	0	0
Northern African:																		
Ethiopians	60	720	0	0	131	0	0	0	19	0	0	374	0	68	340	35	33	0
Northern Moroccans	186	802	95	22	107	0	6	0	61	19	0	357	19	221	0	37	55	0
Central Moroccans	174	782	49	0	172	14	15	0	53	6	0	358	28	227	0	69	10	0
Saharans	118	771	66	0	131	30	89	0	47	7	0	431	12	92	0	71	23	0
European/Middle Eastern:																		
Yemenite Jews	76	764	41	0	195	5	0	0	50	0	0	269	0	78	340	22	0	0
Samaritans	80	716	13	0	50	0	0	0	7	0	131	19	0	250	439	0	0	92
Druze	140	821	37	0	176	16	0	0	73	0	0	191	0	230	228	0	21	29
Adygei	106	746	0	0	198	0	0	0	57	0	0	390	0	223	78	54	0	0
Catalans	176	770	34	0	293	19	7	0	52	0	0	256	11	270	0	46	6	6
Basque	190	797	57	16	308	54	16	0	70	0	0	206	1	235	0	37	5	0
Russians	92	767	0	0	304	0	0	0	148	0	4	245	0	237	38	24	0	0
Finns	70	745	14	0	293	0	0	0	307	0	0	209	0	177	0	0	0	0
Danes	96	759	34	0	290	12	0	10	125	0	0	256	0	275	0	0	0	0
Irish	136	741	0	0	294	0	0	8	155	0	0	317	0	219	0	0	7	0
European North Americans	176	778	35	0	292	33	0	7	74	4	0	272	6	232	46	0	0	0
Eastern Asian:																		
San Francisco Chinese	96	503	21	0	21	0	0	10	63	0	0	94	0	104	0	688	0	0
Taiwanese Chinese	94	454	22	0	0	0	0	0	32	0	0	106	0	107	11	723	0	0
Hakka	82	505	0	0	0	12	0	0	98	12	0	98	0	98	0	683	0	0
Japanese	84	422	14	0	24	0	0	0	34	0	0	97	0	83	0	748	0	0
Ami	80	566	13	0	0	0	0	0	0	0	0	196	0	29	154	609	0	0
Atayal	82	314	0	0	0	0	0	0	0	0	0	133	0	25	25	816	0	0
Cambodians	46	790	65	0	140	0	0	0	56	0	0	205	0	251	0	283	0	0
Kachari	30	595	33	0	133	0	0	0	0	0	0	174	0	592	67	0	0	0
Pacific:																		
Nasioi	44	756	23	0	0	0	0	0	380	74	0	234	0	62	0	182	45	0
Micronesians	66	736	47	0	0	0	0	30	146	20	0	423	0	110	0	222	0	0
Yakut	98	748	13	0	140	0	0	13	131	0	0	424	0	97	24	158	0	0
North American:																		
Cheyenne	110	587	0	0	91	0	0	0	291	0	0	564	0	9	0	0	45	0
Arizona Pima	88	634	0	11	210	0	0	0	246	0	0	511	0	0	0	0	23	0
Mexican Pima	106	658	9	0	166	13	0	0	481	10	0	286	0	35	0	0	0	0
Maya	94	508	13	92	23	0	0	0	117	0	0	683	11	11	51	0	0	0
South American:																		
Ticuna	130	306	0	0	69	0	0	0	108	0	0	823	0	0	0	0	0	0
Rondonian Surui	76	197	0	0	26	0	15	0	66	0	0	893	0	0	0	0	0	0
Karitiana	100	523	12	11	227	0	11	0	32	0	0	649	0	0	0	0	58	0

NOTE.—All values of frequencies and estimated heterozygosities are multiplied by 1,000.

^a Expected haplotype heterozygosity.

^b Haplotypes with a frequency <5% in all populations.

^c Haplotype names in the present article are derived from the alleles of the six sites in order (5'→3'), from top to bottom, on the chromosome (cf. table 3): *ADH1C* EcoRI, *ADH1C* HaeIII, *ADH1C* Ile349Val, *ADH1B* Arg47His, *ADH1B* RsaI, and *ADH1B* Arg369Cys.

^d Haplotype in the schema in figure 3.

^e The 212111 haplotype is ancestral (i.e., each of the six sites is represented by the ancestral allele).

^f Haplotypes with the high-efficiency *ADH1B* allele are indicated in boldface italic.

^g Haplotype with the *ADH1B**369Cys allele.

Table 5

F_{st} Values in Analyses with and without Populations from Eastern Asia and Siberia

POPULATIONS TESTED ^a	<i>F_{st}</i> VALUE FOR					
	<i>ADH1C</i>			<i>ADH1B</i>		
	<i>EcoRI</i> (Intron 2)	<i>HaeIII</i> (Exon 5)	Ile349Val	Arg47His	<i>RsaI</i> (Intron 3)	Arg369Cys
With eastern Asians (<i>n</i> = 33)	.10	.16	.17	.48	.41	.22
Without eastern Asians (<i>n</i> = 25)	.08	.13	.14	.32	.13	... ^b

^a The eastern Asian populations include the San Francisco Chinese, Taiwanese Chinese, Hakka, Japanese, Ami, Atayal, Cambodian, and Yakut; *n* refers to the number of populations in the test.

^b Not calculated, because the *ADH1B* Arg369Cys site is not polymorphic in eastern Asia.

notable patterns among geographic regions occur for two of the expressed sites, the *ADH1B* Arg47His and Arg369Cys sites.

These data confirm the well known high frequency of the *ADH1B**47His (high-activity) allele in eastern-Asian populations and its presence in some European and southwestern-Asian populations. We now document the presence of the *ADH1B**47His allele in northern-African populations for the first time. In southwestern-Asian samples, the frequency of the *ADH1B**47His allele is greater than that previously reported in other populations from the region. We found frequencies as high as 0.68 (in Samaritans), 0.27 (in Druze), 0.41 (in Yemenite Jews), and 0.38 (in Ethiopian Jews). Previous reports of this allele in populations from this general region showed much lower frequencies: 0.20 (in Israeli Jews) (Neumark et al. 1998) and 0.125 (in Turks) (Goedde et al. 1992). In all other populations studied from Europe, the Middle East, and Africa, the *ADH1B**47His allele was observed at frequencies no higher than 0.13 (in Adygei) and was usually either much lower or absent. These are similar to frequencies that have previously been reported for populations in these regions (e.g., frequencies of 1%–5%, in central and northern Europe; see Goedde et al. 1992). We find the site to be monomorphic for *ADH1B**47Arg in sub-Saharan African populations except for Ethiopians and to be rarely heterozygous in Native American populations.

Few populations have previously been found to have the *ADH1B**369Cys allele; our results are similar to those that have previously been reported, but we now show that the allele is present in all the African samples that we examined except the Ethiopians. Indeed, this allele is essentially an African-specific allele. Bosron et al. (1983) first reported the observation, on the basis of protein electrophoresis, of the *ADH1B**369Cys allele (then called “*ADH2**3”) in a sample of African Americans from Indianapolis. Wall et al. (1997) did report a very low frequency (0.063) of this allele, in a sample of Amerindians from southern California. It has not generally been tested for in other samples. We observed the *ADH1B**369Cys allele not only in the African American

sample that we studied but also in all but one of the African samples that we studied (mean frequency of the *ADH1B**369Cys allele is 0.11 in the 11 African populations). There were also individual occurrences of that allele in five non-African samples—the European American, Druze, Catalan, Basque, and Mayan samples—but not elsewhere. Its presence in both the Amerindian sample that Wall et al. (1997) studied and the Mayan sample that we studied can be explained by recent migration and/or admixture. In the southwestern-Asian and European populations, the allele may have persisted at a low frequency (estimated at 0–3%) since modern humans arrived but may be only rarely observed, as in our samples of the Druze and European Americans, by happenstance. Alternatively, occurrence of the allele in these few sampled individuals may reflect more recent gene flow from Africa (e.g., by northern Africans migrating into the Iberian peninsula).

At the *ADH1C* Ile349Val site, the ancestral, lower-activity allele (*ADH1C**349Val or *ADH1C**2) is the more common allele in all but two populations (Finns and Mexican Pima), and the site has heterozygosities >0.2 in all northern-African, European, Pacific, and Amerindian (except for the Surui) populations. Heterozygosities are generally lower in the sub-Saharan African populations and the eastern-Asian populations.

The allele-frequency distribution of the noncoding *ADH1B* *RsaI* (intron 3) site shows close parallels with the nearby *ADH1B* Arg47His site in eastern-Asian, Pacific, and Native American populations but not in African, southwestern-Asian, and European populations. The *RsaI* site is fixed (or nearly fixed) only for the Native American populations. The allele-frequency distribution for the noncoding *ADH1C* *HaeIII* site very closely tracks that for the *ADH1C* Ile349Val site, which is 5.3 kb away. The pattern for the *ADH1C* *EcoRI* site is similar to—but not as highly correlated as—the other two *ADH1C* sites, even though it is closer to the *HaeIII* site (2.7 kb) than the *HaeIII* site is to the Ile349Val site. These allele-frequency correlations among the diverse population samples are a reflection of—indeed, a measure of—linkage disequilibrium.

Table 6

Linkage Disequilibrium for Five Class I *ADH* Sites, for Six Class I *ADH* Sites, and for the Segment between the Six Class I *ADH* Sites and the *ADH7 Styl* Site

POPULATION	FIVE SITES ^a		SIX SITES ^b		ADH7 CLASS I SEGMENT	
	ξ	<i>P</i>	ξ	<i>P</i>	ξ	<i>P</i>
African:						
Southeastern Bantu-speakers	.68	<.001	.62	.004	-.04	.649
!Kung San	.45	.006	.50	.018	-.15	.951
Biaka	1.00	<.001	1.15	<.001	.03	.285
Mbuti	1.63	<.001	1.72	<.001	.04	.299
Yoruba	3.72	<.001	3.75	<.001	.06	.574
Ibo	2.32	<.001	2.54	<.001	-.03	.574
Hausa	1.67	<.001	1.66	<.001	-.02	.527
African Americans	3.29	<.001	3.32	<.001	.09	.072
Ethiopians	2.22	<.001	2.20	<.001	.58	.005
Northern Moroccans	.98	<.001	1.11	<.001	.01	.359
Central Moroccans	2.47	<.001	2.98	<.001	.22	.005
Saharans	1.71	<.001	2.20	<.001	.09	.167
Non-African:^c						
Yemenites	2.62	<.00108	.279
Samaritans	3.24	<.00164	<.001
Druze	2.50	<.00107	.176
Adygei	3.68	<.00105	.231
Catalans	2.82	<.00103	.250
Basque	3.16	<.00102	.330
Russians	3.72	<.00102	.343
Finns	2.46	<.00123	.027
Danes	2.69	<.00115	.059
Irish	2.46	<.001	-.01	.438
European North Americans	3.48	<.001	-.03	.625
San Francisco Chinese	3.01	<.00100	.466
Taiwanese Chinese ^d	2.36	<.001	-.05	.757
Hakka	3.32	<.00108	.154
Japanese	2.50	<.00114	.088
Ami ^d	.52	.00113	.068
Atayal ^d	.90	<.00109	.086
Cambodians	2.86	<.001	-.03	.525
Kachari	3.01	<.001	-.04	.491
Nasioi	1.97	<.00135	.032
Micronesians	2.60	<.00163	.001
Yakut	3.30	<.00101	.416
Cheyenne	2.28	<.00118	.007
Arizona Pima	1.71	<.00106	.195
Mexican Pima	3.34	<.001	-.04	.619
Maya	2.36	<.001	-.02	.508
Ticuna	2.20	<.00120	.002
Rondonian Surui	1.52	<.00119	.026
Karitiana	2.22	<.00121	.010

^a *ADH1C EcoRI*, *ADH1C HaeIII*, *ADH1C Ile349Val*, *ADH1B Arg47His*, and *ADH1B RsaI*.

^b *ADH1C EcoRI*, *ADH1C HaeIII*, *ADH1C Ile349Val*, *ADH1B Arg47His*, *ADH1B RsaI*, and *ADH1B Arg369Cys*.

^c The *ADH2 Arg369Cys* site generally does not vary in non-African populations; therefore, the ξ values will usually not vary between the five- and six-site polymorphisms in non-African populations.

^d Alcoholic individuals comprise ~50% of these samples.

Population frequencies of the other Class I *ADH* sites (*ADH1B RsaI*, *ADH1C EcoRI*, *ADH1C HaeIII*, and *ADH1C Ile349Val*) generally cluster by region. The exceptions to strong geographic patterning primarily appear to be populations that have probably undergone strong

founder effects. For example, some of the southwestern-Asian samples (Samaritans, Yemenite Jews, and Ethiopian Jews) have high frequencies of the *ADH1B*47His* allele. The Samaritans have a noticeably lower frequency of the *ADH1C EcoRI* site-present allele than do other south-

Table 7

Pairwise Linkage Disequilibrium, $|D'|$, between the *ADH7* *StyI* Site and Each of the Class I Sites

POPULATION	$ D' $ BETWEEN <i>ADH7</i> <i>StyI</i> AND					
	<i>ADH1C</i> <i>EcoRI</i>	<i>ADH1C</i> <i>HaeIII</i>	<i>ADH1C</i> <i>Ile349Val</i>	<i>ADH1B</i> <i>Arg47His</i>	<i>ADH1B</i> <i>RsaI</i>	<i>ADH1B</i> <i>Arg369Cys</i>
Southeastern Bantu-speakers	.37	.13	.2474	.28
!Kung San	.56	.14	.3547	1.00**
Biaka	.20	.14	.3350	.88*
Mbuti	.23	.22	.4213	1.00
Yoruba	.26	.27	.2724	.54*
Ibo	.55	.31	.31	...	1.00	.75*
Hausa	.04	.33	.3317	.36
African Americans	.08	.33	.25	1.00	.40	.21
Ethiopians	.41	.43	.62*	.83**	.62	...
Northern Moroccans	.21	.05	.32	.79*	.05	1.00*
Central Moroccans	.37*	.13	.22	1.00**	.34*	1.00*
Saharans	.14	.17	.10	.67	.56*	1.00**
Yemenites	.85*	.86*	.86	.59	.36	...
Samaritans	1.00	1.00	1.00*	1.00**	1.00**	...
Druze	.02	.10	.07	.47*	.43	1.00
Adygei	.14	.20	.20	.37	.26	...
Catalans	.07	.07	.00	1.00**	.14	1.00
Basque	.04	.05	.13	.16	.09	1.00**
Russians	.27	.12	.12	.14	.53	...
Finns	.38*	.06	.0836	...
Danes	.14	.09	.0241	...
Irish	.51*	.25	.2620	...
European North Americans	.12	.16	.04	1.00	.03	1.00
San Francisco Chinese	.11	.51	.78	.52*	.64	...
Taiwanese Chinese	1.00	.77	.77	.01	.24	...
Hakka	1.00	.77	.77*	.32	.58	...
Japanese	1.00*	1.00*	1.00**	.32	.80**	...
Ami	...	1.0022	.08	...
Atayal51	.71*	...
Cambodians	1.00	.78	.79*	.38	.44	...
Kachari	.50	.50	.50	1.00	.40	...
Nasioi	1.00**	.54	.83	.54	.15	...
Micronesians	.23	.40	.45*	1.00**	.80**	...
Yakut	.29	.42*	.47	.53	.36	...
Cheyenne	.48	.87**	.76**	...	1.00	...
Arizona Pima	1.00*	.36	.26
Mexican Pima	.68	.25	.25	1.00	.20	...
Maya	.60	.50	.52	1.00	1.00	1.00
Ticuna	1.00*	1.00**	1.00**
Rondonian Surui	1.00	1.00**	1.00*
Karitiana	.86**	.87**	.57*

NOTE.—Ellipses indicate when comparison was not possible.

* $P \leq .05$.** $P \leq .01$.

western-Asian populations. The Finns, the Nasioi, and the Mexican Pima have distinctly higher frequencies of the *ADH1C* *HaeIII* site-absent and *ADH1C**349*Ile* alleles than do their neighboring populations. The unusually high frequency of the *ADH1B**47*His* allele in the African Americans (graphed as a low frequency of the *ADH1B**47*Arg* allele in fig. 2) is not explainable by either founder effect or European admixture, since it is higher than that seen in Europe. There may be other African populations with high frequencies of this high-activity allele that have not yet been identified.

F_{st} Values

The high F_{st} values for the *ADH1B* *Arg47His* and *RsaI* (intron 3) site polymorphisms clearly place these two sites as outliers in the distribution of global variation that can be attributed to random genetic drift among populations. The high F_{st} values reflect the large frequency differences, for these sites, between eastern-Asian populations and most other populations. With the eastern-Asian allele frequencies at these two sites included in the calculation, the F_{st} value for the *ADH1B* *RsaI* site is three times as

high as it is when the frequencies for these populations are omitted; the F_{st} value for the *ADH1B* Arg47His polymorphism is also much higher when calculated including, rather than when calculated excluding, the eastern-Asian populations. We have not observed such a large difference—nor such high F_{st} values across the set of 33 populations—for any of the 86 other non-*ADH* sites that our laboratory has studied (Pakstis et al. 2002) and are not familiar with such high values for studies in the literature of samples from a range of global populations. Interestingly, the changes in F_{st} value for the three *ADH1C* polymorphisms when the eastern-Asian populations are removed are not very large—even though the Ile349Val polymorphism, like the *ADH1B* Arg47His polymorphism, encodes proteins with different metabolic efficiency for the breakdown of ethanol, and the other sites are generally in disequilibrium with it, as suggested in figure 2.

Haplotype Distribution and Evolution

The ancestral haplotype must be the one with ancestral alleles at all sites, although not all occurrences of this haplotype are necessarily identical by descent with the ancestral haplotype. This haplotype is named “212111,” on the basis of a binary system of allele names (cf. tables 3 and 4). Random genetic drift could explain this pattern of ancestral haplotype frequencies. A possible concomitant factor would be regeneration by recombination of the ancestral combination of alleles at the individual sites. There is only one common haplotype with the *ADH1C* *HaeIII* site-absent allele, 112111, and it differs from the ancestral haplotype only at the *ADH1C* *EcoRI* site. The common haplotypes with the *ADH1C* *EcoRI* site-present allele differ from the ancestral haplotype at two or three other sites, including the *ADH1C* *HaeIII* site. Thus, only two heterozygotes that are likely to occur commonly, 112111/221111 or 112111/221121, would be able to regenerate the ancestral haplotype by recombination, and the crossovers would have to be between the two *ADH1C* sites, only 2.7 kb apart. This seems unlikely to be a systematic “force” but could have regenerated a few instances that random genetic drift raised to modest frequencies. It seems more parsimonious, pending possible clarification from data on additional sites, especially sites closely flanking these Class I sites, to assume that this distribution of the ancestral haplotype is the consequence of random genetic drift.

The haplotypes that are common (i.e., those with a frequency $\geq 10\%$ of the chromosomes) in any African population and in most non-African populations can be accounted for by a simple tree of mutations starting from the ancestral haplotype (fig. 3). These haplotypes account for between 76.5% (Samaritans) and 100% (Adygei and Ticuna) of the chromosomes in these 41 populations. The

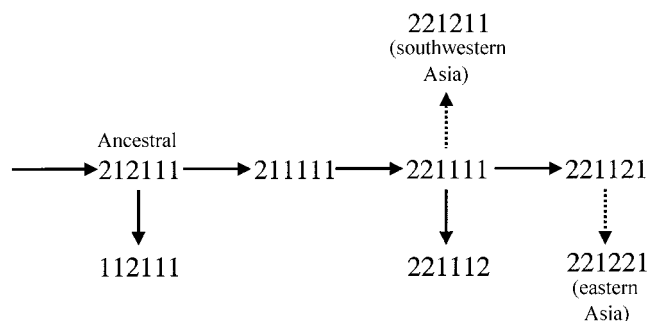


Figure 3 Class I *ADH*-haplotype evolutionary tree. Haplotype names are listed in table 4. All haplotypes in the figure are observed at frequencies $>5\%$ in one or more African samples. Each solid arrow represents a single base mutation. The two dashed arrows cannot both be single mutations if recurrent mutation to *ADH1B**47His is excluded; one of the two presumably arose by recombination. The 221221 haplotype is the one most common in eastern Asia and associated with the protective effect against alcoholism. The 221112 haplotype is the only one with the African-specific *ADH1B**369Cys allele.

other haplotypes in table 4, none of which exceeds 10% anywhere, require recombination if recurrent mutation is assumed not to have occurred. From the ancestral haplotype, two independent derivatives seem likely. One involves mutation of the *ADH1C* *EcoRI* site to generate the 112111 haplotype now common in Europe and seen in most populations around the world. An independent mutation of the *ADH1C* Ile349Val site on the ancestral haplotype generates the 211111 haplotype. This haplotype is rarely seen today but is present in the !Kung San, Biaka Pygmies, and African Americans. The single haplotype that is most common around the world involves a mutation that starts from this currently uncommon haplotype of the *ADH1C* *HaeIII* site to generate the 221111 haplotype. The *ADH1B**369Cys allele, found only in African populations, is commonly seen on a single haplotype and, thus, likely represents a single mutation on this common haplotype to generate the 221112 haplotype. The rare occurrences of this allele on other haplotypes indicate that recombination events have occurred.

At this level of analysis, gene conversion cannot be distinguished from ordinary crossing-over, and, with one exception, the other haplotypes can be explained by a single crossover between two of the common noncrossover haplotypes in figure 3. The exception is the 222211 haplotype, which can arise from a crossover between 221111 and 212211, which is itself a crossover product. Collectively, the haplotypes in table 4 require inference of crossovers in every interval except the last. As noted above, the African-specific *ADH1B**369Cys allele does exist on other haplotypes in the residual class. The 221122 haplotype (with frequencies of 1.5% among southeastern Bantu-speakers and 0.8% among African Americans) could be the result of a crossover in the last interval be-

tween the common 221121 and 221112 haplotypes, and the 211112 haplotype (with a frequency of 2.6% among Biaka) could be the result of a crossover between the haplotypes 211111 and 221122 that are common in the Biaka.

In eastern Asia, the 221221 haplotype (*ADH1C* *EcoRI* site present, *ADH1C* *HaeIII* site present, *ADH1C**349Ile, *ADH1B**47His, *ADH1B* *RsaI* site present, and *ADH1B**Arg369) is estimated at frequencies >60%—as high as 82% in the Atayal. There may be a unique shared evolutionary history of the *ADH1B**47His-containing haplotypes in the populations of eastern Asia, north Africa, Iberia, and southwestern Asia, especially in light of the fact that the *ADH1B**47His allele in the Middle Eastern samples occurs primarily in a haplotype observed in northern Africa and Iberia and was only slightly different from the haplotype common in eastern Asia. These two common haplotypes (indicated by the dotted arrows in fig. 3) cannot be jointly explained without requiring recurrent mutation or recombination.

Because it occurs in essentially all parts of the world on a specific haplotype, the derived allele at the *ADH1B* *RsaI* site seems likely to have arisen on the common 221111 haplotype to generate the now reasonably common 221121 haplotype. The *ADH1B**47His allele does not fit simply into such a scheme since it occurs as part of two different common haplotypes in Asia (221221 and 221211) that differ only at the *RsaI* site, 906 bp downstream from the *ADH1B* Arg47His site. If we exclude recurrent mutation as being very unlikely, then two schemes, both involving recombination, could explain the origins. Ethiopian Jews have the *ADH1B**47His allele, suggesting that it may have arisen in northeastern Africa before the expansion out of Africa. This would be analogous to the large-normal (CTG)_n alleles, at the locus causing myotonic dystrophy (*DM*), that exist in northeastern African and non-African populations but not in sub-Saharan populations (Tishkoff et al. 1998). If the haplotype common in Ethiopians and southwestern-Asian populations, 221211, is the original haplotype that carries the *ADH1B**47His allele, then the haplotype that is common in eastern Asia must have arisen by recombination with the common 221121 haplotype, the only common haplotype for the *RsaI* site-present allele.

This recombination event in southwestern Asia or northeastern Africa with a subsequent increase in frequency of the 221221 haplotype in eastern Asia seems most compatible with the spread of modern humans out of Africa and then, from west to east, across Asia. The alternative—that the *ADH1B**47His allele arose on the haplotype with the *RsaI* site-present allele (i.e., 221121) and that the 221211 haplotype arose by recombination—seems less likely but certainly cannot be excluded. The occurrence of the 221221 haplotype

in northwestern Africa could be considered an argument for this scenario. A proper statistical evaluation of these alternatives would require historical demographic and frequency data, which are not available. Additional polymorphic sites downstream from the existing six Class I markers may help clarify this aspect of haplotype evolution.

Selection on the ADH1B Arg47His Polymorphism?

The common eastern-Asian haplotype, which has the *ADH1B**47His allele, could have attained such a high frequency through genetic drift or the effects of selection. Drift seems to be an insufficient explanation, for several reasons. The high F_{st} values for this and the adjacent *RsaI* site are clearly outliers, showing more genetic variation among populations than is seen for any other markers. It would take a strong population bottleneck and/or strong subsequent random genetic drift within eastern Asia for this rare allele to become frequent. The *aldehyde dehydrogenase 2* (*ALDH2*) locus provides additional evidence that drift may not be the sole factor determining the frequency of this haplotype. *ALDH2* is functionally monomorphic in most populations, but not in those of eastern-Asian descent (Goedde et al. 1992; Peterson et al. 1999). The protein produced by this eastern-Asian allele acts as a dominant null allele that dramatically reduces the ability to breakdown acetaldehyde, the by-product of ADH metabolism of ethanol. In heterozygous individuals, only ~1/16 of the *ALDH2* enzyme tetramers would be functionally active. Heterozygosity and homozygosity for the null *ALDH2* allele combined with alleles for high-efficiency ADH enzymes should result in a high level of acetaldehyde, a toxin, in the body following ethanol ingestion. It seems unlikely that alleles of unlinked genes in two gene families in the same metabolic pathway that both increase acetaldehyde levels would both have high frequencies in the same populations by random genetic drift alone. Again, although it cannot be proven that the high frequency of this ADH haplotype is primarily due to selection, it seems unlikely that random genetic drift is solely responsible. We hope that future studies will help our understanding of the causes behind the high frequency of this haplotype in only one region of the world.

Extent of Linkage Disequilibrium

In all population samples that we studied, there was significant evidence of linkage disequilibrium across the five Class I ADH sites. This is expected in the non-African samples on the basis of our other studies of multiple populations (Tishkoff et al. 1996; Kidd et al. 1998, 2000), since the five sites polymorphic outside of Africa span a small region that is slightly larger than 30 kb. It also agrees with other empiric studies on more restricted sets of populations (Reich et al. 2001). It does not agree

with expectations for some theoretical models (Kruglyak et al. 1999) nor some results for individual loci, such as lipoprotein lipase (*LPL*), where a recombination hotspot disrupts linkage disequilibrium across a short segment (Templeton et al. 2000; Jeffreys et al. 2001). Also, in contrast to data at several loci we have studied in the same population samples (Pakstis et al. 2000), in the African samples linkage disequilibrium at this locus has a wide range of ξ values ($0.45 < \xi < 3.72$) and, in several cases, is average or above average in comparison to non-African samples. One African population, the Yoruba, has the same ξ value as the maximum observed in all other samples. As a rule, based on analyses of data from multiple loci (Pakstis et al. 2000 and unpublished data from Kidd Lab), values of ξ and other measures of linkage disequilibrium are generally low in African populations and less than those observed in samples from other parts of the world.

Interestingly, pairwise linkage disequilibrium, as measured by D' , between the *ADH7* *StyI* and some Class I sites is observed at moderate values and is significant in some samples. For the segment test of linkage disequilibrium between the *ADH7* site and the six Class I *ADH* sites, very few populations had significant evidence of linkage disequilibrium, although some did in certain pairwise combinations of sites. It may be that some pairwise tests are significant while the segment test is insignificant for these samples when some specific pairwise site combinations tested are nonrandom (linkage disequilibrium) but the other specific pairwise site combinations are random (linkage equilibrium) and “outweigh” the nonrandom combinations. Because there are no clear overall patterns of variation or linkage disequilibrium between the non-Class I *ADH7* site and the Class I sites, studies of the role that the Class I *ADH* genes play in alcoholism may need to examine the degree of linkage disequilibrium across large regions on a population-by-population and site-by-site basis to eliminate any effects of linkage disequilibrium with a nonmember of the Class I *ADH* cluster.

Since linkage disequilibrium between causative and noncausative sites has resulted in positive association studies of alcoholism in samples from eastern Asia for those sites that appear not to be causative (Osier et al. 1999) and since linkage disequilibrium may extend over large regions in this gene cluster, the range of possible locations of sites that could be truly causative is much larger than had previously been anticipated. The range of sequence that could be suspected encompasses at least the *ADH1C* and *ADH1B* genes and all intergenic sequence, a distance of >40 kb. It is possible that even though the *ADH1B* Arg47His polymorphism incorrectly appears to be causative by association studies because of linkage disequilibrium with another, truly functional variant, possibly in the promoter region. Also, if other functional sites

show positive associations in populations outside eastern Asia, then it will be important to ensure that such results cannot be accounted for by linkage disequilibrium with yet other, possibly undiscovered, sites, perhaps in other members of the *ADH* cluster.

The need to screen for effects of non-Class I *ADH* polymorphisms is especially true for the Japanese, for whom samples have been extensively studied for association between Class I *ADH* sites and alcoholism. We observed significant pairwise linkage disequilibrium between the *ADH7* *StyI* site and four of five Class I sites polymorphic in the Japanese, even though the segment test between *ADH7* and the six Class I sites was not significant. The cause for this pattern of linkage disequilibrium in eastern Asia and any effect on association studies in the Japanese will need to be pursued. The contrasting pattern of linkage disequilibrium between the Japanese and other eastern-Asian samples is not due to the informativeness of individual markers, since the heterozygosities for individual sites in non-Japanese eastern-Asian samples are generally comparable to or higher than those observed in the Japanese sample. This pattern of linkage disequilibrium also means that it is not valid to assume that all eastern-Asian populations are homogeneous in genetic studies of the *ADH* genes and possibly in studies of other genes for which the assumption of homogeneity has not been tested.

To date, almost all case-control association studies of alcoholism in populations of European ancestry have used only the *ADH1C* Ile349Val polymorphism because of its higher heterozygosity. Those studies have had largely negative results but may have had low power to detect an indirect effect on the *ADH1C* Ile349Val site through linkage disequilibrium with some other, as-yet-undetected, polymorphism in the cluster. Use of haplotypes in such a study would help overcome this problem (Kidd et al. 1996) and may resolve the conflict between positive linkage studies to the region and negative association studies with known variants in European samples.

A significant effort is under way to design generalized linkage disequilibrium maps that are based on “haplotype blocks” punctuated by recombination hotspots (Daly et al. 2001; Jeffreys et al. 2001; Johnson et al. 2001), so that only the sites distinguishing common haplotypes need be studied. Most of these studies focus on samples of Europeans and only one or two non-European samples. In the global samples that we studied, we found strong, but incomplete, linkage disequilibrium across the six Class I sites. In any one region of the world, the most common haplotypes (i.e., those that account for a minimum of 80% of the chromosomes in the region) can be distinguished by two or three of the sites. However, different regions of the world require different combinations of sites. Globally, five of the six sites are essential.

The *ADH* genes are an interesting exception to the

patterns of variation and linkage disequilibrium observed at other loci in the human genome. Untangling the forces at play in the evolutionary history of the ADH genes in humans presents a unique challenge. The resulting knowledge will provide insight into how to refine the design and interpretation of studies of the role that the ADH genes play in the protection against alcoholism in different populations.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

ALFRED, <http://alfred.med.yale.edu/alfred/>
 dbSNP, <http://www.ncbi.nlm.nih.gov/SNP/>
 GenBank, <http://www.ncbi.nlm.nih.gov/Genbank/> (for 4q21-23 [accession numbers AP002026, AP002027, AP002028, and AC097530])
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for *ADH1A* [MIM 103700], *ADH1B* [MIM 103720], *ADH1C* [MIM 103730], *ADH7* [MIM 600086], *ADH6* [MIM 103735], *ADH4* [MIM 103740], and *ADH5* [MIM 103710])

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