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Genetics and Epidemiology of Gallbladder Disease in New World Native Peoples

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SUMMARY

Native peoples of the New World, including Amerindians and admixed Latin Americans such as Mexican-Americans, are highly susceptible to diseases of the gallbladder. These include cholesterol cholelithiasis (gallstones) and its complications, as well as cancer of the gallbladder. Although there is clearly some necessary dietary or other environmental risk factor involved, the pattern of disease prevalence is geographically associated with the distribution of genes of aboriginal Amerindian origin, and levels of risk generally correspond to the degree of Amerindian admixture. This pattern differs from that generally associated with Westernization, which suggests a gene-environment interaction, and that within an admixed population there is a subset whose risk is underestimated when admixture is ignored. The risk that an individual of a susceptible New World genotype will undergo a cholecystectomy by age 85 can approach 40% in Mexican-American females, and their risk of gallbladder cancer can reach several percent. These are heretofore unrecognized levels of risk, especially of the latter, because previous studies have not accounted for admixture or for the loss of at-risk individuals due to cholecystectomy. A genetic susceptibility may, thus, be as "carcinogenic" in New World peoples as any known major environmental exposure; yet, while the risk has a genetic basis, its expression as gallbladder cancer is so delayed as to lead only very rarely to multiply-affected families. Estimates in this paper are derived in part from two studies of Mexican-Americans in Starr County and Laredo, Texas.

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INTRODUCTION

Among the effects of Westernization are a rise in the frequency of diseases of the gallbladder (GBD), specifically a tendency to form gallstones (cholelithiasis) and its complications, as well as cancer of the gallbladder (GBCA) and other diseases of the biliary tract system [1]. This has clearly been observed in Europe, North America [2], and more recently in Africa [2] and Japan [3] and seems to be a general response to some aspect(s) of diet, although at present the relevant aspects of exposure are not clearly understood.

Even considering this worldwide trend, there has been, at least since World War II, an epidemic of GBD among Amerindians [4–9] and peoples genetically related to them [10–14], which is of a nature and proportion different from the pattern seen elsewhere. The evidence from before the war is sparse, but suggests that GBD in these peoples was much less common. Today, there is a consistent gradient of prevalence from more-admixed to less-admixed populations, which cannot be explained by any known aspects of the diet or environment and which, along with other epidemiological data, suggests that there is a genetic susceptibility in New World (NW) peoples distinct from that which attaches generally to Westernization. Here, the term NW peoples will be used to refer to Amerindians and their relatives, but not to Eskimos, in whom the evidence is still equivocal.

The existing evidence that this epidemic has a genetic basis is largely circumstantial, but comes from a wide variety of populations and from epidemiological, pathological, and physiological studies. The purpose of our study is to show that from the available evidence: (1) the pattern of GBD and associated morbidity in NW peoples is more consistent with a genotype-by-environment interaction than simply with the effects of a changed environment [15], and (2) if this assessment is correct, the bearers of susceptible genotypes may be at much higher risk of GBD, including cancer, than has been thought. Data relevant to the problem will be presented from new studies of GBD among the Mexican-Americans of Texas, an important European-Amerindian hybrid group, in which we will quantify GBD risk as a function of the rate of admixture with the NW genes.

MATERIALS AND METHODS

The new results presented are derived from studies of Mexican-Americans in Laredo and Starr County, both on the Texas-Mexico border. The Laredo study is a complete genealogical study of the city, now of 92,000 people, over the past century [16–18]. In addition to the extensive genealogical data, all 42,864 hospital inpatient discharge records from this city for the period 1910–1945 have been used, as well as all cholecystectomy records for the year 1980. To compute age- and sex-specific disease rates from these data, the U.S. census of Webb County was used; this is a Standard Metropolitan Statistical Area that includes only Laredo and its sparsely settled rural outlying county. Only hospitalizations to Laredo residents were included.

Data are also presented that were gathered in Starr County, Texas [19]. Starr County is a rural area located 100 miles down the Rio Grande River from Laredo, whose ethnic composition is 97% Mexican-American. The data used here are anamnestic (i.e., by recall interview) and stem from a household survey of GBD and/or gallbladder surgery in approximately 10% of the county, yielding a sample of 617 males and 1,050 females. Complete details can be found elsewhere [19].

Here, special cumulative incidence values have been computed to estimate lifetime risk or to show the effect of differential disease susceptibility in various subpopulations on the lifetime risk to a member of one of the groups. These are computed from age-specific incidence rates by actuarial methods [20] modified for the present purposes. In particular, a tandem double-decrement problem has been addressed in order to estimate the cancer risk to a genetically susceptible individual not undergoing cholecystectomy in an admixed population. The mathematical details are given in the APPENDIX.

GALLBLADDER DISEASE OTHER THAN CANCER

Gallbladder disease seems to have been rare in Amerindians prior to World War II, based on the few systematic reports of Amerindian health. In these reports, GBD is either absent or is noted by the authors to be rare [21–24]. We know of no published data on Mexican-Americans. However, in Laredo, which grew in size from about 25,000 to 55,000, only 20 cholecystectomies and 42 cholecystotomies were performed in the entire period from 1910–1945. By the 1960s, and steadily since then, there have been about 275 cholecystectomies performed *annually* in Laredo. It is possible that this is due partly to improved diagnosis, reporting, and surgery, but the trend seems to be a real one, for reasons to be discussed below.

Since about 1950, there have been many reports documenting very high rates of GBD in Amerindians across the American continents, from Alaska to parts of South America; the result of this epidemic is that in Amerindians GBD has become one of the leading causes of hospitalization, especially among females [6, 7, 9, 25–32]. Because of high positive correlations among obesity, adult-onset diabetes mellitus, and GBD in NW populations [15], it can probably be inferred that GBD is highly prevalent in those tribal groups for whom only a high rate of diabetes has so far been documented, and this encompasses most of North America [7–9, 33–35]. In addition to hosptial-record surveys, the high rate of GBD in Amerindians has been documented by studies of autopsy series [29, 31, 32], and by direct bile-sample studies documenting the development of cholesterol-saturated ("lithogenic") bile in young adult Amerindian females in two tribal areas [36, 37].

The available evidence suggests that there is a general correspondence between risk of GBD and Amerindian admixture. In a small study of Minnesota Chippewa females over age 20, Thistle et al. [6] found that GBD prevalence was correlated with known degree of Amerindian ancestry. On a larger population scale, GBD is common in those Latin American populations in which there is a substantial degree of European-Amerindian admixture. These include Mexicans [12], Bolivians [10, 14], Chileans [11], Peruvians [38], and others [13]. In a similar way, GBD in Mexican-Americans is typically elevated [38–40], with age-specific prevalence rates that are two to three times those found in non-Hispanic Caucasians living in the same place and time, compared with five- to sixfold increases in Amerindians.

Figure 1 shows the prevalence of diagnosed GBD by age 55 in females of several populations, indicating the general trend from high to low risk associated with NW genetic admixture, based on data from several different studies [5, 19, 40–42]. While the data from the populations shown are not strictly comparable

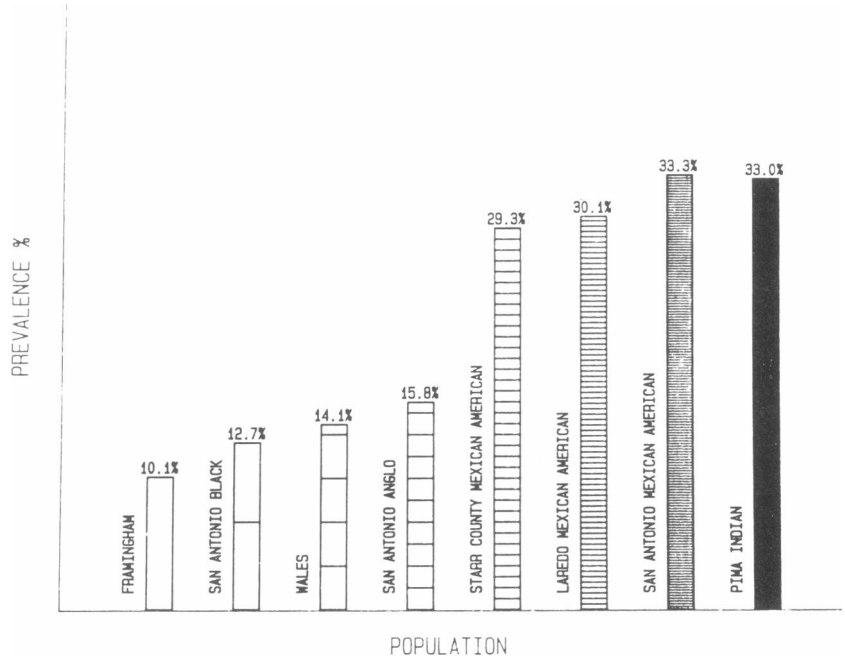

FIG. 1.—Prevalence of clinically diagnosed gallbladder disease by age 55 in individuals surveyed in various populations. Sources: Pima [5], San Antonio [40], Laredo (this paper), Starr County [19], Wales [41], Framingham [42]. Exact ages of subjects vary, but center on age 55 in all studies. Data in this figure are for females only.

in the methodologies employed, the pattern is clear; namely, Amerindians and Mexican-Americans have much higher rates than do Caucasians and blacks.

We noted above that very few cholecystectomies were performed in Laredo before 1945. A complete enumeration of cases in selected years has shown that the per-capita rate of surgery for individuals over age 15 in Laredo was 0.0052 in the 1960s, and has remained about the same, being 0.0046 in 1980; in addition, the age and sex pattern of cases has been similar since the early 1960s (our unpublished data, 1984). Therefore, there has been a stable pattern of surgery for at least 25 years. This is also reflected by a similar pattern of prevalences of cholecystectomy in Starr County [19]. The age-specific rates of cholecystectomy for Laredo are given in table 1. The lack of an increase in population rates of surgery with age is due to the age-specific decrease in the fraction of the censused population that is still at risk by virtue of having an intact gallbladder.

The Pima, who have doubtlessly been studied more extensively than any other group, are not completely without European admixture [43, 44]. Therefore, the lack of a risk gradient from Amerindian to Mexican-American in figure 1 may not reflect a true lack of such a gradient. Further, this figure was based on clinically diagnosed disease in the Pima; however, a cholecystographic study in the Pima [4] showed a 90% prevalence of gallstones in women by age 65. No data are as

yet available from ultrasound or other cholecystrographic methods in Mexican-Americans, but their prevalence can hardly exceed this value. A gradient of GBD rates occurs in tricultural New Mexico, where the highest rates were found in the Amerindians and intermediate rates in Hispanics [39], and in Bolivia [14].

Prevalence in males is lower in all populations, and there are fewer data available, so they were not included in figure 1. However, by age 55, prevalences in male Caucasians range from 3% to 5% [41, 42]. in Mexican-Americans, 5% to 15% [19, table 1]; and in the Pima, 12.3% for diagnosed disease [5], but 68% by cholecystography [4]. This is consistent with the female preference shown by GBD in all populations. However, in NW peoples, there is a higher female:male ratio than in other populations. This is about 2–3 to 1 in Caucasians [40–42], but 4–5 to 1 in NW peoples [5, 26, 29, 31, 45, 46]. In Laredo, the sex ratio for cholecystectomy is about 4.4 to 1, and in Starr County, it is 4.1 to 1 [19].

Gallstones are known to be associated with or exacerbated by pregnancy, and family size in NW peoples has traditionally been larger than in other U.S. groups. However, physiological studies [36, 37] have shown that NW women tend to produce lithogenic bile very early, before they have born any, or many, children. Cholecystectomies in very young adults, and even in teenagers, are frequent in Starr County and in Laredo, and the mean age of clinical GBD in NW peoples is 40 or less [27, 31, 45] as compared with about 50 in Caucasians [40–42]. Mean age is about 45 in Laredo. In addition, GBD rates have remained high while fertility rates have systematically declined in NW populations over the past generation. Further, among those NW populations in which it has been studied, the correlation between parity and risk is inconsistent [4, 6, 25]. For these reasons, it does not seem likely that high parity is the major explanation for elevated rates of GBD in NW peoples. This is also shown by the fact that GBD is substantially elevated in males as well as females. In Starr County, the relative risk of GBD, relative to the Framingham population, is 2.0 for females but about 6.0 for males [19].

TABLE 1

ESTIMATED AGE-SPECIFIC INCIDENCE OF GALLSTONE DISEASE IN LAREDO, TEXAS (PER THOUSAND)

	Female	Male
15–24	3.19 (32)	0.10 (1)
25–34	5.55 (41)	0.29 (2)
35–44	5.24 (27)	0.91 (4)
45–54	4.70 (22)	1.87 (7)
55–64	6.53 (24)	4.16 (12)
65–74	7.53 (22)	1.90 (4)
75–84	8.52 (13)	7.91 (8)

NOTE: Rates computed as hospital cases/census population, based on enumeration of 1980 cases to Laredo residents and the U.S. 1980 census. There were nine cases over age 85. Sample sizes in parentheses.

CANCER OF THE GALLBLADDER (GBCA)

The most strongly associated risk factor for GBCA, in all populations, is the presence of cholesterol gallstones. The nature of the carcinogenic mechanism is not known; it may involve the physical irritation of gallbladder mucosa by the stones or the carcinogenic activity of concentrated bile in gallbladder stasis [38, 47–53], selected from an extensive literature). Regardless of the causal mechanism, there is documentation, largely derived from Mexican-Americans and Amerindians, of the cytopathological evolution of carcinoma from hyperplasia and carcinoma in situ (CIS) to cancer in the gallstone-affected gallbladder [54–57]. Studies have consistently reported evidence of prior or coexisting gallstones in tissue removed for cancer of the gallbladder [48], and carcinoma has been found preferentially to arise in tissue areas affected by large stones [47]. In NW peoples, the incidence of coexisting gallstones is always found to be high and has approached 100% in some clinical series [48]. The only evidence for a biochemical difference is a reported deficiency in Bolivians in the ability of their liver to sulfate lithocholic acid returned to it via the enterohepatic circulation [13, 58]; lithocholic acid, a secondary bile acid formed in the gut, has been shown to be a promotor of carcinogenesis and to be mutagenic and mitogenic in some test systems [59–62]. Whether or not this can be replicated, it is probably incidental to the epidemiology of primary GBD because it is unlikely to be the primary cause of gallstones.

As one would expect in populations predisposed to early gallstone formation, GBCA occurs more frequently in NW peoples. These include Amerindians in Alaska [8], Canada [63], and the U.S. [9, 26, 29, 30, 32, 38, 39, 64, 65], and admixed Latin Americans from Bolivia [10, 14], Peru, [38], and elsewhere [13], as well as Mexicans [11] and Mexican-Americans [38, 48, 66]. Table 2 gives standard mortality or morbidity ratios (SMRs) [20] for cancers of the gallbladder and biliary system for various NW peoples and several comparison populations. The latter include Latin Americans with little NW admixture, and Asians, sharing remote common ancestry with NW peoples (i.e., at a time prior to the peopling of the Americas). While small sample effects can be seen, especially in regard to males, these SMRs show the higher risks generally found in peoples with substantial NW genetic admixture. The high rate in Japan is interesting and not yet explained, but note that it involves males much more than females, which has not been observed in NW populations; this may be at least partially related to the combining of bile duct and gallbladder tumors in data reporting. The same kind of pattern can be seen in age-adjusted (i.e., directly standardized) incidence or mortality rates from comparable populations (e.g., [10, 13, 67]), including the gradient in risk correlated with admixture both in New Mexico [39] and in Bolivia [14]. The epidemiological pattern of GBCA reflects the presence of gallstones, and, because there are more data on cancer than on gallstones, can be used to expand the area known to be affected by a high prevalence of GBD.

There is evidence that GBCA in NW peoples may be more than just a reflection of their higher prevalence of gallstones. In other populations, less than 1% of untreated gallstone cases lead to GBCA [47]. In Caucasians, the frequency of asymptomatic GBCA or CIS of the gallbladder discovered at cholecystectomy

TABLE 2

STANDARDIZED MORTALITY OR MORBIDITY RATIOS FOR GBCA IN SELECTED NW AND COMPARISON POPULATIONS

POPULATION	GALLBLADDER		BILIARY DUCTS	
	Males	Females	Males	Females
US white*	1.00	1.00	1.00	1.00
New Mexico Anglo	0.83	0.54	0.89	0.71
New Mexico Hispanic	2.40	4.05	1.48	1.92
Texas Hispanic:				
Laredo	0	2.38	1.14	0
Rio Grande Valley	0.83	2.97	1.25	1.79
San Antonio	3.70	4.32	2.07	1.09
El Paso	2.59	2.41	2.04	2.27
California Hispanic	1.90	2.77	1.47	2.34
New Mexico Amerindians	8.34	7.16	0.52	4.39
Miscellaneous Amerindians	5.16	4.81	1.22	3.95
Alaska Amerindians	3.26	6.12	6.67	10.00
Alaska Eskimo	2.33	5.56	0	0
Taiwan Chinese	0.41	0.18	...	...
Japanese	6.00	1.70	...	...
Hawaii Filipinos	1.25	0.27	1.86	1.05
Puerto Rico	0.71	0.77	1.96	2.10

NOTE: Japanese data from [90], Chinese data from [91], and Hawaii data from [92] are standardized morbidity, not mortality, ratios. To account for small samples, Alaska data are weighted average of data in [86, 87], except for biliary ducts, for which only the first report included data. The remaining data in this table were presented at the symposium "Cancer Incidence in Low Risk Populations," held at Snowbird, Utah, 1980, and sponsored by the National Cancer Institute; data were presented by the following investigators: New Mexico, Charles Key; Amerindians, Mozart Spector; California Hispanics, James Enstrom; Puerto Ricans, I. Martinez; Texas data, K. Weiss, updated from [16]. Standard population was United States, 1970. All data for circum-1970.

performed for symptomatic gallstones is about 0.5 to 1.5% [47, 57, 68, 69]. In contrast to this, in several clinical and autopsy studies in Mexico, where gallstones occur often and at young ages in females, the rate of cancerous or precancerous lesions in gallbladders removed for gallstones has been found to range between about 3% to 5% [12, 54, 56]; CIS was found adjacent to carcinoma of the gallbladder in most of the cases in these studies. In Amerindians, cancer or precancerous lesions have been found in from 2% to 4.5% of gallbladders removed for stones [8, 29–31, 45, 46, 57, 70].

The pattern of GBCA in Mexican-Americans, for which there are some data showing longitudinal trends, may help to demonstrate that the epidemic of GBD is a post-World War II one. In Laredo, only six of 75 cases occurred prior to 1940, and only five more by 1950; currently, about eight cases occur annually. More substantial evidence comes from the pattern of GBCA in Texas in the period 1944–66 [71], which shows a steady increasing trend in rates for both sexes (but especially for females) in Mexican-Americans, but a steady or only slightly increasing trend in blacks and Caucasians in the state.

Cumulative Incidence of Diagnosed GBCA in NW Peoples

Carcinoma of the gallbladder has a poor prognosis, and less than 10% of patients are alive 5 years after the diagnosis. The tumor is typically asymptomatic

until it has already become large enough to block the passage of bile into the duodenum and/or has metastasized to the liver and other adjacent tissues. The mean ages of cases are given in table 3 for various populations. Although the numbers are small for some of these, it appears that in men there is very little difference among the groups, but that in NW females, the onset pattern is shifted downward by about 5 years.

A sense of the impact of GBCA on NW populations can be obtained from cumulative incidence in various populations. This is shown in table 4, based on cancer registry data from [67]. These figures show that by age 75 there is several times the risk of GBCA in NW individuals, especially in females, and that the probability of disease can exceed 1%. Although based on small numbers, the risks appear to increase rapidly after age 75. We have estimated elsewhere [73] that there are about 270 cases of GBCA among U.S.-censused Amerindians and Mexican-Americans every year, and that by the time the currently living population now under age 40 have reached age 70 they will have experienced about 18,000 cases of this generally fatal disease. This subgroup comprises only about 5% of the U.S. population, but will incur roughly 20% of all cases.

Cancer Risk to Those Not Undergoing Cholecystectomy

Approximately 15% of gallbladder cancer cases are discovered as occult tumors in the course of cholecystectomy to treat symptomatic gallstones [57]. The remaining 85% of cases must occur in that fraction of the population that still has a gallbladder at risk. Because registry-based cancer rates are determined by dividing cases by total population, the risk of GBCA in NW populations, to individuals actually at risk, must be considerably higher than the registry rate. By adjusting the total population to include only those still at risk, corrected cumulative incidences can be computed using methods described in the APPENDIX, part 2. These are given in table 4 for a population subject to cholecystectomy at the Laredo rate (cholecystectomy rates for an Amerindian population are not available). The risk of GBCA to such a female by age 85, that is, if she has not undergone a prior cholecystectomy, is about 3%.

These calculations were based on adjusting the tumor rate to account for the tumors discovered incidentally in the course of cholecystectomy. If by age 85, 40% of women have had a cholecystectomy, and in these, 3% were found to have occult cancer or CIS, this represents a risk of about 1.2% to the cohort. The estimates in table 4 indicate that in the remainder of the population the risk of

TABLE 3

MEAN AGE AND SEX DISTRIBUTION OF GBCA CASES IN SELECTED POPULATIONS (%)

	LAREDO	NEW MEXICO		
		Hispanic	Amerindians	Anglo
Male	68.9 (17)	71.0 (55)	68.0 (10)	70.3 (37)
Female	69.1 (58)	68.0 (186)	67.3 (29)	73.8 (36)

NOTE: Sample sizes in parentheses. Laredo data original for this paper. New Mexico data from [67].

TABLE 4

CUMULATIVE PROBABILITY OF GBCA IN SELECTED POPULATIONS (%)

DISEASE/POPULATION	BY AGE 55	65	75	85
A. In the whole population				
Cholecystectomy:				
Laredo males	03.2	07.3	09.2	16.9
Laredo females	18.4	24.8	32.2	40.4
Gallbladder cancer:				
Connecticut females	0	00.1	00.2	00.6
Puerto Rico females	0	00.1	00.3	00.5
New Mexico Anglo females	0	0	00.2	00.4
New Mexico Hispanic males	0	00.1	00.5	00.9
New Mexico Hispanic females	00.1	00.4	01.0	01.7
New Mexico Amerindian males	0	00.2	01.3	01.3
New Mexico Amerindian females	00.3	01.1	02.6	04.1
B. Gallbladder cancer among the at risk				
Mexican-American males	0	00.1	00.5	01.0
Mexican-American females	00.1	00.4	01.3	02.8

NOTE: All data from [67] except Laredo is new to this paper, and Caucasian cholecystectomy data, used in section A, from [42]. The values in section B assume the entire population is equally susceptible.

GBCA may be about 3%, that is, the cancer risk of "silent" gallstones—or whatever else may be the risk factor in NW peoples—is more than twice that due to symptomatic stones, yet the former cases will be more advanced when discovered. In that sense, in NW peoples, it appears that the ubiquitous saturated bile and gallstones in NW females [4, 6, 28, 37] may not be as benign as gallstones seem to be in Caucasian males [74, 75]. Even without adjusting for the "at risk," GBCA is one of the more common tumors in NW peoples [10, 13, 14, 54, 67, 73, 76].

GENETIC EPIDEMIOLOGICAL CONSIDERATIONS

The pattern of genotypic variability in an admixed population is a function of the gene frequencies of the original contributing parental populations, the number of loci involved in a trait of interest, the mating pattern relative to those loci, and the amount of admixture. For example, if susceptiblity is due to a dominant allele at a single locus, with frequency p in the admixed population, then with random mating a fraction $1 - (1 - p)^2$ will bear at least one copy of the susceptibility allele. For a threshold phenomenon based on a polygenic distribution of susceptibility, the fraction of the admixed population exceeding the threshold will be a function of the threshold value in both parental populations and the mixing proportion (admixture) in the hybrid population.

To illustrate the importance of this, we assume that susceptibility to GBD in NW peoples has a genetic basis, that the gene frequency in unadmixed Amerindians was 1.0 based on its high prevalence in at least some tribal groups [4], and that

the susceptibility genotype did not exist in Europeans. These assumptions lead to epidemiologically reasonable results.

We do not have good genetic admixture rates from Amerindian groups for whom we also have good data on GBD. However, such data do exist for Mexican-Americans. Several estimates have been made of Amerindian admixture in Mexican-Americans in California and Texas, based on gene frequencies [77–79] and skin reflectance [80]. The admixture estimates from these studies vary, ranging from about 15% to about 40% Amerindian genes.

Because we assume an ancestral Amerindian gene frequency of 1.0, the admixture rate represents the Amerindian gene frequency in an admixed population. If susceptibility to GBD is of a dominant single locus type, then a locally varying fraction $[1 - (1 - p)^2]$, or from 28% to 64% of Mexican-Americans are susceptible. For a susceptibility threshold, assuming all Amerindians exceeded the threshold and no Europeans did so, the admixture proportion is itself the fraction of the hybrid population exceeding the threshold, that is, 15%–40%.

If the genetic hypothesis is correct, then only a subset of individuals in NW populations is specially at risk, even in a provocative environment; the remainder are at the normal risk. The cases of disease would, thus, be concentrated in the more susceptible subpopulation. It is of interest to estimate what the risk specific to a susceptible genotype might be, that is, the biological effect of the genotype itself as a risk factor.

To make these computations, we begin with a cohort of individuals at mixed risk, with proportions dependent on an assumed admixture level. This cohort is then subjected to the double decrements of cholecystectomy and gallbladder cancer at rates appropriate to each subgroup. At each age, the proportion of the population still at risk is computed, and the whole-population disease registry rates adjusted so as to be specific to those actually at risk.

To make these computations, the age pattern of rates of cholecystectomy and GBCA in a pure European population and in a pure NW population are needed. For Caucasians, cholecystectomy rates from the Framingham study [42] and GBCA rates from the Connecticut tumor registry were used. For the NW, as in computing table 4, the Laredo cholecystectomy rates and the New Mexico Hispanic tumor registry rates were used. Cumulative incidences were computed by the methods given in the APPENDIX, part 3. The results are shown in table 5. As in table 4, the risk of GBCA by age 85 to a woman not undergoing prior cholecystectomy is about 2.8% even if Amerindian admixture were 100%. However, if a lesser fraction of Mexican-Americans are susceptible, the risk can be much higher; if only about 50% (corresponding to a gene frequency of .3) are susceptible, the cumulative incidence in the susceptible subgroup is about 7%. This is a very high risk by any standard, comparable, for example, to that of lifelong heavy smoking [81], and shows that the possession of a susceptible genotype can be very "carcinogenic" in today's environment. Regardless of the mechanism by which the genotype(s) lead to GBD and GBCA, if the genetic hypothesis is correct, the penetrance of the genotype(s) in the form of either GBD or GBCA seems to be very high, approaching 1.0 in females. It should be noted that the foregoing conclusions regarding disease distribution and risk in the susceptible

TABLE 5

CUMULATIVE PROBABILITY OF CLINICAL GBD TO GENETICALLY SUSCEPTIBLE FEMALES OF A NW POPULATION AS A FUNCTION OF FRACTION SUSCEPTIBLE (IN %)

	SUSCEPTIBLE FRACTION (%)				
AGE	20	40	60	80	100
Cholecystectomy:					
55	48.0	29.8	23.5	20.4	18.4
65	62.3	39.3	31.3	27.3	24.8
75	79.9	51.1	40.7	35.4	32.2
85	97.5	64.8	51.5	44.6	40.4
Gallbladder cancer:					
55	00.7	00.3	00.2	00.1	00.1
65	03.3	01.2	00.7	00.6	00.4
75	14.9	03.8	02.3	01.7	01.3
85	90.1	08.8	04.8	03.5	02.8

NOTE: Uses Laredo 1980 cholecystectomy rates, Framingham cholecystectomy rates [42], Connecticut and New Mexico tumor registry rates [67], and methods described in text. Last column is for whole population at susceptible risk level, corresponding to values in table 4. Correspondence between susceptible fraction and admixture depends on genetic model (see text). These values differ from [74] in using 1980 rather than 1965 Laredo cholecystectomy rates and better estimates of Caucasian risk.

subgroup are essentially the same whether this susceptibility is of a polygenic or single-locus type.

Here, no consideration was given to assortative mating. However, obesity is associated as an early-adult manifestation of process(es) that lead to gallstone formation, and a tendency to become obese in this population may be associated with, or due to, the same physiology responsible for lithogenic bile. Thus, it is possible that body type, one criterion affecting mate choice, is genetically non-random with respect to the risk of gallstones. To the extent that this occurs in NW peoples, the risk genotypes make up a smaller, more homozygous, subset of the population than computed above under Hardy-Weinberg assumptions, and the risk to susceptible genotypes may be greater.

Familial Gallbladder Disease

The hypothesis that the pattern of GBD in NW peoples is genetic is largely based on the association of disease with NW genes on a population basis. However, results from the Starr County study document excess risk within families in an admixed population [19]. There, the risk of GBD in first-degree relatives of affected probands was 1.8 times the probability of a random individual being affected, in a sample of 183 affected probands. That this was not simply due to shared family environments is suggested by the fact that the relative risk of GBD concordance in spouse pairs was 0.99 in a sample of 345 pairs; that is, 8.1 GBD + / + pairs were expected on the basis of population disease rates and eight were observed [19].

Familial Gallbladder Cancer

If GBCA is mainly a matter of long-term exposure to environmental risk factors, one might expect to find very little evidence of excess risk in sibs of affected

probands, whereas if there are genes involved, familial patterns of risk would be a sine qua non of such risk. In Laredo, where there had been 76 cases up to 1980, there is no documentable instance of a multiply-affected nuclear family, although families multiply affected with obesity and diabetes, which are associated diseases [15], are common. In the literature, there are only two Hispanic families from northern New Mexico [48, 66] and two affected Choctaw brothers from Arizona [65] reported with GBCA. If, as we argue, GBCA in NW peoples is due to a genetic susceptibility, why is there no obvious evidence of familial risk?

In order for a sibship of Mexican-Americans to manifest two cases of GBCA, for example, it must have produced at least two survivors (especially females) without early cholecystectomy and who have been over age 60 since the time GBCA has been more reliably diagnosed and/or more common (circa 1945). As shown elsewhere [73], the probability of this, given the demography of the times, is very small—only one sibship in 143,000 would be doubly affected, several times the number of NW sibships of eligible age in New Mexico, for example.

Gallbladder cancer is the last stage in a pathogenic process that takes place over decades. While its precursor, gallstones, are quite common and are familial in NW peoples, demographic factors make it unlikely that the final phenotype will appear to be familial. We have referred to this kind of phenomenon as "phenotype amplification" [73]; as a consequence, studies of the genetics of susceptibility to late-stage disorders like GBCA should be based on earlier manifestations, such as in bile physiology, as a more relevant phenotype.

DISCUSSION

In other populations of the world, a rise in GBD has resulted from "Westernization," which has been attributed to the consumption of high-calorie, high-fat, low-fiber diets and insufficient exercise (e.g., [1, 2]). However, not only do the geographic patterning and high prevalence of GBD in NW peoples suggest a genetic etiology, but other facts do so as well. First, in NW peoples, GBD is associated with high rates of obesity and noninsulin-dependent diabetes mellitus in a way that indicates a shared, genetic etiology [15]. Second, the diseases of Westernization also include elevated rates of ischemic heart disease and of tumors of the breast, endometrium, colon, rectum, and prostate [1]. Yet, all of these diseases occur at lower frequency in NW peoples, who, to judge by GBD and diabetes rates, have been exposed to the relevant environmental risk factors for at least 40 years [15]. The pattern of response to those risk factors, whatever they be, seems to be different in NW peoples. Third, the lower disease rates in blacks, who have typically shared similar socioeconomic status with NW peoples, suggests that this is not simply a problem of poverty. Fourth, it seems unlikely that NW peoples share with each other, on a continental scale, exposures that they do not also share with blacks and Europeans; indeed, it is not clear that NW peoples typically have the kind of diet and lifestyle that has been suggested [2] as a cause of GBD. Fifth, the association between rates of colon cancer and cholecystectomy, which has been reported in some, but not all, studies in other populations (e.g., [82–85]) does not occur.

Insofar as our inferences are correct, the geographic epidemiology of GBD suggests a susceptibility in NW peoples not shared by their Asian ancestral relatives. The Americas were first settled by immigration associated with the raising of the Bering land bridge from Asia during glacial epochs. Descendants of these original Amerindians expanded to occupy the remainder of the hemisphere in a few thousand years, a short time on an evolutionary time scale. These are the ideal conditions for the evolution of a genetic variant(s), in response to some demographic or selective conditions, which would be found on a hemispheric scale in the descendants of a small founding population. It will be important to establish whether Eskimos, who arrived in the Americas much later than the Amerindians, and who are related to them only by virtue of their prior common northern Asian origins, share the susceptibility found in Amerindians. Eskimos have experienced recently increased rates of GBD [7, 8], although this increase seems to have been later, and (so far) smaller, than that experienced by Alaskan Amerindians [8, 86, 87]. If this result bears out, the origin of this genetic susceptibility will be placed early in, or shortly before, the peopling of the Americas; if Eskimos are shown to share the susceptibility with Amerindians, its probable origin would be pushed back to some earlier time in northern Asia.

GBD is probably a result of acculturation to nonaboriginal environments, because the genetic basis of the diseases as they are now manifest would have had to rise in frequency in the face of some negative selection. It is possible, considering the nature of the relationship among obesity, parity, puberty, and the initial development of gallstones, that today's susceptibility reflects genes positively selected for the efficient utilization and storage of nutrients by females. In aboriginal times, especially in the climate of the Bering land bridge, food supplies may have been unpredictable and a fertility and lactation advantage would accrue to females with such genes. Males also could have benefited from such genes. This is basically Neel's "thrifty gene" hypothesis visited [88] and once revisited [89] to explain the high incidence of diabetes in the human species, except that it is applied to a particular population group known to have passed through a period of population expansion in an Arctic environment in which the occurrence of "thrifty genes" could have been important. The evolution of those genes is reflected in their current descendants, in whom their original selective value no longer applies and, indeed, is a medical detriment. Alternatively, it is possible that this NW susceptibility has evolved strictly as a function of genetic drift.

The apparently highly carcinogenic result of gallstones and/or bile in NW peoples is of considerable concern. Not only are a high proportion of susceptibles at substantial cancer risk, but it is presently not possible to identify which individuals these are. In addition, the rates of biliary tract disease, including cancer, which is not clearly associated with gallstones, are elevated in NW peoples, as are some other digestive system cancers; some cases may be related to compounds present in bile or to biliary stasis and reflux [15].

If our calculations based on the admixture estimates are even approximately correct, a substantial fraction of the population is at high risk, and this group warrants attention. The use of genetic or physiological markers may provide the basis for identifying them by virtue of the information provided for determining

individual admixture rates. In this context, markers then take on significance beyond that generally considered in linkage investigations and, in fact, provide relevant information on the genetics of disease even in the absence of linkage, as well, perhaps, as on the ancestral relationships of NW peoples, including Eskimos.

Since it is not clear whether a bile component is unusually carcinogenic in NW peoples [13, 58] or whether this is simply due to very high rates of subclinical gallstones [3, 4], it is not currently possible to suggest practical preventive measures for cancer of the gallbladder, although early detection of gallstones by ultrasonography may at least indicate those who would be at risk by virtue of stones. We have in progress a study of the bile constituents of cholecystectomy patients in Laredo and Starr County to determine if enzymatic differences in bile-acid production from cholesterol can be detected, but preliminary results are inconclusive. In addition to direct clinical benefits, gallbladder disease in NW peoples provides a rare opportunity to combine the approaches of physiology, genetics, epidemiology, and anthropology to the understanding of a major epidemic.

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APPENDIX
CALCULATION METHODS

Following are the mathematical methods used to compute the tables and figures for this paper. A reference on the basic actuarial methods used is [20]. The methods have general applicability.

(1) Cumulative Probability for a Single Trait

Let $h(x)$ be the incidence, or "hazard," rate at age x for a trait in question. This is estimated as the number of cases occurring in a given time period over the at-risk population in that time period, at the specified age. A cohort of individuals exposed to this hazard will have a fraction

$$l(t) = e^{-\int_0^t h(x)dx} \tag{1}$$

unaffected by age t. $H(t) = 1 - l(t)$ we define as the "cumulative probability" of the trait. If there are unrelated causes, such as competing causes of death, then $l(t)$ is the probability that a member of the cohort unaffected by the competing cause is unaffected by the trait, and $H(t)$ is the fraction of those not removed from risk by the competing cause who are affected; this is true if, as in the case of competing mortality, $h(x)$ applies only to those at risk (alive) at age x.

Here, we use the discrete-time analogue of equation (1). First, the hazard is converted to a probability of manifesting the trait in an w-year period, $Q(x)$, by the conversion relationship

$$Q(x) = \frac{wh(x)}{1 + \frac{w}{2}h(x)}. \tag{2}$$

This is approximate in that it does not include the presence of causes of death in the denominator [18], but the error is quite small in this case because much of GBD occurs at ages when death rates are very low. From equation (2), following life-table methods, the probability of being affected by age T is

$$H(T) = 1 - l(T) = 1 - \prod_{x=0}^{T-w} [1 - Q(x)] , \quad (3)$$

where, as hereafter, the iteration is in w-year intervals: 0, w, $2w$, . . . , $T - 2w$, so that the final term carries the results through the interval $T - w$ to T. In this paper, we have used $w = 5$.

The value of $H(T)$ from equation (3) is used to compute the Laredo cumulative probabilities of GBD, from the $h(x)$ values in table 1, for figure 1. If there are no deaths by age T, which is approximately true for ages up to about 60, then $H(T)$ is roughly equal to the lifetime prevalence of the trait in a sample of individuals age t, that is, the probability that such an individual has now, or has ever had, the trait.

(2) Cumulative Probability in the Presence of a Nonfatal Competing Cause

Let $r(x)$ be the hazard function for a nonfatal competing "cause," here referring to cholecystectomy, which removes them from risk of the trait in question but which does not remove individuals from the census population. Then the estimated risk, if based on census-population data, is not the true hazard to those at risk since many in the census are not actually at risk.

Converting the hazards $r(x)$ and $h(x)$ to values $Q_r(x)$ and $Q_h(x)$, via equation (2), and assuming them to be stochastically independent sources of decrement at any given age, the probability of reaching age $x + w$ unaffected (survivorship) can be written as a function of the hazards and the survivorship at age x:

$$l(x + w) = l(x)[1 - Q_h(x)][1 - Q_r(x)] . \quad (4)$$

The independence assumption is mathematically necessary without externally derived information on the relationships between the rates [18], but is probably not a numerically important problem in this context. Indeed, it may be nearly correct, given the very high prevalence of gallstones and/or lithogenic bile in NW females (see text); in such a person, the mechanisms that determine whether the condition will develop symptoms of stones or will develop symptomatic cancer are different. Individuals will fall into three categories: (1) unaffected by either decrement, with probability given by $l(x + w)$, (2) affected by the trait only, with probability $A(x + w) = l(x)[1 - Q_r(x)]Q_h(x)$, and (3) a remainder affected by the cause with or without the trait, $C(x + w) = l(x)Q_r(x)$.

The cumulative probability by age T, for the trait only (i.e., not including those doubly affected with trait and cause) is the sum of the terms $A(x)$ over all ages up to T divided by this sum plus those still unaffected by either cause or trait:

$$H(T) = \frac{\sum A(x)}{l(T) + \sum A(x)} = \frac{\sum l(x)[1 - Q_r(x)]Q_h(x)}{l(T) + \sum l(x)[1 - Q_r(x)]Q_h(x)} . \quad (5)$$

This is computed, as for table 5, by iteration, starting with a radix of $l(0) = 1.0$ at age $x = 0$. We omit those affected by both the trait and the cause since, here, we are interested in cancers not found in the course of gallstone surgery; otherwise, a set of terms equal to $l(x)Q_h(x)Q_r(x)$ should be added to numerator and denominator of equation (5).

(3) Cumulative Probability for a Trait with Nonfatal Competing Cause in a Subgroup of an Admixed Population

This is an extension of equation (5) but includes two subpopulations each subject to the trait as well as the cause, at subgroup-specific hazard rates. Here, we will denote the subpopulation of main interest (e.g., NW natives) with natural symbols, and the other (e.g., Europeans) with primes ('). We denote the fraction of the population at high risk by m (corresponding to admixture), and that at normal risk by n ($= 1 - m$).

Usually, available data are in the form of whole-population rates, computed by dividing new cases by the census population. In the case of European cholecystectomy and gallbladder rates, these are available, as in Framingham and in the tumor registries or other sources. But there are no data for "pure" Amerindian populations since there are no such populations, or at least none have been studied for GBD. Yet, there are data from admixed populations. The disease rate in such a population may be expressed as a function of the pure subgroup rates and the mixing proportions: $p(x) = \text{cases}(x)/\text{pop}(x) = mh(x) + nh'(x)$, from which we can estimate the pure-population risk as

$$h(x) = \frac{p(x) - nh'(x)}{m} . \tag{6}$$

From a similar relationship, we can estmate the value of $r(x)$. Actually, at age $x = 0$, equation (6) applies with $n = (1 - m)$, but thereafter the fractions of high- and low-risk individuals still at risk (i.e., who have not yet suffered gallstones or GBCA) decreases. We must write these as $m(x)$ and $n(x)$, and they do not sum to 1.0 since some of the population is at zero risk but is still counted in the census. Thus, we must recursively compute the values of $m(\ )$ as

$$m(x + w) = m(x)[1 - Q_h(x)][1 - Q_r(x)] \tag{7}$$

and analogously for $n(\ )$.

In equation (6) we assumed that the mixed-population and one subgroup rates are known and that we need only solve for the rate in the other subpopulation [otherwise, equation (6) would be indeterminate]. However, typically, even the rates applicable to the "known" subgroup are whole-population rates. For example, Caucasian GBCA rate data do not take into account those individuals counted in the census but who no longer have their gallbladders. Hence, in an admixed situation, we can say that these rates, call them $h''(x)$ and $r''(x)$, apply to the whole $1 - m$ fraction of the admixed population. Really what we want are rates $h'(x)$ and $r'(x)$ applicable to the fraction of size $n(x)$, which is still at risk [such that $n(x)/(1 - m)$ is the fraction of the original subpopulation that is still at risk]. The desired rate, for example, $h'(x)$, is computed as

$$h'(x) = \frac{(1 - m)h''(x)}{n(x)} . \tag{8}$$

From these, substituting $m(x)$ for m and $n(x)$ for n in equation (6), we can compute the age-specific values of $h(x + w)$, $h'(x + w)$, $r(x + w)$, and $r'(x + w)$, and using equation (2), the values of $Q_h(x + w)$, $Q_h'(x + w)$, $Q_r(x + w)$, and $Q_r'(x + w)$. This forms a recursive system, with the survivorship at age $x + w$ given as

$$\begin{aligned} l(x + w) = {} & m(x)[1 - Q_h(x)][1 - Q_r(x)] \\ & + n(x)[1 - Q_h'(x)][1 - Q_r'(x)] . \end{aligned} \tag{9}$$

Counting those in the first subgroup who are or have been affected by the trait only (i.e., cancer without gallstone surgery), the cumulative probability is given by $A(x) = m(x)[1 - Q_r(x)]Q_h(x)$ and

$$H(T) = \frac{\sum A(x)}{m(T) + \sum A(x)} . \tag{10}$$

These are the values plotted in figure 2. These recursion relationships are quite easy to computerize and can be applied to any similar situation involving admixed populations.

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