It was not known until recently whether the endemic of cholesterol gallstones among certain southwestern American Indian tribes was unique among this ethnic group. With use of ultrasonography of the gallbladder and standard diagnostic criteria, gallstones are now found in epidemic proportions in 13 diverse American Indian tribes and communities living in Arizona, Oklahoma, and the Dakotas. We speculate that this predisposition is polygenic involving “thrifty” genes that conferred survival advantages when Paleo-Indians migrated from present-day Siberia to the Americas during the last Great Ice Age approximately 50,000 to 10,000 years ago. A reasonable hypothesis is that functioning of these genes promoted more efficient calorie utilization and storage in the form of adipose tissue. Beneficial results would have been operative during the isolation of Paleo-Indians in the Bering Strait land bridge (Beringia) when thrifty genes would have ensured sufficient fat reserves for survival of prolonged winters, successful pregnancy outcomes, and extended lactation periods. The authors’ conjoint work on genetics of experimental cholesterol cholelithiasis in inbred mice promises help in pinpointing orthologous genetic loci (LITH genes) in the human genome. Moreover, the shared environments and homogeneity of American Indian tribes and communities should facilitate discovery of the ensembles of their common and rarer cholesterol gallstone genes. It is anticipated that knowledge of expression, polymorphisms, and functionality of LITH genes will help resolve the molecular mechanisms of this complex heterogeneous trait and thereby provide targets for novel therapies to prevent cholesterol cholelithiasis worldwide. (HEPATOLOGY 2002;36:781-791.)

In 1962, Sievers and Marquis at the US Public Health Service Indian Hospital in Phoenix labeled gallstone disease the “American Indian’s burden.” They were not alone in being impressed by the frequent clinical evidence of both gallstones and their ominous complication, gallbladder cancer, among American Indian tribes living in southwestern Arizona. Franz J. Ingelfinger, the doyen of American gastroenterology at the time, became acquainted with the high prevalence of gallstones among southwestern American Indian tribes from Dr. John S. Fordtran, now of Dallas, TX. For the several years preceding his arrival at Boston University Medical Center in 1960 to begin his fellowship in gastroenterology, Fordtran was in the Public Health Service at the Navajo Medical Center in Fort Defiance, AZ. He recalls that he saw an astonishing number of men and women with gallstones among Navajo, Zuni, and Hopi Indians who lived in surrounding mesas. He recollected that while most American Indians were obese, many were not, and “you did not have to be obese to have gallstones.” Fordran told his former mentor about the extraordinarily high prevalence of gallstones among the Fort Defiance Indians. Ingelfinger did not believe him and decided to see for himself. So he and Fordtran traveled to Fort Defiance, where they spent several days mulling over patients’ charts, interviewing two surgeons who did the gallbladder surgery, and seeing patients. It was only then that Franz Ingelfinger became convinced of the enormity of the American Indian gallstone problem. As he said of himself, “...but sinners, when they see the light, feel with greater violence than do saints.” With the obsession of a convert, Ingelfinger vigorously promulgated gallstone research for-
ever after; he even carried around an American Indian gallstone, “a large cholesterol solitaire,” which he brought to Bethesda, MD, to highlight the enormity of the American Indian problem to the officials at the NIH. Dr. Donald M. Small recalls that this is what got Franz Ingelfinger “his first big gallstone grant.”

In his 1968 position paper, Ingelfinger penned a bold manifesto: “The study of gallstones has been grossly neglected.” He also noted that gallstones were an illness characteristic of four recent US presidents and that removing the gallbladder was big business, sometimes with unhappy results such as the “double cystic duct” mishap involving a well-known British Foreign Secretary. Moreover, Ingelfinger called for precise surveys of both asymptomatic and symptomatic gallstone patients, claiming that such an exercise would define the magnitude of the problem and identify risk factors such as habits, diet, and genetics. Thinline in his editorial was the fact that he had already catalyzed such a study in Pima Indians through the NIH, employing an English rheumatologist named Dr. Peter H. Bennett. In addition, he had also sent Dr. Donald Small to the Pasteur Institute in Paris (1963-1965) to Dr. Dikran Dervichian, head of the Service de Biophysique, to learn by means of a thorough phase equilibrium study of model biliary lipid systems how gallbladder bile could become supersaturated with cholesterol.

The classic Pima study was an age- and sex-stratified randomized sample of 596 American Indians, with prevalence rates of gallstones inferred from history of cholecystectomy plus positive oral cholecystography. True prevalence rates (Fig. 1) showed that, although infrequent during adolescence, gallstones appeared explosively during the early 20s reaching a maximum in the third and fourth decades in women and somewhat later in men, with as many as 80% of women and 70% of men cumulatively affected. Clinical prevalence, i.e., symptoms or prior cholecystectomy, in the same population was 50% in women and 20% in men. This landmark study found no association of gallbladder disease with obesity (present in 84%), diabetes mellitus (34%), or parity (median 3-4 children per family). Since at about the same time it was established that relative, as opposed to absolute, content of cholesterol was the quotient that resulted in supersaturation of bile, an enormous flowering of studies on the pathophysiology of “lithogenic” bile as well as risk factors and true prevalence rates of gallstones followed worldwide (summarized in Paigen and Carey). However, the lithogenic potential of the Pima appeared unique; the biles of these individuals, irrespective of gender, became markedly supersaturated with cholesterol during puberty. In another American Indian population (Chippewa), lithogenic bile was more common and severe among adolescent American Indian women with normal cholecystograms than in matched white controls. It was established in the Pima that the physical stages of stone disease, i.e., nucleation, crystal formation, and stone growth, were delayed approximately 7 to 10 years after puberty. Symptoms, if they occurred, did not appear for another 5 to 10 years.

Several important demographic insights on gallstones are revealed by these and later epidemiologic studies. The classic Pima study was an age- and sex-stratified randomized sample of 596 American Indians, with prevalence rates of gallstones inferred from history of cholecystectomy plus positive oral cholecystography. True prevalence rates (Fig. 1) showed that, although infrequent during adolescence, gallstones appeared explosively during the early 20s reaching a maximum in the third and fourth decades in women and somewhat later in men, with as many as 80% of women and 70% of men cumulatively affected. Clinical prevalence, i.e., symptoms or prior cholecystectomy, in the same population was 50% in women and 20% in men. This landmark study found no association of gallbladder disease with obesity (present in 84%), diabetes mellitus (34%), or parity (median 3-4 children per family). Since at about the same time it was established that relative, as opposed to absolute, content of cholesterol was the quotient that resulted in supersaturation of bile, an enormous flowering of studies on the pathophysiology of “lithogenic” bile as well as risk factors and true prevalence rates of gallstones followed worldwide (summarized in Paigen and Carey). However, the lithogenic potential of the Pima appeared unique; the biles of these individuals, irrespective of gender, became markedly supersaturated with cholesterol during puberty. Symptoms, if they occurred, did not appear for another 5 to 10 years.

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in men. Nonetheless, apart from the Pima, no true gallstone prevalence data for other North American Indian tribes or communities were available until recently.

In an earlier issue of this Journal, James Everhart et al. determined true prevalence rates together with associated risk factors of gallbladder disease among American Indian subjects who participated in the Strong Heart Study, in which gallstones were detected by ultrasonography plus self-reporting of cholecystectomy. This study involved 13 American Indian tribes and communities at three sites in Arizona, Oklahoma, and the Dakotas. Participants numbered 3,296 subjects and included Pima, Maricopa, and T’ohono O’odham (Arizona); Apache, Caddo, Comanche, Delaware, Fort Sill Apache, Kiowa, and Wichita (Oklahoma); Oglala Sioux and Cheyenne River Sioux (South Dakota); and Spirit Lake (North Dakota) tribes and communities, with cohorts evenly divided among sites. Ascertainment of the degree of American Indian heritage was based on self-reporting of the heritage of the participants’ grandparents. Although this method has been validated independently against genetic markers, it contrasts with the Chilean study in which investigators determined ABO blood group distributions and mitochondrial DNA polymorphisms in Chilean Mapuche, Hispanic, and Maori tribes. In the recent study, female and male participants with 100% American Indian heritage varied from 46% and 44% in the Dakotas, to 74% and 71% in Oklahoma, to 93% (both genders) in Arizona. Overall, 100% American Indian heritage was present in 72% of women and 68% of men.

Because of selection bias in the Strong Heart Study, all subjects were 45 years or older. But, as inferred from the original Pima study (Fig. 1), this age range should provide maximum gallstone prevalence rates in women and a close approximation in men. Intersite differences were small, and cumulative prevalence rates increased to 74% in women 65 years or older (age-standardized prevalence, 66%) and 44% in men 65 years or older (age-standardized prevalence, 31%). These frequencies, which were found across the three geographically and culturally diverse regions, are significantly higher than the maximum gallstone prevalence rates in Chilean Mapuche women (50%) and especially Mapuche men (13%) despite their 88% American Indian heritage. In the study by Everhart et al., a strong positive correlation existed between risk of gallstones and the degree of North American Indian heritage when other factors were simultaneously controlled for. The authors’ conclusion that gallstone disease is found in epidemic proportions in American Indian populations and “not limited to a small group of related tribes of a single region” (Fig. 1) is timely and important information.

Apart from the association of gallstones with American Indian heritage and advancing age, multivariate logistic regression analysis revealed that waist circumference was associated with gallstones among American Indian men, whereas diabetes mellitus (type 2) and parity were associated in women. However, body mass index was associated only with prevalence of gallstone disease in women in an age-adjusted analysis. Similar to the original Pima study, the prevalence rates of diabetes mellitus and obesity were so high that they may be very important determinants of gallstone risk at the genetic or phenotypic levels, but the association is not easily quantified because of their high frequencies. In addition to the sizable morbidity and mortality rates associated with cholecystectomy, it is known that among Pima the death rate attributable to malignancies, especially gallbladder cancer, is 7 times higher in tribal members with gallstone disease. However, similar information is not available for the other tribes and communities in the latest study.

Pathophysiology of Gallstone Formation in American Indians

One clearly would like to know details of the gallstone phenotypes in American Indians and how their high prevalence rates of diabetes mellitus and severe obesity might contribute. Several interesting epidemiologic features concerning biliary cholesterol lithogenicity in American Indians compared with whites have been noted. (1) Cholesterol “solitaires” are much more common than the 8% found in whites (hence the “typicality” of Franz Ingelfinger’s litho-talisman). (2) Pigment gallstones are very rare, possibly related to the American Indians’ high degree of cholesterol supersaturation, which engenders phase-separated vesicles in bile that act as calcium bilirubin binders and decrease biliary saturation with calcium bilirubinate. (3) Symptomatic gallstones are considerably more frequent not only in female but also in male American Indians.

After recruitment in the early 1970s from the Rockefeller University in New York, Dr. Scott Grundy and his colleagues moved to Phoenix, AZ, to quantify the pathophysiology of cholesterol gallstone formation in American Indians of the US Southwest. Employing an innovative duodenal marker-perfusion technique, they measured individual secretion rates of all 3 biliary lipids in subjects with and without gallstones. They found major increases in biliary cholesterol secretion and significant decreases in bile salt secretion rates in American Indian gallstone patients. Both abnormalities combined to result in the production of the most marked cholesterol supersatura-
ations observed in human bile. Reuben et al. in London, UK, and Shaffer and Small in Boston, MA, performed similar studies in obese whites with this in vivo technique. Their findings revealed that the major abnormality in obese subjects, both with and without gallstones, was hypersecretion of cholesterol that was not compensated for by increased bile salt secretion rates, implying that there was a fixed level of bile salt synthesis and enterohepatic cycling of the bile salt pool in obesity. In mice, increased hepatic cholesterol synthesis drives cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in bile salt synthesis (via the neutral pathway), through the action of the oxysterol nuclear receptor liver-X-receptor α (LXRα). In obese humans, however, the increased total body synthesis rate of cholesterol is not necessarily coupled with increased CYP7A1, as shown by measurement of bile salt kinetics in obesity; so it is not surprising to find that the promoter region of human CYP7A1 does not contain a response element for LXRα. Differential gender frequencies of cholesterol gallstones in men and women may be related to CYP7A1 activity in that its gender-related expression may explain the male predominance of cholesterol gallstones in most susceptible inbred mice.

In contrast, in humans there is an appreciably higher female-to-male gender frequency of gallstones, as in American Indians and especially in whites. In mice, gallstones form only in response to massive cholesterol intake; the sterol level in a lithogenic diet corresponds to approximately 50% of mouse strains are totally resistant to cholesterol gallstones in most susceptible inbred mice. In 1966, R. B. McConnell, an astute Liv- ersdal physician, analyzed clinical incidences of gallstones and gallstone patients and concluded that liability to gallstone formation was most likely polygenic. This view was articulated by several others in the intervening years to explain the hepatic origins of lithogenic bile, but as we will show below, new data in mice suggest that at least obesity per se plays only an indirect role in cholelithogenesis via shared obesity and lithogenic genes.

Possible Genetic Origins of Cholesterol Gallstones Involving “Thrifty” Genes

A mid-19th century monograph on cholelithiasis by a notable French physician, Victor Albans Fauconneau-Dufresne claimed that cholelithiasis is often “hereditary”; half a century later, Lord Moynihan of Leeds articulated a similar opinion with only anecdotal documentation. In 1966, R. B. McConnell, an astute Liverpudlian physician, analyzed clinical incidences of gallstones in families, twins, and the relatives of young gallstone patients and concluded that liability to gallstone formation was most likely polygenic. This view was articulated by several others in the intervening years to explain the hepatic origins of lithogenic bile, but a genetic component was first proven by the elegant studies of William van der Linden on an extensive series of families, especially those with young probands with gallstones in northern Sweden. He opined that the genetic basis of gallstones could not get an airing among contemporary (1980s) “cholanologists,” because studies on the physical chemistry and pathophysiology of bile were so glamorous. In recent years, abundant evidence has accrued, especially from family and population studies, for a strong 30% genetic component to cholesterol gallstone disease.
In this secluded area several times the size of a 20,000-year period, spanning the nadir of the last glacial period, there were big game hunters who became trapped by mountainous cliffs on one side and glaciers on the other. Within the present-day Alaska there was scant food, and survival depended on hunting-gathering strategies. It has been suggested that this bleak, desolate, and inhospitable environment saw 10 to 11 months of harsh winter and was unlike any climate known on Earth today. By modern-day societies, Paleo-Indians developed ingenious methods for trapping and killing these large animals to feed their families. For a large family, victuals may not have been secured by male hunter-gatherers more than once a year.

As extrapolated from pre-Columbian skeletal remains, average life expectancy in these prehistoric times was no longer than late adolescence to early adulthood. Moreover, current thinking is that the highly seasonal nature of food sources led to the likelihood that successful survival, and indeed reproduction, depended on genes that produced a favorable advantage principally by efficient fat storage. The hypothesis that “thrifty” genes facilitated survival in the Pleistocene era, whereas today the same genes cause cholesterol gallstones, obesity, and non-insulin-dependent diabetes mellitus, is intriguing.

Certainly genetic evolution is most likely to have been accelerated by “scattered families, a population bottleneck favoring genetic drift, possibly a founder effect; and relatively sparse food supply and stringent environmental conditions.” As articulated elegantly by Weiss et al., these “thrifty” genes appear primarily to have involved lipid physiology, hepatobiliary function, and metabolism. Since digestion and absorption is so highly efficient in all Homo sapiens, it is likely that the major effect of those “thrifty” genes was to promote calorie storage in the form of adipose tissue for energy needs during extended winters. A key event for survival of a population with a shortened life span would naturally be early puberty, possibly facilitated by leptin from adipocyte secretion, and sufficient fat reserves to go through a successful pregnancy and nursing period as well. Good evidence that the scarcity of food favored the accumulation of “thrifty” (gallstone) genes in Paleo-Indians during their Beringian seclusion is the infrequent occurrence of cholesterol gallstones in present-day Siberians.

Recent prevalence rates of cholelithiasis by ultrasonography in a sample of 6,676 Europeoids (Russians and Ukrainians) and 997 Mongoloids (Evenks and Hakases) revealed 2.9% in southern and 1.5% in northern Mongoloids, compared with 4.5% and 8.8% for Europeoids. These prevalence rates are orders of magnitude less than in any North or South American Indian tribe or community today.

With Beringian deglaciation, which probably began 20,000 to 12,000 years BP, an inviting, narrow ice-free corridor opened between the Cordilleran and Laurentine ice sheets in northwest Canada. As the sea level rose as much as 300 feet, Paleo-Indians fled from their vast domain in Beringia, and within approximately 1,000 to 2,000 years, had populated all of North, Central, and South America. This rapid expansion into the Americas occurred principally by land, but there is emerging paleo-archaeological evidence that there was also a western sea route, because settlers appeared in coastal and inland...
Monte Verde, a part of southern Chile, as early as 12,500 BP, preceding settlement of some more proximal regions of Central and South America. Early American Indians experienced a sustained hunting-gathering lifestyle in the Americas for many millennia during which they diverged culturally and linguistically. However, in the case of the southwestern Indians, most notably the Pima, their subsistence depended on an agricultural economy for at least the last 2,000 years. Moreover, throughout the continent, their diets were apparently invariant for thousands of years, yet the diet changed radically in the mid-20th century, possibly with the appearance of the US Department of Agriculture’s (USDA’s) Commodity Food Distribution Program on Indian Reservations (http://www.commodityfoods.org), which included pre-packaged high-caloric diets composed of meat, poultry, fruits, vegetables, grains, vegetable oils, and peanut and dairy products.

One well-documented but not unique exception to this is the case of the Tarahumara Indians, who inhabit the Sierra Madre Mountains in the state of Chihuahua, Mexico. The diet of these Uto-Aztecan people, who are closely related to the Pima, still consists largely of legumes, tubers, berries, fruits, and nuts. Moreover, both men and women value running and exercise constantly, often traveling over rugged mountainous terrain for distances of 10 to 30 miles per day. The Tarahumares (the name means “fleat of foot”) are remarkably free of obesity and diabetes mellitus and reputedly do not suffer from gallstones. The NIH have also been studying the Mexican Pima, also called Mountain Pima (nearby cousins of the Tarahumara) residing in the Sierra Madre Mountains of northern Mexico. They also live on a subsistence economy with traditional dietary habits (corn and beans) and are lean and extremely active. Dr. Peter Bennett believes that they, like the Tarahumara Indians, probably do not have gallstone disease. Possibly the best evidence for the “sensitivity” of American Indian “thrifty” genes to the dietary environment is an on-site experiment performed on Tarahumara Indians by Dr. William E. Connor and colleagues over a decade ago. These investigators challenged 13 fit and lean Tarahumara Indians (5 women and 8 men including one adolescent) with an affluent Western diet (4,100 kcal/d) for a short period of 5 weeks. The recipients were encouraged to continue their normal lifestyle, but they gained weight rapidly (average 7%) and elevated their serum low-density lipoprotein cholesterol levels by 39% and their very-low-density lipoprotein triglyceride levels by 18%. Although not investigated, we would wager that their bile became lithogenic! Dr. William E. Connor has voiced the opinion, “I suspect that they (Tarahumares) will remain a population without the diseases that their US counterparts are developing because of their poverty. When the crops failed over the past couple of years (pre-1997), there was outright starvation.”

An anecdotal report suggests that obesity may have been sporadic in American Indians for some centuries. In the early 18th century when the Jesuit missionary, Padre Eusebio Francisco Kino (born “Chini” in the Tyrolean village of Segno in the Italian Alps) made his sixth visit to the Pima (1702), his American Indian friends had become so “stocky” (rechoncho) that he hardly recognized them. Moreover, by the end of the 19th and early 20th century, Pima obesity was documented frequently in photographs, especially of Pima women. They can be found in a classic work, “The Annual Report of the Bureau of American Ethnology to the Secretary of the Smithsonian Institution for 1904-05” (see also Fig. 2). In a science feature that appeared in the New York Times in 1980, Ms. Jane Brody made the fanciful prediction that if Father Kino could see the Pima today, “he would be even more astonished,” which is certainly true according to Peter Bennett, who has studied the Arizona Pima for over 40 years. Although the caloric intake of the Pima is not much different from American whites, 80% are grossly obese, presumably because of the aggressive nature of their many “thrifty” obesity genes that cause efficient use of calories.

Paleopathological evidence from pre-Columbian Indian burial sites is the most persuasive available that gallstones were probably once quite rare in American Indians. These excavations and autopsies at several sites in North and South America have described infrequent...
“small, multiple and pearly cholesterol stones.” Stone prevalence rates in mummified remains range from 2 of 75 Chileans, dating from 1,900 to 1,700 years BP, to 6 of 1,000 Ohioans at a late Woodland site, dating from 1,000 to 800 years BP, and autopsies in over 300 Peruvians dating from 2,600 to 320 years BP (the latter date being in the late 17th century) failed to show even one example of gallstones. An ethno-botanical compendium of medicines used by Mesquakie Indians, which contains a total of 300 herbal remedies, mentions neither gallbladder disease nor any remedy for it. Moreover, at the beginning of the 20th century, a published list of the 28 diseases commonly seen in the Pima made no mention of gallstones, nor biliary pain, nor jaundice. Furthermore, in their vast repertoire of “medicine songs,” which covers every conceivable ailment afflicting the Pima tribe, and indeed is their largest class of folksongs, not one refrain refers to gallstones or gallbladder disease. Therefore, the preponderance of clinical and ethnologic evidence supports the concept that “thrifty” human cholesterol gallstone alleles (LITH genes) lay dormant for thousands of years in the ancestors of today’s American Indians. However, in the middle of the 20th century, possibly the decade immediately preceding and the decade during and after World War II, the genes became unmasked by the radical environmental change previously alluded to, possibly the donation of USDA prepackaged commodity foods, and the American Indians’ lipid metabolism responded explosively to this perturbation. This stands in contrast to whites, who putatively evidenced milder responses to a similar environmental challenge possibly during the Industrial Revolution a century earlier. Most American Indians became very obese and acquired diabetes mellitus, and cholesterol gallstones formed rapidly and frequently. The “chololithogenic” environment of the latter 20th century Western diets seems to have been specifically consumed foods rich in refined sugar and fat-containing foods with very low fiber content. Moreover, since hardship farming became less common, physical activity markedly decreased. In his Bureau of American Ethnology Report (1908), Russell opined that even then some young Pima men acquired a degree of obesity that “is in striking contrast with the tall and sinewy Indian conventionalized in popular thought.” More recent evidence from large-scale surveys in whites has suggested that sedentary lifestyles in both genders correlate positively with risk of cholecystectomy, a surrogate for symptomatic cholelithiasis, as well as the possibility of new gallstone formation. In the case of “thrifty” American Indian genes, the new diseases may be not only at the genetic level, but also at the metabolic level: in humans, diabetes mellitus and obesity are factors predisposing to gallstones, and marked obesity predisposes to diabetes mellitus.

Genetics of Experimental Cholelithiasis in Inbred Mice

Our conjoint work for nearly a decade has focused on identifying cholesterol gallstone susceptibility loci (Lith alleles) in inbred mice. As is the case for the human disease, cholesterol gallstones in the mouse are a complex polygenic trait. Although there is no assurance that LITH genes in humans and Lith genes in mice will be the same, the conservation of mammalian genomes for tens of millions of years is reassuring. In 1995, we published use of quantitative trait loci (QTL) mapping, a powerful genetic technique, to locate the first cholesterol gallstone gene (Lith1) on mouse chromosome 2. Subsequent studies determined its canalicular transporter expression on hepatocytes, fine mapping of the locus, and hepatobiliary phenotypes, resulting in the bile salt export protein (BSEP, officially ABCB11) being a candidate gene for Lith1. In the same cross, Lith2 was discovered on mouse chromosome 19 and may be identical to the multidrug resistance-related protein, isoform 2 (MRP2, officially ABCC2), which is the conjugate organic anion transporter responsible for much of bile salt–independent bile flow. Since summarizing our work on 9 QTLS located on 19 murine autosomes plus the X chromosome in January 2001, we have completed an extensive strain survey involving most of the genetically diverse mouse strains (Paigen B, Bouchard G, Carey MC, http://aretha/jax.org pub-cgi/phenome/mpa cgi? rtu= studies&details id = 29). We found that somewhat less than half of the strains were susceptible, often favoring males in contrast to the female gender predominance in American Indians (Fig 1) and whites. We then chose 4 of the most genetically susceptible and resistant strains to outcross with 4 gallstone resistant strains, which were arranged (on paper) with alternating susceptible and resistant strains in a “daisy chain” configuration; experimentally each strain was outcrossed with both of its neighbors. Although these experiments are still ongoing, it appears that Lith genes (either main or interacting genes) may be present on essentially all murine chromosomes. With the use of modern genetic strategies to define the genetic regions, the entire ensemble of Lith loci in the mouse is capable of being located. Table 1 lists main QTL (Lith) loci numbers 1 and 5-9 from different crosses in inbred mice and whose candidate genes for Lith alleles appear relevant to American Indian cholesterol gallstone formation. The table also suggests how the orthologous genes in humans could explain dysfunction of several key proteins in forming abnormal bile both chemically and pathophysiologically in post-pubertal Ameri-
Table 1. Selected Quantitative Trait Loci (QTLs of Lith Alleles) for Cholesterol Gallstones in Inbred Mice That Might Be Relevant to American Indian Cholesterol Gallstone Formation

<table>
<thead>
<tr>
<th>QTL</th>
<th>Chr</th>
<th>Ref</th>
<th>Candidate Genes*</th>
<th>Candidate Function</th>
<th>Predicted Pathophysiological and Biochemical Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lith1</td>
<td>2</td>
<td>60-63</td>
<td>i. Nr1h3 (Lxrα) †</td>
<td>Oxysterol nuclear transcription factor: activates transcription of many cholesterol homeostatic genes (e.g., Cyp7a1, Abca1, Abcg5/Abcg8, Lpl)</td>
<td>i. Unlikely to influence bile salt synthesis via CYP7A1 but may augment cholesterol secretion rate.</td>
</tr>
<tr>
<td>Lith5</td>
<td>9</td>
<td>64</td>
<td>i. Lpc</td>
<td>Hepatic lipase: involved in both chylomicron remnant and high density lipoprotein cholesterol uptake</td>
<td>i. May increase exogenous and endogenous cholesterol delivery to liver.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ii. Scap</td>
<td>SREBP-cleavage activating protein: regulates proteolytic activation of SREBPs, transcription factors whose targets include cholesterol and bile acid synthetic genes (e.g., Hmgcr and Cyp8b1, respectively)</td>
<td>i. May up-regulate cholesterol synthesis and contribute to defective bile salt synthesis via acidic pathway.</td>
</tr>
<tr>
<td>Lith6</td>
<td>6</td>
<td>63-65</td>
<td>i. Pparg</td>
<td>Peroxisome proliferator-activated receptor: ligand-activated nuclear transcription factor involved in lipid synthesis and storage; associated with hyperlipidemia, obesity, insulin resistance, type 2 diabetes, and CYP7A1 activity indirectly</td>
<td>i. May contribute to diabetes type 2 and obesity, thereby increasing cholesterol synthesis. May inhibit CYP7A1 and block bile salt synthesis via the neutral pathway.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ii. Apobec1</td>
<td>Apolipoprotein B mRNA editing complex 1 converts full-length ApoB100 to ApoB48</td>
<td>ii. May contribute to more rapid delivery of chylomicron cholesterol to liver and hence biliary cholesterol hypersecretion.</td>
</tr>
<tr>
<td>Lith7</td>
<td>10</td>
<td>65</td>
<td>i. Nr1h4 (Fxr)</td>
<td>Bile acid nuclear transcription factor: controls feedback inhibition of bile acid synthesis via CYP7A1, activates transcription of bile salt transporters including Abcb11, Abcc2, Slc10a1 (Ntcp), and Fabp6 (Hlpb)</td>
<td>i. May inhibit bile salt synthesis via the neutral pathway.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ii. Scp2</td>
<td>Sterol carrier protein 2: involved in intracellular cholesterol transport, and possibly biliary lipid secretion</td>
<td>ii. May promote biliary cholesterol hypersecretion.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>iii. Lepr</td>
<td>Leptin receptor: may influence biliary secretion of plasma-derived cholesterol</td>
<td>iii. May promote coupling of cholesterol to other biliary lipids.</td>
</tr>
<tr>
<td>Lith8</td>
<td>4</td>
<td>65</td>
<td>i. Nrd1b2 (Shp1)</td>
<td>Short heterodimer partner 1: nuclear transcription factor that inhibits bile acid synthesis by interaction with LRH-1, the competence factor for Cyp7a1 transcription</td>
<td>i. May inhibit bile salt synthesis via the neutral pathway.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ii. Lepr</td>
<td>Leptin receptor: may influence biliary secretion of plasma-derived cholesterol</td>
<td></td>
</tr>
<tr>
<td>Lith9</td>
<td>17</td>
<td>65</td>
<td>i. Abcg5/Abcg8</td>
<td>Canalicular (and intestinal) half-transporters for cholesterol and neutral sterols</td>
<td>i. May greatly augment biliary cholesterol secretion.</td>
</tr>
</tbody>
</table>

Abbreviations: Chr, chromosome on which the QTL is located; Ref, reference.
*Official gene symbol with common gene symbol in parentheses.
†Although Abcb11 (Bsep) remains a viable candidate gene for Lith1, the gene listed here is also a candidate gene within the Lith1 locus and is further ruled in by an independent intercross.65

American Indians. In one of these crosses65 (Table 1), three major Lith QTLs (Lith7, Lith8, Lith9) were found. They may be caused by polymorphisms of genes leading to up-regulation of ABCG5 and ABCG8 (Lith9), the putative canalicular half-transporters responsible for transporting much of cholesterol into bile, and up-regulation of FXR (Lith7), the bile salt nuclear receptor that down-regulates cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme in de novo bile salt synthesis via the neutral pathway.11 With respect to Lith8,65 the putative genes involved may lead to inhibition of bile salt synthesis, augment intracellular cholesterol traffic and hepatic uptake of lipoproteins, and couple cholesterol to biliary lipids for bile secretion (H. Wittenburg, MA Lyons, B. Paigen, M.C. Carey, unpublished observations, 2002). It will certainly be worth scrutinizing whether these genes are major gallstone genes in American Indians since many of the biochemical and pathophysiological characteristics of the Pima11,24 are similar to the phenotypes in these strains of mice.62-65

In connection with centripetal obesity typical of American Indians11 (Fig. 1), we examined susceptibility to cholesterol gallstone formation in three polygenic and five monogenic strains of overweight mice.66 Compared with background strains, some murine models of obesity increased while others decreased cholesterol gallstone susceptibility.66 Therefore, cholesterol gallstone formation in overweight mice is not simply a secondary result of obesity per se, but rather, certain obesity genes impact cholesterol gallstone risk while others have no effect. Thus, it is possible that in American Indians obesity genes and gallstone genes are agonist or antagonist in the stone diathesis, irrespective of the magnitude of obesity per se. Indeed, among American Indian gallstone populations,19 we are provided with a rich population base to perform such studies to differentiate effects of obesity genes from cholesterol gallstone genes on the gallstone phenotype, as well as gene-environmental interactions. We hope to be guided by results of inbred mouse studies, which will, in the interim, have revealed the entire Lith allele ensemble.11
Will all this work eventually have any relevance to gallstone prevention and new treatments? We believe the answer is unequivocal yes. The late James V. Neel, originator of the “thrifty” gene hypothesis and a leading founder of modern human genetics, wrote, “Much more effective and cost-efficient improvement to human well-being can be made by tailoring the environment to the genome rather than the genome to the environment: Lifestyle interventions are more practical than genetic ones.” It is highly unlikely that this admonition can be applied easily to American Indians to alleviate their cholesterol gallstone epidemic, because the environmental changes that interacted with thrifty genes to produce the gallstone diathesis are not readily identifiable. Moreover, lifestyle changes are notoriously difficult to implement and sustain, especially among impoverished peoples. Besides, such incursions would depend critically on much fundamental and difficult-to-obtain information of gene-gene as well as gene-environmental interactions. However, the new science of nutrigenomics might stimulate a careful search for micronutrients in the original American Indian diets that may bring unexpected results to reverse or ameliorate this rocky state of affairs. Nonetheless, we are more inclined to think that it is the surfeit of both micro- and macronutrients in American Indian diets for over half a century that is at least partially responsible for the gallstone epidemic and other “thrifty” gene dysfunctions; it was a salutary lesson to appreciate that it took only 5 weeks for gene-environmental interactions to become evidenced phenotypically by plasma lipid profiles in the Tarahumara Indians following consumption of a high-calorie Western diet.

On the other hand, targeting genes to normalize their function would not necessarily involve making “transgenic humans,” especially for a disease that is not often fatal. A good recent example is the widespread use of thiazolidinediones for treating type 2 diabetes mellitus, which has heralded a new era of clinical pharmacogenomics. These drugs activate the peroxisomal proliferator-activated receptor (PPARγ), a nuclear receptor that regulates the expression of multiple genes involved in lipid metabolism. PPARγ has been considered the “ultimate thrifty gene,” and it has been proposed that a maladapted “thrifty” response coordinated by PPARγ contributes to the recent rise in the prevalence among whites of obesity, atherosclerosis, insulin resistance, type 2 diabetes mellitus, and perhaps gallstones (at least in the inbred mouse; see Lith6 in Table 1). This fits well with the possibility that PPARγ or another master regulator, such as FXR as suggested by our mouse studies, may also be involved in the gallstone epidemic among American Indians (Table 1). Hence, because mapping of regulatory genes in the inbred mouse allows us to look for the orthologous loci on the human genome, then clearly American Indian Lith6 alleles will be discovered. It is not too far-fetched to believe that one day physicians responsible for the health of American Indian peoples will be prescribing agonists or antagonists for several hepatic lipid transporters (Table 1) such as ABCG5 and ABCG8, or nuclear receptors (LXRα, FXR, PPARγ) and other transcription factors and rate-limiting enzymes (e.g., CYP7A1), to prevent gallstones totally and hence eradicate a major contributor to the American Indian’s burden.

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