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# Major loci influencing serum triglyceride levels on 2q14 and 9p21 localized by homozygosity-by-descent mapping in a large Hutterite pedigree

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**Serum triglyceride (TG) level is a well-known risk factor for cardiovascular disease, a leading cause of morbidity and mortality in Western countries. Although genome-wide scans for TG have been conducted in several populations, few loci have shown strong evidence for linkage. The Hutterites are a founder population, which practices a communal lifestyle that includes a uniformly high-fat, high-cholesterol diet. We measured serum TG in 485 Hutterites ≥14 years old and performed a genome-wide scan to find genetic determinants of the observed variation in TG levels, using mapping methods that take advantage of the extensive inbreeding and linkage disequilibrium (LD) in this single, 1623-member pedigree. We report two highly significant associations with TG levels, alleles at D2S410 on 2q14 (locus $P = 5.8 \times 10^{-6}$, genome-wide $P = 0.005$) and at *IFNA* on chromosome 9p21 (locus $P = 4.3 \times 10^{-5}$, genome-wide $P = 0.024$). In each case, homozygosity at the locus is associated with low TG levels, suggesting that alleles at nearby loci may protect against high TG levels.**

## INTRODUCTION

Hypertriglyceridemia is an important risk factor for cardiovascular disease (1–5). Variation in serum triglyceride (TG) levels among individuals results from both environmental and genetic factors (6–9), with heritability estimates of approximately 40% in diverse groups (7,9,10). Eight previous genome scans have been performed for TG (10–17), but in only one did a locus reach genome-wide levels of significance (12).

Because of the multifactorial and complex nature of the phenotype, we have focused our genetic studies on the Hutterites (18), a young founder population of European descent. The Hutterites are a religious sect that originated in the Tyrolean Alps in the 1500s. Between the mid-1700s and mid-1800s, while in Russia, the population grew in size from 120 to >1000 members (19). In the 1870s, 900 of these members migrated to what is now South Dakota, and approximately half settled on three communal farms. The population has since expanded dramatically, with >35 000 Hutterites living in >350 communal farms (i.e. colonies) in the northern USA and western Canada. Genealogical records trace all extant Hutterites to <90 ancestors who lived in the early 1700s to early 1800s (20). The relationships among these ancestors are unknown, and some of them may have been related (21). The three original South Dakota colonies have given rise to the three major subdivisions of the modern Hutterite population, the Schmiedeleut, Dariusleut and Leherleut. Members of each subdivision have remained reproductively isolated from each other since 1910 (22). The subjects of our study are Schmiedeleut Hutterites who live in nine colonies in South Dakota and are descendants of 64 of the 90 Hutterite ancestors. The 485 individuals in this study are related to each other in a single, 1623-person, 13-generation pedigree with only 64 founders (23).

The Hutterites offer several advantages for complex trait mapping. First, the limited number of ancestors should minimize genetic heterogeneity. That is, the number of alleles influencing variation in complex phenotypes should be reduced compared with outbred populations. Second, the relatively recent origins of the Hutterites results in extensive linkage disequilibrium (LD), allowing the use of genome-wide association-based tests for localizing disease genes. For example, in a study of STRPs on two chromosomes unselected for disease, over 70% of locus pairs separated by up to 4 cM

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**Table 1.** Median (25–75% quartiles) lipid levels and BMI by age. According to NCEP guidelines (31), the normal ranges for each phenotype are: TG (30–149 mg/dl), total cholesterol (120–199 mg/dl), LDL cholesterol (60–129 mg/dl), HDL cholesterol (40–80 mg/dl), BMI (<25 kg/m$^2$). Median values that exceed normal ranges are underlined

| Age group | TG | Total cholesterol | LDL cholesterol[a] | HDL cholesterol | BMI |
|---|---|---|---|---|---|
| 14–24 ($n=153$) | 78 (61–108) | 176 (156–190) | 103 (87–123) | 49 (42–62) | 21.7 (19.6–24.4) |
| 25–44 ($n=224$) | 109 (74–173) | 200 (176–228) | 127 (106–152) | 44 (37–54) | 26.4 (23.3–29.7) |
| 45–64 ($n=76$) | 146 (108–230) | 226 (202–248) | 147 (132–165) | 44 (35–52) | 28.5 (26.3–32.6) |
| 65–89 ($n=32$) | 153 (116–218) | 232 (211–256) | 149 (139–166) | 40 (36–50) | 27.9 (26.4–32.8) |
| All ($n=485$) | 106 (73–168) | 195 (170–228) | 124 (101–149) | 46 (38–55) | 25.5 (21.8–29.2) |

[a]LDL cholesterol was not calculated for 33 individuals with TG >300 mg/dl (2 age <25, 18 age 25–44, 9 age 45–64, 4 age >65).

showed significant levels of LD (24). Third, the Hutterites live communally. Of particular interest, smoking is prohibited and all meals are prepared and eaten in communal kitchens (18,25). All colonies use traditional recipes, which are high in saturated fat, protein and sodium and high in energy intake (26,27). Men and women participate in traditional gender roles on the colony, and individuals retire at age 45, after which their lifestyle is relatively sedentary (19). This communal lifestyle ensures that environmental risk factors, including diet, are remarkably uniform. Such homogeneity should reduce the confounding effects of environmental risk factors and enhance the role of genetic variation on disease risk. Furthermore, Hutterites living in three South Dakota colonies had elevated weight and blood pressure and died an average of 10 years younger compared with a control population living in Sioux Falls (28). In the nine colonies that participated in this study, cardiovascular disease, including myocardial infarction, stroke and hypertension, accounted for 51% of deaths among Hutterites whose cause of death was known and who died after age 40 (unpublished data). Cancer, the second leading cause of death in this age group, accounted for only 23%. The remaining deaths were attributed to diabetes mellitus (2%), infectious causes (8%), brain tumors (3%), Alzheimer's disease (3%), and old age (10%).

We previously estimated the heritability ($h^2$) of TG in the Hutterites as 0.37 (29), similar to the other populations cited above. However, estimates in the Hutterites more closely reflect the genetic component than previous estimates because the confounding effects of household environment are minimized in the Hutterites, as relationships between all individuals in the 1623-person pedigree are considered (18). Thus, heritability of TG in the Hutterites may actually be higher than in other populations. Here we report the results of a genome-wide scan for TG in the Hutterites, using two homozygosity-by-descent (HBD) mapping methods that take advantage of the extensive inbreeding in the population and consider the genealogical relationships between all pairs of individuals (30). We identified two genomic regions that were associated with TG levels and met genome-wide levels for significance.

## RESULTS

Because our sampling strategy was population-based and included all individuals ≥14 years, the average age was young (mean = 34.5) compared with other genome screens. Nonetheless, lipids and body mass index (BMI) were elevated, on average, even in the younger age groups (Table 1). For example, half of all the individuals in the 25–44 year age group had elevated total cholesterol (≥200 mg/dl), half had elevated LDL cholesterol (≥130 mg/dl), one-third had elevated TG levels (≥150 mg/dl), and 64% were overweight (BMI ≥ 25 kg/m$^2$). By age 65, 56% of individuals had high TG levels. The distribution of TG in the sample is shown in Figure 1. Overall, 144 individuals (30%) had TG values exceeding the recommended normal range (31).

TG levels were significantly correlated ($P < 0.001$) with total cholesterol ($r = 0.328$), LDL cholesterol ($r = 0.320$), HDL cholesterol ($r = -0.533$) and BMI ($r = 0.350$); however, none of the most significant results of genome screens overlapped between any of these phenotypes (discussed in 18 and unpublished data).

In our genome screen for TG, one locus reached genome-wide (GW) significance using the locus-specific HBD analysis. This method tests for association with HBD across all alleles at a locus, and is therefore a test of linkage. The major (highest) linkage peak was on chromosome 2q14 at 131 cM (LOD = 3.54, $P = 5.4 \times 10^{-5}$; GW $P = 0.019$) and is clearly the most significant linkage peak in the genome scan (Fig. 2). The second largest peak is on chromosome 9p21 (LOD = 1.83, $P = 0.0036$; GW $P = 0.734$), which does not meet the criteria for suggestive linkage (see methods). The genomewide results are available for both HBD and ASHBD analyses on our website (http://www.genes.uchicago.edu/TGresults).

Two loci reached genome-wide significance using allele-specific HBD analysis (ASHBD). This method tests for associations with HBD for specific alleles at each locus (Table 2). The major loci identified were on chromosome 2q14 (D2S410 at 125 cM; $P = 5.8 \times 10^{-6}$; GW $P = 0.005$) and chromosome 9p21 (*IFNA* at 36 cM; $P = 4.3 \times 10^{-5}$, GW $P = 0.024$). A second, adjacent marker on 2q14 reached suggestive significance (D2S1328 at 132 cM; $P = 1.9 \times 10^{-4}$; GW $P = 0.102$). Both D2S410 and D2S1328 lie within the most significant linkage peak, and the *IFNA* microsatellite locus lies within the second most significant linkage peak on 9p (Figure 2). In all of these cases, HBD for the significant allele at these loci were associated with low TG levels.

The genome scan was repeated using age$^2$, or age$^2$ and BMI, in addition to age and gender, as covariates, similar to other studies (10,12–15). Including age$^2$ in the model increased the estimate of $h^2$ to 0.43, and adding BMI increased $h^2$ to 0.49. However, the linkage peak on chromosome 2 was unchanged when age$^2$ and/or BMI were included in the linkage analysis (Fig. 3). Including age$^2$ in the ASHBD analysis also had little

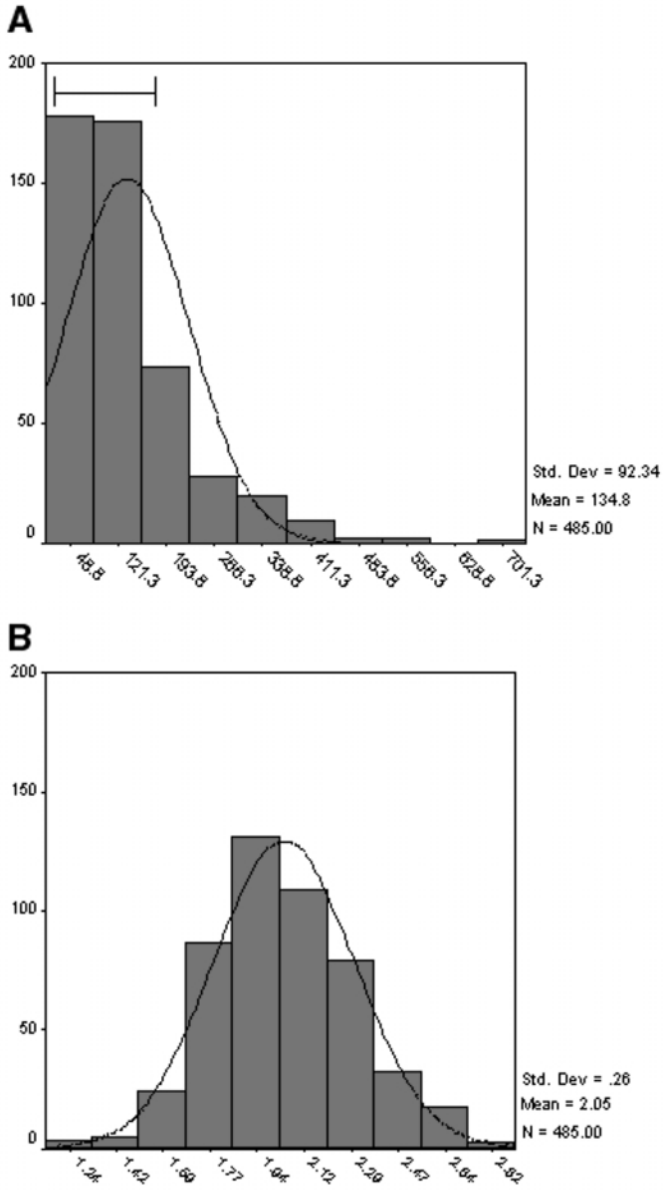


**Figure 1.** Histograms showing the distribution of TG levels in the Hutterites. Normal curves are overlaid for comparison. (a) Untransformed TG, bar indicates normal TG range; (b) $\log_{10}$(TG).

effect on the significant associations (data not shown); however adding BMI reduced the significance of the associations with both 2q and 9p loci. The 170 bp allele at D2S410 remained significant at the genome-wide level ($P = 3.0 \times 10^{-5}$; GW $P = 0.019$), but the 150 bp allele at the *IFNA* locus did not ($P = 2.7 \times 10^{-4}$; GW $P = 0.121$). No new loci reached genome-wide significance by either method.

## DISCUSSION

We report two novel, and perhaps major, loci that contribute to variation in serum TG levels: one on 2q14 and another on 9p21. Only one previous genome screen identified a major (genome-wide significant) locus influencing TG levels, which was on 15q in Mexican-Americans (LOD 3.88) (12). Neither our study nor six other genome screens detected linkage to this region. Other genome screens in different population samples reported evidence for suggestive linked loci on chromosomes 2p (13), 7q (10,12), 9q (17), 12q (16), 13q (15), 17q (16) and 20 (14). None of these loci appear to have strong effects in the Hutterites. Overall, there is little consensus among studies or between our studies in the Hutterites and the previously reported genome scans in Mexican American families (12), Pima Indian families (13), Framingham families (10), Rochester (Minnesota) families (15), German myocardial infarction families (17), obese families (16), hypertensive sib pairs (14), or Finnish familial combined hyperlipidemia families (11). It should be noted, however, that our methods only test under a recessive genetic model. Thus, although we had increased power to detect recessive alleles, we may have had little power to detect dominant or additive alleles (30).

Nonetheless, we detected two chromosomal regions with major effects on serum TG levels in the Hutterites, neither of which was detected in other genome scans. Although founder populations have been valuable for mapping single-gene, Mendelian traits, their utility for mapping complex phenotypes, such as TG levels, has been proposed but not proven. In theory, the reduced genetic heterogeneity and increased LD should facilitate mapping of genes that contribute to phenotypes with multifactorial etiologies (32–34). In addition, the relatively homogeneous environment that characterizes most founder populations should reduce the non-genetic factors that contribute to variation. The Hutterites in particular are exposed to a very uniform environment. Not only do the Hutterites have relatively small interpersonal differences in diet, but their high-fat diet is both universal and lifelong. Further, after age 45 these individuals have minimal physical activity. Thus, genotypes influencing TG levels should be highly penetrant because nearly all individuals are exposed to a high-risk lifestyle. This may be reflected in the young age at which elevated cholesterol and TG levels are observed in the Hutterites (Table 1). In this context, it is particularly interesting that the two most significant findings in this study were associations with *low* TG levels, suggesting that homozygosity for alleles at these loci may protect against high TG levels even when exposed to a high-risk lifestyle. If so, linkages to these regions might not be detected in studies of families ascertained on the basis of high TG or cholesterol levels or the presence of cardiovascular disease (11,14,15,17). Nonetheless, identifying alleles that protect against elevated TG levels, particularly in the presence of a high-risk lifestyle, could have significant implications for both population screening and identifying novel therapeutic targets.

Our strongest linkage and association are with loci on chromosome 2q. Note that this region remained significant when BMI was added as a covariate, indicating that the underlying variant does not act indirectly through body size. A drop of 1 LOD from the top of the linkage peak defines a region of 10 cM (128–138 cM from p-ter), which contains ~45 genes (UCSC Human Genome Project Working Draft, June 2002 assembly, http://genome.cse.ucsc.edu/). D2S410 lies 3 cM to the left of this narrowly defined linkage peak, and D2S1328 falls within the peak. Because D2S410 shows a stronger association with TG than D2S1328 by ASHBD, we

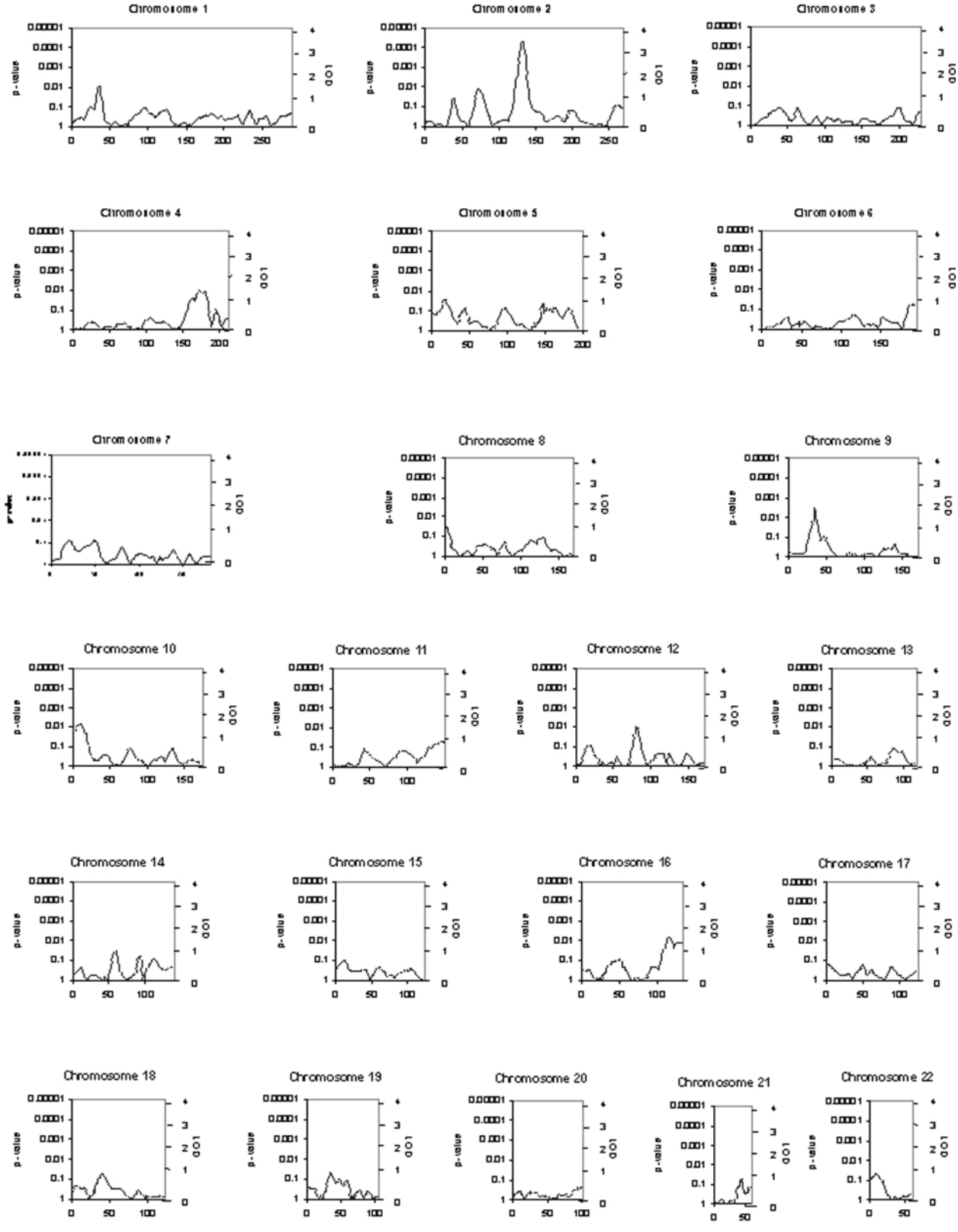


**Figure 2.** Results of a genome-wide scan for TG linkage using the locus-specific HBD model. Distance in cM is indicated on the *x*-axis of each plot.

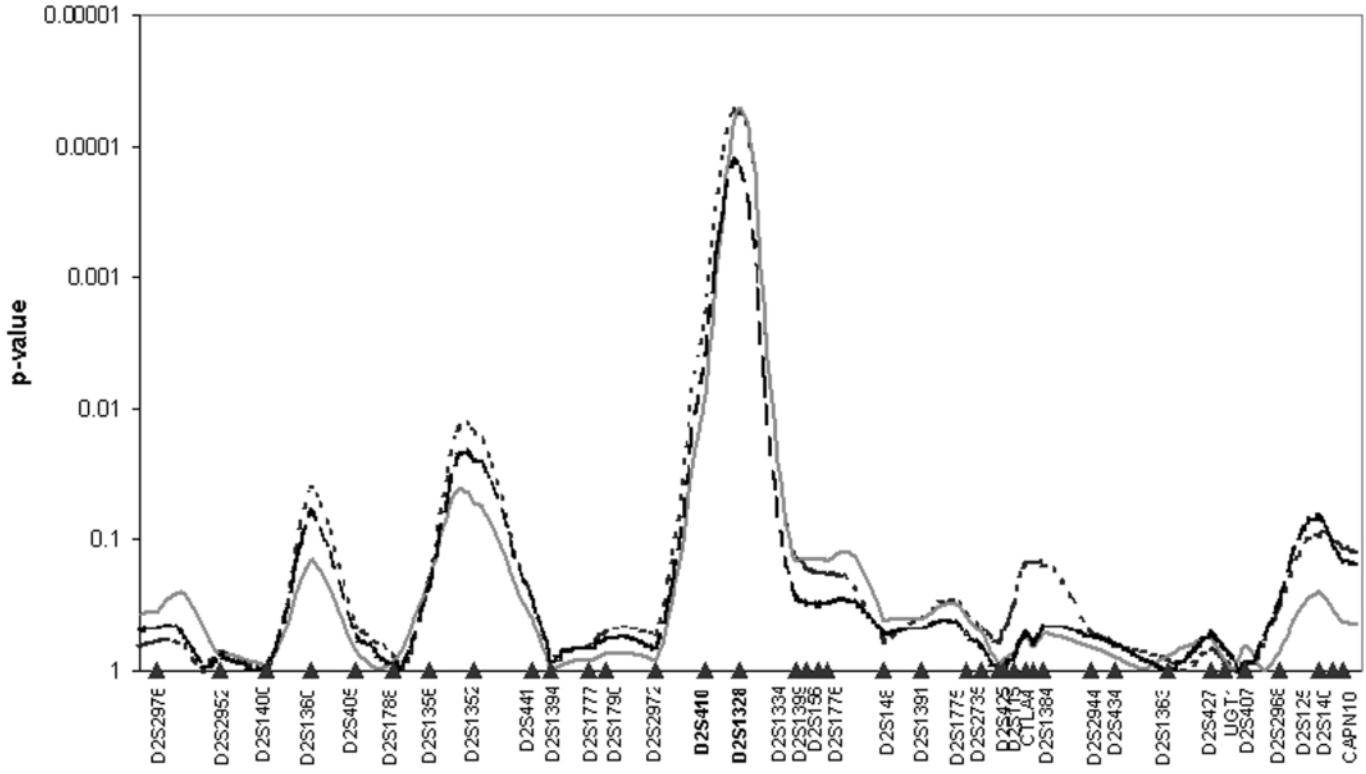


**Figure 3.** Effect of adding age$^2$ and BMI as covariates to the HBD linkage analysis of chromosome 2. Covariates included: age and gender (dotted line); age, age$^2$ and gender (dashed line); age, age$^2$, gender and BMI (solid line). Positions of genotyped markers are indicated by labeled triangles along the bottom of the graph.

**Table 2.** Results of genome-wide association using ASHBD. All STRP loci with alleles that were associated with TG with $P < 6.7 \times 10^{-4}$ (see Methods) are shown. The direction indicates whether homozygosity is associated with high (+) or low (−) TG levels

| Locus | Chromosome band | Distance from p-ter (cM) | Allele (bp) | Direction of effect | Calculated *P*-value[a] | GW *P*-value[b] |
|---|---|---|---|---|---|---|
| D2S410 | 2q14 | 125 | 170 | − | $5.8 \times 10^{-6}$ | 0.005 |
| D2S1328 | 2q14 | 132 | 158 | − | $1.9 \times 10^{-4}$ | 0.102 |
| *IFNA* | 9p21 | 36 | 150 | − | $4.3 \times 10^{-5}$ | 0.024 |

[a]Bonferroni-corrected (see Methods).
[b]Based on permutation test (see Methods).

hypothesize that the gene of interest is on the proximal side of the peak. Although this decreases the number of potential genes, the region still needs to be narrowed before initiating positional cloning studies.

The linkage and association on 9p are located in the same region that showed linkage to HDL cholesterol in Mexican Americans (35). The linkage results for HDL cholesterol in the Hutterites generally track closely to the results for TG, including a modest peak at this location on 9p (at 36 cM, LOD = 1.20, $P = 0.019$; GW $P = 0.998$). However, unlike the results for TG, there were no significant associations with HDL at this or nearby loci (data not shown). Therefore, the effect of this variant on HDL cholesterol level may be due to the correlation between these two phenotypes. Further, the reduction of the TG association by the addition of BMI as a covariate suggests that the underlying variant may have a broader effect on metabolism than a simple direct effect on TG levels.

The TG-associated 9p marker is located in the type 1 interferon (*IFN*) gene cluster (36). This is a particularly interesting candidate region because elevated levels of interferon α in AIDS patients may be responsible for their high TG levels (37), and interferon α therapy for chronic hepatitis C is associated with a significant increase in TG levels (38). The mechanism by which interferon affects TG appears to be indirect, through lowering of lipoprotein lipase, hepatic triglyceride lipase and cholesterol esterase transfer protein activities (39). Further investigation is needed to determine how genes in the *IFN* cluster of 9p may affect TG levels in the general population, but these collective data suggest that variation in one or more type 1 *IFN* genes plays a role.

Although these susceptibility loci have been identified in a founder population, we do not expect their significance to be limited to this population. In general, allele frequencies at STRP loci in the Hutterites are similar to those of outbred

populations, and nearly all common alleles that are present in outbred families are also present in the Hutterites. For example, a comparison of 32 autosomal STRP loci showed that 90% of alleles with frequencies >0.10 in the CEPH families are present in the Hutterites, with their frequencies highly correlated ($r^2 = 0.759$, $P < 0.001$). Of those alleles that were absent in the Hutterites, 94% had frequencies <0.10 in the CEPH families (40). Based on these observations, we expect that associations with common disease alleles that are present in the Hutterites are also likely to be present in outbred populations and to occur at similar frequencies. This expectation was met in our studies of variation at the *IL4RA* locus, which revealed similar frequencies of disease-associated variants in the Hutterites and in outbred Caucasian families (41). Additionally, in a recent study of 65 polymorphisms in 36 genes that have been associated with cardiovascular disease phenotypes, the associations in the Hutterites were similar to associations in outbred populations. For example, cholesterol ester transfer protein (*CETP*) promoter polymorphisms were associated with HDL cholesterol level ($P = 0.00001$), the *APOE* E2 allele was associated with low LDL cholesterol ($P = 0.001$), and the angiotensin (*AGT*) thr235 allele was associated with high blood pressure ($P = 0.03$) (42), similar to studies in other populations. Thus, it is likely that the protective or susceptibility alleles discovered in the Hutterites will also influence TG levels in the general population, but owing to their uniform environment and reduced genetic heterogeneity are easier to detect in the Hutterites.

## METHODS

### Subjects and clinical evaluation

We studied 522 individuals over the age of 14, during field trips to nine Hutterite colonies in South Dakota in 1996–1997. Our population-based sampling strategy resulted in nearly all individuals living in these colonies being included in our studies.

Height was measured by use of a plastic stadiometer, to the nearest 3 mm, with the subject in stocking feet; weight was measured using a Tanita model TBF105 scale, with the subject wearing light clothing. BMI was calculated as weight (in kilograms) divided by the square of height (in meters). We determined total cholesterol, HDL cholesterol and TG levels in blood samples drawn after an overnight fast by measurement with an automated Kodak Ektakem DT 60 Unit following manufacturer's instructions. LDL cholesterol levels were calculated by the Friedewald formula: LDL = total cholesterol − [HDL + (triglycerides/5)]. LDL was not calculated for 33 subjects with TG > 300 mg/dl. Our analyses are validated by the College of American Pathologists, based upon the national reference methods performed by the Clinical Chemistry Standardization Section, Centers for Disease Control and Prevention, Atlanta, Georgia. At the same time we obtained blood for DNA extractions from all members of the colonies who were >5 years old. Thirty-seven subjects who were taking hypercholesterolemia medication or exogenous estrogens at the time of sampling were excluded from these analyses. The remaining 485 individuals were 49% male and 51% female. The study protocol was approved by the University of Chicago and the University of South Dakota institutional review boards.

### Genotyping

DNA samples from 693 individuals were genotyped by the Mammalian Genotyping Service (Marshfield, WI, USA), using 386 STRP markers spaced throughout the genome (screening set 9), yielding a 9.1 cM map. In addition, we typed >300 additional markers in regions of interest and in candidate genes for other studies in our laboratory (e.g. asthma, atopy) using a variety of genotyping methods (25) and yielding a final map with average spacing of 5 cM, although some regions are more densely mapped than others. Map distances are based on the Marshfield map (http:/research.marshfieldclinic.org/genetics).

### Statistical analysis

Genome scans were performed using two HBD mapping methods, and significance was assessed using a novel permutation-based method. These methods are described in detail elsewhere (18,30), but will be reviewed here. The probability of HBD at a locus (irrespective of allele) and for specific alleles is estimated using information on all loci and complete genealogical relationships. These HBD estimates are included as a main effect in a linear model where influences due to the polygenic background are taken into account by means of additive and dominance variance components. The locus HBD is a test of linkage, although it can be thought of as a test for association with HBD across all alleles at a locus. Both methods take advantage of the extensive LD in the Hutterites.

These methods rely on the existence of regions that are homozygous by descent (HBD) in inbred individuals to detect quantitative trait loci that act in a recessive manner. For estimates of locus HBD, we use each individual's multilocus genotype information and the complete pedigree information to estimate the individual's conditional probability of HBD at arbitrary positions in the genome via a hidden Markov model method. The significance of HBD as a predictor in the model is assessed for each point at all locations in a fine grid throughout the genome. In other words, we identify loci where individuals with high probability of HBD have a phenotypic value that is different from the mean. The allelic association (or LD) mapping method, allele-specific HBD (ASHBD), uses an extension of our HBD calculation to determine the probability of HBD for specific marker alleles. In this case, each allele at each locus is tested for significance as a predictor in the model for the quantitative trait. Both HBD methods are multipoint because the estimates of HBD at each point use information on all loci.

For both methods we assess empirical locus (or allele-) specific and GW significance for each locus using a Monte Carlo permutation based test. Our permutation test preserves the covariance structure due to relatedness among individuals and allows us to assess significance in the presence of multiple, dependent tests and to guard against deviations from normality in the data. Note that no follow-up markers were placed in regions with a strong signal for this data set, so our estimations of GW significance are valid. The observed locus *P*-values for

the ASHBD method are also Bonferroni corrected to adjust for multiple tests when several alleles were present at a marker. The Bonferroni corrected *P*-values tended to be very close to the locus-specific permutation-based *P*-values (i.e. the difference is usually within the range of sampling variability of the Monte Carlo procedure). Thus, because only 1000 permutations are performed at each locus, we report the Bonferroni corrected *P*-values here for the ASHBD method. Although likelihood ratios were not calculated, we can assign an equivalent one degree of freedom LOD score to each of our *P*-values using the formula $\mathrm{LOD} = 0.217\,F^{-1}(1-P)$, where $F^{-1}$ is the inverse cumulative distribution function of a $\chi^2$ random variable with one degree of freedom. To assess GW significance, we used the smallest *P*-value over the genome in each of the 1000 permutations. From these 1000 *P*-values we compute the GW *P*-value by counting the fraction that are less than or equal to the *P*-value determined from the real data. GW significance is met if the GW *P*-value is $\leq 0.05$. Suggestive significance was met if the *P*-value at a locus was less than the average of these 1000 minimum *P*-values ($2.5 \times 10^{-3}$ for HBD, $6.7 \times 10^{-4}$ for ASHBD).

TG values were log-transformed to establish a normal distribution (Fig. 1). In our analysis of TG the dominance variance component was insignificant (29) and removed from the analysis. Age, age$^2$, gender and BMI were found to have significant correlations with TG level. Analyses were performed separately with age and gender; age, age$^2$ and gender; or age, age$^2$, gender and BMI as covariates.

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