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Original Research Article

Apolipoproteins (Apoproteins) and LPL Variation in Mennonite Populations of Kansas and Nebraska

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ABSTRACT Apoproteins (also known as apolipoproteins) have been studied extensively because of their role in lipid transport, association between specific genotypes and elevated serum lipid levels, and increased risk of heart disease. There is considerable genetic variation in the geographic distributions of these markers, with a north-south cline of the APOE*4 allele observed in Europe by Lucotte et al. ([1997] Hum Biol *69*:253–262). This study compares the frequencies of seven APO (APOA1 −75 bp, APOA1 +83 bp, APOB Ins/Del, APOB *Xba*I, APOC3 *Sst*I, and APOE) and LPL loci in Mennonite populations from Kansas and Nebraska. In total, 277 individuals were sampled from Goessel, Meridian, Garden View, and Lone Tree in 2002–2004. In addition, DNA samples that were collected in 1981 from Henderson, Nebraska, were genotyped for the seven APO and LPL loci. Of the seven APO and LPL loci tested, only one locus, APOB *Xba*I, departed significantly from Hardy-Weinberg equilibrium, with an unexpected excess of observed heterozygotes. The frequencies of the several APO loci are unique among the Mennonites, separating them from other European populations. A bidimensional scaling representation of Reynold's coancestry distances based on allelic frequencies of the seven APO and LPL markers in five Mennonite congregations fails to represent schematically the known patterns of fission. It is unclear whether the observed patterns are due to selection operating on these loci or whether genetic drift, small populations sizes, or a lack of statistical power of these biallelic loci distort the observed genetic relationship among congregations. Am. J. Hum. Biol. 17:593–600, 2005.

Genetic studies indicate that common variants in genes coding for apoproteins have significant influence on the interindividual variation of plasma lipid levels, and may affect the risk for cardiovascular disease in some populations (Tall et al., 1997; Kamboh et al., 1999; Ellsworth et al., 1999). Apoproteins, the protein moiety of the lipid transport system, serve a variety of functions affecting lipid metabolism: they stimulate the synthesis of lipoproteins, serve as cofactors for enzymes lipoprotein lipase (LPL) and lecithin-cholesterol acyltransferase (LCAT), and act as lipoprotein-binding proteins for cell receptors. Moreover, variation in the expression of these apoproteins is affected by environmental covariates such as gender (Ordovas, 2002) and dietary intake (Vincent et al., 2002). To better define the biological significance of variants of apoproteins and the LPL polymorphisms, it is essential to document their distribution in diverse ethnicities living under a variety of different environmental conditions. Popu-lation studies provide an additional approach to clarifying the role genetic polymorphisms might play in the expression of complex diseases when examined from the perspective of variation of genotypic frequencies, phenotypic amplification, and divergence (Weiss, 1993).

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Extensive association studies examined the relationship of apoproteins to plasma lipids. However, the results were not always consistent. This inconsistency may reflect the effects of genetic epistasis, gene-by-environment interaction, or the application of inadequate sample sizes needed to capture a minimal effect of the polymorphism on a lipid phenotype. In contrast to this project on a rural, agricultural population, most studies of apoproteins reported in the literature are based on clinical samples of patients.

Here, we report on the distribution of six apoprotein polymorphisms (APOA1 *Msp*I −75 and +83, APOB insertion/deletion and *Xba*I, APOC3 *Sst*I, and APOE *Hha*I) and the LPL $Ser_{447stop}$ mutation in Mennonite populations residing on the central plains of Kansas and Nebraska. We investigated the observed variation in small, genetically isolated series of populations, in order to document the patterns in rural farming communities with high longevity (i.e., females have a life expectancy of 86 years of age, and males, 82), much greater than US averages.

We also applied these genetic polymorphisms to examine the genetic architecture, level of genetic homogeneity among congregations, level of inbreeding, and consequences of evolutionary forces acting on the gene pool of these populations (Crawford et al., 1989; Crawford, 2000a). The statistical relationship between these polymorphisms and variation in lipid levels of the Mennonite population will be addressed in a subsequent article.

SUBJECTS AND METHODS

Population background

Two distinct groups of Mennonites participated in this study: the General Conference and the Halderman Mennonites. The General Conference Mennonites (from Goessel, Kansas, and Henderson, Nebraska) had a relatively heterogeneous origin during the Reformation, being primarily converts from the Netherlands, Prussia, and Switzerland. They emigrated from Germany to Russia in 1788 and formed a congregation in the Molotschna region of the Ukraine, called Alexanderwohl, in honor of the Russian czar. They remained there until the 1870s when the Russian crown withdrew their exemption from service in the military. During their habitation in Russia, they experienced increased inbreeding ($F = 0.02$), which was followed by population fission along familial lines after their relocation to the United States in the 1870s (Rogers, 1984). The descendants of the original Alexanderwohl congregation are relocated in Goessel, Kansas, and Henderson, Nebraska. (Crawford et al., 1989; Crawford, 2000b). The Halderman Mennonites were converts from various Anabaptist groups, such as the Amish and Mennonites. These more conservative Mennonites followed a charismatic leader to Central Kansas and have been numerically expanding rapidly to form new colonies. Meridian was the first Halderman community to be sampled by our group in 1981. Garden View and Lone Tree are offshoots from the original Meridian community.

Sample and data collection

This study was approved by the University of Kansas Advisory Committee on Human Experimentation, and carried out after informed consent was obtained from the participants. The researchers met with the congregations in their respective churches and explained the nature of the research. Samples of volunteers attended clinics that were held the week after the community meetings. There is no evidence for bias, either regional or by sex. More than 50% of all adults from each congregation participated in the study.

Blood samples were collected by venipuncture from fasting adult individuals of both sexes residing in the five Mennonite congregations. Samples were spun down, and plasma was separated immediately and frozen on site. The sample utilized in this analysis consisted of 41 individuals from Goessel, 53 from Meridian, 57 from Lone Tree, and 48 from Garden View, all congregations located in the state of Kansas. The fifth sample, 76 individuals from the Henderson congregation, in the state of Nebraska, was obtained in 1981 as part of a study of biological aging. These samples have been stored at $-80^{\circ}C$ since their collection.

DNA extraction and genotyping

DNA was extracted from the buffy coats of blood samples, using a Super Quick Gene Kit (Analytical Genetic Testing Center, Denver, CO). Variations in genotypes were determined by means of the polymerase chain

reaction (PCR), using protocols described by Anderson et al. (1997) for APOB insertion/deletion and *Xba*I, by Maekawa et al. (1995) for APOE *Hha*I, by Hixson et al. (1991) for APOC3 *Sst*I, by Kamboh et al. (1996) for APOA1 *Msp*I −75 and +83, and by Kuivenhoven et al. (1997) for the $Ser_{447stop}$ mutation in LPL.

Statistical analysis

Genotypic and allelic frequencies at each locus were calculated by the gene-counting method. Statistical analyses for testing Hardy-Weinberg equilibrium were done using the Arlequin software package (Schneider et al., 1997) with a modified version of the exact test described by Guo and Thomson (1992). Arlequin was also used to calculate pairwise genetic distances between congregations, using the estimator proposed by Reynold et al. (1983), and the exact test of population differentiation (Raymond and Rousset, 1995) to test the homogeneity of genetic distribution among Mennonite congregations.

Genetic resemblances between the Mennonites of Kansas and Nebraska and other European populations based on APOE, APOB *XbaI*, and APOB insertion/deletion polymorphism distribution were explored by means of correspondence analysis, using the program NTSYS 2.11S (Exeter Software). This technique simultaneously compares populations and genetic markers in a dimensionally reduced geometric space. For this purpose, we utilized the allelic frequencies compiled by Corbo et al. (1999).

The actions of systematic vs. nonsystematic evolutionary processes on Mennonite congregations were investigated using the model of Harpending and Ward (1982), which proposes that a linear relationship exists in subdivided populations between mean per locus heterozygosity (H_o) and distance from the centroid of distribution (r_{ii}). Thus the relative roles of stochastic processes or nonsystematic pressures (genetic drift or founder effect) and systematic pressures (migration and selection) on the genetic structure of populations may be assessed by regressing r_{ii} against H_o. Subpopulations that have high r_{ii} and low heterozygosity most likely experienced stochastic processes. On the other hand, subpopulations with high mean per locus heterozygosity (H_o) and low r_{ii} likely experienced high systematic pressure in the form of migration (gene flow) and/or selection. In small populations with reproductive isolation, migration rather than selection is the most likely explanation for the observed high H_o and low r_{ii} values.

RESULTS

The genotypic and allelic frequencies in the seven loci for the total Mennonite sample examined in this study are presented in Table 1. In addition, Table 1 contains the results for the Hardy-Weinberg test for genetic equilibrium. Significant departure from equilibrium was found only in the locus APOB *Xba*I, where the number of observed heterozygotes exceeded expectation. Given the historical and demographic background of this population, one would expect deviations from equilibrium based on an excess of homozygotes. This is an unexpected result, because the Mennonite population underwent considerable inbreeding and population fission along familial lines. More recently, inbreeding was reduced markedly to $F = 0.0062$, with Mennonites from adjoining communities marrying into Alexanderwohl (Crawford, 2000a). In general, the distribution of genotypes indicates an absence of reproductive isolation and a low level of inbreeding, as already reported in earlier studies utilizing traditional genetic markers (Crawford et al., 1989; Crawford, 2000a).

The distributions of apoprotein allelic frequencies in Mennonites, however, present some unique features when compared to other European populations. For example, the incidence of the APOB*X− allele (0.295) is below the range found in Europe (0.445 and 0.606 in Sweden and Italy, respectively). The same is true for the APOE*4 allele, with the Mennonites exhibiting a frequency of 0.055, while the variation in Europe is between 0.069 and 0.208 in Turkey and Finland, respectively (Corbo et al., 1999). APOE*4 in the Dutch and Germans (parental populations for the Mennonites) are a respective 0.154 and 0.145 (Slooter et al., 2001). On the other hand, the incidence of the APOC3*S2 allele in Mennonites (0.152) is above the range of (0.08–0.11) found in the USA, The Netherlands, southern Italy, and Belgium (Dammerman et al., 1993; Hoffer et al., 1998; Garasto et al., 2003). Distributions of the other polymorphic markers fall within the range of variation observed in European populations (Paul-Hayase et al., 1992; Kuivenhoven et al., 1997; Baroni et al., 2003).

TABLE 1. Genotype and allele frequencies, and Hardy-Weinberg equilibrium exact tests, using a Markov chain[1]

Locus	N	Genotype	Observed	Expected	Allele	Frequency
APOA1 −75 bp	274	G/G	193	196.4	G	0.847
		G/A	12	71.1	A	0.153
		A/A	3	6.4	HW eq. $P = 0.15 \pm 0.001$	
APOA1 +83 bp	274	C/C	252	252.4	C	0.960
		C/T	22	21.1	T	0.040
		T/T	0	0.4	HW eq. $P = 1.000 \pm 0.000$	
APOB Ins/Del	277	Ins/Ins	143	136.6	Insertion	0.702
		Ins/Del	103	115.9	Deletion	0.298
		Del/Del	31	24.6	HW eq. $P = 0.111 \pm 0.001$	
APOB *Xba*I	276	+/+	122	137.1	XbaI+	0.705
		+/−	145	114.9	XbaI−	0.295
		−/−	9	24.1	HW eq. $P = 0.001 \pm 0.001$	
APOC3 *Sst*I	277	S1/S1	199	199.4	S1	0.848
		S1/S2	72	71.2	S2	0.152
		S2/S2	6	6.4	HW eq. $P = 0.823 \pm 0.001$	
APOE	247	E2/E2	0	2.1	E*2	0.093
		E2/E3	42	39.2	E*3	0.852
		E2/E4	4	2.5	E*4	0.055
		E3/E3	181	179.4		
		E3/E4	17	23.0		
		E4/E4	3	0.7	HW eq. $P = 0.072 \pm 0.001$	
LPL $Ser_{447stop}$	275	S/S	215	215.6	S	0.885
		S/X	57	55.8	X	0.115
		X/X	3	3.6	HW eq. $P = 1.000 \pm 0.000$	

[1]Chain length, 100,000; dememorization, 1,000. HW eg., Hardy-Weinberg equilibrium.

Genetic resemblance between the Mennonites of Kansas and Nebraska and other European populations, based on APOE, APOB *Xba*I, and APOB insertion/deletion polymorphisms, explored by means of correspondence analysis, are presented in Figure 1. Comparative frequencies for these alleles, as used in this analysis, were compiled by Corbo et al. (1999). The first and second axis account for 72.6% and 25.9% of the total variation, respectively. A clear differentiation of the Mennonites from other European populations can be observed, due principally to their low incidence for *Xba*I− and E*4 alleles. A north-south gradient in the frequencies of the E*4 allele, described earlier by Lucotte et al. (1997), can be seen in Figure 1, with the separation of Italy, Southern Europe, and Central Europe from Denmark, Sweden, and Finland along the first axis. There is a reduction in frequency of the APOE*4 allele from north to south, and an inverse in E*3 from south to north.

Variation between congregations

The exact test of population differentiation indicates that significant genetic variation exists among congregations ($P < 0.001$). A locus-by-locus analysis reveals that differences are significant for APOE, APOB insertion/deletion, and APOA1 *Sst*I −75 polymorphism distribution. Pairwise comparisons show significant differences in 7 of the 10 comparisons, with only Goessel-Meridian, Goessel-Garden View, and Henderson-Meridian nonsignificant. Bidimensional scaling representation of the coancestry distances of Reynold et al. (1983), representing genetic resemblances among Mennonite congregations, are presented in Figure 2. Henderson clusters with Meridian, and Goessel clusters with Garden View, whereas Lone Tree appears alone, distant from the other congregations. The first axis separates Goessel and Garden View from Henderson, Meridian, and Lone Tree.

Systematic vs. nonsystematic processes

Figure 3 compares r_{ii} and H_o among the five Mennonite congregations. Meridian has the highest H_o and lowest r_{ii} values, suggesting recent immigration, or a highly heterogeneous recent origin. Garden View exhibits low H_o but also low r_{ii}, suggesting that the

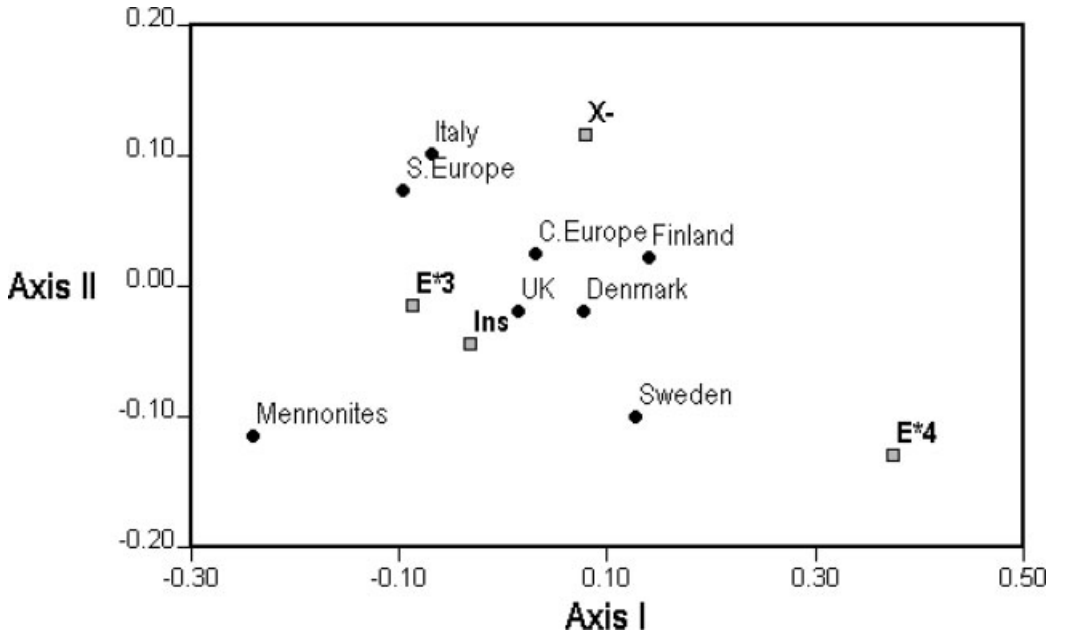

Fig. 1. Genetic resemblances between Mennonites of Kansas and Nebraska and other European populations based on APOE, APOB *Xba*I, and APOB insertion/deletion polymorphism distribution explored by means of correspondence analysis.

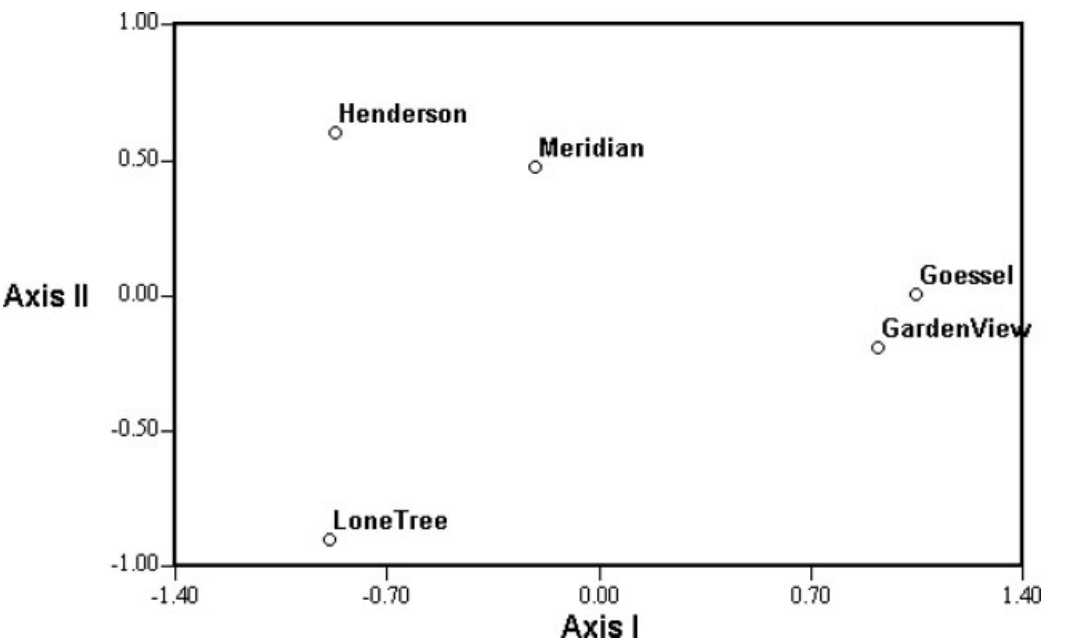

Fig. 2. Bidimensional scaling representation of coancestry distances of Reynold et al. (1983), depicting genetic resemblances among Mennonite congregations, based on apolipoprotein and LPL frequencies variation.

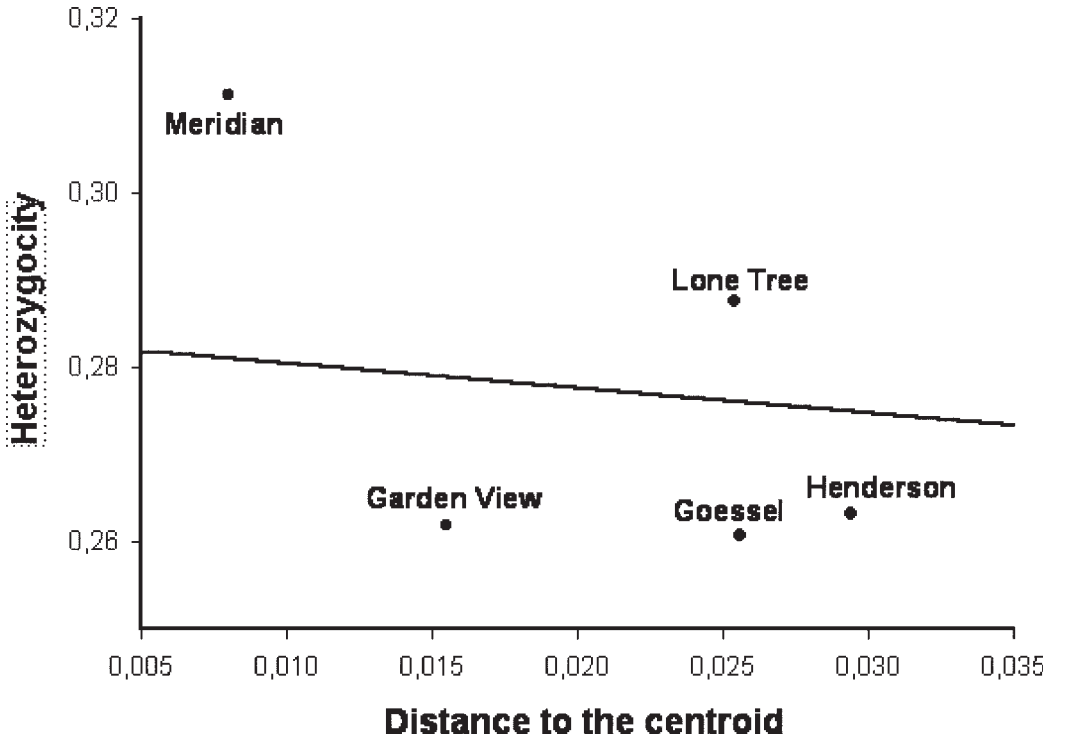

Fig. 3. Regression plot of average heterozygosity on distance of each population from gene frequency centroid (R_{ii}) of Mennonite populations and theoretical regression line.

reduction in heterozygosity was not accompanied by significant genetic drift. Lone Tree, Goessel, and principally Henderson exhibit high r_{ii}, suggesting a relatively high differentiation from the hypothetical ancestral population. However, Lone Tree exhibits a level of heterozygosity above that predicted from the model, suggesting that differentiation could be due to external immigration. On the other hand, the position of Goessel and Henderson, below the regression line, suggests possible evidence of gene drift.

DISCUSSION

In this study, we did not find evidence of the genetic effects of inbreeding among the Mennonites of Kansas and Nebraska. As reported before (Crawford, 2000a), since the 1930s, reproductive isolation and levels of inbreeding have been declining markedly among these Mennonites. In addition, there has been greater gene flow into these communities, mainly from other Mennonite populations. With the establishment of a Mennonite college in close proximity to Goessel and Meridian, mate selection among students expanded to more distant Mennonite colonies.

The apoprotein gene frequencies appear to be less useful in the reconstruction of the evolutionary history of the Mennonite congregations than are traditional markers, such as blood groups, protein variants, or DNA markers with high heterozygosity. In part, the apoprotein markers may contain less statistical power because 6 of the 7 loci are biallelic, with frequencies of p ranging from 0.7–0.9. Loci containing multiple alleles with frequencies of p ranging between 0.4–0.6 are much more informative statistically. The highly documented history of the Mennonite congregations predicts that Henderson and Goessel should cluster together. These two communities split from the Alexanderwohl congregation in the Molotschna region of Ukraine in the 1870s, when they relocated in the United States. Blood-marker frequencies cluster these two communities with Western Europe

and separate them from Meridian (Crawford, 2000a). In contrast, Meridian, Garden View, and Lone Tree should cluster together, given their past historical connections. Historically, Garden View and Lone Tree split from Meridian fairly recently. Unfortunately, we were unable to sample these two communities during the earlier study because they had not split from Meridian; therefore, Mantel tests cannot be utilized to correlate the distance matrices based on standard markers vs. apolipoprotein frequencies.

On the other hand, the degree of genetic differentiation among congregations, calculated as the mean value of r_{ii} (R_{ST}, an estimate analogous to F_{ST}; Harpending and Jenkins, 1973), is 0.02, which is unexpectedly high considering the close genetic relationships between populations. One explanation for such a value can be found in the existence of fission processes among Mennonite congregations, generally in the form of kinship-structured migrations. The evolutionary consequence of this practice is to increase genetic variation among populations, with its effects being greatest in small populations (Fix, 2004).

The interesting question is, why do genetic relationships between Mennonite populations differ from known historical reconstructions and from cluster analyses based on standard genetic markers? Does selection distort the expectations predicted from genetic drift and migration patterns? It would be useful to sequence these regions and search for the molecular signature of selection. The excess of heterozygotes for APOB *Xba*I, instead of the predicted excess of homozygotes usually found in small, highly isolated populations, would support the selection explanation. Another possible explanation is differential migration into these congregations, with migrants having different APOB variants. There may be less statistical power in these biallelic loci with frequencies close to fixation.

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