A Locus Influencing Total Serum Cholesterol on Chromosome 19p
Results From an Autosomal Genomic Scan of Serum Lipid Concentrations in Pima Indians

Giuseppina Imperatore, William C. Knowler, David J. Pettitt, Sayuko Kobes, John H. Fuller, Peter H. Bennett, Robert L. Hanson

Abstract—A genome-wide linkage study was analyzed to identify loci that influence serum lipid concentrations in Pima Indians. Linkage analyses were conducted for total cholesterol measured in 998 siblings from 292 nuclear families, for total triglycerides in 547 siblings from 188 families, and for high density lipoprotein (HDL) cholesterol in 590 siblings from 201 families. Genotypes were generated for 516 autosomal microsatellite markers. Multipoint variance components methods were used to assess linkage. The strongest evidence for linkage with total cholesterol was on chromosome 19p (lod score 3.89), in the vicinity of the marker D19S1034, which is near the low density lipoprotein receptor gene. The strongest evidence for linkage with HDL cholesterol was on chromosome 3q (lod score 2.64) near D3S3053. For triglycerides, the strongest evidence for linkage was on chromosome 2p near D2S1788 (lod score 1.70) and on chromosome 3p near D3S2406 (lod score 1.77). This genomic scan provides evidence for a locus influencing total cholesterol concentration on chromosome 19p. It also suggests a locus influencing HDL cholesterol on chromosome 3q. (Arterioscler Thromb Vasc Biol. 2000;20:2651-2656.)

Key Words: cholesterol ■ HDL cholesterol ■ linkage ■ genetics ■ triglycerides

Elevated total cholesterol and low HDL cholesterol concentrations are well-established risk factors for coronary heart disease. Elevated triglyceride level may also be a risk factor for cardiovascular disease. Family and twin studies indicate that genetic factors account for a substantial proportion of interindividual variability in serum lipid concentrations. However, genetic factors involved in lipid disorders have been identified mainly for monogenic familial disorders, and these account only for a small proportion of coronary heart disease. For most lipid abnormalities, the genetic determinants remain unknown. Given the complexity of lipid metabolism, genome-wide linkage studies are an appealing strategy to localize genes influencing serum lipid levels. Such studies, in contrast to those of candidate genes, can potentially detect regions that contain genes that influence lipid metabolism but whose function is currently unknown.

In the present study, genome-wide linkage analyses to detect loci influencing total cholesterol, HDL cholesterol, and total triglyceride concentrations were conducted among Pima Indians. The prevalence of diabetes mellitus among Pimas is very high, with >50% of individuals aged >40 years affected. Because diabetic individuals tend to have higher total cholesterol and triglyceride concentrations and lower HDL cholesterol concentrations than do nondiabetic persons, linkage analysis of serum lipids in this population is complicated by the high prevalence of diabetes. If the same loci influence lipid levels in diabetic and nondiabetic individuals, then power would be maximized by pooling these individuals. On the other hand, if loci that are important in diabetic persons are different from those in nondiabetic individuals (eg, as a result of a genetically determined response to hyperglycemia), then separate linkage analyses should be conducted. To determine the extent to which potential genetic determinants of serum lipid levels in diabetic persons overlap those in nondiabetic persons, familial resemblance of lipid levels among diabetic siblings, nondiabetic siblings, and siblings discordant for diabetes was assessed before performing linkage analysis.

Methods

Subjects and Phenotypes
Since 1965, the Pima Indians from the Gila River Indian Community have participated in a longitudinal survey in which each resident aged ≥5 years is asked biennially to participate in a standardized...
medical examination. The examination has included a 75-g oral glucose tolerance test. Participants have been asked to fast overnight before the examination, but the glucose tolerance test has still been administered even if the individual did not fast. Serum samples were centrifuged at 1440g for 10 minutes at room temperature and stored at −20°C for 1 to 5 days before measurements. Total serum cholesterol was determined with a colorimetric method from 1965 to March 1992 and with an enzymatic method subsequently. Total triglyceride and HDL cholesterol concentrations have been measured since 1993 by enzymatic methods. Accuracy of the lipid assays has been monitored and verified by the Centers for Disease Control Laboratory Program Office, the College of American Pathologists Surveys Program, or the American Association of Bioanalysts.

For the present analyses, measurements of total serum cholesterol, triglycerides, and HDL cholesterol were taken from the last available examination for each individual. For statistical procedures, all variables were logarithmically transformed to reduce skewness. The logarithmically transformed values were adjusted for age, age2, and sex and, among diabetic individuals, for the duration of diabetes by multiple linear regression analysis. These adjusted values were standardized to a mean of 0 and an SD of 1 before genetic analyses. Separate regression and standardization analyses were conducted for diabetic and nondiabetic individuals, thereby adjusting for diabetes. Only individuals aged ≥20 years were included in the analyses.

Quantitative Genetic Analyses

Before performing linkage analysis, quantitative genetic analyses were conducted to estimate the extent of overlap between familial determinants of total cholesterol, HDL cholesterol, and triglyceride concentrations in nondiabetic individuals and in diabetic individuals. Familial resemblance for each phenotype was assessed among diabetic siblings, nondiabetic siblings, and siblings discordant for diabetes. These analyses included all nuclear families from the population study in which ≥2 siblings had been examined. This resulted in 1232 sibships composed of 3900 participants, of whom 1667 individuals had diabetes and 2233 did not. Because data for HDL cholesterol and triglyceride concentrations were available from only more recent examinations and because triglyceride concentrations were measured only in individuals who were fasting, families informative for HDL cholesterol were a subset of those informative for total cholesterol, and families informative for triglycerides were yet a further subset. There were 1552 sibships in 553 families in the analysis of HDL cholesterol concentrations and 1454 sibships in 526 families in the analysis of triglyceride concentrations.

Statistical analyses were based on the variance components method for quantitative traits. This method involves fitting a linear “mixed” model in which the phenotypic variance is partitioned into various components. Typically, one estimates the trait mean (μ), and the variance is partitioned into a “polygenic” component (σ2G), which reflects overall familial effects (both genetic and shared environment), and an “environmental” component (σ2E), which reflects effects unique to the individual. Under the assumption of multivariate normality, the phenotype covariance-matrix (Ω) for individuals in a pedigree is Ω = Φσ2G + σ2E, where Φ is a matrix of the expected proportion of alleles shared that are identical by descent (IBD) for pairs of relatives, and I is an identity matrix. Parameters are estimated by maximum likelihood methods, and the ratio of σ2G to the total variance (σ2G + σ2E) provides an estimate of the proportion of phenotypic variance potentially attributable to additive genetic factors.

To test whether familial determinants of lipid concentrations were the same in diabetic and nondiabetic individuals, this model was extended to incorporate components of covariance. In these analyses, separate mean and variance parameters were estimated in diabetic (μd, σ2Gd, and σ2Ed with subscript d indicating diabetic) and nondiabetic (μnd, σ2Gnd, and σ2End with subscript nd indicating nondiabetic) siblings, and a “polygenic” covariance component between siblings discordant for diabetes (σGnd-d) was also estimated (all parameters were estimated simultaneously among all siblings). The σGnd-d parameter reflects the correlation in the trait between pairs of siblings discordant for diabetes; a high value reflects shared familial determinants between diabetic and nondiabetic persons.

Under the assumption that familial resemblance among siblings is due to additive effects of polygenes, degree of overlap can also be quantified by calculating the “correlation” (ρd) between the familial determinants in diabetic and nondiabetic persons as a function of the estimated variance components [ρd = σGnd-d / (σGd + σGnd)2/2]. The null hypothesis of no overlap in familial determinants of lipid concentrations (ρd = 0) was assessed by the likelihood ratio test.

Linkage Analyses

As previously described, an autosomal genomomic scan to detect loci linked to type 2 diabetes and related traits has been conducted in 1338 individuals in 112 extended pedigrees from this population. In all 1338 individuals, 516 autosomal microsatellite markers were typed; the proportion of samples that failed to amplify was not >15% for any marker (median 2.5%). Median heterozygosity was 68%, and median distance between adjacent markers was 6.4 cM (range 0 to 25.6 cM). Marker allele frequencies and genetic map locations were estimated in these subjects as previously described. The Pima map agreed well with the Marshfield map; one exception was ATAA9053, which was originally (but no longer) placed at the p-terminus of chromosome 19 in the Marshfield map, which could not be mapped to chromosome 19 in the present sample. Among the 1232 families with ≥2 siblings with lipid measurements, 292 families had ≥2 siblings with total cholesterol and genotypic data, providing 998 siblings; 201 families had ≥2 siblings with HDL cholesterol and genotypic data, containing 590 siblings; and 181 families had ≥2 siblings with triglyceride and genotypic data containing 548 siblings.

Linkage analyses were conducted by the variance components method. In this method, the mixed model, described above, is extended to include a monogenic component of variance (σ2M) influenced by a locus linked to the region of interest, in addition to “polygenic” (σ2G) and “environmental” (σ2E) components. The dative variance-covariance matrix is Ω = Φσ2G + σ2M + σ2E, where Φ is a matrix of the proportion of IBD alleles shared, estimated from genotypic data, and Φ and I are defined as above. Multipoint estimates of IBD were obtained as described by Fulker et al. At any point on the chromosome, this method estimates the proportion of IBD alleles shared as a weighted average of IBD at each individual marker. Estimates of IBD for individual markers were obtained by the method of Curtis and Sham, which was implemented by use of the FASTLINK program. This method uses genotypic information from all relatives to infer IBD for sibling pairs. To avoid the need for a more complex model to account for polygenic correlations among extended relatives, analyses of phenotypic data were restricted to sibships.

Parameters were estimated by use of the MIXED procedure of SAS (SAS Institute). Linkage was assessed by the likelihood ratio test comparing the full model to one in which σ2M was constrained to equal 0. The lod score was calculated by dividing the likelihood ratio test for linkage by 2 log10(10).

Results

Table 1 shows results of covariance components analyses in families from the population study. For total cholesterol, for example, the estimated additive genetic component accounted for 48% of the total variance among nondiabetic siblings, for 32% of the variance among diabetic siblings, and for 37% of the variance among siblings discordant for diabetes. All 3 traits were significantly (P<0.0001) correlated among siblings discordant for diabetes. Estimated correlations between familial determinants in diabetic and nondiabetic siblings

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TABLE 1. Proportion of Total Variance Accounted for by Components of Covariance (With SEs) for Lipid Concentrations Among Diabetic and Nondiabetic Siblings

<table>
<thead>
<tr>
<th>Diabetic Parameter*</th>
<th>Total Cholesterol</th>
<th>HDL Cholesterol</th>
<th>Triglycerides</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>$\sigma_{\text{tot}}^2$</td>
<td>0.52±0.05</td>
<td>0.68±0.08</td>
</tr>
<tr>
<td></td>
<td>$\sigma_{\text{ed}}^2$</td>
<td>0.48±0.05</td>
<td>0.32±0.07</td>
</tr>
<tr>
<td>Yes</td>
<td>$\sigma_{\text{ed}}^2$</td>
<td>0.68±0.06</td>
<td>0.66±0.10</td>
</tr>
<tr>
<td></td>
<td>$\sigma_{\text{sd}}^2$</td>
<td>0.32±0.06</td>
<td>0.34±0.10</td>
</tr>
<tr>
<td>Discordant</td>
<td>$\sigma_{\text{sd1+d}}^2$</td>
<td>0.37±0.05</td>
<td>0.45±0.09</td>
</tr>
<tr>
<td>$R^2$†</td>
<td></td>
<td>0.96</td>
<td>1.35</td>
</tr>
<tr>
<td>$P$‡</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are proportion±SE. *See methods for definition of parameters. SEs were estimated from likelihood ratio test.

$R^2$ represents “correlation” between the familial determinants of lipid concentrations in nondiabetic persons and those in diabetic persons ($R^2=\frac{\sigma_{\text{tot}}^2-(\sigma_{\text{ed}}^2+\sigma_{\text{sd}}^2)}{\sigma_{\text{tot}}^2}$). Because estimates of $\sigma_{\text{ed}}^2$ were not constrained to be $\leq\sigma_{\text{sd}}^2$, correlations, >1 are possible; none of the estimates of $\sigma_{\text{sd1+d}}^2$ was significantly different from the value that gives $R^2=1$.

†$P$ value for null hypothesis $\sigma_{\text{sd1+d}}^2=0$.

(R^2) were ≈0.96 for each trait, suggesting that familial determinants of total cholesterol, total triglyceride, and HDL levels are largely the same in diabetic and nondiabetic individuals. Therefore, linkage analyses were performed jointly in diabetic and nondiabetic persons.

Characteristics of subjects in the genomic scan are reported in Table 2. In ≈40% of sibling pairs, both siblings had diabetes, whereas in ≈20% of sibling pairs, both siblings were nondiabetic, and ≈40% of sibling pairs were discordant for diabetes.

In Table 3, all chromosomal regions with a lod score $>1.18$ ($P<0.01$, which is of sufficient magnitude to replicate strong linkage signals seen in other populations$^{18}$) with total cholesterol, triglycerides, or HDL cholesterol are reported. The only region that reached a lod score $>3$ was on chromosome 19p at the location of the marker D19S1034 in the analyses of total cholesterol. Multipoint linkage results for chromosome 19 are shown in Figure 1. The 1-lod support interval for the location of the putative gene influencing total cholesterol covers the region from 0 to 20 cM and includes the gene for the LDL receptor (LDLR). The highest lod score for HDL occurred on chromosome 3q at D3S3053. For total triglycerides, the strongest evidence for linkage was on chromosome 2p near D2S3788 and on chromosome 3p near D3S2406. Figure 2 shows the results of multipoint linkage analyses for chromosomes 2 and 3. The 1-lod support interval for the HDL linkage on chromosome 3q encompasses a 23-cM region from 162 to 185 cM. The 1-lod support interval for triglycerides on chromosome 2p extends for 23 cM from 35 to 58 cM; that on chromosome 3p covers 30 cM from 76 to 106 cM. Additional regions with some evidence for linkage were seen on chromosome 5 with triglycerides and on chromosomes 7 and 20 with HDL cholesterol (Table 2). To assess whether the effects of these loci differed according to the presence of diabetes, regions shown in Table 2 were analyzed by using an extension of the covariance components model that estimates separate monogenic components for diabetic siblings, nondiabetic siblings, and siblings discordant for diabetes. There was no significant difference among the monogenic components for any region ($P>0.05$).

**Discussion**

An autosomal genome-wide linkage study was analyzed to detect loci influencing serum concentrations of total cholesterol, HDL cholesterol, and triglycerides in Pima Indians. The results provide strong evidence (lod score 3.89) that a gene on chromosome 19p influences total cholesterol levels and weaker but suggestive evidence (lod score 2.64) for a locus influencing HDL cholesterol concentration on chromosome 3q. For triglyceride levels, linkage evidence was weaker; the highest lod scores were seen on chromosomes 2p and 3p (lod scores 1.70 and 1.77, respectively).

The present study has also examined the extent to which genetic determinants of lipid concentrations in diabetic per-
sons overlap those in nondiabetic persons. Because the prevalence of diabetes among the Pimas is high, it is important to examine this potential overlap to determine whether separate or joint linkage analyses are indicated. This was assessed by estimating familial resemblance for each phenotype among nondiabetic siblings, diabetic siblings, and siblings discordant for diabetes. For all 3 phenotypes, resemblance among siblings discordant for diabetes was similar to that among siblings concordant for diabetes. This is consistent with the hypothesis that genetic determinants of lipid levels in nondiabetic persons and in diabetic persons are largely shared. Thus, linkage analyses were conducted by pooling diabetic and nondiabetic individuals, but with adjustment for diabetes. The adjustment for diabetes will generally enhance power to detect loci influencing lipid levels, unless the major genes influencing lipid levels also influence susceptibility to diabetes. This is unlikely, inasmuch as none of the regions identified as potentially linked with lipid levels showed linkage with diabetes in these families.13

The variance components method is a powerful tool for assessing genetic linkage. The method does not require specification of mode of inheritance, which makes it useful for analyzing complex traits for which the mode of inheritance is usually unknown, but it is somewhat less powerful than correctly specified parametric analyses. In addition, sibships rather than sibling pairs are analyzed, relaxing the assumption of independence among pairs of siblings inherent in sib-pair methods of linkage analysis. However, the method is potentially sensitive to individuals with extreme phenotypic values. To determine whether a few such individuals accounted for the present results, chromosomes showing linkage were reanalyzed after the exclusion of individuals whose trait values were >3 SDs from the mean; in each case, substantial evidence for linkage remained.

The variance components method assumes multivariate normality, and statistical evidence for linkage may be inflated if the trait distribution deviates radically from a normal distribution.19 To reduce skewness, a logarithmic transformation was applied to each of the lipid variables. One can also impose a normal distribution by ranking individuals according to the quantitative trait and applying an inverse gaussian transformation; when this procedure was applied to the present data, results similar to those presented above were obtained. This suggests that the present lod scores are not inflated because of violations of the assumption of multivariate normality. In the absence of such violations, probability values associated with lod scores are well approximated by a $\chi^2$ distribution with 1 df (with use of a 2-tailed test for a small sample and a 1-tailed test for a large sample).20 With this assumption, the 1-tailed $P$ value associated with linkage of the normalized total cholesterol concentration to chromosome 19p is 0.00002, that associated with HDL cholesterol on chromosome 3q is 0.0004, that associated with triglycerides

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Marker* (cM)</th>
<th>Total Cholesterol, lod (cM)†</th>
<th>HDL Cholesterol, lod (cM)†</th>
<th>Triglycerides, lod (cM)†</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>D2S1788 (40.6)</td>
<td>0.22 (44.0)</td>
<td>0.02 (44.0)</td>
<td>1.70 (44.0)</td>
</tr>
<tr>
<td>3</td>
<td>D3S2406 (97.0)</td>
<td>0.00 (92.0)</td>
<td>0.00 (92.0)</td>
<td>1.77 (92.0)</td>
</tr>
<tr>
<td>3</td>
<td>D3S3053 (181.1)</td>
<td>0.00 (175.0)</td>
<td>2.64 (175.0)</td>
<td>0.00 (175.0)</td>
</tr>
<tr>
<td>5</td>
<td>D5S1720 (127.1)</td>
<td>0.0 (127.0)</td>
<td>0.69 (127.0)</td>
<td>1.59 (127.0)</td>
</tr>
<tr>
<td>7</td>
<td>D7S2846 (53.1)</td>
<td>0.00 (54)</td>
<td>1.37 (54.0)</td>
<td>0.00 (54.0)</td>
</tr>
<tr>
<td>19</td>
<td>D19S1034 (0.0)</td>
<td>3.89 (0.0)</td>
<td>0.00 (0.0)</td>
<td>0.00 (0.0)</td>
</tr>
<tr>
<td>20</td>
<td>D20S1012 (91.6)</td>
<td>0.00 (91.0)</td>
<td>1.51 (91.0)</td>
<td>0.00 (91.0)</td>
</tr>
</tbody>
</table>

* Marker closest to linkage peak. Locations on Pima map (cM) are given in parentheses. †Location of peak multipoint lod score.

**Figure 1.** Multipoint linkage results for chromosome 19. Markers used in genomic scan are shown across top. Location of LDLR gene is indicated by arrow.

**Figure 2.** Multipoint linkage results for chromosomes 2 and 3. Markers used in genome-wide scan are shown across top.
on chromosome 2p is 0.0023, and that associated with triglycerides on 3p is 0.0033.

Criteria for statistical significance in genetic linkage studies have been controversial. For mendelian traits, a lod score >3.0 has traditionally been considered to represent a high probability of linkage, whereas a lod score of 2.0 to 3.0 is suggestive of linkage but is associated with an unacceptably high type I error to be considered conclusive. Although some authors have proposed more stringent criteria for complex traits, the criteria originally proposed for mendelian traits appear to be applicable to complex inheritance.25 By these criteria, the lod score of 3.89 observed on chromosome 19 for total cholesterol represents significant evidence for linkage, whereas the lod score of 2.64 on chromosome 3 is suggestive of a locus influencing HDL cholesterol. However, regardless of the magnitude of the lod score, it is possible for a result from a single study to be spurious, and the present results need to be replicated in other subjects to distinguish a statistical artifact from a true linkage.

The chromosome 19p region identified as linked with total cholesterol concentrations contains the LDLR gene. Because LDL particles transport most of the cholesterol in the plasma, total cholesterol concentrations largely reflect LDL cholesterol. A large number of rare mutations in the LDLR gene are responsible for the autosomal-dominant disorder familial hypercholesterolemia (MIM 143890). This fact and the known role of LDLR in lipid metabolism give rise to the hypothesis that more common polymorphisms in this gene may influence lipid levels. Consequently, a number of candidate gene studies have examined linkage and association of polymorphisms in the vicinity of LDLR with lipid phenotypes. Polymorphisms in the LDLR gene are associated with total or LDL cholesterol levels in some populations, but not in others.22,23 Weak evidence for linkage in this region has been observed with LDL cholesterol concentrations in Hutterites (lod score 0.8).23 Linkage has also been observed with LDL size distributions, which reflect atherogenicity of the LDL particles, in families with coronary artery disease (lod score 1.3)26 and in families in which the proportion of LDL cholesterol contained in small dense particles segregates in a mendelian fashion (lod score 4.1).27 In contrast, this region of chromosome 19 was not linked to LDL particle size or triglyceride concentrations in dizygotic female twins.28 Recently, genome-wide linkage analysis in Mexican American families suggested a locus influencing cholesterol concentration in LDL-1, a fraction containing large LDL particles, 16 cM centromeric from LDLR (lod score 2.3).29 None of these studies assessed linkage with total cholesterol concentration; nonetheless, they lend support to the hypothesis of a locus influencing lipid metabolism on chromosome 19p.

Although the LDLR gene is a strong positional candidate to influence cholesterol levels, the present findings could reflect other genetic elements in the region. Additional potential candidate genes in this region include those coding for the insulin receptor and the third component of complement (C3). Acylation-stimulation protein is a fragment of C3 that promotes clearance of lipoproteins from the circulation by increasing uptake of free fatty acids into the adipose tissue.

In this genomic scan, no region reached the level of significant linkage for HDL cholesterol concentrations, but a region on chromosome 3q showed a lod score of 2.64, which is suggestive of linkage. Complex segregation analyses suggest that there may be strong genetic influences on HDL levels,30,31 but linkage studies with candidate genes have not identified these determinants.30 A genomic scan of Mexican Americans did not show linkage of chromosome 3 with concentrations of subfractions of HDL cholesterol but identified strong evidence for linkage with concentrations of the 2a subfraction to chromosomes 8 and 15,32 neither of which showed evidence for linkage with total HDL cholesterol in the present study. The chromosome 3q region identified in the present study as linked to HDL cholesterol was also modestly linked to fasting insulin concentrations in nondiabetic Pimas (lod score 1.2).33 Low HDL concentration and low insulin sensitivity are features of the insulin resistance syndrome, and alteration of a common genetic pathway may be responsible for this association.34 Potential candidate genes in this region of chromosome 3q include peroxisome bifunctional enzyme, which is involved in peroxisomal fatty acid oxidation, and liver glucose transport protein-2.

In the present analysis, only weak evidence of linkage for total triglycerides was detected. Although serum triglyceride levels are heritable, it is unknown whether the genetic component comes from a large number of genes with small effects or from a single gene with large effects. The region on chromosome 2p that was linked to triglyceride levels in the present analyses is near the gene for apoB, which was modestly linked to triglyceride concentrations in dizygotic female twins (lod score 1.0),29 and is near a region linked with serum leptin levels in Mexican Americans (lod score 5.0).35 A recent genomic scan of serum triglyceride levels in Mexican Americans did not detect linkage in any of the regions that were tentatively linked in the present study but showed significant evidence for linkage (lod score 3.9) on chromosome 15q, where there was no evidence for linkage in the Pimas.36 Loci with small or moderate effects are difficult to detect with linkage analysis, and detection of such loci requires a larger sample size than is available in the present study.

Fine-mapping studies and studies of candidate genes in the chromosome 19p region are required to identify the genetic elements responsible for the observed linkage with total cholesterol levels. Identification of the genetic mechanisms influencing lipid concentrations will help investigators to better understand lipid metabolism. This will improve understanding of the pathophysiology of the atherosclerotic process and, ultimately, may lead to better treatment and prevention of cardiovascular disease.

Acknowledgments

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Electronic Database Information

References
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