Adrenocortical Lipid Depletion Gene (ald) in AKR Mice Is Associated with an Acyl-CoA:Cholesterol Acyltransferase (ACAT) Mutation*

Vardiella L. Meiner‡‡, Carrie L. Welch¶, Sylvaine Cases¶, Heather M. Myers, Eric Sande, Aldons J. Luis§, and Robert V. Farese, Jr.***‡‡

From the Gladstone Institute of Cardiovascular Disease, ‡Cardiovascular Research Institute, and **Department of Medicine, University of California, San Francisco, California 94141-9100 and the ¶Department of Microbiology and Molecular Genetics, Department of Medicine, and Molecular Biology Institute, University of California, Los Angeles, California 90095

(Received for publication, August 26, 1997, and in revised form, October 17, 1997)

‡‡ To whom correspondence should be addressed: Gladstone Institute of Cardiovascular Disease, P. O. Box 419100, San Francisco, CA 94141-9100. Tel.: 415-695-3759; Fax: 415-285-5632; E-mail: bob_farese.gicd@quickmail.ucsf.edu.

Ald, a recessive allele in AKR inbred mice, is responsible for complete adrenocortical lipid depletion in postpubertal males, which appears to be androgen dependent. Two recent observations (adrenocortical lipid depletion in acyl-CoA:cholesterol acyltransferase-deficient (Acact−/−) mice and the mapping of Acact to a region of chromosome 1 containing the ald locus) prompted us to ask whether adrenocortical lipid depletion in AKR mice results from an Acact mutation. Refined genetic mapping of Acact and ald was consistent with colocalization of these loci. Crossing Acact−/− with AKR (ald/ald) mice yielded postpubertal male offspring characterized by adrenocortical lipid depletion, indicating that these loci are not complementational and are therefore allelic. Immunoblotting of preputial gland homogenates demonstrated that AKR mice had an ACAT protein with a lower molecular mass than other mouse strains. Analysis of Acact cDNA from AKR mice revealed a deletion of the first coding exon and two missense mutations. Despite these coding sequence differences, the ACAT protein from the ald allele catalyzed cholesterol esterification activity at levels similar to that of wild-type protein. We speculate that the adrenocortical lipid depletion resulting from the ald mutation is caused by an altered susceptibility of the mutant protein to modifying factors, such as androgen production at puberty, in an as yet undetermined manner.

About a dozen naturally occurring mutations that affect lipid metabolism in mice have been described (1). In most cases, the mutant genes have not yet been cloned. One such mutation, adrenocortical lipid depletion (ald),1 is expressed as a recessive trait in inbred AKR mice and was first described in 1955 by Arnesen (2, 3). The ald phenotype is characterized by spontaneous lipid depletion in the zona fasciculata at the time of sexual maturation (2, 4). The lipid depletion results from a loss of cholesterol esters, which are normally abundant in the adrenocortical cells, but not of free cholesterol or triacylglycerols. In male ald/ald mice, the lipid depletion is nearly total and appears to be androgen-dependent (5). Male ald/ald mice younger than 30 days have normal adrenocortical lipid content, but cholesterol ester stores are rapidly lost between 30 and 40 days, and the depletion is essentially complete by 50 days (5). In female mice, the lipid depletion is subtotal and variable (4). Prepubertal orchietomy prevents the ald phenotype in male AKR mice, and the phenotype has been reversed by postpubertal orchietomy (6) or by the administration of adrenocorticotropic hormone (2) or cortisone (7). The ald locus was mapped to chromosome 1 in 1976 (8). The DBA/2 strain is characterized by a similar adrenocortical lipid depletion phenotype, which appears to be due to a different genetic locus (6).

We have recently generated mice in which we have disrupted the gene encoding acyl-CoA:cholesterol acyltransferase (ACAT) (9), an enzyme responsible for catalyzing the formation of cholesterol esters from free cholesterol and fatty acids (10–12). ACAT-deficient (Acact−/−) mice have tissue-specific reductions in cholesterol esters, including near-total depletion of cholesterol esters in the adrenal cortex in both males and females (9). Coincident with these observations, we (13) and others (14) mapped the Acact gene to chromosome 1 near the ald locus. These observations prompted us to ask whether ald results from an Acact mutation.

In the current study, we examined whether ald and Acact are allelic through genetic crosses of AKR and Acact−/− mice and by molecular analysis of the Acact mRNA, protein, and enzyme activity levels in AKR mice. The results indicate that ald is indeed due to a naturally occurring mutant Acact allele but that the ald protein is capable of catalyzing cholesterol esterification normally. This finding suggests that the expression of the adrenocortical cholesterol ester depletion phenotype in AKR mice is caused by an altered susceptibility of the mutant protein to modifying influences.

MATERIALS AND METHODS

Mice—All inbred strains including AKR were obtained from The Jackson Laboratory (Bar Harbor, ME). Acact−/− mice (9) were of mixed C57BL/6 and 129/Sv genetic background and were genotyped by HPTLC, high-performance thin-layer chromatography; PCR, polymerase chain reaction; bp, base pair(s); RACE, rapid amplification of cDNA ends.

* This work was supported by the J. David Gladstone Institutes, American Heart Association, California Affiliate Grant-in-aid 95-239 (to R. V. F.), and National Institutes of Health Grants HL30568 and HL42488 (to A. J. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 The abbreviations used are: ald, adrenocortical lipid depletion; ACAT, acyl-CoA:cholesterol acyltransferase; Acact, acyl-CoA:cholesterol acyltransferase mouse gene; CHO, Chinese hamster ovary;
with the polymerase chain reaction (PCR) as described (9). Mice were housed in a pathogen-free barrier facility operating on a 12-h light/12-h dark cycle and were fed rodent chow (Ralston Purina, St. Louis, MO).

Genetic Mapping of ald and Acact—Colocalization of ald and Acact was confirmed by typing microsatellite markers flanking a small region of chromosome 1 presumed to contain both loci. For Acact, a (C57BL/6J × Mus spretus)F1 × C57BL/6J interspecific backcross mapping panel (15) was typed, and for ald, a set of (AKXL)RI strains was typed. RI strain and progenitor control DNAs were purchased from The Jackson Laboratory. Genomic DNA was amplified with PCR primers for microsatellite markers (Research Genetics, Huntsville, AL) as described (16). Amplification products were separated on 5% polyacrylamide gels and detected by autoradiography. Variant alleles were scored, and the segregation patterns were entered into a Map Manager version 2.6.5 (17) data base containing either backcross (13) or RI strain data. The backcross panel has been typed for ~400 markers and the AKXL strains for 343 markers (17) distributed throughout the genome. Map positions were determined by minimizing the number of double and multiple recombination events. These mapping data have been deposited in the Mouse Genome Data Base (accession number MGD-JNUM-42073).

Tissue Lipid Analysis—For neutral lipid staining, adrenal glands were perfusion fixed in 3% paraformaldehyde in Tyrode’s solution, dissected from surrounding tissues, and frozen. Frozen transverse cut sections were stained with Oil Red O (18). For chromatographic analysis, lipids were extracted from adrenal gland homogenates with the Bligh-Dyer method (19), and high-performance thin-layer chromatography (HPTLC) was performed as described (20).

Immunoblot Analysis—Tissues were homogenized in 20 mM Tris, 150 mM NaCl, and 0.1% Triton X-100 (pH 7.4). An equal volume of homogenate containing 10% SDS was then added to protein aliquots (final concentration, 5% SDS); the samples were heated to 65 °C for 10 min and protein aliquots were size fractionated by SDS-polyacrylamide gel electrophoresis with 10% gels. The separated proteins were transferred to nylon membranes and immunoblotted with a rabbit anti-acetoacetate oxidase generated against a glutathione S-transferase/mouse ACAT fusion protein containing the amino-terminal 120 residues of the mouse ACAT protein (21). The low density lipoprotein receptor-related protein was detected with a rabbit antisemur (22). Binding of the antibodies was detected with an enhanced chemiluminescence kit (Amersham Corp.).

RNA and cDNA Analysis—RNA was prepared from tissues by standard techniques (23). For Northern blots, 10-μg samples were analyzed with a 32P-labeled 510-bp mouse ACAT cDNA fragment (nucleotides 804–1304 (14)) or 283 primer (24) as hybridization probes. cDNA was synthesized from RNA samples with a first-strand cDNA kit (Stratagene, La Jolla, CA). 5’-rapid amplification of cDNA ends (RACE) experiments were performed with an antisense primer (5’ GAGAAGGTTGTGAGTGCACA 3’) and a 5’-RACE kit (Life Technologies, Inc.). For reverse transcriptase-PCR, cDNA was amplified with sense (5’ 9778 (14)) and antisense (5’ 9777 (14)) markers flanking a small region containing both loci, utilizing DNA from backcrossed or recombinant inbred strains. Acact and ald localization was confirmed by hybridization to the AKXL specific microsatellite marker panel (15) was typed, and for ald loci, a set of (AKXL)RI strains was typed for mapping and for ald mapping, Recombination frequencies and genetic distances (centimorgan × S.E.) are indicated between loci.

RESULTS
We previously mapped Acact to a region of mouse chromosome 1 (13) containing the ald locus (8). Because the phenotype of Acact+/− mice included cholesterol ester depletion in the adrenal cortex of both male and female mice (9), we performed refined mapping of Acact and ald with microsatellite markers flanking the region containing both loci, utilizing DNA from backcrossed or recombinant inbred strains. Acact and ald were localized within the proximal to distal interval D1Mit1346-A13 (Fig. 1). The segregation pattern of Acact exhibited no recombination with D1Mit14 or D1Mit33 among 62 or 55 backcross progeny, respectively. ald was localized proximal to D1Mit33 but within the 95% confidence interval for the location of Acact, indicating that the Acact and ald loci were indistinguishable as determined within the resolution of these genetic markers.

To determine whether Acact and ald were allelic, we performed two genetic experiments designed to test whether the ald locus would complement the Acact− locus. We crossed Acact+/− mice (mixed C57BL/6 and 129Sv background) with ald/ald (AKR) mice and assessed the adrenal neutral lipid content in Oil Red O-stained adrenal sections of male postpubertal F1 offspring. Whereas Acact+/− and Acact+/− control male mice had abundant neutral lipids in their adrenal cortex, male postpubertal F1 offspring (carrying one ald and one Acact− allele) exhibited marked adrenocortical lipid depletion, similar to that in Acact−/− controls (Fig. 2A). In a separate experiment, we crossed Acact−/+ × mice with ald/ald mice and analyzed the adrenal gland cholesterol ester content of the postpubertal male F1 offspring by HPTLC. All F1 offspring that carried an Acact− allele and an ald allele had marked cholesterol ester depletion (Fig. 2B), similar to that observed in Acact−/− controls and less than that observed in ald/ald controls. In contrast, control littermate F0 offspring carrying an Acact+ allele and an ald allele had cholesterol ester levels equivalent to or greater than that in Acact−/− controls (Fig. 2B).
The failure of the ald allele to complement the Acact allele in both experiments indicated that the two loci were allelic.

Immunoblotting of preputial gland homogenates from adult male mice of 10 inbred strains demonstrated that the Acact protein from AKR mice migrated at a lower molecular mass (42 kDa) on SDS-PAGE than the 45-kDa protein in the other mouse strains (Fig. 3A). Similarly, a protein of reduced molecular mass was observed in adrenal gland homogenates from male and female AKR mice and in ovary homogenates from female AKR mice (not shown). Quantitative analysis of the Acact protein in adrenal homogenates of AKR mice demonstrated that its levels in males and females were reduced to ~33 and ~46%, respectively, of the levels in control male wild-type mice (C57BL/6 × 129/Sv).

Northern blotting of RNA from the preputial glands of adult AKR mice revealed that the Acact mRNA was less abundant and slightly smaller than that of control mice (Fig. 4A). Similar findings were observed in RNA samples from kidney and testes of AKR mice (data not shown). Because the immunoblotting and Northern blotting experiments suggested that the ald protein may be truncated, we examined the cDNA sequences of the Acact mRNA and observed several differences as compared with the wild-type Acact cDNA (14). Initial 5′-RACE experiments followed by cDNA sequencing demonstrated that the ald cDNA contained a 118-bp deletion spanning part of the 5′ untranslated region and the initial coding sequences (cDNA nucleotides 790–897 (14)). To further investigate this finding, we performed reverse transcriptase-PCR experiments using primers located on either side of the deleted region (Fig. 4B). Whereas a 312-bp wild-type product was consistently amplified from cDNA of control mice, a 194-bp product (reflecting the 118-bp deletion) was amplified from all tissues tested of both male and female AKR mice; the 194-bp product was also observed at very low levels in the cDNA from the control mice. DNA sequencing of this amplification product demonstrated that it lacked the identical 118 bp identified in the 5′-RACE

**Fig. 2.** Lack of complementation for the ald and Acact loci as shown by Oil Red O staining of adrenal cortices (A) and HPTLC of adrenal lipid extracts from postpubertal male offspring (B). For A, AKR (ald/ald) mice were crossed with Acact +/+ mice, and the lipid content of adrenal glands of the F1 male postpubertal offspring was analyzed by Oil Red O staining. For B, AKR (ald/ald) mice were crossed with Acact +/+ mice, and adrenal lipid extracts of male postpubertal F1 offspring were analyzed by HPTLC. All F1 offspring mice were genotyped for the + or − allele at the Acact locus as described (9). Oil Red O staining of frozen sections and HPTLC analysis of lipid extracts were as described under “Materials and Methods.”

**Fig. 3.** Mutant ACAT protein in AKR mice demonstrated by immunoblotting. A, immunoblots of preputial gland homogenates from 10 different inbred strains of mice demonstrating an ACAT protein of reduced molecular mass specific for the AKR strain. Whole-tissue homogenates from postpubertal male mice were prepared, and protein samples (75 μg) were analyzed by immunoblotting with an antiserum that recognizes the mouse Acact protein (21). B, immunoblots of adrenal homogenates demonstrating that the ACAT protein is reduced in molecular mass and detected in lower amounts in both female and male AKR mice. Trace amounts of a protein migrating identically to the reduced mass protein were sometimes observed in homogenates from wild-type mice. Indicated amounts of protein samples from whole cell homogenates of adult mice were analyzed by immunoblotting for Acact as described above. Sample aliquots were also immunoblotted with an antiserum that recognizes the low density lipoprotein receptor-related protein (LRP) (22) as an internal standard. Densitometric scanning of the autoradiogram and comparison of ACAT and LRP bands revealed that the mutant ACAT protein in male and female AKR mice was detected at ~33 and ~46%, respectively, of the levels in control male wild-type mice (C57BL/6 × 129/Sv).
ald Gene Is Associated with an ACAT Mutation

To confirm that the protein expressed by the ald allele could catalyze cholesterol esterification, we transiently expressed ald and wild-type Acact cDNAs in cultured ACAT-deficient (AC29) CHO cells (27) and measured cholesterol esterification activity in membrane homogenates. The transfection of the ald cDNA resulted in the expression of an Acact protein of lower molecular mass than that obtained with wild-type cDNAs, confirming the results of the immunoblot experiments in tissues from AKR mice. Membranes prepared from cells transfected with ald cDNA catalyzed cholesterol esterification at levels much greater than membranes of untransfected cells and similar to that of membranes from cells transfected with wild-type cDNA (Fig. 6). Normal cholesterol esterification activity was also observed in a pulse cholesterol esterification assay performed on intact transfected AC29 cells (data not shown).

**DISCUSSION**

**ald**, a naturally occurring mutation in the AKR inbred mouse strain, is associated with cholesterol ester depletion in the adrenal cortex, which becomes manifest at the time of sexual maturation (3). In the current study, we provide genetic and molecular evidence indicating that ald is associated with a naturally occurring mutant Acact allele. The AKR strain carries mutations at the Acact locus that result in an amino-terminal deletion in the Acact protein and two amino acid changes compared with the wild-type protein. Despite these differences, the ACAT protein from the ald allele catalyzed cholesterol esterification at levels similar to that of the wild-type protein. This finding suggests that the adrenocortical cholesterol ester depletion phenotype due to the ald mutation results from an altered susceptibility of the mutant ald protein to modifying influences present in vivo.

**experiments. DNA sequencing from C57BL/6 genomic DNA revealed that the 118 bp deleted in the ald cDNA corresponded to an exon containing the AUG translation initiation codon. In addition to the deleted sequences, the ald cDNA contained two missense mutations: A → G at cDNA nucleotide 1248, resulting in Ile → Val at amino acid 147, and C → T at nucleotide 1422, resulting in His → Tyr at amino acid 205). UT, untranslated.

To determine whether the protein product of the ald allele could esterify cholesterol, we performed cholesterol esterification activity assays on adrenal membranes harvested from AKR mice and controls. The adrenal membranes from adult male AKR mice had activity levels (121 ± 11 pmol/mg protein/min) similar to those of membranes from wild-type control (C57BL/6 × 129/Sv) male mice (111 ± 17 pmol/mg protein/min) and much greater than those of Acact−/− controls (3.8 ± 0.5 pmol/mg protein/min) (Fig. 5A). Membranes from female AKR mice exhibited −3–4-fold greater activity (351 ± 171 pmol/mg protein/min) than those from male mice (consistent with the higher cholesterol ester in adrenal glands of female mice (26)). Because these assays were performed at Vmax, with saturating concentrations of cholesterol substrate (20 nmol), we hypothesized that the activity levels of the ald protein might be altered at lower substrate concentrations owing to altered affinity of the protein for cholesterol substrate. We therefore analyzed activity levels of postpubertal male AKR adrenal membranes in response to various concentrations of cholesterol. At each cholesterol concentration tested, however, the cholesterol esterification activity in membranes from AKR mice was equal to or greater than that of wild-type control membranes (Fig. 5B).
Values are the mean ± SD for two samples of adrenal glands pooled from four mice each and assayed in duplicate.

**B**

Cholesterol esterification activity of male AKR mice in response to different concentrations of cholesterol substrate. Cholesterol esterification activity as described under "Materials and Methods." Data are shown for duplicate samples from a single transfection experiment; the experiment was repeated once, and the identical result was obtained. The same membrane samples (75 μg) were immunoblotted with an antisera that recognizes Acact (21) to demonstrate protein expression (inset).

**Fig. 5. Normal cholesterol esterification activity in adrenal gland membranes from AKR mice.** A, cholesterol esterification activity of adrenal membranes from adult male and female AKR mice compared with male Acact+/+ and Acact−/− mice. Adrenal membranes were prepared as described under "Materials and Methods," and samples (200 μg) were assayed for cholesterol esterification activity. Exogenous cholesterol substrate was provided as cholesterol/phospholipid liposomes. Values are the mean ± SD for two samples of adrenal glands pooled from four mice each and assayed in duplicate. B, cholesterol esterification activity of male AKR mice in response to different concentrations of cholesterol substrate. Cholesterol esterification assays were performed as described above, except that the cholesterol was added as an acetone solution. Each data point is the mean of pooled adrenal glands from four mice in duplicate experiments.

Two lines of genetic evidence from our studies indicate that ald and Acact are allelic. First, refined genetic mapping demonstrated colocalization of the two loci to the same region of chromosome 1. Second, a cross between Acact−/− and AKR (ald/ald) mice demonstrated a lack of complementation between the ald and Acact− alleles with regard to adrenocortical lipid depletion in male postpubertal mice. Male postpubertal F1 offspring from this cross with an ald/+ genotype were characterized by cholesterol ester depletion in the adrenal cortex. If ald and Acact were alleles of different loci, then this cross would result in heterozygosity at each locus (i.e. Acact+/− and ald/+), and male postpubertal offspring would be expected to have abundant cholesterol esters in the adrenal cortex at levels similar those in Acact+/− mice or +/ald mice. The fact that DNA from AKR mice could not correct the adrenocortical lipid depletion caused by the Acact− allele provides near-definitive evidence that the ald mutation results from a mutant Acact allele.

The RNA and immunoblot analyses demonstrate clearly that the ald allele results in the production of a mutant Acact protein. Of 10 inbred strains surveyed, an Acact protein of lower molecular mass was found only in the AKR strain. The analysis of Acact cDNA from AKR mice demonstrated that the truncated Acact protein lacks the first coding exon containing the AUG translation initiation codon. Further experiments will be necessary to identify the genomic mutation that leads to the missing exon. The lack of this exon in the ald mRNA results in an amino-terminal deletion of at least the first 30 amino acids of the Acact protein. We hypothesize that translation of the Acact protein from the ald mRNA begins at the AUG codon at amino acid 34; this AUG is in the correct reading frame and is located in a Kozak consensus sequence for translation initiation (28). Supporting this hypothesis, the expression of the ald cDNA in cultured cells resulted in a truncated protein of identical molecular mass to that observed in tissues from AKR mice. The mutant protein was detected at lower levels than the wild-type Acact protein by immunoblotting, both in homogenates from male and female AKR mice and in cultured cells expressing equal amounts of wild-type or mutant Acact cDNAs. These immunoblot results may truly reflect lower levels of the ald protein, possibly due to a lower rate of translation originating from the alternate AUG start codon. However, it is also possible that the amino-terminal deletion in the ald protein resulted in altered reactivity of this truncated protein with the...
polyclonal antiserum (which recognizes epitopes at the amino terminus of ACAT) and that the immunoblotting data reflect this difference.

Despite the alterations, the ald protein catalyzes cholesterol esterification at levels equivalent to or greater than those of the wild-type Acact protein. This finding was observed both in adrenal tissues from adult male and female postpubertal AKR mice and in cultured cells expressing the mutant protein. Similar findings indicating that the ald protein can catalyze cholesterol esterification have been observed in pulse assays of cultured peritoneal macrophages from AKR mice. We observed normal or supranormal activity levels in assays carried out both at $V_{\text{max}}$ and at a range of cholesterol substrate concentrations. Thus, it is clear that the ald protein can catalyze cholesterol esterification, at least under the conditions utilized in our experiments. These data provide important structure-function information indicating that the Acact protein can still catalyze cholesterol esterification at normal levels despite an amino-terminal truncation (likely of 33 amino acids) and the amino acid changes at residues 147 and 205. Although this finding was somewhat surprising given the cholesterol ester depletion phenotype, it was not totally unexpected, inasmuch as the adrenal glands of AKR mice contain normal levels of cholesterol esters before sexual maturation. It is possible that the ald protein is associated with reduced ACAT activity in intact male AKR adrenal glands and that we were unable to reproduce the conditions or modifying factors that inhibit the activity in adrenal glands in our in vitro assays.

How does the ald allele contribute to cholesterol ester depletion at the time of puberty? The answers to this question are largely speculative. It is clear that alterations of different proteins involved in cholesterol metabolism can cause adrenocortical lipid depletion. Phenotypes of adrenocortical lipid depletion have been observed for knockouts of Acact (9) and Apoa1 (26) and in the naturally occurring ald mutations in AKR and DBA/2 (6) mice. In contrast to the cholesterol ester depletion in both male and female Acact−/− and Apoa1−/− mice, however, the ald phenotype in AKR and DBA/2 mice is conditional and is manifested only in the presence of modifying influences, such as androgen production at the time of puberty. Supporting the hypothesis that adrenocortical lipid depletion can result from the interactions of different factors, preliminary studies in DBA/2 mice suggest that the primary defect is not due to the Acact locus and that at least two loci are responsible for the phenotype. In AKR mice, we speculate that an increase in androgens affects or interacts with the ability of the ald protein to esterify cholesterol or to facilitate the storage of cholesterol esters. One possibility is that the amino-terminal deletion in the ald protein affects protein-protein interactions or the localization of the ald protein in intracellular compartments or in cell membranes and that these alterations influence the susceptibility of the protein to androgen-associated factors. Identifying factors that modulate the adrenocortical lipid depletion phenotype could provide a better understanding of cholesterol metabolism in adrenocortical cells and intracellular cholesterol metabolic pathways.

Acknowledgments—We thank Jim McGuire and Dale Newland for technical assistance, Dr. T. Y. Chang for the AC29 cell line, Dr. Joachim Herz for advice and the LRP antiserum, Drs. Joseph Goldstein and Ira Tabas for helpful discussions, Amy Corder and John Carroll for graphics, Stephen Ordway and Gary Howard for editorial support, and Angela Chen for manuscript preparation.

REFERENCES

1. Reeve, K., and Doolittle, M. H. (1996) J. Lipid Res. 37, 1387–1405
2. Arnesen, K. (1955) Acta Endocrinol. 18, 396–401
3. Arnesen, K. (1974) Acta Pathol. Microbiol. Scand. Suppl. 248, 15–19
4. Arnesen, K. (1963) Acta Pathol. Microbiol. Scand. 58, 212–218
5. Molne, K., and Brabrand, G. (1968) Acta Pathol. Microbiol. Scand. 72, 475–490
6. Deering, C. H., Shire, J. G. M., Kessler, S., and Clayton, R. B. (1973) Biochem. Genet. 8, 101–111
7. Arnesen, K. (1964) Acta Pathol. Microbiol. Scand. 60, 487–492
8. Taylor, B. A., and Meier, H. (1976) Genet. Res. 26, 307–312
9. Meiner, V. L., Cases, S., Myers, H. M., Sande, E. R., Bellosa, S., Schambelan, M., Pittas, R. E., McGuire, J., Herz, J., and Farre, R. V., Jr. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14041–14046
10. Chang, T.-Y., and Doolittle, M. H. (1983) in The Enzymes (Boyer, P. D., ed) Vol. 16, pp. 523–539, Academic Press, New York
11. Suckling, K. E., and Stange, E. F. (1985) J. Lipid Res. 26, 647–671
12. Goodman, D. S. (1965) Physiol. Rev. 45, 747–789
13. Welch, C. L., Xia, Y.-R., Shechter, I., Farre, E., Mehrabian, M., Mehdizadeh, S., Warden, C. H., and Luisi, A. J. (1986) J. Lipid Res. 27, 1406–1421
14. Oelmen, P. J., Oka, K., Sullivan, M., Chang, C. C. Y., Chang, T. Y., and Chan, L. (1995) J. Biol. Chem. 270, 26192–26201
15. Warden, C. H., Mehrabian, M., He-K., Yoon, M.-Y., Diep, A., Xia, Y.-R., Wen, P.-Z., Svenson, K. L., Sparkes, R. S., and Luisi, A. J. (1993) Genomics 18, 295–307
16. Dietrich, W., Katz, H., Lincoln, S. E., Shin, H.-S., Friedman, J., Dracopoli, N. C., and Lander, E. S. (1992) Genetics 131, 423–447
17. Manly, K. F. (1993) Mamm. Genome 4, 303–313
18. Coalson, R. E. (1981) in Staining Procedures (Clark, G., ed) 4th Ed., pp. 228–229, Williams & Wilkins, Baltimore
19. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
20. Kozak, M. (1989) J. Cell Biol. 116, pp. 523–539, Academic Press, Oxford
21. Bligh, E. G., and Dyer, W. J. (1959) Can. J. Biochem. Physiol. 37, 911–917
22. Kowal, R. C., Herz, J., Goldstein, J. L., Eiser, V., and Brown, M. S. (1989) Proc. Natl. Acad. Sci. U. S. A. 86, 5810–5814
23. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Biochemistry 18, 5294–5299
24. Barbu, V., and Dautry, F. (1989) Nucleic Acids Res. 17, 7113
25. Erickson, S. K., Shrewsbury, M. A., Brooks, C., and Meyer, D. J. (1988) J. Lipid Res. 29, 930–941
26. Plump, A. S., Erickson, S. K., Weng, W., Partin, J. S., Breslow, J. L., and Williams, D. L. (1996) J. Clin. Invest. 97, 2660–2671
27. Cadigan, K. M., Heider, J. G., and Chang, T.-Y. (1988) J. Biol. Chem. 263, 274–282
28. Mark, M. (1989) J. Cell Biol. 186, 229–241

2 I. Tabas, personal communication.
3 C. Welch, unpublished observations.