

Apolipoprotein A-I Q[-2]X Causing Isolated Apolipoprotein A-I Deficiency in a Family with Analphalipoproteinemia

Dominic S. Ng, Lawrence A. Leiter, Camilla Vezina, Philip W. Connelly, and Robert A. Hegele
Department of Medicine, St. Michael's Hospital, University of Toronto, Ontario M5B 1A6, Canada

Abstract

We report a Canadian kindred with a novel mutation in the apolipoprotein (apo) A-I gene causing analphalipoproteinemia. The 34-yr-old proband, product of a consanguineous marriage, had bilateral retinopathy, bilateral cataracts, spinocerebellar ataxia, and tendon xanthomata. High density lipoprotein cholesterol (HDL-C) was < 0.1 mM and apoA-I was undetectable. Genomic DNA sequencing of the proband's apoA-I gene identified a nonsense mutation at codon [-2], which we designate as Q[-2]X. This mutation causes a loss of endonuclease digestion sites for both BbvI and Fnu4HI. Genotyping identified four additional homozygotes, four heterozygotes, and two unaffected subjects among the first-degree relatives. Q[-2]X homozygosity causes a selective failure to produce any portion of mature apoA-I, resulting in very low plasma level of HDL. Heterozygosity results in approximately half-normal apoA-I and HDL. Gradient gel electrophoresis and differential electroimmunodiffusion assay revealed that the HDL particles of the homozygotes had peak Stokes diameter of 7.9 nm and contained apoA-II without apoA-I (Lp-AII). Heterozygotes had an additional fraction of HDL₃-like particles. Two of the proband's affected sisters had documented premature coronary heart disease. This kindred, the third reported apoA-I gene mutation causing isolated complete apoA-I deficiency, appears to be at significantly increased risk for atherosclerosis. (*J. Clin. Invest.* 1994. 93:223-229.) Key words: atherosclerosis • genetics • lipoproteins • coronary heart disease • reverse cholesterol transport

Introduction

Plasma HDL level is inversely correlated with the risk of coronary heart disease (CHD)¹ and has consistently emerged as a powerful predictor of risk (1-5). ApoA-I is the major protein component of HDL. While the actual mechanism for the protective effect of HDL remains poorly understood, a hypothesis that has received much attention relates to the role of HDL in

“reverse cholesterol transport” (6), a major mechanism for removal of excess cholesterol from peripheral tissues. HDL is a heterogeneous class of lipoprotein particles with regard to both size and hydration density and these different classes may have different associations with disease. For example, HDL₂, a major density subfraction, may be the crucial component for prevention of CHD (7). However, this has been challenged by a recent prospective study (8). Alternatively, immunoaffinity chromatography can be used to divide HDL particles into two major subfractions: (a) particles with only apoA-I (Lp-AI) and (b) particles with both apoA-I and apoA-II (Lp-AI/AII) (9). Although Lp-AI has been shown to be the component responsible for efficient cholesterol efflux from peripheral cells (10), other data suggest comparable efficiency for both Lp-AI and Lp-AI/AII with regard to efflux (11). Other putative “cardio-protective” roles of HDL are suggested by in vitro evidence of an antioxidant effect on LDL oxidation (12, 13) and prevention of the self-aggregation of LDL (14). These latter effects were felt to be directly related to the apoA-I component.

In normal human plasma, > 90% of apoA-I is found in HDL. The plasma concentration of apoA-I is also a predictive factor of CHD (7). Mature apoA-I is a 243-amino acid single polypeptide chain synthesized by the liver and intestine. The apoA-I gene is localized to the long arm of chromosome 11 and is at the 5' end of the apoA-I-CIII-AIV gene complex. The apoA-I mRNA translates the 267-amino acid prepropeptide, which undergoes intracellular cleavage leading to a 249-amino acid propeptide. The propeptide is secreted and undergoes extracellular proteolysis giving rise to mature apoA-I.

Genetically determined diseases characterized by complete HDL deficiency can help to understand the mechanism of the cardioprotective role of HDL. A number of kindreds with hereditary analphalipoproteinemia (AAL) have known defects in the apoA-I gene (15-23) that result in virtual absence of plasma HDL. In some other kindreds, the molecular defect either does not involve the apoA-I gene (24, 25) or has yet to be identified (26-29). Interestingly, despite a nearly complete absence of HDL in some of the kindreds, there is highly variable susceptibility to premature CHD. Possible reasons include the small sample size of each kindred, confounding associated apolipoprotein deficiencies (15-18), and presence of abnormal circulating apoA-I variants (21, 22). Therefore, it is important to examine the prevalence of premature CHD in kindreds with isolated apoA-I deficiency. Two such kindreds have been identified (20, 23). Unfortunately, the information that can be learned from these is limited because only one member had xanthomata and premature CHD in one kindred (20) and in the other kindred, the proband was only 5 yr old (23).

In this paper, we report a kindred with an isolated apoA-I deficiency caused by a structural mutation in the apoA-I gene, with five adult homozygotes. This allowed us to examine the clinical and biochemical sequelae of an isolated apoA-I deficiency in one of the largest affected kindreds yet studied.

Address correspondence to Dr. Robert A. Hegele, DNA Research Laboratory, Room 126WA, St. Michael's Hospital West Annex, 38 Shuter Street, Toronto, ON M5B 1A6, Canada.

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1. Abbreviations used in this paper: AAL, analphalipoproteinemia; CHD, coronary heart disease; LCAT, lecithin:cholesterol acyltransferase; Lp-AI, HDL with apoA-I only; Lp-AI/AII, HDL with both apoA-I and apoA-II;

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Methods

Subjects

Proband. The proband was a 34-yr-old Caucasian woman who was ascertained through the Lipid Clinic at St. Michael's Hospital in June 1992 with undetectable plasma HDL. She was the product of a consanguineous marriage. She initially presented for medical attention at the age of 30 yr with xanthelasma. In the same year, the patient was diagnosed to have bilateral cataracts requiring cataract extraction on the right eye. She also had bilateral subretinal lipid deposition with exudative proliferative retinopathy complicated by bilateral retinal detachments, which were treated surgically. She had a long-standing history of mild imbalance. She had no history of diabetes mellitus or hypertension, or any symptom suggestive of coronary or vascular insufficiency. Other history of past health included tonsillectomy at age 13 yr. She smoked one pack of cigarettes a day for 20 yr. On physical examination, she had a body mass index of 25.4 kg/m², bilateral punctate xanthelasmata of the upper eyelids, mildly thickened Achilles tendons, mild midline cerebellar ataxia, and asymmetric bilateral neurosensory hearing loss.

Initial biochemical evaluation before referral included determination of plasma lipid and lipoprotein as follows: total cholesterol was 6.88 mM, triglycerides were 2.87 mM, HDL-cholesterol (HDL-C) was 0.06 mM, and LDL-cholesterol (LDL-C) was 5.76 mM. She was initiated on gemfibrozil but was switched to lovastatin because of lack of efficacy. Fasting glucose, glycosylated hemoglobin, serum electrolytes, liver function tests, serum protein electrophoresis, and thyroid function tests were all normal. Both resting electrocardiogram and stage 5 Bruce protocol stress electrocardiogram were normal. Computed tomography scan of the head had normal results. Audiometry showed mild bilateral asymmetrical neurosensory hearing loss.

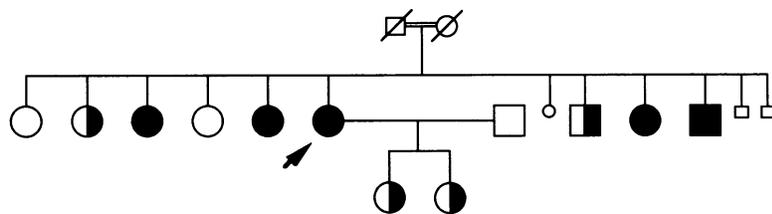
First-degree relatives. The proband's father died suddenly due to a myocardial infarction at the age of 64 yr with no antecedent ischemic

symptoms. Her mother died of stomach cancer at age 51. Nine of the proband's eleven siblings were studied biochemically. One sister (II-c, Fig. 1), aged 38 yr, suffered a myocardial infarction at age 34. She underwent two-vessel coronary bypass surgery 3 yr later. Other risk factors include a 25 pack-year smoking history and "hypercholesterolemia" for several years. She had bilateral xanthelasmata, Achilles tendon xanthomata, and planar xanthomata in the web spaces of the hands, cubital and popliteal fossae. She also had a mild degree of cerebellar ataxia. Her tonsils were normal. A second sister (II-b, Fig. 1), aged 39 yr, had hypertension, obesity, "hyperlipidemia" for several years and angina with documented reversible myocardial ischemia on thallium stress test. She also had cerebellar ataxia. The remaining sibs, aged from 24 to 41 yr, were asymptomatic for CHD, neuropathy, or visual impairment.

Lipid analyses

Plasma lipids and lipoproteins. The plasma lipid and lipoprotein profiles were determined in the J. A. Little Lipid Research Laboratory using the Lipid Research Clinics' protocol (30). Blood samples were obtained from subjects after a 12–14-h fast and collected into tubes containing Na₂-EDTA. Plasma was separated after centrifugation at 3,000 rpm at 4°C for 30 min. VLDL was isolated by ultracentrifugation of plasma at 45,000 rpm for 16.5 h at 10°C using a model 50.3Ti rotor in an L8-80 centrifuge (Beckman Instruments, Inc., Fullerton, CA). HDL was isolated from the infranantant with dextran sulfate precipitation. HDL₁ (*d* = 1.063–1.21 g/ml), HDL₂ (*d* = 1.063–1.125 g/ml), and HDL₃ (*d* = 1.125–1.21 g/ml) were prepared by sequential ultracentrifugation at 45,000 rpm for 40 h at 10°C. The total plasma and lipoprotein cholesterol and triglycerides were measured by automated enzymatic procedures using the model RA1000 (Technicon Instruments, Tarrytown, NY). Free cholesterol and phospholipid were determined by gas chromatography (31).

Apolipoproteins and Lp(a). Apolipoprotein AI, AII, B, CII, CIII and E were measured using ELISA techniques (32, 33). Apo AI and



IDENTIFICATION	II-a	II-b	II-c	II-d	II-e	II-f	III-a	III-b	II-f-s	II-g	II-h	II-i	II-j	II-k	II-l
Age	41	39	38	37	35	34	16	14	39	32	28	26			
Total cholesterol (mM)	4.85	6.06	5.43	6.34	5.11	5.77	4.47	3.33	3.81	5.09	4.64	4.23			
Triglyceride (mM)	1.01	2.09	1.10	3.61	1.22	2.53	1.94	0.73	2.04	1.46	1.45	1.81			
HDL-cholesterol (mM)	0.81	0.56	0.10	0.80	0.08	0.08	0.55	0.57	0.86	0.72	0.09	0.10			
LDL-cholesterol (mM)	3.68	5.02	5.18	4.39	4.70	5.00	3.39	2.58	2.41	3.88	3.89	3.78			
VLDL-cholesterol (mM)	0.36	0.48	0.15	1.15	0.33	0.69	0.53	0.18	0.54	0.49	ND	0.35			
VLDL-C/TG ratio	0.16	0.10	0.06	0.14	0.12	0.12	0.12	0.11	0.12	0.15	ND	0.08			
free/total cholesterol	0.28	0.31	0.32	0.27	0.30	0.33	0.28	0.26	0.31	0.27	ND	0.33			
apoA-I (g/L)	1.06	0.70	<0.05	1.15	<0.05	<0.05	0.72	0.61	1.14	0.86	<0.05	<0.05			
apoB (g/L)	1.21	1.29	1.49	1.72	1.53	1.77	1.15	0.81	0.92	1.30	1.40	1.21			
apoA-II (g/L)	0.21	0.17	0.13	0.23	0.07	0.07	0.18	0.21	0.27	0.24	0.09	0.08			
apoC-II (μg/mL)	16.2	23.5	19.5	42.1	16.6	32.1	32.4	17.4	28.3	32.9	12.3	10.4			
apoC-III (μg/mL)	68.9	73.4	46.4	166.2	47.9	71.0	105.5	47.6	105.1	98.5	40.4	41.9			
apoE (μg/mL)	49.3	57.2	64.9	152.8	39.1	74.9	46.1	32.0	35.8	48.2	44.1	33.0			
apoE isotype	4/3	3/3	3/3	4/4	3/3	3/3	3/3	3/3	3/3	3/3	4/4	4/3			
apoA-I genotype	AA	Aa	aa	AA	aa	aa	Aa	Aa	AA	Aa	aa	aa			

Figure 1. Biochemical features in subjects from pedigree AI Q[-2]X. Proband is indicated by the arrow. Small symbols indicate subjects not sampled. ND indicates analysis not done. Plasma apoA-I concentrations shown were determined nephelometrically. The proband's plasma apoA-I concentration determined by ELISA was < 0.01 g/liter. ApoA-I genotypes shown indicate the presence of either homozygosity for the wild-type allele (AA), heterozygosity (Aa), or homozygosity for the AI Q[-2]X mutation allele (aa) as determined by Fnu4HI RFLP analysis. Reference values for plasma apolipoprotein levels, based on 87 normal control subjects and expressed as mean±SD, are apoA-I, 1.55±0.22 g/liter; apoB, 1.22±0.32 g/liter; apoA-II, 0.33±0.06 g/liter; apoCII, 29±15 μg/ml; apoCIII, 116±44 μg/ml; and apoE, 50±21 μg/ml.

apo B levels were also measured by nephelometry (Behring, Montreal, Canada). Plasma Lp (a) was determined by ELISA (Terumo Medical Corp., Elkton, MD).

Electrophoretic procedures

Agarose gel electrophoresis, SDS-glycerol electrophoresis, isoelectric focusing, and immunoblotting procedures were performed as previously described (34–36). HDL particle sizes were determined by non-denaturing gradient PAGE using precast 4–30% gels (Pharmacia LKB Biotechnology, Uppsala, Sweden) (37). In order to visualize the particles, aliquots corresponding to 10–20 μ g of HDL protein were applied to the gel. Well sizes were adjusted to accommodate the larger volumes of HDL required for the subjects with severe HDL deficiency. Proteins were stained with 0.025% Coomassie Brilliant Blue G-250 (Eastman Kodak Co., Rochester, NY), and the gels were scanned with a densitometer (Helena Laboratories, Beaumont, TX). The gels were calibrated with a standard protein mixture (HMW Calibration Kit, Pharmacia LKB Biotechnology) consisting of thyroglobulin (hydrated particle size 17.0 nm), ferritin (12.2 nm), lactate dehydrogenase (8.16 nm), and bovine serum albumin (7.1 nm). Migration distances of peaks were measured relative to bovine serum albumin. Particle sizes of HDL fractions were calculated from the calibration curve of peak migration distance values against hydrated particle size of the standard protein mixture with nonlinear regression curve fitting software (GraphPad Software, San Diego, CA).

Lp-AI particles in plasma, HDL, HDL₂, and HDL₃ subfractions were measured by differential electroimmunodiffusion assay (Sebisa, Issy-les-Moulineaux, France) (38).

DNA analyses

PCR amplification. Genomic DNA was prepared from the proband and 11 first-degree relatives. Based on genomic sequence information (39, 40), we designed oligonucleotide primers (Table I) to amplify genomic regions spanning exons 2, 3, and 4 of the apoA-I gene using the Taq PCR. Reactions were carried out in a 100- μ l volume and contained 1 μ g of DNA, 200 μ M of dNTPs, 0.1 mM of primers, and 2.5 U of Taq polymerase (Gibco BRL, Gaithersburg, MD). Cycling was performed with a Biosycler oven (BIOS Corp., New Haven, CT) as follows: DNA was initially denatured at 94°C for 10 min followed by the addition of the reaction mixture and Taq polymerase and three cycles of 93°C (1 min), 60°C (30 s), and 72°C (30 s) followed by 30 cycles of 93°C (20 s), 60°C (20 s), and 72°C (30 s) incubation.

Cloning and sequencing of the PCR-amplified DNA. The PCR product was purified by extraction with phenol followed by precipitation with 7.5 M NH₄OAc and absolute alcohol. The purified DNA was then sequentially digested with BamHI and EcoRI (New England Biolabs, Beverly, MA), gel purified, ligated into pUC 19, and subcloned into *E. coli* DH5 α as previously described (41). Sequencing of the denatured

double-stranded DNA was carried out using T7 polymerase (United States Biochemicals, Cleveland, OH) according to the supplier's protocol.

Apo A-I genotyping. PCR-amplified exon 3 of the apoA-I gene with primers 3 and 4 from the proband and 11 family members were digested with BbvI or Fnu4HI (New England Biolabs) for 4 h. The digestion conditions were those recommended by the supplier. The digestion reaction was then electrophoresed on a 4% polyacrylamide gel, stained with ethidium bromide, and subsequently visualized under UV light. The PCR-amplified exon 3 from a series of 75 normolipidemic controls were genotyped with Fnu4HI digestion in a similar manner.

Apo E genotyping. Apo E isotyping was determined for the proband and 11 family members using HhaI digestion of PCR amplified apoE gene (42).

Statistical analysis

Correlation of plasma apoA-I with apoA-II was performed with standard linear regression.

Results

Plasma lipids and apolipoproteins. Concentrations of plasma lipids and apolipoproteins for members of this kindred are presented in Fig. 1. The proband and four of her 11 siblings (II-c, e, i, j) had severe deficiency of HDL (HDL < 0.1 mM) and nondetectable plasma apoA-I. Agarose gel electrophoresis of these subjects showed absence of the α -migrating band, establishing the biochemical diagnosis of AAL. IEF and immunoblotting of the proband's plasma also showed no evidence of any apoA-I isoform (data not shown). Two siblings (II-b, h) and both of the proband's two daughters (III-a, b) had reduced plasma HDL to 41–52% of age- and sex-matched median. Their plasma apoA-I levels were 0.61–0.86 g/liter. Two other sibs and the proband's husband had reduced plasma HDL (56–58% of median for age and sex) but near normal plasma apoA-I levels (1.06–1.15 g/liter).

The proband, despite ongoing lovastatin therapy, had total cholesterol exceeding the 90th percentile and LDL-cholesterol exceeding the 95th percentile, and triglyceride exceeding the 95th percentile. She also had elevated plasma apoB, markedly reduced but detectable apoA-II and normal apoC-III.

Plasma LDL-cholesterol levels exceeded the 90th percentile in three of the four siblings (II-c, e, j) with AAL. Two of the remaining sibs (II-b, d) and one daughter (III-a) also had LDL-C exceeding the 90th percentile. Triglyceride levels were normal in the four siblings with AAL but exceeding the 90th percentile in the two sibs (II-b, d) and one of the two daughters (III-a). Lp(a) levels of the members ranged from 0.8 to 7.8 mg/dl and were within the normal range.

Plasma apoA-II levels also varied significantly among the family members. Subjects with AAL had markedly reduced but detectable plasma apoA-II. The remaining subjects had moderately reduced plasma apoA-II and the plasma apoA-II levels correlated positively with plasma apoA-I levels ($r = 0.87$; slope = 0.158 with 95% confidence interval: $-0.001-0.317$).

Mean plasma level of apoC-III was 49.5 μ g/ml (SD = 12.4 μ g/ml) for subjects with AAL, 81.2 μ g/ml (SD = 26.33 μ g/ml) for members with intermediate plasma apoA-I (IIb, h; III-a, b), and 113.4 μ g/ml (SD = 49.2 μ g/ml) for those with near normal plasma apoA-I (II-a, d; II-f-s). The ratio of free cholesterol/total cholesterol ranged from 0.30 to 0.33 in AAL subjects and from 0.26 to 0.28 in the remaining members.

DNA analysis. Sequencing of three separate clones showed a single C \rightarrow T substitution at codon [-2] of the apoA-I gene in

Table I. Sequence and Position of Oligonucleotide Primers

Primer	Sequence	Position*
1.	5'-ATAGAATTCTTCTGCATGCTGAAGGCA-3'	634
2.	5'-ATAGGATCCGCCAGTGAGAAACCTGCT-3'	845
3.	5'-ATAGAATTCAGCAGGTTTCTCACTGGC-3'	828
4.	5'-ATAGGATCCAGTCTGGCTTCAACATCA-3'	1144
5.	5'-ATAGAATTC ^u ACTGGAAATGCTAGGCC-3'	1565
6.	5'-ATAGGATCC ^u TCTCCTTGAGAGCCTCA-3'	2100
7.	5'-ATAGAATTC ^u AGCTGCACGAGCTGCAA-3'	1946
8.	5'-ATAGGATCC ^u ATTCTGAGCACCGGAAG-3'	2318

* The position number is that of the nucleotide underlined, which corresponds to the 5' end of the genomic part of the primer. The numbering system is according to Shoulders et al. (40). Each primer includes a 5' nongenic 9-mer sequence for ligation.

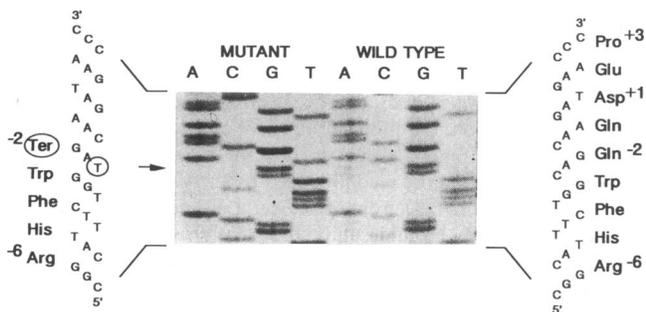


Figure 2. ApoA-I Q[-2]X DNA sequence. The autoradiogram shows the nucleotide change from C to T at codon -2, which resulted in the change of a codon encoding for the amino acid glutamine (Q) to a premature termination codon (X).

exon 3 (Fig. 2). This mutation was detected in all three clones sequenced which suggested homozygosity. The mutation, designated Q[-2]X, transforms a codon at position [-2] relative to the first amino acid of circulating mature apoA-I. The normal sequence at this position encodes glutamine, but the mutated codon encodes premature termination. Cloned sequences of exons 2 and 4 of the proband's DNA were normal. Endonuclease digestions of the proband's DNA exon 3 fragment with BbvI and Fnu4HI each showed complete loss of a digestion site as predicted by the mutant sequence, confirming the mutation and the homozygosity.

ApoA-I genotypes using Fnu4HI were determined in the family members (Fig. 3) and in a series of 75 normolipidemic Caucasian controls. All 75 control subjects showed homozygosity for the apoA-I wild isotype. All AAL subjects showed homozygosity for the apoA-I Q[-2]X allele. The proband's husband and two sibs (II-a, d) showed homozygosity for wild type apoA-I. The proband's two obligate heterozygote daughters (III-a, b), and two other sibs (II-b, h) showed heterozygosity for the wild-type and mutant alleles.

Gradient gel electrophoresis. Gradient gel electrophoresis of the homozygotes is illustrated in Fig. 4. The HDL fraction is dominated by a population of very small particles with peak diameter of 7.9 nm. In the heterozygotes, in addition to the particles of size 7.9 nm in diameter, a second population with peak diameter of 8.4 nm can also be seen, which closely approximate the sizes of HDL₃ subfractions seen in normal sub-

jects. In heterozygotes there is no HDL seen with peak size in the HDL₂ range.

Differential electroimmunodiffusion. Lp-AI particles in whole plasma, HDL, HDL₂, and HDL₃ subfractions were separated by differential electroimmunodiffusion. Lp-AI particles were detected in the plasma, HDL, HDL₂, and HDL₃ fractions among the unaffected members (data not shown). For the heterozygotes, the same particles were detected in plasma, HDL, HDL₃, but not the HDL₂ subfractions (data not shown). For the homozygotes, Lp-AI was undetectable (data not shown).

Discussion

We have identified a Canadian kindred with a novel nonsense mutation of the apoA-I gene which we designate as Q[-2]X. Homozygosity for Q[-2]X causes a selective failure to produce any portion of the mature apoA-I. This results in nondetectable plasma apoA-I and a very low level of HDL-C. Heterozygosity for Q[-2]X results in intermediate levels of apoA-I and HDL-C, and the unaffected members have the highest HDL-C and apoA-I levels. This suggests the genetic trait is being transmitted in an autosomal co-dominant fashion. This mutation distinguishes itself from previously reported cases of genetically determined HDL deficiencies. Of the nine siblings ascertained, five were homozygous for the mutation. This is the largest number of adult homozygotes in any single kindred with apoA-I deficiency. There is a high prevalence of premature CHD among affected subjects.

The clinical manifestation appears highly heterogeneous among the homozygotes. The proband had clinical findings of cerebellar neuropathy, bilateral premature cataracts, subretinal lipid deposition complicated by retinal detachment, and tendon xanthomata. The first two of these signs have not been previously described in any genetically determined HDL deficiency. Their relevance to the apoA-I mutation in this family remains to be clarified. Retinopathy related to subretinal lipid deposition observed in the proband is unique among kindreds with genetically determined apoA-I deficiency. Retinal involvement has only been reported in two cases of patients with lecithin:cholesterol acyltransferase LCAT deficiency (43). The clinical manifestations of one homozygote sister (II-c) included arcus cornealis, planar xanthomata, and premature CHD. Although planar xanthoma and/or corneal abnormalities appeared to distinguish subjects with severe HDL defi-

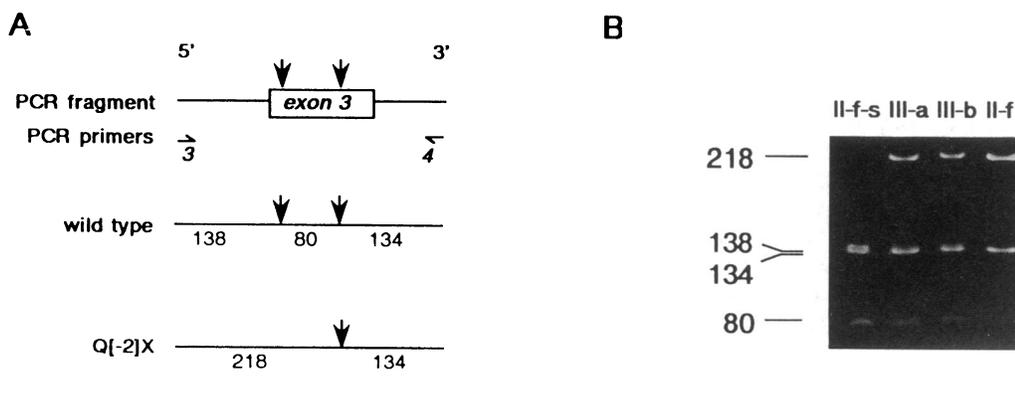


Figure 3. (A) Restriction map for exon 3. The position of Fnu4HI restriction sites are indicated with vertical arrows. Fnu4HI fragment sizes (in bp) are shown for alleles containing the normal apoA-I gene exon 3 sequence ("wild type") and the mutant apoA-I gene exon 3 sequence ("Q[-2]X"). The regions of the genomic DNA sequence corresponding to PCR primers 3 and 4 are indicated. (B) Segregation of Fnu4HI

in a partial Q[-2]X pedigree. The unaffected member (II-f-s), the proband's husband showed the wild-type Fnu4HI RFLP. The Q[-2]X allele obliterates a Fnu4HI site, creating a larger band (218 bp) in the proband (II-f). The two daughters, who are obligate heterozygotes, showed combined wild-type and Q[-2]X Fnu4HI RFLP.

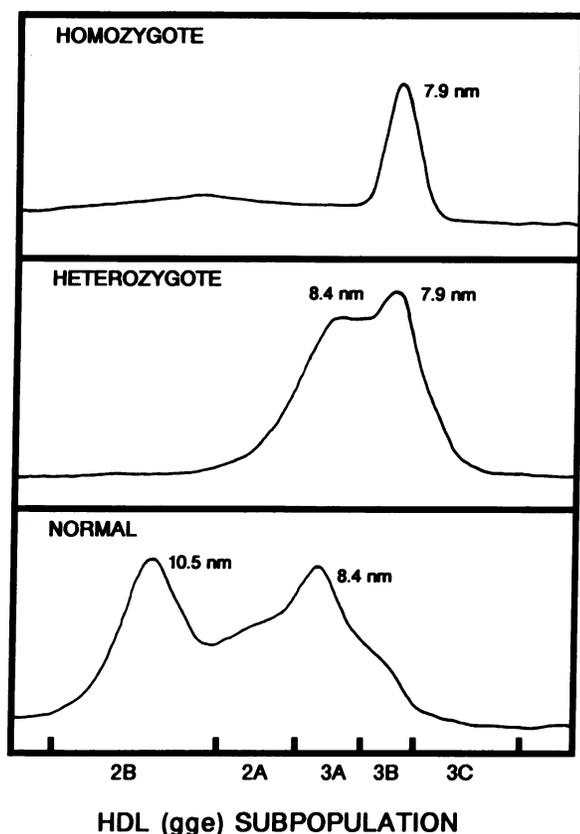


Figure 4. HDL particle size profiles. Densitometric scans after non-denaturing 4–30% PAGE of HDL isolated from an AI Q[–2]X homozygote (*top*), heterozygote (*center*), and a normal subject (*bottom*). HDL subpopulation intervals are shown below the scan profile and peak particle diameters are given in nanometer.

ciency among a number of other kindreds reported (15–23, 26), this appears not to be the case in our kindred. All of the remaining homozygotes, though not available for detailed clinical examination, were contacted and had no complaints related to CHD, neuropathy, or visual impairment. Among the heterozygotes, one sister (II-b) had reversible CHD on stress thallium testing but this was confounded by other CHD risk factors including cigarette smoking, hypertension, and obesity. She also complained of mild imbalance. The remaining heterozygotes and unaffected siblings had no complaints related to the above illnesses.

The affected members of the kindred not only had a reduced number of HDL particles but also an abnormal HDL size distribution as shown by the gradient gel electrophoresis. The HDL among homozygotes was dominated by a fraction of very small HDL particles with peak diameter of 7.9 nm. The heterozygotes had two populations of HDL particles, one with peak diameter of 7.9 nm and the other with peak diameter of 8.4 nm, resembling HDL₃ particles in normal individuals. On the other hand, the heterozygotes were virtually devoid of any HDL particles in the HDL₂ flotation range. Among the unaffected relatives, the HDL particle distribution appeared normal.

Differential electroimmunodiffusion assay of the HDL fractions of the homozygotes showed the presence of apoA-II but not apoA-I, which suggests that the small HDL particles

seen in gradient gel electrophoresis were Lp-AII. Detection of Lp-AII particles have been described in several reports of apoA-I deficiencies (22, 26, 28). In transgenic mice overexpressing apoA-II (44), human apoA-II-only particles of 8 nm diameter were observed. Taken together these observations further support the stable formation of human apoA-II-only particle of a distinct size when apoA-II is unable to interact with human apoA-I due either to the absence of apoA-I or structural abnormalities of apoA-I, or in human apoA-II transgenic mice containing only murine apoA-I. These observations also reinforce the concept that apoA-I is the major determinant of HDL structure as well as plasma level.

The plasma levels of apoC-III were only moderately reduced in the AAL subjects which suggests absence of a gross functional defect of the apoC-III gene associated with the apoA-I mutation. The reduction in plasma apoC-III is presumably due to a lack of circulating HDL particles to accept transfer of apoC-III from the triglyceride-rich particles, resulting in increased catabolism. This further confirms that the present mutation results in a selective failure to produce any portion of the mature apoA-I. The free cholesterol/total cholesterol ratio was normal for both affected and unaffected members. This is in agreement with previous findings that apoA-I is not essential for LCAT activity (45).

The LDL-C in 7 of 11 members in the kindred exceed the 90th percentile after adjustment for age and gender. Four of these seven subjects have concurrent elevation of triglycerides over the 90th percentile (type IIb). Such findings are not uniform among reported cases of genetically determined HDL deficiency and are unlikely to be pathogenetically related to the apoA-I deficiency. The cause of the hyperlipidemia remains to be elucidated.

The severe HDL deficiency clearly predisposes some of the young members in this kindred to premature CHD. One of the five homozygotes (II-c) suffered a myocardial infarction due to coronary atherosclerosis at age 34, whereas the other homozygote sibs so far had no signs of CHD. One heterozygote (II-b) had documented reversible myocardial ischemia which was confounded by the presence of numerous coronary risk factors including elevated LDL-C, hypertension, cigarette smoking, and obesity. The father, an obligate heterozygote, died of massive MI at the age of 64 yr with no antecedent symptoms. The incidence of CHD among the homozygotes is lower than that of homozygous familial hypercholesterolemia (46). There is no history of early CHD related mortality in this kindred and the homozygous subjects have all lived to their reproductive age. These observations appear to parallel the lack of fulminant atherosclerosis during the early stage of development in mice after apoA-I deletion by homologous recombination (47) although there are fundamental differences in the major lipoprotein classes between mice and human that make the direct comparisons difficult.

It appears that inability to synthesize apoA-I or assemble HDL particles may be the common denominator among the kindreds predisposed to CHD. Two kindreds with combined deficiency of apoA-I and apoC-III, one due to apoA-I and apoC-III gene inversion defect (15, 16) and the other due to deletion of the entire apoA-I-CIII-AIV gene complex (17, 18), rendering the individuals unable to synthesize apoA-I and apoC-III in the former case and additional apoA-IV synthetic defect in the latter case, appeared most susceptible to premature CHD. A Japanese family with an apoA-I gene codon 84

nonsense mutation (19, 20) and a presumed HDL assembly defect also appeared to be susceptible. Our kindred, with an isolated apoA-I synthetic defect also confirms this association with CHD. The opposite was true for subjects with homozygous apoA-I_{202:fs} (21), heterozygous apoA-I_{Seattle} (22), and apoA-I_{Iowa} (48) defects, all of which manifest circulating apoA-I variants. Clinically, the former also had added planar xanthoma, but the latter three had arcus cornealis, corneal clouding and systemic amyloidosis respectively. Furthermore, patients with Tangier disease (29) have unique abnormal deposition of lipids and hypercatabolism of apoA-I and are spared from increased risk of CHD. LCAT deficiency causes severe HDL deficiency and premature atherosclerosis in addition to hemolytic anemia, corneal deposit and glomerulopathy (25). A variety of structural defects of the LCAT gene have been reported (49, 50). Fish eye disease (51, 52) patients also have severe HDL deficiency, putatively due to specific loss of LCAT-HDL interaction as a result of specific structural mutations of the LCAT gene. Fish-eye disease subjects are spared from premature CHD. It is probable that specific lipid-tissue interaction plays a major role in modulating an individual's susceptibility to CHD.

In summary, we have reported a large Canadian kindred with a genetic mutation Q[-2]X in the apoA-I gene causing specific failure of production of apoA-I resulting in AAL. Clinically, the five homozygotes had uniformly low HDL levels but were highly heterogeneous with regard to clinical features. The retinal lipid deposits described in the proband are unique. There was a marked increase in prevalence of premature CHD in this kindred, attributable to the unique Q[-2]X mutation. The large size of this kindred, the diversity of clinical manifestations, and the established molecular defect of isolated absent apoA-I, should facilitate the search for critical determinants responsible for the diverse clinical phenotypes, with particular reference to the development of premature CHD.

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