# A novel apolipoprotein C-III variant, apoC-III(Gln38 → Lys), associated with moderate hypertriglyceridemia in a large kindred of Mexican origin

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**Abstract** Apolipoprotein C-III (apoC-III) is a major protein component of very low density lipoproteins (VLDL), chylomicrons, and a minor component of high density lipoproteins (HDL). Studies of naturally occurring human variants of apoC-III will help in adding to our understanding of the physiological function of this apolipoprotein. Using isoelectric focusing (IEF) of VLDL fractions we screened over 2500 lipid clinic patients and have identified an individual with a novel apoC-III variant. DNA sequencing revealed the variant to be a Lys for Gln exchange at amino acid residue 38 due to an A for C substitution in exon 3. This was confirmed by $NH_2$-terminal protein sequence analysis. The mutant Lys38 variant was present in VLDL at about the same level as the normal form although the total amount of apoC-III was increased by 34%. The proband, a 16-year-old boy of Mexican origin, had a plasma level of total triglycerides above the 95th percentile for his age. Family studies revealed a further 16 individuals who were heterozygous for this apoC-III(Gln38 → Lys) variant. Compared to 21 unaffected relatives, the 17 heterozygous subjects had a statistically significant 32% elevation of their plasma levels of triglycerides when adjusted for age, sex, body mass index, and lifestyle. Other lipid and lipoprotein values were unaffected. The presence of an additional positive charge at residue 38 suggests that this residue is involved in the function of apoC-III. The elevation of plasma levels of triglycerides supports the view that apoC-III is involved in the regulation of the catabolism of triglyceride-rich lipoproteins.—**Pullinger, C. R., M. J. Malloy, A. K. Shahidi, M. Ghassemzadeh, P. Duchateau, J. Villagomez, J. Allaart, and J. P. Kane.** A novel apolipoprotein C-III variant, apoC-III(Gln38 → Lys), associated with moderate hypertriglyceridemia in a large kindred of Mexican origin. *J. Lipid Res.* 1997. **38:** 1833–1840.

**Supplementary key words** apolipoprotein variants • genetic variants • lipolysis • hypertriglyceridemia • triglyceride-rich lipoproteins • chylomicrons • triacylglycerol

ApoC-III is the most abundant protein in VLDL comprising approximately 40% of the protein mass. Based on ultracentrifugation, the concentration of this 8.8 kDa glycoprotein in plasma is about 12 mg/dl, of which 60% is in HDL, 20% in VLDL, 10% in LDL, and 10% in IDL (1). Isoelectric focusing reveals three isoforms, apoC-III-0, apoC-III-1 and apoC-III-2, with, respectively, 0, 1 and 2 residues of sialic acid per molecule. The concentrations of these three isoforms are 14%, 59%, and 27%, respectively, of the total C-III in plasma (2). The apoC-III gene codes for a 99-residue prepeptide (3, 4). Removal of the signal sequence gives rise to the mature 79-residue circulating form of the protein.

Little is known about the function of apoC-III in lipoprotein metabolism. It has been shown to inhibit lipoprotein lipase (LPL) (5, 6) and hepatic lipase (7) in vitro with the major inhibitory effect apparently residing in an $NH_2$ terminal domain, although there are a number of hydrophilic sequences within the peptide that also interact with LPL (8). ApoC-III does not compete with apoC-II at the apoC-II activation site on LPL (8). Glycosylation of apoC-III does not seem to be important for LPL inhibition (8). It has also been shown to inhibit the hepatic uptake of chylomicrons (9, 10). It has been proposed that the physiological role of apoC-III involves the regulation of catabolism of triglyceride-rich lipoproteins by inhibiting their uptake (2).

Transgenic mice expressing the human gene have elevated levels of VLDL triglyceride with greater than

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Abbreviations: VLD1, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; IEF, isoelectric focusing; PCR, polymerase chain reaction; BMI, body mass index; LPL, lipoprotein lipase.

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double the normal amount of apoC-III per particle (11, 12). These VLDL particles have a larger diameter. It is likely that the observed hypertriglyceridemia is a result of the low fractional catabolic rate of VLDL in these animals and low clearance rate of chylomicron remnants. This low rate is probably due to a combined effect of the increased amount of apoC-III and the decreased amount of apoE on VLDL. These studies suggest that apoC-III interferes in the apoE-mediated clearance of triglyceride-rich lipoproteins. This notion was strengthened by the observation of a return to normal levels of triglycerides when these mice were crossed with those overexpressing apoE (13). ApoC-III-deficient mice have lower fasting levels of triglycerides with an absence of post-prandial lipemia and an increased rate of chylomicron clearance (14). Unfortunately, the interpretation of these findings is complicated by the accompanying decrease in the intestinal expression of the apoA-I and apoA-IV genes in these animals.

Four structural variants of apoC-III have been reported to date. One was due to oversialylation at residue 74 (15). Lack of sialylation was observed in another variant, apoC-III(Thr74 → Ala) (16). The concentration in VLDL of the variant apoC-III(Asp45 → Asn) was found to be double that of the normal peptide in heterozygotes (17). However, the lipid and lipoprotein profiles of apoC-III(Asp45 → Asn) heterozygotes were no different from those of unaffected family members. The fourth variant, apoC-III(Lys58 → Glu), was found in two heterozygotes to be present at a lower level in VLDL and HDL than the normal form (18). These individuals had unusual HDL with some large apoE-rich HDLc-like particles.

We report here a novel apoC-III variant associated with elevated triglycerides. The 17 heterozygotes in a large kindred of Mexican ancestry were unaffected with respect to cholesterol levels and levels of HDL lipids.

## MATERIALS AND METHODS

### Experimental subjects

A boy of Mexican ancestry was referred at age 16 with hypertriglyceridemia. Though moderately obese he was free of apparent systemic disorders including diabetes, hypothyroidism, and renal dysfunction. The pedigree of his family is presented in **Fig. 1.** Isoelectric focusing of apoVLDL from the proband revealed an abnormal pattern (see Fig. 2). Blood samples were obtained from 11 relatives in the San Francisco Bay Area and subsequently from a further 29 relatives living in Mexico. The proband's maternal grandfather was deceased.

### DNA and lipoprotein isolation

Genomic DNA was prepared from each family member (19). An additional sample of blood was drawn after a 14-h fast for lipid analyses as described previously (19, 20). EDTA (0.05%, w/v), sodium azide (0.05%, w/v), and benzamidine (0.03%, w/v) were added in order to minimize degradation of lipoproteins. VLDL ($d < 1.006$ g/ml), LDL ($1.006 < d < 1.063$ g/ml), and HDL ($1.063 < d < 1.21$ g/ml) were prepared from plasma by sequential ultracentrifugation (21) for each of the first 11 family members except for subject III-18 from whom too little blood was obtained. Total cholesterol and triglycerides in plasma and in lipoprotein fractions were measured using an automated chemistry analyzer (Hoffman-La Roche). For the other 29 individuals plasma levels of HDL cholesterol and HDL triglyceride were determined after precipitation of apolipoprotein B-containing lipoproteins with $MgCl_2$ and dextran sulfate. Levels of LDL cholesterol were calculated according to the formula of Friedewald, Levy, and Fredrickson (22). Plasma levels of total triglycerides, total cholesterol, LDL cholesterol, and HDL cholesterol were adjusted for age (to 25 years to provide a common baseline) and gender, by non-linear regression analysis using data in the Lipid Research Clinics Population Studies Data Book (23). These lipid values were also adjusted for lifestyle differences between people of Mexican origin living either in the US or in Mexico, using recently published data (24). In addition, plasma levels of triglycerides were adjusted for BMI. For subjects 20 years or older, regression coefficients reported by Cowan et al. (25) were used and for those under 20, data by Glueck et al. (26) were used.

### Delipidation, desialylation, and isoelectric focusing of VLDL

IEF was performed on delipidated VLDL, using tube gels, as previously described (27). In addition, slab IEF gels (9% T, 2.67% C, 8 M urea and 2% ampholines, pH 3.5–7) were run using a mini-Protean II slab cell apparatus (Bio-Rad, Richmond, CA). Gels were stained with Coomassie brilliant blue G.

Where appropriate, the VLDL were treated with neuraminidase to desialylate the proteins. To 1 mg of apoVLDL in 1.5 ml of sodium acetate (100 mM, pH 5.0) was added 0.1 units of neuraminidase from *Clostridium perfringens* (Boehringer Mannheim, Indianapolis, IN) and the sample was incubated at 37°C for 90 min. The desialylated VLDL was then delipidated.

Slab IEF gels, stained with Coomassie blue, were

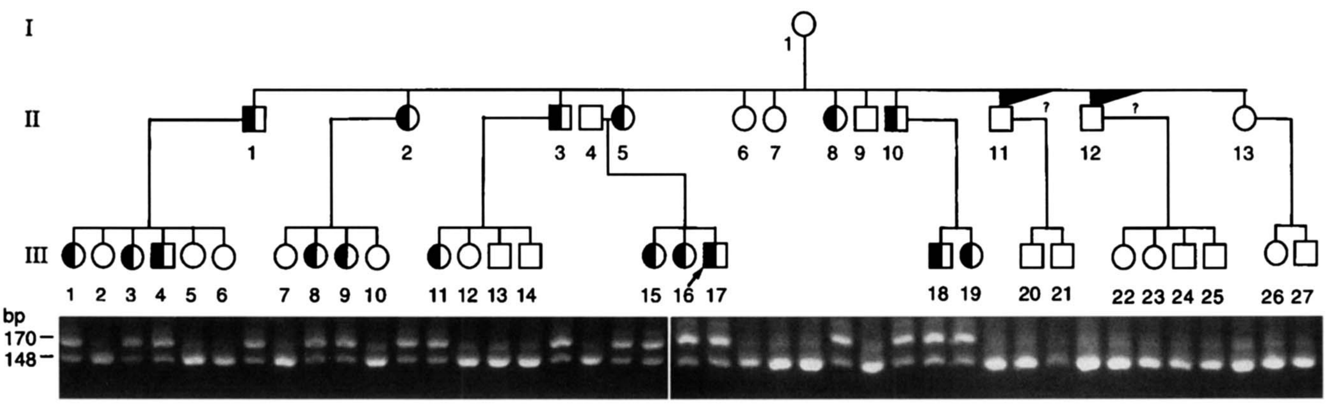

**Fig. 1.** Pedigree of the kindred studied; ◧, and ◐, indicate males and females heterozygous for the presence of the apoC-III(Gln38 → Lys) variant. Beneath the symbols is an agarose gel of PstI digested DNA, amplified using a primer containing a mismatch that allows identification of the underlying mutation. The artificially created PstI site is deleted by the mutation, a C to A transition at nucleotide 1240 (Genebank accession no. X03120).

scanned in reflective mode using a flat-bed computer scanner and analyzed on a Macintosh 6100/66 computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available from zippy.nimh.nih.gov or from the National Technical Information Service, Springfield, VA).

### $NH_2$-terminal sequence analysis

Slab gels of delipidated VLDL were electrotransferred to Immobilon polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA) to allow the $NH_2$-terminus of the apoC-III0 band to be sequenced on a model 473A protein sequencer (Applied Biosystems, Foster City, CA).

### DNA amplification and direct sequencing of the apoC-III gene

Exons 3 and 4 of the apoC-III gene, which code for the mature circulating peptide, were amplified from genomic DNA, using oligonucleotide pairs nos. 78/79 and nos. 81/83 (listed in **Table 1**), respectively. The reactions were performed in 50 mM Tris-HCl, pH 9 (at 25°C), 20 mM $(NH_4)_2SO_4$, 1.5 mM $MgCl_2$, 200 μM of each dNTP, and using 100 ng of each primer in a total volume of 50 μl. After initial denaturation at 96°C for 2 min, 1 unit of Hot Tub polymerase (Amersham Life Science Inc, Cleveland, OH) was added at 80°C and 'touchdown' PCR (28) was carried out at an initial annealing temperature of 62°C declining in 1°C steps to 56°C for a total of 36 cycles. For each cycle the denaturing and elongation steps were 96°C for 30 s and 72°C for 120 s, respectively. A Perkin-Elmer thermal cycler (Perkin-Elmer Corp, Norwalk, CT) was used.

Dideoxysequencing was carried out on the double-stranded DNA by cycle sequencing, using Thermosequenase and the internal labeling protocol with $[\alpha\text{-}^{35}S]$dATP (Amersham Life Science Inc, Cleveland, OH). For each exon both strands were sequenced using primers nos. 241 and 242 for exon 3 and primers nos. 243 and 244 for exon 4.

TABLE 1. ApoC-III gene oligonucleotides used in this study

| | Sequence | Site |
|---|---|---|
| No. 78 | 5′ GCGGATCCACCCCACTCAGCCCTGCTCTTTC 3′ | 1088–1118, sense |
| No. 79 | 5′ TGCCCGGGGGGATGGGGAGGGAGGCCAGCGG 3′ | 1254–1284, antisense |
| No. 81 | 5′ TTCGCATGCCCTGCTCTGTTGCTTCCCCTGAC 3′ | 3062–3093, sense |
| No. 83 | 5′ AGGAGCTCGCAGGATGGATAGGCAGGTGGAC 3′ | 3239–3269, antisense |
| No. 241 | 5′ GCCGATCCACCCCACTC 3′ | 1088–1104, sense |
| No. 242 | 5′ TGGCAGGGGGGATGGGGAGGG 3′ | 1264–1284, antisense |
| No. 243 | 5′ TTCTCATGCCCTGCTCTGTTG 3′ | 3062–3082, sense |
| No. 244 | 5′ AGGAGCTGGCAGGATGGATAG 3′ | 3249–3269, antisense |
| No. 253 | 5′ GCAGGAGTCCCAGGTGGCCCtG 3′ | 1218–1239, sense |
| No. 254 | 5′ GGAAAGAGGAGGCTGAAGAGGC 3′ | 1366–1387, antisense |
| No. 263 | 5′ TGACAAAGGCCCTGTGAG 3′ | 1387–1404, antisense |

Lower case lettering in no. 253 indicates change made to introduce an artificial PstI restriction site at nucleotide 1237. Numbering is from the apoC-III gene sequence Genebank accession number X03120.

TABLE 2. Lipid and lipoprotein profiles of affected individuals

| Subject | C-III Residue 38 | Age | Sex | TC | Adjusted TC | TG | Adjusted TG | VLDL TC | LDL TC | Adjusted LDL-TC | HDL TC | Adjusted HDL-TC | HDL TG | BMI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| II-1 | Q/K | 43 | M | 212 | 195 | 149 | 82 | 30 | 149 | 120 | 33 | 46 | 9 | 26.3 |
| II-2 | Q/K | 41 | F | 199 | 198 | 129 | 96 | 26 | 127 | 115 | 45 | 48 | 18 | 32.5 |
| II-3 | Q/K | 45 | M | 234 | 214 | 104 | 55 | 21 | 179 | 143 | 34 | 48 | 8 | 27.0 |
| II-5 | Q/K | 28 | F | 243 | 243 | 270 | 216 | 44 | 147 | 149 | 52 | 42 | 26 | 39.5 |
| II-8 | Q/K | 35 | F | 174 | 168 | 79 | 81 | 8 | 128 | 122 | 38 | 30 | 15 | 28.6 |
| II-10 | Q/K | 36 | M | 221 | 195 | 108 | 83 | 15 | 151 | 128 | 54 | 53 | 12 | 26.5 |
| III-1 | Q/K | 9 | F | 152 | 179 | 75 | 87 | 15 | 98 | 110 | 39 | 45 | 16 | 22.7 |
| III-3 | Q/K | 12 | F | 197 | 235 | 57 | 67 | 11 | 133 | 152 | 52 | 60 | 13 | 20.8 |
| III-4 | Q/K | 14 | M | 149 | 185 | 79 | 98 | 16 | 87 | 102 | 46 | 56 | 11 | 19.0 |
| III-8 | Q/K | 14 | F | 162 | 197 | 63 | 65 | 13 | 108 | 125 | 41 | 48 | 10 | 23.9 |
| III-9 | Q/K | 9 | F | 160 | 189 | 115 | 173 | 23 | 97 | 109 | 40 | 46 | 14 | 12.8 |
| III-11 | Q/K | 4 | F | 193 | 229 | 59 | 39 | 12 | 131 | 146 | 50 | 55 | 14 | 30.9 |
| III-15 | Q/K | 8 | F | 181 | 195 | 103 | 185 | 13 | 102 | 114 | 67 | 56 | 17 | 14.3 |
| III-16 | Q/K | 13 | F | 179 | 199 | 114 | 145 | 13 | 124 | 143 | 41 | 35 | 20 | 26.2 |
| III-17 | Q/K | 16 | M | 148 | 173 | 184 | 195 | 26 | 81 | 95 | 41 | 37 | 19 | 32.0 |
| III-18 | Q/K | 9 | M | 149 | 164 | 62 | 87 | 12 | 81 | 94 | 56 | 45 | 26 | 20.8 |
| III-19 | Q/K | 7 | F | 154 | 166 | 44 | 82 | 6 | 93 | 103 | 55 | 46 | 18 | 15.1 |
| Mean | | 20.2 | | 182.6 | 195.5 | 105.5 | 107.9[a] | 17.9 | 118.6 | 121.7 | 46.2 | 46.7 | 15.6 | 24.6 |
| ±SE | | 3.5 | | 7.5 | 5.7 | 13.6 | 13.0 | 2.3 | 6.8 | 4.6 | 2.2 | 2.0 | 1.3 | 1.7 |

All lipid values are in mg/dl.

[a] $P = 0.05$ compared to values of unaffected family members (see Table 3).

### DNA screening for the apoC-III(Gln38 → Lys) variant

The apoC-III gene exon 3 was amplified from Genomic DNA with oligonucleotides no. 78 and no. 263. The PCR conditions were as above except a cold start was used with a GeneAmp PCR system 9600 (Perkin-Elmer Corp, Norwalk, CT) and 33 cycles of: 96°C for 15 s; 62°C 15 s; 72°C 40s. Using 1 μl of this reaction product, a nested amplification was then performed with primers no. 253 and no. 254 under the same conditions except at an annealing temperature of 60°C. Primer no. 253 has a base mismatch (see Table 1) that introduces an artificial PstI site at nucleotide 1237. This site is missing in alleles that code for the apoC-III variant. Aliquots were digested with PstI and run on 4% MetaPhor agarose gels (FMC BioProducts, Rockland, ME) using ethidium staining.

## RESULTS

A novel apoC-III charge-change variant, apoC-III(Gln38 → Lys), was found to be associated with a statistically significant 32% elevation of plasma triglycerides when adjusted by nonlinear polynomial regression for age, sex, BMI, and lifestyle ($P = 0.05$) (**Tables 2 and 3**). No other differences in lipid or other parameters were statistically significant. The proband of the large kindred of Mexican origin (Fig. 1) was a boy age 16 (II-17) who presented with a level of triglycerides in plasma of 184 mg/dl. This is above the 95th percentile for his age (23). His level of total cholesterol was normal (148 mg/dl). Isoelectric focusing of apoVLDL from this subject, his mother and his sister (**Fig. 2**) showed a higher amount of material at the position on the gel of apoC-III-0, less at the position of apoC-III-2 and an additional band at the −1 position, with a pI of about 5.3, between the positions of apoC-III-0 and apoE. The concentrated non-apoB apolipoproteins associated with LDL fractions from three heterozygotes were also subjected to IEF. Although the overall amounts of the C-III isoforms were greatly decreased, the distribution was similar to the VLDL fraction (data not shown). IEF gels of HDL fractions were more difficult to assess due to overlap of the C-III bands with other HDL proteins.

Sequencing of apoC-III gene exons 3 and 4, by dideoxysequencing after PCR of the patient's DNA, revealed only one mutation. This was a CAG → AAG transition in the codon for residue 38 of the mature protein in exon 3, a lysine for glutamine substitution (**Fig. 3**). The anti-coding strand is shown in Fig. 3, which reveals the mutation as a G to T transition. This mutation represents an electrostatic charge change that is consistent with the pattern seen by isoelectric focusing. $NH_2$-terminal sequence analysis of the material focusing at the normal apoC-III-0 position revealed only apoC-III sequence

TABLE 3. Lipid and lipoprotein profiles of unaffected individuals

| Subject | C-III Residue 38 | Age | Sex | TC | Adjusted TC | TG | Adjusted TG | VLDL TC | LDL TC | Adjusted LDL-TC | HDL TC | Adjusted HDL-TC | HDL TG | BMI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I-1 | Q/Q | 64 | F | 240 | 200 | 129 | 66 | 26 | 175 | 127 | 39 | 37 | 15 | 39.7 |
| II-4 | Q/Q | 43 | M | 152 | 129 | 85 | 61 | 11 | 92 | 74 | 50 | 49 | 15 | N/A |
| II-6 | Q/Q | 33 | F | 181 | 177 | 50 | 37 | 3 | 123 | 119 | 55 | 43 | 12 | 41.8 |
| II-7 | Q/Q | 19 | F | 179 | 197 | 41 | 58 | 2 | 126 | 144 | 51 | 43 | 15 | 19.4 |
| II-9 | Q/Q | 29 | M | 170 | 161 | 170 | 183 | 23 | 108 | 99 | 38 | 38 | 17 | 21.5 |
| II-11 | Q/Q | 40 | M | 217 | 203 | 69 | 47 | 14 | 164 | 135 | 39 | 55 | 8 | 21.6 |
| II-12 | Q/Q | 40 | M | 189 | 177 | 151 | 82 | 30 | 122 | 100 | 37 | 52 | 12 | 26.9 |
| II-13 | Q/Q | 29 | F | 168 | 182 | 82 | 84 | 16 | 107 | 107 | 45 | 49 | 9 | 23.1 |
| III-2 | Q/Q | 11 | F | 133 | 159 | 69 | 87 | 14 | 79 | 90 | 40 | 46 | 10 | 19.2 |
| III-5 | Q/Q | 16 | F | 165 | 202 | 88 | 92 | 18 | 103 | 120 | 44 | 51 | 14 | 24.6 |
| III-6 | Q/Q | 17 | F | 166 | 202 | 87 | 80 | 17 | 102 | 119 | 47 | 53 | 12 | 29.3 |
| III-7 | Q/Q | 16 | F | 142 | 173 | 104 | 111 | 21 | 81 | 94 | 40 | 45 | 12 | 25.0 |
| III-10 | Q/Q | 7 | F | 163 | 190 | 67 | 117 | 13 | 112 | 124 | 38 | 43 | 17 | 11.1 |
| III-12 | Q/Q | 11 | F | 139 | 166 | 74 | 69 | 15 | 83 | 94 | 41 | 47 | 17 | 28.0 |
| III-13 | Q/Q | 18 | M | 163 | 209 | 86 | 95 | 17 | 107 | 126 | 39 | 51 | 13 | 19.7 |
| III-14 | Q/Q | 19 | M | 178 | 226 | 62 | 67 | 12 | 122 | 142 | 44 | 58 | 8 | 20.4 |
| III-20 | Q/Q | 9 | M | 141 | 168 | 48 | 57 | 10 | 100 | 116 | 31 | 35 | 13 | 18.9 |
| III-21 | Q/Q | 8 | M | 175 | 209 | 62 | 78 | 12 | 128 | 151 | 35 | 39 | 13 | 18.3 |
| III-22 | Q/Q | 18 | F | 169 | 204 | 72 | 85 | 14 | 111 | 128 | 44 | 51 | 8 | 20.0 |
| III-23 | Q/Q | 14 | F | 169 | 205 | 74 | 78 | 15 | 106 | 123 | 48 | 55 | 13 | 24.2 |
| III-24 | Q/Q | 12 | M | 194 | 236 | 65 | 75 | 13 | 116 | 134 | 65 | 76 | 20 | 20.4 |
| III-25 | Q/Q | 7 | M | 185 | 222 | 51 | 92 | 10 | 111 | 134 | 64 | 71 | 19 | 13.2 |
| III-26 | Q/Q | 6 | F | 145 | 169 | 82 | 106 | 16 | 104 | 115 | 25 | 28 | 9 | 22.2 |
| III-27 | Q/Q | 7 | M | 170 | 204 | 85 | 61 | 17 | 118 | 142 | 35 | 39 | 11 | 25.9 |
| Mean |  | 20.1 |  | 170.5 | 190.4 | 81.4 | 82.0 | 15.0 | 112.5 | 119.1 | 43.0 | 48.1 | 12.9 | 23.2 |
| ±SE |  | 3.0 |  | 5.0 | 5.1 | 6.3 | 5.9 | 1.3 | 4.5 | 3.9 | 1.9 | 2.2 | 0.7 | 1.4 |

All lipid values are in mg/dl.

with the simultaneous presence of glutamine and lysine at residue 38, confirming that the material in this band was a mixture of the normal and variant peptides.

We have since collected blood samples, prepared DNA, and carried out lipoprotein and lipid analyses on a total of 41 members of this kindred. A total of 17 persons were found to be heterozygous for this mutation using PCR with a modified primer and digestion with PstI (Fig. 1). Heterozygosity for the C to A transition in the first base of codon 38 is revealed by the presence of bands at both 170 bp and 148 bp. This screening was

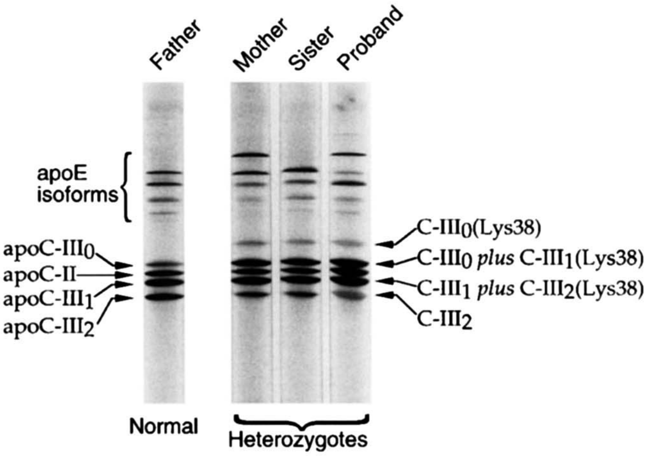

**Fig. 2.** Analytical isoelectric focusing gels of VLDL from the proband, his mother, father, and sister. The positions of normal apoC-III and variant apoC-III bands, apoC-II and the apoE isoforms are indicated.

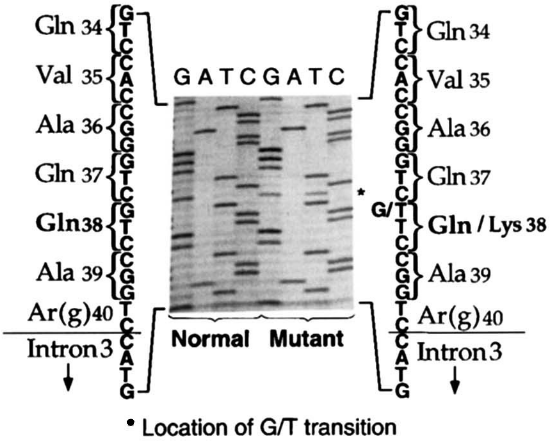

**Fig. 3.** DNA sequencing gel showing the single nucleotide change leading to the apoC-III(Gln38 → Lys) variant. The ladders show part of the anticoding strand of exon 3 and intron 3 of the apoC-III gene. An asterisk indicates the T for G substitution in the codon for amino acid residue 38.

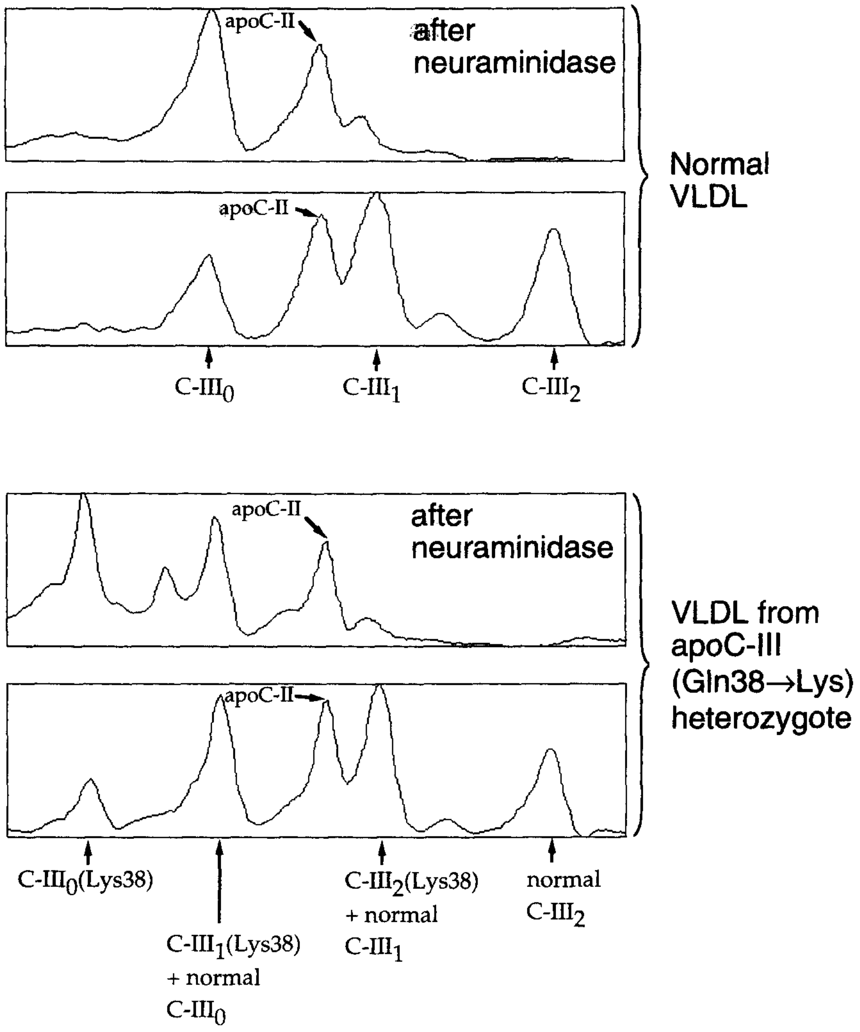

**Fig. 4.** Densitometric scan of Coomassie blue-stained slab isoelectric focusing gels of apoVLDL from an individual heterozygous for the apoC-III(Gln38 → Lys) variant and from a normal individual. The scans are of untreated and neuraminidase-treated samples.

in all but one case (subject III-18, from whom insufficient blood could be obtained) confirmed by isoelectric focusing of apoVLDL (results not shown).

Scanning of the slab IEF gels in the region of the C-II and C-III apolipoproteins revealed the abnormal distribution of material at the C-III-0, C-III-1, and C-III-2 positions compared to normal VLDL with the presence of the additional band at a pI of 5.3 (**Fig. 4**). After treatment with neuraminidase, normal VLDL shows essentially all the apoC-III in the C-III-0 band (Fig. 4). VLDL from an affected subject showed a large increase in the intensity of the additional band with a pI of 5.3. This is the desialylated variant peptide.

The apoC bands on slab IEF gels were scanned and quantified for 9 heterozygotes and 20 unaffected relatives and the results are shown in **Table 4**. This shows that there is 34% ($P = 0.05$) more total apoC-III (normal and variant isoforms) in the VLDL fraction from the affected subjects compared to their normal relatives. The ratio of C-III to C-II in VLDL is also increased ($P = 0.02$). On the IEF gels from heterozygotes, there are no peptides overlapping with the C-III-0 variant (at the −1 position) or with the normal C-III-2. As can be seen from Table 4 there is 83% of the C-III-0 variant compared to the amount of normal C-III-0 in the normal samples (0.37 versus 0.44 mg/dl). Similarly, there is 80% of normal C-III-2 in the heterozygotes compared to that in the normals (0.62 versus 0.78). Hence there is the same amount of normal and variant C-III in the VLDL from the heterozygotes.

TABLE 4. Plasma concentrations of apoC-III isoforms and apoC-II in very low density lipoproteins

| | Heterozygotes (Gln/Lys) (n = 9) | Normals (Gln/Gln) (n = 20) | *P* |
|---|---|---|---|
| | *mg/dl plasma* | | |
| ApoC-III | | | |
| Relative position on gel | | | |
| −1 | 0.37 ± 0.06 | | |
| 0 | 0.86 ± 0.09 | 0.44 ± 0.05 | |
| +1 | 0.92 ± 0.08 | 0.85 ± 0.07 | |
| +2 | 0.62 ± 0.08 | 0.78 ± 0.08 | |
| Total apoC-III | 2.77 ± 0.30 | 2.07 ± 0.19 | 0.054 |
| ApoC-II | 0.69 ± 0.08 | 0.60 ± 0.06 | 0.361 |
| ApoC-III/apoC-II | 4.04 ± 0.16 | 3.52 ± 0.12 | 0.016 |

## DISCUSSION

Little has been learned from classical biochemical studies about the function of apoC-III in lipid metabolism. The results of gene targeting and transgenic studies in animals present fascinating effects, but are difficult to interpret. Detection of naturally mutant alleles of candidate genes and their consequent effects on lipoprotein metabolism can be expected to provide important clues to the biochemical roles of these gene products. New causes of atherogenic dyslipidemia will be revealed in the search for mutations in proteins involved in lipid metabolism.

To date, apart from a deletion associated with major derangement of the A-I/C-III/A-IV locus (29) there have been reported only four apoC-III structural variants (15–18), none of which have been shown clearly to have any effect on lipoprotein metabolism. ApoC-III variants are thought to be extremely rare in the German population (17). This is confirmed in our studies in the San Francisco Bay Area. Although a number of apolipoprotein E and apolipoprotein C-II variants have been detected in this laboratory, the apoC-III(Gln38 → Lys) variant reported here is the first apoC-III mutation to be detected by isoelectric focusing of VLDL prepared from more than 2500 patients attending the UCSF Lipid Clinic.

VLDL from the heterozygous subjects identified with the ApoC-III(Gln38 → Lys) variant had the same amount of the variant apoC-III compared to the normal peptide. This is in contrast to the 2-fold increase seen with apoC-III(Asp45 → Asn) heterozygotes (17). This latter variant and the variant reported in the present study were not associated with changes in the levels of HDL cholesterol unlike the apoC-III(Lys58 → Glu) variant, which was associated with elevated levels of HDL cholesterol (18).

Despite the lack of evolutionary conservation of the apoC-III sequence, as pointed out by Lüttmann et al (17), the human, bovine, macaque, and porcine peptides contain a glutamine at the position corresponding to residue 38 of the human sequence. The mouse and rat sequences have valine at this position and the dog has arginine.

The presence of an additional positive charge at residue 38 of the human sequence causes a moderate, but statistically significant, 32% elevation of plasma levels of triglycerides and supports the view that apoC-III is involved in the regulation of the catabolism of triglyceride-rich lipoproteins, probably by inhibiting their clearance.

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