A rapid and reliable method for direct genotyping of codon 360 in the human apolipoprotein A-IV gene

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Abstract Human apolipoprotein A-IV exhibits a polymorphism, first investigated at the protein level, that is caused by a single amino acid substitution of glutamine to histidine at codon 360. Detection of this polymorphism requires polymerase chain reaction (PCR) and direct sequencing of the amplified products, radiolabeled allele-specific oligonucleotides (ASOs) technique, or restriction enzyme analysis of the amplified products. However, these techniques involve the use of radioactivity and/or are not well suited to the rapid processing of a large number of samples. In this paper, we propose a new technique, a bispecific-allele primer amplification, in which a simple electrophoresis of PCR products is used for typing the variation at codon 360. The 3' primer of PCR hybridizes with one or other homologous sequence in the apoA-IV gene, depending on the presence or the absence of the mutation. This differential hybridization of the primer is used for typing the variation. In order to demonstrate the validity of this system, 120 individuals phenotyped by two-dimensional electrophoresis and genotyped by ASO were analyzed by this new technique. The results obtained by the latter method are in agreement with those found by the other techniques. However, this method is simple, more reliable, and will facilitate population studies without using radioactive materials. —**Zaiou, M., S. Visvikis, A. Visvikis, and G. Siest.** A rapid and reliable method for direct genotyping of codon 360 in the human apolipoprotein A-IV gene. *J. Lipid Res.* 1992. **33:** 1061–1066.

Supplementary key words apolipoprotein A-IV • polymorphism • two-dimensional electrophoresis • bispecific-allele primer • allele specific oligonucleotide • polymerase chain reaction

Apolipoprotein A-IV (apoA-IV) is a glycoprotein with a molecular mass of 46,000 daltons, synthesized predominantly in the small intestine (1–3). It is a major component of postprandial chylomicrons and high density lipoprotein (HDL) particles (4). The exact physiological function of apoA-IV was, until now, not clear. However, it has been demonstrated that this apolipoprotein is an important element in the metabolism of chylomicrons and HDL (5). It has also been shown that apoA-IV can function as a cofactor for the enzyme lecithin:cholesterol acyltransferase (LCAT) (6) and that it may act as an activator of a plasma factor involved in the conversion of HDL subclasses (7). It is also implicated in reverse cholesterol transport by promoting efflux of cholesterol from cells (8).

The gene coding for apoA-IV resides in the region 11q13-qter on the long arm of chromosome 11 and is in close linkage with the apoA-I and apoC-III genes (9, 10).

The plasma apoA-IV has been shown to be polymorphic (11). This polymorphism has been detected by two-dimensional electrophoresis (12, 13) or by isoelectric focusing (IEF) (7, 14). The "normal" allele, apoA-IV-1 has a frequency of 0.943, and the "mutant" allele, apoA-IV-2, a frequency of 0.057 (15). Association studies have demonstrated a relationship between this polymorphism and interindividual variability in plasma HDL cholesterol, triglyceride, and glucose levels in supposedly healthy populations (11, 15–17).

It was therefore of interest to study which mutation causes this apoA-IV polymorphism. Recently, after sequencing of the apoA-IV gene, we identified two polymorphic sites, each resulting in an amino acid substitution: glutamine (CAG) is converted to histidine (CAT) at codon 360 and threonine (ACT) to serine (TCT) at codon 347 (18). Among these polymorphisms, the glutamine to histidine substitution of amino acid 360 corresponds to the apoA-IV polymorphism reported at the protein level (19). Tenkanen et al. (20) reported that the two alleles present a differential effect on LCAT activation, apoA-IV-1 being more efficient than the apoA-IV-2 isoform. But

Abbreviations: apo, apolipoprotein; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; IEF, isoelectric focusing; EDTA, ethylenediamine tetraacetic acid; PCR, polymerase chain reaction; ASO, allele specific oligonucleotide; DMSO, dimethyl sulfoxide; SDS, sodium dodecyl sulfate; bp, base pair(s).

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their results are contradictory to data reporting that apoA-IV-2 is *more efficient in activating* LCAT *than* apoA-IV-1 (21). As this polymorphism is located at residue 360, in the center of a 22-mer amphipathic helix (aminoacids 351-373), and as the activating capacity of apolipoproteins (especially of apoA-I) is thought to be due to their amphipathic helices, population and case control studies regarding the effect of this polymorphism on LCAT activation must be performed. However, to realize this kind of study, based on the phenotyping or genotyping of a large number of samples, a simple and reliable technique is desirable, if not obligatory.

Detection of the G to T mutation at codon 360 in the apoA-IV gene requires the polymerase chain reaction (PCR) and direct sequencing of the amplified products (18, 19) or hybridization of the amplified products with radiolabeled allele-specific oligonucleotides (ASOs). However, these techniques involve the use of radioactivity and are not well suited for the rapid processing of a large number of samples. Recently, restriction isotyping of human apolipoprotein A-IV was proposed for studying this polymorphism (22, 23). In order to simplify the detection of the G to T substitution we have used an alternative approach in which a simple electrophoresis of PCR products is used for typing the variation at codon 360. We used an oligonucleotide as one of the PCR primers, specific for two different sites in the apoA-IV gene: one site including the mutation, the second site showing a high homology with the first and localized 24 bp upstream of the studied mutation. Comparison with other techniques detecting this polymorphism and restriction analysis of the amplified products improved the accuracy, sensitivity, and specificity of bispecific-allele hybridization.

MATERIALS AND METHODS

Samples

Blood samples were collected, from 33 presumed healthy nuclear families taking part in systematic health examination at the Center for Preventive Medicine in Nancy, France, into ethylenediaminetetraacetate (EDTA) Vacutainers. After spinning, the plasma and buffy coat were separated and immediately frozen in liquid nitrogen until analysis. The analyses investigating the frequencies of the codon 360 polymorphism were carried out on a sub-sample of 66 unrelated adults (33 men and 33 women) from the total sample of 33 families.

Two-dimensional electrophoresis

Three μl of plasma was used for each two-dimensional gel. The two-dimensional electrophoresis was carried out as previously described (24). Individual protein moieties appeared as spots on the gel. The two-dimensional gels were typed concordantly and independently by two experienced observers. Mapping of apoA-IV to our two-dimensional gel pattern was done with the aid of an anti-apoA-IV antibody (15).

DNA extraction

Genomic DNA was purified from buffy coat by the method of Miller, Dykes, and Polesky (25).

PCR amplification for Allele Specific Oligonucleotide hybridization (ASO)

The two oligonucleotide primers used for the PCR reaction (26) were purchased from Appligene (France) and were synthesized according to the published apoA-IV gene sequence (10, 27). They were both 20 nucleotides in length. Their nucleotide sequences were: primer 1: 5'-CGGGTGGAGCCCTACGGGGA-3' for the 5' primer, primer 2: 5'-TGGGGCCAGTGCACCAGGGG-3' for the 3' primer.

The PCR amplification of the target region (300 bp) was carried out in a final volume of 100 μl containing approximately 1 μg of genomic DNA template, 200 ng of each primer, 200 mM of each dNTP, 50 mM KCl, 10 mM Tris-HCl (pH 8.0), 1.5 mM $MgCl_2$, 0.01% gelatin, and 5% DMSO. Samples were overlaid with mineral oil and incubated at 95°C for 5 min to denature initially the template DNA. Subsequently, 1 unit of Taq polymerase (Perkin-Elmer Cetus) was added. Samples were processed through 30 cycles consisting of 1 min at 94°C (denaturation) and 3 min at 59°C (annealing and extension). To assure the successful amplification of the targeted region in the apoA-IV gene, the PCR product (10 μl) was subjected to nondenaturing electrophoresis in 8% polyacrylamide gels at 90 V for 4 h. The amplified DNA fragments were visualized after staining of the electrophoresed gel by ethidium bromide under UV transilluminator. A molecular mass marker was included in each gel (ΦX174 DNA digested with *Hae*III) to calculate the size of the amplified fragments.

Allele Specific Oligonucleotide hybridization (ASO)

Ten μl of the amplified DNA was added to 190 μl of denaturing solution (0.5 N NaOH/1.5 M NaCl). This mixture was transferred directly onto Zeta-probe membrane (Bio-Rad) and blotted with a slot-blot apparatus (Schleicher & Schuell).

The filter was prehybridized for 2 h at 54°C in a solution containing, per liter, 0.9 mol of NaCl, 0.09 mol of EDTA, 0.25 g of denatured herring sperm DNA, and 0.1% (w/v) of SDS. The oligonucleotide probes, both 15-mers were designed according to the sequence of the apoA-IV gene (10, 27) as follows: "Normal" probe: 5'-AACAGCAGCAGGAGC-3', "Mutant" probe: 5'-AACAGCATCAGGAGC-3'.

Oligonucleotides were labeled at the 5' terminus with [γ-^{32}P]ATP to a final specific activity of 5 μCi/pmol

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(1 Ci = 37 GBq) using T4 polynucleotide kinase. After purification in a Nensorb 20 cartridge (NEN), 10 pmol of each ASO, both end-labeled, were applied separately onto the filter. Hybridization was performed in the same solution as for prehybridization, at 54°C, in which [γ-^{32}P]-end-labeled ASO probes were added. The hybridization was performed overnight. The filter was washed twice in 1 × SSC (NaCl, 0.15 mol/l; sodium citrate 0.015 mol/l) for 5 min at room temperature and then at 56°C for 2 min (in 1× SSC). The filter was exposed to Kodak XAR-5 film for 2-4 h. After autoradiography, the filter was stripped of the bound probe by washing in 0.4 N NaOH solution for 30 min at 42°C and in SDS 0.5%, SSC 0.1×, Tris-HCl 0.2 mol/l, pH 7.5, for 30 min at the same temperature. The filters were then hybridized with the other probe.

Allele bispecific primer

For the direct detection of the substitution of Gln to His in codon 360, we have used allele bispecific amplification. Amplification of the human apolipoprotein A-IV target sequence was carried out by PCR using the oligonucleotides 5'-CGGGTGGAGCCCTACGGGGA-3' as the 5' primer [previously used for PCR amplification for allele specific oligonucleotide hybridization (ASO)] and 5'-GCTCCTGCTGCTGCTCCAGC-3' as the 3' primer (allele-bispecific primer). This last primer of 20 nucleotides was initially chosen from the published apoA-IV gene structure (10, 27) at the end of the target DNA sequence. The point mutation of interest occurs at the last base on the 3' end of this 3' primer. We have introduced an A/A mismatch three bases from the 3' end. The PCR conditions used were the same as described above.

Restriction analysis of the amplified products (using allele-bispecific primer)

The *Ava*II, *Tth*111I, and *Hind*II restriction sites were analyzed separately. Ten µl of amplified DNA corresponding to two individuals carrying fragments of 244 and 220 bp was digested with 10 units of the appropriate restriction enzyme (Boehringer Mannheim) at 37°C for 6 h. The digestion products were visualized directly with ethidium bromide after electrophoresis on 8% polyacrylamide gel at 120 V for 4 h.

RESULTS

Our technique is based on the high homology observed between sequences **a** and **b** (**Fig. 1**) of the human apolipoprotein A-IV gene. The 5' primer is specific for the beginning of the gene region in which our mutation is included (in grey, Fig. 1). Sequence **a** (in grey, Fig. 1) served us for 3' primer synthesis. To choose these primers, the second element which we considered was that if a primer is mismatched at the 3' last nucleotide, then amplification would not occur. Therefore, we chose the 3' last nucleotide as our point mutation (G-T). According to the presence or absence of the mutation, the primer will be hybridized with one or other homologous DNA sequence, localized as already shown in Fig. 1, 24 bp upstream the base where the mutation occurs. To ensure the allele-

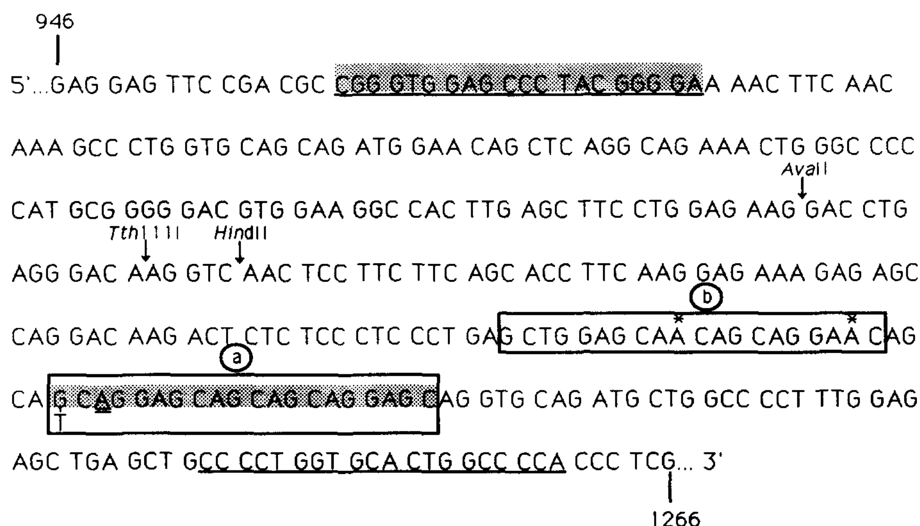

Fig. 1. Partial nucleotide sequence (nucleotide 946 to nucleotide 1266) of the human apoA-IV gene (27) in which the G to T substitution is indicated. The locations of the 1 and 2 primers used in PCR amplification of the 300 bp sequence for analysis using ASOs are underlined. Primers 5' and 3' used for bispecific-allele amplification are indicated in grey. Homologous sequences **a** and **b** are boxed. The introduced mismatch A/A is double underlined. Asterisks indicate the mismatches between the 3' primer and **b** sequence. Restriction sites of *Ava*II, *Hind*II, and *Tth*111I endonucleases are shown by arrows.

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specificity of the 3' primer, we introduced additional destabilization by substituting A for T (double underlined in Fig. 1), thus generating an A/A mismatch three bases from the 3' end of the primer. One hundred and twenty individuals already phenotyped by two-dimensional electrophoresis and genotyped by slot-blot hybridization using allele-specific oligonucleotides (results not shown) were typed by this new technique. **Fig. 2** shows the results obtained when analyzing DNA of five individuals. Three different patterns were observed. Lanes 5 and 6 show a 244 bp amplification product corresponding to the target sequence chosen. This product was obtained from the hybridization of the 3' primer with sequence **a** (Fig. 1). The A/A mismatch introduced into the synthesized primer did not prevent amplification. In addition, binding of the primer with the **a** sequence is more favorable than binding to the **b** sequence as higher homology exists (95% versus 90%). Lanes 3 and 4 (Fig. 2) show both amplification products of 220 bp. In this case, the 3' primer was hybridized with the **b** sequence. Due to the mutation (G–T) a mismatch is generated at the 3' end of the 3' primer and thus, amplification of the 244 fragment cannot occur. The second mismatch introduced (A/A) enhances this process. In this case the 3' primer hybridizes only with the highly homologous (90%) **b** sequence (asterisks (*) in Fig. 1 show the differing nucleotides between **b** sequence and 3' primer), and therefore these individuals are homozygous for the mutation. Lane 2 of Fig. 2 shows amplification products corresponding to 244 bp and 220 bp. In this case, the 3' primer was annealed at both sequences **a** and **b** (Fig. 1) indicating that the substitution occurred on only one of the two alleles. This individual is therefore heterozygous for the mutation. Restriction enzyme analysis with *Ava*II, *Tth*111I, and *Hind*II confirmed the identity of the resulting PCR products.

The 33 nuclear families studied in this work served us for determining the inheritance of the apoA-IV alleles. Using allele counting, we determined the frequencies of each isoform. In the sub-sample of 66 unrelated adults there were 59 individuals who have genotype A-IV-1/A-IV-1, and 7 with genotype A-IV-1/A-IV-2. No adult presented the A-IV-2/A-IV-2 genotype (however we found children with A-IV-2/A-IV-2 genotype). The relative frequency of the A-IV-1/A-IV-1 genotype in this sample is 89.4% and the relative frequency of the apoA-IV-1/A-IV-2 is 10.6%. The apoA-IV-I allele was the most frequent allele in the sample, with a frequency of 94.7%. The relative frequency of the apoA-IV-2 was 5.3%.

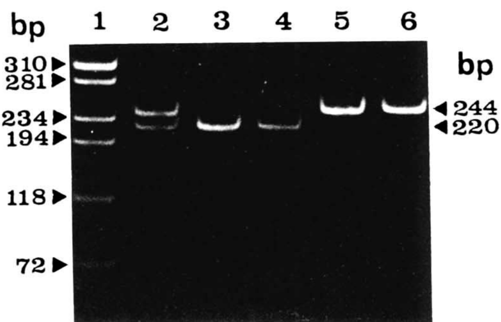

Fig. 2. Direct detection of the Gln^{360} to His^{360} substitution in the human apoA-IV gene by PCR using the bispecific primer. Lane 1: molecular size marker (ΦX174 DNA digested with *Hae*III). Lanes 2 to 6 show the amplification products of five different individuals.

DISCUSSION

Recently, it has been proved that the protein polymorphism of apoA-IV is the consequence of a single base substitution on the apoA-IV gene that leads to a change of the amino acid 360 (19). Among existing techniques: IEF, two-dimensional electrophoresis, ASOs, or restriction isotyping after PCR and direct sequencing of amplified products, IEF and restriction isotyping seem to be the least time- and money-consuming.

However, techniques detecting polymorphisms at the protein level present limitations, especially with regard to posttranslational modifications or to the plasma concentration of the apolipoprotein. Previously, we have reported the complete absence of apoA-IV on two-dimensional silver-stained gels (28). This absence after ASOs analysis, was shown to be due either to a posttranslational modification or to a low concentration of the apolipoprotein in the plasma. Concerning restriction isotyping after PCR, although the nucleotide changes responsible for the Gln to His substitution alter cleavage sites for *Fnu*4HI, this restriction enzyme cut frequently in apoA-IV sequences (22). Hixson and Powers (23) proposed, for studying the same polymorphism, based on restriction isotyping, a PCR realized with a mismatched primer introducing a *Pvu*II site. Even this method seems expensive and necessitates a restriction endocunclease analysis step.

We therefore concluded that the existence of a technique enabling a more rapid detection of this polymorphism (G–T substitution) on the apoA-IV gene could be an important tool for genetic studies. After direct DNA sequence analysis and identification of 29 individuals (18), we used the ASO technique to type 120 individuals for the 360 codon polymorphism. Although the ASO method is a powerful diagnostic tool, it requires gel-purified high specific activity probes and several days of manipulation. The short oligomer probes (in our case 15 bases long) can also hybridize to other genomic sequences so that restriction endonuclease digestion and gel electrophoresis are required to separate the target sequence from the bulk of the genomic DNA. In the case of the G–T polymorphism, we frequently observed a nonspecific signal (on samples

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already typed by two-dimensional electrophoresis) which, though low in intensity, could provoke ambiguous results, especially if ASO was the only method used for typing. Therefore, determination of the optimal conditions for the hybridization and washing steps remain a limitation for application of the ASO technique.

Given these limitations, and in order to overcome these problems, we designed a new method: bispecific allele primer amplification, for the detection of the 360 mutation. The technique depends on the absence of a 3'-exonucleolytic proofreading activity associated with the Taq DNA polymerase (29) and on the presence of a homologous sequence in the apoA-IV gene (Fig. 1). Depending on the presence or the absence of the mutation, the 3' primer of PCR hybridizes with one or the other homologous sequence. This differential hybridization of the 3' primer was used for the detection of G-T substitution at codon 360. In order to demonstrate the validity of this new system, 120 individuals phenotyped by two-dimensional electrophoresis and genotyped by ASO were analyzed by the bispecific-primer technique. The results obtained by the latter method are in agreement with those found by the other techniques.

Bispecific-allele PCR has the capacity to detect expediently the mutation that changes glutamine to histidine at codon 360 of the apoA-IV gene. It allows a rapid and reliable genotyping of codon 360 polymorphism as shown in Fig. 2. It involves only two steps: PCR and polyacrylamide gel electrophoresis. The simplicity of this methodology will also eliminate collateral costs associated with the others. In conclusion, this new simple, rapid and reliable methodology, should facilitate the study of codon 360 substitution in the apoA-IV gene.

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REFERENCES

1. Beisiegel, U., and G. Utermann. 1979. An apolipoprotein homolog of rat apolipoprotein A-IV in human plasma. Isolation and partial characterization. *Eur. J. Biochem.* **93:** 601–608.
2. Green, P. H. R., R. M. Glickman, C. D. Saudek, C. B. Blum, and A. R. Tall. 1979. Human intestinal lipoproteins: studies in chyluric subjects. *J. Clin. Invest.* **64:** 233–242.
3. Green, P. H. R., R. M. Glickman, J. W. Riley, and E. Quinet. 1980. Human apolipoprotein A-IV. Intestinal origin and distribution in plasma. *J. Clin. Invest.* **65:** 911–919.
4. Weinberg, R. B., and A. M. Scanu. 1983. Isolation and characterization of human apolipoprotein A-IV from lipoprotein-depleted serum. *J. Lipid Res.* **24:** 52–59.
5. Bisgaier, C. L., and R. M. Glickman. 1983. Intestinal synthesis, secretion, and transport of lipoproteins. *Annu. Rev. Physiol.* **45:** 625–636.
6. Steinmetz, A., and G. Utermann. 1985. Activation of lecithin:cholesterol acyltransferase by human lipoprotein A-IV. *J. Biol. Chem.* **260:** 2258–2264.
7. Menzel, H. J., P. M. Kovary, and G. Assmann. 1982. Apolipoprotein A-IV polymorphism in man. *Hum. Genet.* **62:** 349–352.
8. Stein, O., Y. Stein, M. Lefevre, and P. S. Roheim. 1986. The role of the apolipoprotein A-IV in reverse cholesterol transport studied with cultured cells and liposomes derived from an ether analog of phosphatidylcholine. *Biochim. Biophys. Acta.* **878:** 7–13.
9. Karathanasis, S. K., P. Oettgen, I. A. Haddad, and S. E. Antonarakis. 1986. Structure, evolution, and polymorphisms of the human apolipoprotein A4 gene (APOA4). *Proc. Natl. Acad. Sci. USA.* **83:** 8457–8461.
10. Elshourbagy, N. A., D. W. Walker, Y-K. Paik, M. S. Boguski, M. Freeman, J. I. Gordon, and J. M. Taylor. 1987. Structure and expression of the human apolipoprotein A-IV gene. *J. Biol. Chem.* **262:** 7973–7981.
11. Menzel, H-J., E. Boerwinkle, S. Schrangl-Will, and G. Utermann. 1988. Human apolipoprotein A-IV polymorphism: frequency and effect on lipid and lipoprotein levels. *Hum. Genet.* **79:** 368–372.
12. Schamaun, O., B. Olaisen, B. Mevâg, T. Gedde-Dahl, Jr., C. Ehnholm, and P. Teisberg. 1984. The two apolipoprotein loci apoA-I and apoA-IV are closely linked in man. *Hum. Genet.* **68:** 181–184.
13. Schamaun, O., B. Olaisen, P. Teisberg, T. Gedde-Dahl, Jr., and C. Ehnholm. 1985. Genetic studies of apolipoprotein A-IV by two-dimensional electrophoresis. *Protides Biol. Fluids.* **33:** 471–474.
14. Utermann, G., G. Feussner, G. Franceschni, J. Haas, and A. Steinmetz. 1982. Genetic variants of the group A apolipoproteins: rapid method for screening and characterization without ultracentrifugation. *J. Biol. Chem.* **257:** 501–507.
15. Visvikis, S., J. Steinmetz, E. Boerwinkle, R. Gueguen, M-M. Galteau, and G. Siest. 1989. Frequency and effects of the apolipoprotein A-IV polymorphism. *Clin. Genet.* **36:** 435–441.
16. Menzel, H-J., G. Sigurdsson, E. Boerwinkle, S. Schrangl-Wills, H. Dieplinger, and G. Utermann. 1990. Frequency and effect of human apolipoprotein A-IV polymorphism on lipid and lipoprotein levels in Icelandic population. *Hum. Genet.* **84:** 344–346.
17. de Knijff, P., M. Rosseneu, U. Beisiegel, W. de Keersgieter, R. R. Frants, and L. M. Havekes. 1988. Apolipoprotein A-IV polymorphism and its effect on plasma lipid and apolipoprotein concentrations. *J. Lipid Res.* **29:** 1621–1627.
18. Boerwinkle, E., S. Visvikis, and L. Chan. 1991. Two polymorphisms for amino acid substitutions in the APO A4 gene. *Nucleic Acids Res.* **18:** 4966.
19. Lohse, P., M. R. Kindt, D. J. Rader, and H. B. Brewer, Jr. 1990. Genetic polymorphism of human plasma apolipoprotein A-IV is due to nucleotide substitutions in the apolipoprotein A-IV gene. *J. Biol. Chem.* **265:** 10061–10064.
20. Tenkanen, H., M. Lukka, M. Jauhiainen, J. Mesto, M. Baumann, L. Peltonen, and C. Ehnholm. 1991. The mutation causing the common apolipoprotein A-IV polymorphism is a glutamine to histidine substitution of amino acid 360. *Arterioscler. Thromb.* **11:** 851–856.
21. Weinberg, R. B., M. K. Jordan, and A. Steinmetz. 1990. Distinctive structure and function of human apolipoprotein variant apoA-IV-2. *J. Biol. Chem.* **265:** 18372–18378.
22. Tenkanen, H. 1991. Genotyping of apolipoprotein A-IV by digestion of amplified DNA with restriction endonuclease

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Fnu4HI: use of a tailored primer to abolish additional recognition sites during the gene amplification. *J. Lipid Res.* **32:** 545–549.
23. Hixson, J. E., and P. K. Powers. 1991. Restriction isotyping of human apolipoprotein A-IV: rapid typing of known isoforms and detection of a new isoform that deletes a conserved repeat. *J. Lipid Res.* **32:** 1529–1535.
24. Visvikis, S., J. Steinmetz, I. Cuvelier, M-M. Galteau, and G. Siest. 1986. Study of apoA-I and apoE polymorphisms using two-dimensional electrophoresis. *In* Recent Progress in Two-Dimensional Electrophoresis. M-M. Galteau and G. Siest, editors. Presses Universitaires, Nancy. 159–165.
25. Miller, S. A., D. D. Dykes, and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* **16:** 1215.
26. Saiki, R. K., D. H. Gelfand, S. Stoffel, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* **239:** 487–491.
27. Yang, C-Y., Z-W. Gu, I. Chong, W. Xiong, M. Rosseneu, H-X. Yang, B-R. Lee, A. M. Gotto, Jr., and L. Chan. 1989. The primary structure of human apolipoprotein A-IV. *Biochim. Biophys. Acta.* **1002:** 231–237.
28. Visvikis, S., J. Steinmetz, E. Boerwinkle, R. Gueguen, and G. Siest. 1990. Apolipoproteine A-IV: frequence des différentes isoformes et variabilité des paramètres liés au métabolisme lipidique. *In* Biologie Prospective, M-M. Galteau, G. Siest, J. Henny, and F. Schiele, editors. Elsever Publications, Cambridge. 193–209.
29. Tindall, K. R., and T. A. Kunkel. 1988. Fidelity of DNA synthesis by the *Thermus aquaticus* DNA polymerase. *Biochemistry.* **27:** 6008–6013.

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