and those using the HiLC instrument showed a mean bias vs the DCCT value of ~1.26% Units. The two values for the mean bias shown in the EQAS by the VaH/a system (~0.33% Units vs the DCCT assigned values and ~0.02% Units vs the instrument’s median values) combined with a mean CV of 3% gave theoretical 95% confidence intervals for a single measurement of approximately ± 0.7% Units and ± 0.2% Units, respectively. Such values for the total error may be regarded as acceptable and fairly good performance for a routine laboratory (18), leading to the conclusion that coupling of a laboratory’s alertness with the manufacturer’s cooperation may produce clinically valid results in routine work. We point out that our performance evaluation results did not stem from an instrument evaluation exercise, but arose mainly from routine work over a 9-month period, supplemented with occasional method comparison exercises. One instrument included in these comparisons gave markedly different results, as it did in the EQAS. Single-point, mathematically simulated recalibration substantially improved the performance, confirming the possibility of improving accuracy through calibration. Two-point calibration is likely to permit even better improvement when significant slope and intercept values are shown by comparison.

We conclude that clinically useful analytical quality can be achieved in the measurement of HbA1c by the use of commercial dedicated HPLC systems and monitoring their performance by means of QC programs with appropriate materials. Occasional method comparison studies may increase the operator’s confidence on performance; appropriate calibration may improve poor performances.

Simonetta Granata (Ospedale Niguarda, Milan, Italy) and Ferruccio Ceriotti (Ospedale S. Raffaele, Milan, Italy) performed the measurements with the alternative systems. Andrea Mosca (Università degli Studi di Milano, Milan, Italy) contributed many suggestions and assistance.

References


Newly Identified Apolipoprotein AV Gene Predisposes to High Plasma Triglycerides in Familial Combined Hyperlipidemia, Josep Ribaltab,*,† Lidia Figuerab,*,† Joan Fernández-Ballart,*,† Elisabet Viellaa,† Manuel Castro Cabezasa,§ Lluis Masana, and Jorge Joven* (1 Unitat de Recerca de Lípidos i Arteriosclerosi, 2 Centre de Recerca Biomèdica, and 3 Unitat de Medicina Preventiva i Salut Pública, Institut de Recerca en Ciències de la Salut, Hospital Universitari de Sant Joan, Universitat Rovira i Virgili, 43201 Reus, Spain; 4 Department of Vascular Medicine, University Medical Center, 3508 GA Utrecht, The Netherlands; * these authors contributed equally to this work; † address correspondence to this author at: Unitat de Recerca de Lípidos i Arteriosclerosi, Facultat de Medicina, Universitat Rovira i Virgili, Sant Llorenç, 21, 43201 Reus, Spain; fax 34-977-75-93-22, e-mail jrv@fmcs.urv.es)

Familial combined hyperlipidemia (FCHL) is the commonest form of hereditary hyperlipidemia (1,2). Its primary defect is increased secretion of hepatic triglyceride (TG)-rich apolipoprotein B (apo B)-containing particles (VLDL) (3) and impaired clearance of postprandial lipoproteins (4), which increases the number of circulating TG-rich lipoproteins. FCHL is present in up to 20% of survivors of myocardial infarction, and it is considered a significant genetic risk factor for developing cardiovascular disease (1,5). The underlying genetic defect is unknown, although the disease has been linked to chromosomes 1 (6) and 11 (7). With regard to the latter, linkage
has been identified in the AI-CIII-AIV cluster, encoded in chromosome 11q23-q24, which has been repeatedly associated with FCHL as a site involved in modulating expression of the disease (8, 9). This is in part explained by the role of apo C-III as a negative regulator of TG hydrolysis. Despite considerable effort, no functional variant has been detected that explains such modulatory action, and the search has been extended to the surrounding regions. In this context, the newly identified apo AV gene (APOAV) (10, 11), adjacent to the AI-CIII-AIV gene cluster and with a clear impact on TG metabolism in animals (10), becomes a candidate. Association of this gene with plasma TG concentrations has also been reported in humans (10). We have explored such an association in FCHL.

According to the nomenclature and methodology used by Pennacchio et al. (10), single-nucleotide polymorphism C/T number 3 (SNP3; 1 is the common allele, 2 is the rare allele) was used as the genetic marker. Genotyping was performed with primers AV-1 (5’-GATTGATTCAGGATGCATTAGGAC-3’) and AV-2 (5’-CCCCAGGAACTG-GAGCGAAATT-3’), which forced a Msel (New England Biolabs) site for enzymatic restriction. Associations were analyzed between the APOAV gene and TG metabolism in a population-based Spanish control group (12) (ESP controls; n = 408), a normolipidemic control group from The Netherlands (13) (NL controls; n = 89), and 16 FCHL families (9) (n = 103) with ≥2 hyperlipidemic and ≥1 normolipidemic first-degree relatives. Normolipidemic controls were selected on the basis of the one lipid measurement if they presented with plasma cholesterol concentrations ≤6.4 mmol/L and/or plasma TGs ≥2.8 mmol/L or increased above the 95th percentile for age and gender in the case of offspring below the age of 19 years. Relatives who did not meet these criteria were assigned the normolipidemic status. All individuals recruited into the study gave fully informed written consent, and the protocol was approved by the local Ethical Committees.

Among the FCHL families, there was a significant association between the APOAV marker and TG-related variables, but when adjusted for age, gender, body mass index, or diet, these associations were not significant in the control ESP group or the normolipidemic NL group (Table 1). Thus, the influence of APOAV on plasma TGs in the general population appears to be fairly limited. In contrast, a much more pronounced effect was seen in FCHL families, among which carriers of the rare allele presented with 30% higher plasma TGs (P = 0.004), 61% higher VLDL-TG (P = 0.008), and 30% higher intermediate-density lipoprotein-TG (P = 0.007). VLDL-cholesterol (34%; P = 0.049) and VLDL-apo B (33%; P = 0.027) were also significantly increased. This fact suggested that APOAV modulates TG concentrations only when there is an altered genetic and metabolic background. To further confirm this point, we analyzed the association between APOAV and TGs in FCHL hyperlipidemic (n = 42) and normolipidemic (n = 61) individuals separately. FCHL hyperlipidemic patients, carriers of the rarer APOAV allele, presented with significantly increased plasma TG concentrations compared with carriers of the common allele [mean (SD), 2.52 (1.34) vs 1.76 (0.90) mmol/L, respectively; P = 0.017], whereas such an association was not present among the FCHL normolipidemic relatives [0.82 (0.47) vs 0.94 (0.44) mmol/L, respectively]. This

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age, years</th>
<th>% males</th>
<th>BMI, kg/m²</th>
<th>TGs, mmol/L</th>
<th>Cholesterol, mmol/L</th>
<th>HDL-C, mmol/L</th>
<th>apo B-100, g/L</th>
</tr>
</thead>
<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>89</td>
<td>33.9 (12.1)</td>
<td>54</td>
<td>22.7 (2.3)</td>
<td>0.88 (0.43)</td>
<td>4.51 (0.93)</td>
<td>1.35 (0.29)</td>
<td>8.4 (2.5)</td>
</tr>
<tr>
<td>1/1</td>
<td>76</td>
<td>34.5 (12.5)</td>
<td>54</td>
<td>22.7 (2.3)</td>
<td>0.88 (0.44)</td>
<td>4.49 (0.95)</td>
<td>1.36 (0.28)</td>
<td>8.3 (2.6)</td>
</tr>
<tr>
<td>1/2</td>
<td>13</td>
<td>30.4 (9.1)</td>
<td>54</td>
<td>22.5 (2.3)</td>
<td>0.90 (0.42)</td>
<td>4.60 (0.82)</td>
<td>1.26 (0.34)</td>
<td>8.8 (2.5)</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Total</td>
<td>408</td>
<td>41.9 (15.2)</td>
<td>51</td>
<td>26.8 (5.0)</td>
<td>1.31 (0.80)</td>
<td>5.27 (1.03)</td>
<td>1.53 (0.38)</td>
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<td>41.8 (15.3)</td>
<td>51</td>
<td>26.9 (5.1)</td>
<td>1.29 (0.76)</td>
<td>5.26 (1.00)</td>
<td>1.55 (0.38)</td>
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</tr>
<tr>
<td>1/2 + 2/2a</td>
<td>53</td>
<td>42.5 (14.2)</td>
<td>51</td>
<td>26.3 (4.5)</td>
<td>1.49 (1.06)b</td>
<td>5.26 (1.18)</td>
<td>1.45 (0.38)</td>
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<tr>
<td>FCHL</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>34.8 (21.3)</td>
<td>50</td>
<td>24.6 (5.1)</td>
<td>1.35 (0.92)</td>
<td>5.14 (1.24)</td>
<td>1.16 (0.29)</td>
<td>9.1 (3.1)</td>
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<tr>
<td>1/1</td>
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<td>48</td>
<td>24.2 (5.0)</td>
<td>1.26 (0.75)</td>
<td>5.06 (1.24)</td>
<td>1.15 (0.29)</td>
<td>8.8 (3.1)</td>
</tr>
<tr>
<td>1/2 + 2/2d</td>
<td>17</td>
<td>42.3 (23.9)</td>
<td>65</td>
<td>26.6 (5.3)</td>
<td>1.82 (1.33)c</td>
<td>5.52 (1.19)</td>
<td>1.16 (0.34)</td>
<td>10.2 (3.2)</td>
</tr>
</tbody>
</table>

a All values are expressed as the mean (SD).
b Control NL, normolipidemic control group; ESP, population-based Spanish control group; 1/1, homozygous for the common SNP3 allele; 1/2 + 2/2, heterozygous or homozygous for the rare SNP3 allele.
c BMI, body mass index; HDL-C, HDL-cholesterol; NA, not available.
d There was only one 2/2 individual.
e 1/2 + 2/2 vs 1/1 control ESP individuals: P = 0.063.
f 1/2 + 2/2 vs 1/1 control FCHL individuals: P = 0.004.
suggested a potential implication of APOAV in the hypertriglyceridemia present in FCHL. This point was further supported by the fact that the frequency of the APOAV variant was significantly \( P = 0.028 \) greater among the FCHL patients than in their relatives (Fig. 1). The frequency of the APOAV variant was 2.7-fold higher in hyperlipidemic FCHL patients than in their normolipidemic relatives and 2-fold higher than in the general population (control ESP, Fig. 1).

Altogether these data suggest that in FCHL families, carriers of at least one rare APOAV allele will have a 3.25-fold higher risk (95% confidence interval for the odds ratio, \( 1.10–9.65 \)) of presenting with the hyperlipidemic phenotype. Once the influence of gender, age, body mass index, and food intake on plasma TG concentrations in these individual is considered, the APOAV genotype could explain up to 30% of the variability observed.

Pennacchio et al. (10) reported negative linkage disequilibrium between markers of the AI-CIII-AIV gene cluster and APOAV, indicating an independent effect of APOAV on plasma TG concentrations. We have also analyzed by means of ANOVA the interaction between APOAV and a marker of the apo C-III gene (APOCIII) with a significant TG-increasing effect in FCHL patients (C1100T; exon 3) (9) and detected a significant interaction \( (P = 0.012) \) between the two. This allows us to speculate that certain haplotypes of APOAV and AI-CIII-AIV might act independently but with additive effects in increasing TG concentrations in FCHL.

It is difficult to speculate about the mechanism by which apo AV contributes to FCHL because very little is known about the protein encoded by APOAV. apo AV is associated with the VLDL and HDL fractions (10, 11), and the mechanism by which apo AV dramatically influences TG concentrations in transgenic and knock-out mice remains unknown (10). Even more recently, apo AV has been linked to liver regeneration in the rat (11). van der Vliet et al. (11) speculated that apo AV might antagonize lipid uptake by the liver, which could explain the association of APOAV with plasma TGs.

Functional characterization of apo AV is required before we can understand the observed associations and gain new insight into the causes of FCHL. However, the fact that APOAV is associated with increased VLDL-apo B but not with total or LDL-apo B suggests that apo AV might be involved in the delay of peripheral TG hydrolysis. At present we report that the polymorphic marker SNP3 of APOAV is overrepresented in FCHL and can be considered as a predisposing factor for this condition. Because of the size of the FCHL group, these results should be considered preliminary. FCHL is a condition associated with cardiovascular risk; therefore, further studies will be necessary to explore whether APOAV is a new candidate marker for cardiovascular disease. Whether such a variant is causative or is in linkage disequilibrium with the actual functional mutation also remains to be studied. We have also shown that the association of APOAV with increased TGs in the general population is limited and completely absent in individuals selected as normolipidemic.

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References

4. Castro Cabezas M, de Bruin TWA, Jansen H, Kock AW, Kortland W, Erkelens...
Frozen sera from 54 patients that had previously been shown to contain M proteins by immunofixation (Sebia), but not by CZE (Paragon 2000™ CZE, software Ver. 1.5; Beckman-Coulter), were assayed by nephelometry (Image, Beckman-Coulter) for FLCs (Freelite). All assays were performed according to the manufacturers’ instructions. The M proteins were of various types and included examples of both intact immunoglobulins and FLCs. The samples were from patients with (a) (chronic) immune stimulation [n = 7; Sjögren syndrome, polyarteritis, pneumonia, *Staphylococcus aureus* sepsis, chronic hepatitis B infection, post-renal transplantation (n = 2)], (b) monoclonal gammopathy of unknown significance (MGUS; n = 8), (c) primary (AL) amyloidosis (n = 1), and (d) B-cell-derived malignant disease (n = 34). The last group included patients with multiple myeloma (n = 20), smoldering myeloma (n = 1), plasma cell leukemia (n = 1), plasmacytoma (n = 3), and lymphoproliferative disease, including Waldenström macroglobulinemia (n = 2), non-Hodgkin lymphoma (n = 6), and chronic lymphocytic leukemia (n = 1). Eleven of the 34 patients with B-cell-derived malignant disease had received a transplant. For four patients, medical records could not be consulted.

Sera from 20 controls were analyzed as well. The free $\kappa$/free $\lambda$ ratios for these samples were within the reference interval as specified by the manufacturer (0.359–1.01).

The results of the free $\kappa$ and free $\lambda$ quantification are shown in Fig. 1. Sera from patients with free $\kappa$ light chain M proteins (n = 9) all showed an increased free $\kappa$/free $\lambda$ ratio (between 9.5 and 793) and increased absolute values for the relevant free $\kappa$ chain. Sera from patients with free $\lambda$ light chain M proteins (n = 12) all showed a low free $\kappa$/free $\lambda$ ratio (between 0.001 and 0.036) and increased concentrations of the relevant free $\lambda$ chain. In 16 of these samples in which monoclonal FLCs were present, quantification of...