Genetic heterogeneity in the apolipoprotein C-III promoter and effects of insulin

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Abstract **In the present study, we have investigated the in vivo and in vitro role of two newly identified variants ($G_{-944}A$ and $A_{-1180}C$) located in the upstream promoter region of the apolipoprotein C-III (apoC-III) gene. These variants were studied in 30 probands diagnosed with FCHL, their relatives, and spouses. The allele frequencies of both variants were not different between the groups. No significant associations between plasma lipid traits and DNA variants were observed. We further analyzed the effect of the presence of these variants in the upstream enhancing region of the apoC-III gene, as five different in vivo occurring haplotypes, on the transcriptional activity of apoC-III in both HepG2 and Caco-2 cells. All five promoter constructs, including the wild type, showed similar enhancing activity of the apoC-III gene. The average transcription efficiency was enhanced 19-fold in HepG2 cells and 11-fold in Caco-2 cells. Previous studies have shown in vitro insulin-dependent down-regulation of the apoC-III gene transcription in HepG2 cells by DNA variation in an insulin response element (IRE) in the apoC-III promoter. We observed a 30% insulin-dependent down-regulation of apoC-III expression that was, however, independent of the presence of the two IRE variants. In contrast, in Caco-2 cells, a more variable insulin-dependent up-regulation was found that was also independent of the presence of the IRE variants. In conclusion, our data suggested that the apoC-III gene transcription in vitro is regulated by insulin but independent of the presence of the two IRE variants at −455 and −482. We were unable to detect associations between these apoC-III variants and plasma lipids and insulin in our FCHL population. This means that in vivo apoC-III transcription not only depends upon insulin but appears to be mediated by other mechanisms.—** Dallinga-Thie, G. M., M. Groenendijk, R. N. H. H. C. Blom, T. W. A. De Bruin, and E. De Kant. **Genetic heterogenicity in the apolipoprotein C-III promoter and effects of insulin. *J. Lipid Res.* 2001.** 42: **1450–1456.**

Supplementary key words gene regulation • familial combined hyperlipidemia • HepG2 • Caco-2 • transcription

The apolipoprotein (apo) genes apoA-I, apoC-III, and apoA-IV are closely linked and tandemly organized within a 15-kb DNA segment of the long arm of human chromosome 11 (11q23-qter) (1–4). ApoC-III is mainly synthesized in the liver and is an exchangeable moiety of chylomicron remnants, VLDL, and HDL particles. ApoC-III plays an important role in the metabolism of triglyceride-rich lipoproteins by inhibiting the function of LPL (5, 6), thereby reducing the capacity to hydrolyze triglyceride-rich lipoprotein particles (7). In hypertriglyceridemic patients, a positive correlation was found between plasma apoC-III levels and triglyceride concentrations (8, 9), whereas LPL activity showed a negative correlation (7). Furthermore, involvement of apoC-III in the processes of binding lipoprotein particles to specific receptors in the liver and in the uptake of lipids in the enterocyte was documented (10–13).

Both naturally occurring disruptions and experimental genetic manipulations of the structure of the apoA-I–C-III–A-IV gene cluster have indicated the importance of the genes in lipid metabolism (14–17). The relevance of more subtle genetic alterations that occur more frequently was evaluated in genetic studies using several DNA polymorphic markers. In a candidate gene approach for FCHL we examined several polymorphisms in the apoA-I–C-III–A-IV gene cluster as separate gene loci and in combinations of haplotypes and characterized their associations with lipid and apolipoprotein phenotypes (18–21). Similar to earlier studies (22–26), we established the involvement of this cluster as a susceptibility region for hyperlipidemia in our FCHL families.

The SstI polymorphism in the 3′ untranslated region of the human apoC-III gene has a modifying effect on plasma apoC-III levels (18). It is in strong linkage disequilibrium with specific polymorphic sites in the 5′ regulatory region of the gene (27–30). Two of these polymorphisms are located within an insulin response element and have been demonstrated to affect the level of transcription in response to insulin treatment of cell cultures in vitro (31). However, no in vivo evidence was found to show a direct relationship

Abbreviations: apo, apolipoprotein; FCS, fetal calf serum; IRE, insulin response element; SSCP, single strand conformation polymorphism.

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of insulin on plasma apoC-III levels and the presence of these two apoC-III variants (28–30).

Regulation of expression of the genes in this cluster occurs primarily at the level of transcription (32). Because the apoC-III gene is encoded in the other strand, its transcription direction is opposite to apoA-I and A-IV **(Fig. 1)**. The distal control elements of apoC-III are of great importance to transcriptional regulation of the apoA-I, apoC-III, and apoA-IV genes (33–35). The effects of genetic heterogeneity on transcriptional regulation of apoC-III has been studied to some extent. In a population of FCHL kindred, we screened for polymorphisms in the upstream region of the apoC-III gene. Polymorphe haplotypes of this region were tested in combination with the proximal promoter of apoC-III in transient expression experiments by monitoring the expression of a reporter gene in HepG2 and Caco-2 cells. Here, we provide evidence that the regulatory region of apoC-III contains additional, previously unpublished, polymorphic sites. This genetic heterogeneity did not affect apoC-III transcriptional regulation either in vitro nor in vivo. Furthermore, the present study provides evidence for an insulin-dependent regulation of apoC-III transcription in vitro that is independent of the presence or absence of polymorphisms in the insulin response element.

MATERIALS AND METHODS

Subjects, polymorphisms, and haplotypes

Dutch Caucasian FCHL probands (n = 30), relatives (n = 476), and spouses (n = 236) were recruited as extensively described (18, 19). All subjects gave informed consent. The Human Investigation Review Committee of the University Medical Center Utrecht approved the study protocol.

DNA was isolated from 10 ml blood following standard procedures (36) and amplified in vitro using the PCR technique with a DNA thermal cycler (Pharmacia, Uppsala, Sweden). Screening of sequence variability in the promoter region upstream of the apoC-III gene in 18 FCHL probands and 32 relatives was performed using single strand conformation polymorphism (SSCP). Six partly overlapping apoC-III sequence regions of 200–300 bp, which span the upstream region up to −1342, were separately amplified and analyzed. The differential electrophoresis mobility of polymorphic fragments was visualized after migration and silver staining on a 12.5% polyacrylamide gel (Phast system; Pharmacia). Running conditions were as follows: prerun 400 V, 10 mA, 2.5 W, 4°C/15°C (100 Vh); application 400 V, 1 mA, 2.5 W, 4°C/15°C (2 Vh); run 400 V, 10 mA, 2.5 W, 4°C/15°C (150–400 Vh). Gels were silver stained according to the manufacturer's procedure. The positions of the polymorphic sites were determined by DNA sequencing based on the Sanger dideoxy chain termination method using commercially available kits (Pharmacia). The $T_{-455}C$ and the $C_{-482}T$ was analyzed as previously described (30). The $G_{-944}A$ was analyzed using allele-specific primers. The following primers were developed: 5′-AGGAGTGA TTCTCTCGTTCA-3′ (forward), 5′-AGTGGTCCAGGAGGGGC CGCTGA CCTT-3′ (wild-type reverse), and 5′-TGCTGGTCCAG GAGGGGCCGCTGAATTACTCGGGGCTG AGATGTCCCC-3′ (mutant reverse). The PCR was a touchdown procedure: 1 min at 95°C followed by 2 cycles each of 45 sec at 95°C for denaturing, 1 min at 68°C for annealing, and 45 min at 72°C for extension. These steps were repeated with a decrease in annealing temperature in each step: 2× 67°C, 3× 66°C, 4× 65°C, 5× 64°C, 6× 63°C, and 25× 62°C and a final extension of 3 min at 72°C. All products were analyzed on 3% agarose gels. The wild-type (11) genotype contained a fragment of 169 bp, and the mutant (22) genotype contained a fragment of 189 bp. The $A_{-1180}C$ was analyzed with the following primers: 5′-TGGAGCCAGGACATCT TAGG-3′ (forward) and 5′-GCTGCTTTGTACTTCTCTATCTC ATTTCCTTTTCAGCACCACTCTGCG-3′ (reverse). PCR conditions were as follows: 4 min at 94°C, 33 cycles of denaturation for 1 min at 94°C, annealing for 1 min at 52°C, and extension for 3 min at 72°C. Restriction digestion was performed using the restriction enzyme AviII (Boehringer Mannheim, Germany) at 37°C for 3 h. The restriction products were analyzed on 3% agarose gels. The wild-type 11 fragment is 168 bp, and the mutant 22 fragments reveal two fragments of 121 and 47 bp, respectively. The haplotypes we selected for cloning were assigned by examining the cosegregation of individual alleles in subjects from FCHL families.

Construction of experimental luciferase reporter plasmids

The apoC-III upstream region between −1342 and +14 was obtained by PCR amplification of genomic DNA using the Expand TM High Fidelity PCR system (Boehringer Mannheim), using the primers 5′-TGTTAGAGGATCCTTCTGCCTG-3′ (sense) and 5′-CTGCCTCCATGGATGAACTGAG-3′ (antisense). The sense and antisense primers contained engineered BamHI and NcoI sites, respectively. Pfu DNA polymerase was used to generate blunt-ended amplification products for cloning into the SrfI restriction site of pCR-Script TM Amp SK(+), as described by the manufacturer (Stratagene). The orientation of the inserts was confirmed by restriction enzyme analysis. Sequence analysis was performed to verify all constructs and to identify by confirmation of the expected variant sites which one of the two possible haplotypes from heterozygous genomic DNA samples was cloned. With the artificial engineered restriction sites in the primer set, fragments from pCR-Script plasmids were obtained and inserted in the multiple cloning site upstream of the luciferase coding sequences into a *Bgl*II-*Nco*I site of the pGL3-basic vector (Promega) to generate the experimental reporter constructs. The distal upstream region of apoC-III promoter in pGL3b–apoC-III constructs was excised as *Sac*I-*Sac*I fragments (−1342 down to −237), yielding the basal apoC-III proximal promoter construct after religation. Excised DNA fragments were isolated from agarose gels using the Easy-Pure TM kit (Biozym). The plasmid DNA used for transfection was isolated using the Qiafilter-plasmid-maxi kit (QIAGEN R).

Cell culture and transient expression assays

Human colon carcinoma (Caco-2) and human hepatoma (HepG2) cells were cultured in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 20% heat-inactivated fetal calf serum (FCS; Boehringer Mannheim), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were grown at 37°C and 5% CO_2. Transfection assays were performed in 24-well cluster plates. Cells were seeded at ~50% confluence and grown in 1 ml of the medium. Before the actual transfection experiments, the cells were washed once with PBS and fed with fresh medium (1 ml). Triplicate wells were transfected using the calcium phosphate DNA coprecipitation method. Each experiment was performed at least three times. The transfection mixture contained a pGL3b construct (1 μg) and the control reporter vector pRL-SV40 (Promega), which was included for normalization of the transcription activity levels (0.01 and 0.1 μg in Caco-2 and HepG2 cells, respectively). Control experiments have shown that the thymidine kinase (TK) vector was less stable than the SV40 vector under our experimental conditions. Furthermore, the TK vector showed a dose-dependent

increase in signal. After 20 h of incubation, the cells were washed with PBS and allowed to recover for 28 h in fresh medium. Forty-eight hours after starting the transfection experiments, the cells were harvested and luciferase activities were measured using a luminometer (Turner), as described in the DUAL-Luciferase TM reporter assay system (Promega). This protocol allows detection of luminescence from firefly luciferase (pGL3) and Renilla luciferase (pRL-SV40) in a single tube. Experiments in the presence of insulin (10^{-6} M) in serum-free medium were performed 22 h after the transfection experiments for 6 h.

Statistical methods

Values given in the text are means ± standard deviation. Statistical significance was calculated using the Student's *t*-test using SPSS version 8.0. The frequencies of the different polymorphisms were calculated with the use of gene counting and allele counting. Deviations of the Hardy-Weinberg equilibrium were tested in spouses with the Chi-square test using the Hardy Weinberg Equilibrium program.

RESULTS

DNA variations in the distal promoter region of apoC-III

DNA isolated from FCHL probands and their relatives was screened for polymorphic sites by SSCP to identify new variants in the 1.3-kb upstream sequence region of the apoC-III gene. In total, 50 subjects, including 18 probands, were analyzed. If abnormalities in the SSCP pattern were observed, then DNA sequence analysis was carried out. Apart from the previously reported variant alleles at positions $T_{-455}C$ and $C_{-482}T$ near the insulin response element (IRE), T_{-625} deletion, $G_{-630}A$, and $C_{-641}A$ (**Fig. 1**) (27, 30), we characterized four new variations: $G_{-944}A$, $A_{-1180}C$, $C_{-1230}A$, and $G_{-1236}T$. The frequencies of the IRE polymorphisms and the newly identified $G_{-944}A$ and the $A_{-1180}C$ variations were tested in 30 FCHL kindred. The three variations upstream from the IRE, T_{-625} deletion, $G_{-630}A$, and $C_{-641}A$, were in almost complete linkage disequilibrium with the IRE variants and were therefore not analyzed in further detail (data not shown). The $G_{-1236}T$ was not investigated further because it is part of an allele-specific *Nco*I recognition sequence that was not compatible with the cloning procedure we followed. The $C_{-1230}A$ was in complete linkage disequilibrium with the *Sst*I polymorphic marker described earlier and therefore does not add additional information on the locus. **Table 1** shows the gene frequencies of the four variants. We did not observe any differences in frequency distribution between affected and unaffected family members and spouses for the IRE variants (30). The frequency of the $_{-944}G$ allele decreased going from probands to hyperlipidemic relatives, normolipidemic relatives, and spouses, but this decrease did not reach the level of significance, whereas the frequency of the C_{-1180} allele was similar in the four groups.

TABLE 1. Allele frequencies of four markers in the apoC-III–apoA-IV intergenic region in familial combined hyperlipidemia

	Probands n = 34	HL rel n = 219	NL rel n = 300	Spouse n = 236
$T_{-455}C$				
1	0.65	0.68	0.65	0.64
2	0.35	0.32	0.35	0.36
$C_{-482}T$				
1	0.66	0.78	0.77	0.76
2	0.34	0.22	0.23	0.24
$G_{-944}A$				
1	0.92	0.81	0.77	0.76
2	0.08	0.19	0.23	0.24
$A_{-1180}C$				
1	0.52	0.64	0.68	0.65
2	0.48	0.36	0.32	0.35

HL rel, hyperlipidemic relatives; NL rel, normolipidemic relatives.

Effect of sequence heterogeneity on transcription efficiency

To investigate whether differences in sequence in the variable upstream region of apoC-III gene promoter affect the transcriptional activation of the apoC-III promoter, we generated five different constructs based on various in vivo occurring haplotypes (**Table 2**). Extensively characterized apoC-III promoter (+14 to −236) and distal regulatory sequences (−237 to −1342) obtained from five genetically different individuals recruited from our FCHL database were used. Constructs containing one of these sequences and the reporter firefly luciferase promoter sequence were used for transient assays in Caco-2 and HepG2 cells. In all experiments, control constructs containing only the apoC-III promoter sequence (+14 to −236) were used and set to 100% transcriptional activity. As shown in **Fig. 2**, all five promoter constructs showed similar enhancing capacity of the minimal apoC-III promoter activity. The average transcription efficiency of the enhancing promoter sequence was 1941 ± 437% in HepG2 cells (Fig. 2A, $P < 0.0001$) and 1155 ± 646% in Caco-2 cells (Fig. 2B, $P < 0.001$). This equals a 19-fold enhancing capacity in HepG2 cells and an 11-fold enhancing capacity in Caco-2 cells in our testing system. In Caco-2 cells, clone C showed a slightly smaller increase in apoC-III transcriptional activity than did clones A and E ($P < 0.05$). From these studies, we can conclude that all the variants present in the enhancing region of the apoC-III promoter have similar additional stimulating effects on apoC-III transcriptional activity in vitro.

In an earlier report, it was found that variations in the insulin response element located between positions −455 and −482 from the start site of the apoC-III gene resulted in defective in vitro down-regulation of apoC-III transcription by insulin (31). In the present study, we investigated whether additional sequence variations in the enhancer region resulted in a different insulin-dependent regulation of apoC-III gene transcription. The effect of insulin on apoC-III promoter activity was tested both in HepG2 and Caco-2 cells using four different promoter constructs (see Fig. 4 legend). Experiments were performed using standard culture conditions (see Materials and Methods), both without FCS and without FCS but with 10^{-6} M insulin. In HepG2 cells, insulin had no apparent effect on the minimal apoC-III promoter activity (**Fig. 3A**). However, in Caco-2 cells, a 2-fold insulin-dependent increase in apoC-

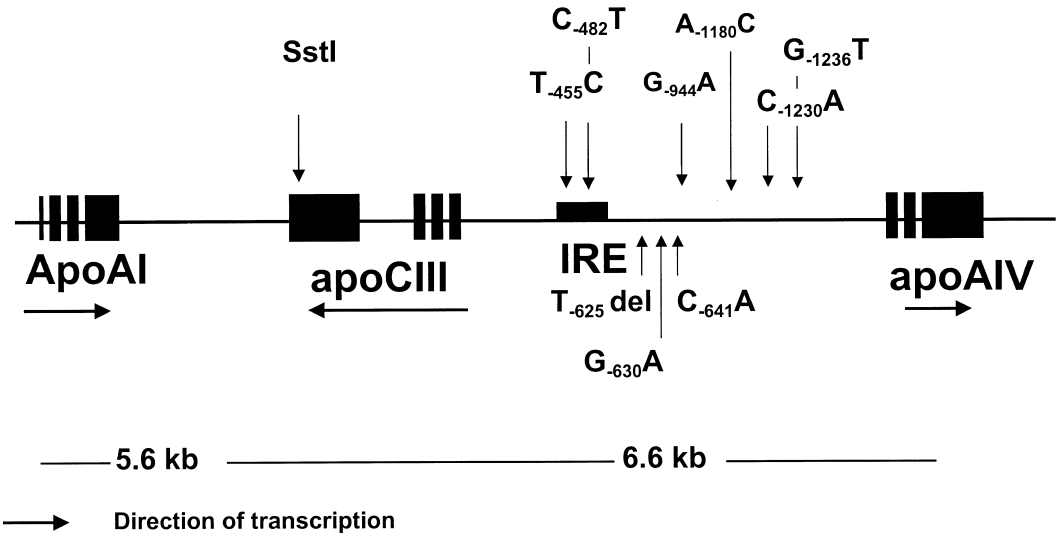

Fig. 1. ApoA-I–C-III–A-IV gene cluster located on chromosome 11.

III minimal promoter activity was observed (Fig. 3B). In HepG2 cells, we observed a 30% insulin-dependent down-regulation of the maximal apoC-III transcriptional expression (**Fig. 4A**, clone A, wild-type haplotype). No additional effect was observed when any of the variants in the enhancer region was present, including the insulin response element variants at −455 and −482 (clones B–D). If the clones were grouped on the basis of the presence or absence of the two insulin variants, as shown in **Table 3**, no difference was observed between the two groups. In contrast, in Caco-2 cells, an insulin-dependent up-regulation of apoC-III transcriptional activation of 10% in clone A, 57% in clone B, 37% in clone C, and 62% in clone D was observed (Fig. 4B), which was again independent of the presence of the IRE variants (Table 3), although it must be noted that the increase in transcriptional activity of apoC-III in the clones containing the IRE variants (clones C and D) reached the level of statistical significance ($P < 0.005$).

DISCUSSION

In the light of an ongoing study on the role of the apoA-I–C-III–A-IV gene cluster in FCHL, we analyzed the distal enhancing region of the apoC-III promoter by SSCP and sequencing to localize new polymorphic sites. Five different polymorphic sites in the enhancer region of the apoC-III promoter have been described (27). Two single

TABLE 2. Common haplotypes used in the present study based on seven different polymorphic sites in the apoC-III promoter region

Clone	$T_{-455}C$	$C_{-482}T$	T_{-625} del	$G_{-630}A$	$C_{-641}A$	$G_{-944}A$	$A_{-1180}C$
A	T	C	T	G	C	G	A
B	T	C	T	G	C	A	C
C	C	T	—	A	A	G	A
D	C	T	—	A	A	G	C
E	T	C	—	A	A	G	C

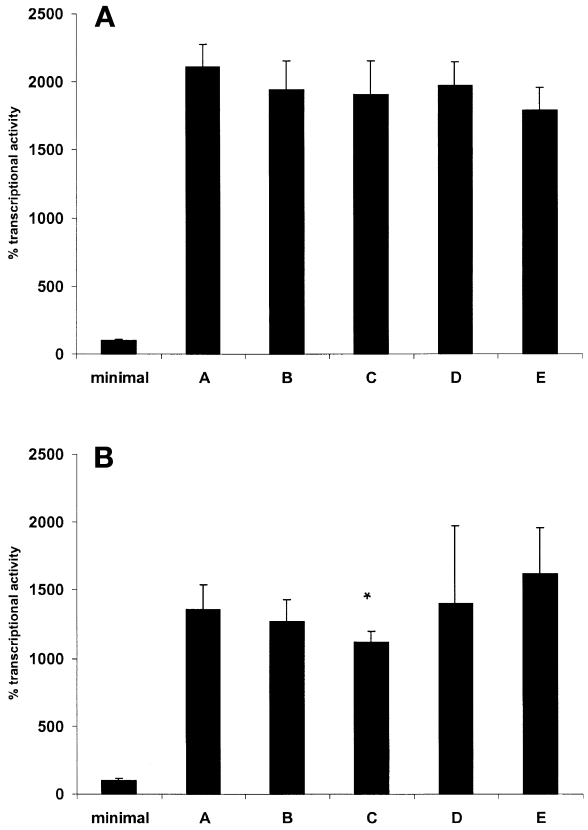

Fig. 2. Effect of variations in the enhancing region of the apoC-III gene on the transcriptional activity of the basal apoC-III promoter in HepG2 cells (A) and Caco-2 cells (B). Composition of the clones is presented in Table 2. The transcriptional activity of the minimal promoter alone is set to 100%. Values are expressed as relative to the levels of the basal promoter and are given as mean ± standard deviation for at least three independent experiments performed in triplicate. All clones showed a statistically significant enhancement versus minimal promoter activity ($P < 0.0001$) in both HepG2 cells and Caco-2 cells. There is no significant difference in the relative increase in transcriptional activities between the clones in HepG2 cells. In Caco-2 cells, clone C showed a significantly lower increase in transcriptional activities than did clones A and E ($P < 0.05$).

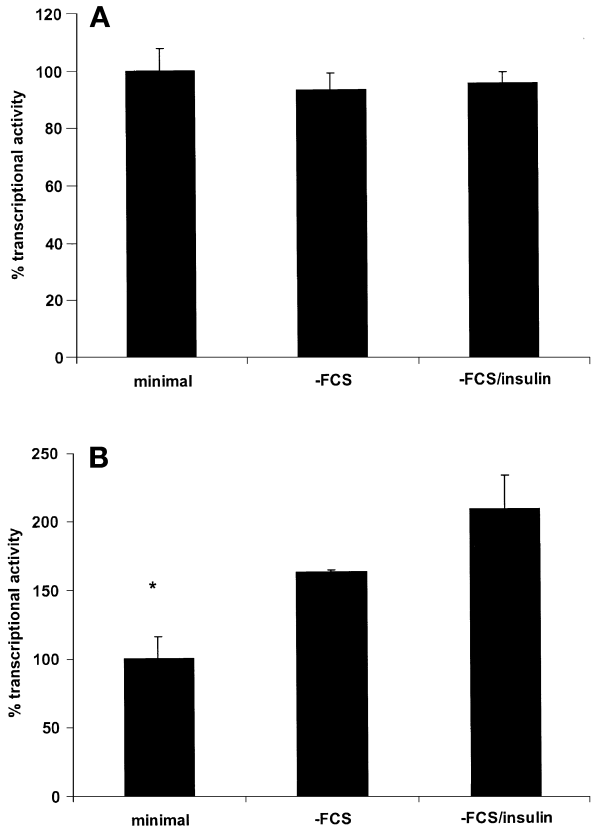

Fig. 3. Effect of insulin on minimal transcriptional activity of the apoC-III promoter. A: HepG2 cells were transiently transfected in medium containing 20% heat-inactivated fetal calf serum (FCS) or in medium depleted with FCS or without FCS but with 10^{-6} M insulin, as indicated in Materials and Methods. B: Caco-2 cells. Cell culture conditions were the same as those described in A. The values are means ± SD for three independent experiments performed in triplicate. * In Caco-2 cells there is a significant increase in minimal promoter activity after incubation with insulin ($P < 0.05$).

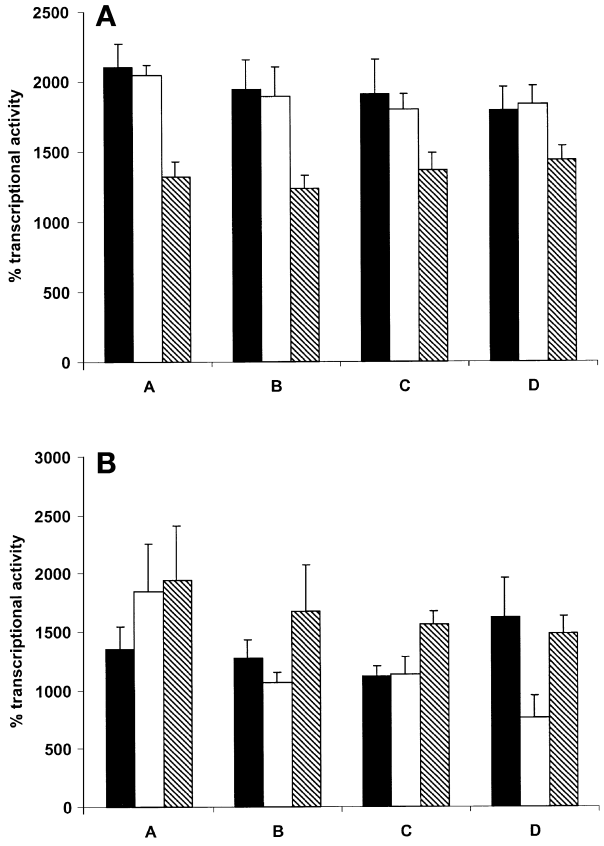

Fig. 4. Effect of insulin on the regulation of the transcriptional activation of apoC-III promoter. The cell culture conditions were the same as those described in Fig. 3. A: HepG2 cells. B: Caco-2 cells. The values are means ± SD for three independent experiments performed in triplicate. The clones were exactly as described in Table 2. Black bars represent standard culture conditions, white bars represent culture medium without FCS, and dashed bars represent culture medium without FCS plus insulin. The decrease in transcriptional activity by insulin in HepG2 cells is statistically significant for all tested clones ($P < 0.05$). In Caco-2 cells, the increase in transcriptional activity by insulin is significant for clones A, B, and C ($P < 0.05$).

base pair changes ($T_{-455}C$ and $C_{-482}T$) were located near a possible IRE (31, 37, 38). In a population analysis, it was shown that the haplotype based on the presence of IRE variants and the S2 allele was associated with increased risk for hypertriglyceridemia. In a more recent study using a large sample of individuals from the ARIC study (29), a similar association was found, but advanced statistical analysis revealed the dominant role of the SstI polymorphism in this haplotype and not the two IRE variants. Similar observations were found in a study on the role of the apoA-I–C-III–A-IV gene cluster in FCHL (30). Analysis of combinations of haplotypes based on the IRE polymorphisms and the SstI and MspI-AI promoter polymorphism provided further evidence for a dominant role of the SstI polymorphism as a major susceptibility locus in FCHL (19). The inclusion of the IRE markers did not improve genetic informativeness in FCHL. No relationship could be observed between plasma insulin and plasma apoC-III and plasma TG levels. It must be mentioned that presence of type 2 diabetes was an exclusion criterion for ascertainment and participation in our study. However, FCHL is also characterized by insulin resistance (39) and compensatory hyperinsulinemia. It is known that insulin resistance is frequently associated with increased risk for hypertriglyceridemia. However, in most studies, data on plasma apoC-III levels are lacking (40, 41). It is therefore not possible to conclude whether or not insulin directly regulates plasma apoC-III levels in vivo. It must be noted that human apoC-III transgenic mice develop hypertriglyceridemia but do not become insulin resistant (42), although on the other hand, in a streptozotocine-treated mouse model with low insulin levels, hepatic apoC-III mRNA is up-regulated (38).

In the present study, we identified four new variants ($G_{-944}A$, $A_{-1180}C$, $G_{-1230}A$, and $G_{-1236}T$) further upstream in this region. Genotype analysis of the two variants $G_{-944}A$ and $A_{-1180}C$ revealed that none of these polymorphic sites have any additional effect on the expression of the FCHL phenotype. Haplotype analysis revealed strong linkage disequilibrium between these polymorphic sites and the previously analyzed SstI and MspI polymorphisms.

The importance of the apoC-III–apoA-IV intergenic re-

TABLE 3. Effect of the presence (+/+) or absence (−/−) of the two variants in the IRE on the insulin-dependent regulation of apoC-III transcriptional activity

Presence of IRE Variants	−	−	+	+
Insulin[a]	−	+	−	+
HepG2	1,969 ± 304	1,282 ± 193*	1,815 ± 228	1,402 ± 210*
Caco-2	1,458 ± 623	1,807 ± 690*	948 ± 332	1,519 ± 214**

All data are presented as means ± SD. Data are presented as percentages of transcriptional activity. The transcriptional activity of the basal apoC-III promoter was set as 100%.

* $P < 0.001$, ** $P < 0.005$ for the effect of insulin treatment on transcriptional activity of apoC-III gene expression.

[a] Insulin is present or absent in the incubation medium.

gion for the transcriptional regulation of the apoC-III gene is well known (43, 44). In this intergenic region, several putative regulatory elements were identified, contributing to the intestinal and hepatic transcription of apoC-III (33). Mutation analysis revealed that different combinations of factors are required for optimal transcription in hepatic and intestinal cells. The minimal apoC-III promoter has been localized in the −25/−160 sequence and contains four different protein binding sites (33, 45), including a hormone response element at −87/−72 (46). Complete activation of the apoC-III gene requires additional elements present in the more distal region between −500/−800 (43), where two additional hormone response elements are present at −673/−648 and −739/−704 (47).

We investigated in vivo occurring human haplotypes, including the FCHL wild-type haplotype (clone A), for their ability to influence transcriptional activity of the apoC-III promoter and to further delineate the in vivo regulation of apoC-III gene expression. ApoC-III is predominantly expressed in adult liver and to a lesser extent in the intestine. Therefore, in vitro experiments were carried out in both HepG2 and Caco-2 cells. By testing different apoC-III haplotypes, including the wild-type haplotype, we demonstrated that apoC-III basal transcriptional activity is enhanced independent of sequence variations within this region. In HepG2 cells a 19-fold increase and in Caco-2 cells a 12-fold increase were observed. Similar results were demonstrated by Li et al. (31) using an apoC-III promoter construct containing the −455, −482, −625, −640, and −641 variants, although the promoter containing only the −455 and −482 variants showed a slightly, but significantly, higher (17%) relative CAT activity than the wild-type construct.

The transcriptional regulation of the apoC-III promoter in vitro is known to be regulated by a variety of agents and several hormones including insulin (38). An IRE was mapped to polymorphic sites (−482 and −455) located in the sequence region that plays a role in the regulation of the apoC-III gene. The homologous apoC-III constructs that we investigated showed concentration-dependent insulin-induced suppression of transcription in HepG2 cells but up-regulation in Caco-2 cells. In contrast to a previous study (31), the presence of a minor allele at both polymorphic sites of the IRE locus did not abolish insulin responsiveness of the apoC-III promoter compared with the wild-type haplotype. It was not the purpose of the present study to elucidate the role of (combinations of) polymorphic sites. Our approach was to select and compare naturally occurring apoC-III gene haplotypes from a highly polymorphic region. In vitro studies such as the present one provide a good basis to explore the potential meaning of genetic heterogeneity and epistasis. However, an in vivo model system is required as well to thoroughly investigate the functional relevance of genetic heterogeneity because this depends on fine modulation in transcription regulation and potentially complex interactions with other factors that may not be present in an in vitro cell culture system. It is therefore not surprising that data obtained in in vitro studies cannot be directly extrapolated to the human situation as in FCHL.

In summary, these studies demonstrate an insulin-dependent but cell-type-specific sensitivity of the basal apoC-III transcriptional activity in vitro that was independent of the presence of sequence heterogeneity in this gene region. Whether an "insulin-resistant" transcriptional regulation of apoC-III can potentially play a role in the initiation of abnormalities in lipid metabolism remains to be determined, but the present data do not contradict an increased apoC-III transcription in hepatocytes under insulin-resistant conditions.

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