

Genotype/Phenotype Relationships in HNF-4 α /MODY1

Haploinsufficiency Is Associated With Reduced Apolipoprotein(AII), Apolipoprotein(CIII), Lipoprotein(a), and Triglyceride Levels

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Hepatocyte nuclear factor (HNF)-4 α is a transcription factor that plays an important role in regulation of gene expression in pancreatic β -cells and in the liver. Heterozygous mutations in the HNF-4 α gene are responsible for maturity-onset diabetes of the young 1 (MODY1), which is characterized by pancreatic β -cell-deficient insulin secretion. HNF-4 α is a major transcriptional regulator of many genes expressed in the liver. However, no liver defect has been identified in individuals with HNF-4 α mutations. In this study, we have identified HNF-4 α target genes that are mainly expressed in the liver, including α 1-antitrypsin, α 1-antichymotrypsin, α -fetal protein, ceruloplasmin, IGF binding protein 1, transferrin, apolipoprotein(AI) [apo(AI)], apo(AII), apo(B), and apo(CIII). Serum levels of these proteins and Lp(a) and triglycerides were measured in 24 members of the HNF-4 α /MODY1 RW pedigree (Q268X mutation), including 12 diabetic patients with HNF-4 α mutations (D-HNF4 $^{+/-}$), 6 nondi-

abetic subjects with HNF-4 α mutations (N-HNF4 $^{+/-}$), 6 normal relatives (N-HNF4 $^{+/+}$), 6 unrelated normal matched control subjects (N-HNF4 $^{+/+}$), and 12 matched diabetic (non-MODY1-5) patients (D-HNF4 $^{+/+}$). Serum levels of apo(AII), apo(CIII), lipoprotein(a) [Lp(a)], and triglyceride were significantly reduced in HNF4 $^{+/-}$ subjects (26.9, 19.8, 12.1, and 72.1 mg/dl, respectively) compared with HNF4 $^{+/+}$ subjects (37.4, 26.5, 45.2, and 124.2 mg/dl, respectively) ($P = 0.00001$, $P = 0.01$, $P = 0.00006$, and $P = 0.000003$, respectively). This reduction was not found when apo(AII), apo(CIII), Lp(a), and triglyceride levels were compared in D-HNF4 $^{+/-}$ versus N-HNF4 $^{+/-}$ or in D-HNF4 $^{+/+}$ versus N-HNF4 $^{+/+}$ subjects, which indicates that HNF-4 α haploinsufficiency rather than hyperglycemia is the primary cause of decreased serum protein and triglyceride concentrations. Furthermore, we determined that genetic or environmental modifiers other than HNF-4 α do not appear to contribute to the observed decrease of HNF-4 α -regulated serum proteins. This study demonstrates that a heterozygous HNF-4 α mutation leads to an HNF-4 α -dependent hepatocyte secretory defect of liver-specific proteins. *Diabetes* 49:832-837, 2000

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AFP, α -fetal protein; apo, apolipoprotein; CV, coefficient of variation; D-HNF4 $^{+/-}$, diabetic patients with HNF-4 α mutations; D-HNF4 $^{+/+}$, diabetic patients who do not have defects in the MODY1-5 genes; EB, embryoid body; ELISA, enzyme-linked immunosorbent assay; ES, embryonic stem; GCK, glucokinase; HNF, hepatocyte nuclear factor; HRP, horseradish peroxidase; IGFBP-1, IGF binding protein 1; LIF, leukemia inhibitory factor; Lp(a), lipoprotein(a); LPL, lipoprotein lipase; MODY, maturity-onset diabetes of the young; MODY-X, early-onset diabetic subjects who do not have defects in the MODY1-5 genes; N-HNF4 $^{+/-}$, nondiabetic subjects with hepatocyte nuclear factor-4 α mutations; N-HNF4 $^{+/+}$, normal control subjects; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; RT, reverse transcription; SABRE, selective amplification via biotin- and restriction-mediated enrichment; SSCP, single-strand conformation polymorphism; VE, visceral endoderm.

Maturity-onset diabetes of the young (MODY), a genetically heterogeneous monogenic disorder responsible for 2-5% of type 2 diabetes, is characterized by an autosomal dominant inheritance, an early age at onset (usually <25 years of age), and abnormal pancreatic β -cell function (1). Heterozygous mutations in the glycolytic enzyme glucokinase (GCK)/MODY2 and in 4 different transcription factors, including hepatocyte nuclear factor (HNF)-4 α /MODY1, HNF-1 α /MODY3, insulin promoter factor 1 (IPF-1/PDX-1)/MODY4, and HNF-1 β /MODY5, have been shown to be responsible for MODY (2-6).

The MODY1 gene is encoded by HNF-4 α , which is a transcription factor that belongs to the steroid/thyroid hormone receptor superfamily. HNF-4 α was first identified by its interaction with *cis*-regulatory sequences of liver-specific gene promoters (7). HNF-4 α plays a critical role in development, cell dif-

TABLE 1
Clinical characteristics of the study groups

Phenotype/ genotype	n	M:F	Age (years)	Age of diagnosis (years)	BMI (kg/m ²)	Fasting glucose (mg/dl)	HbA _{1c} (%)	Fasting insulin (ng/ml)
HNF4 ^{+/-}	18	10:8	32 ± 3		24.0 ± 1.0	92.2 ± 4.9	6.0 ± 0.3*	0.11 ± 0.01
D	12	6:6	37 ± 4	14 ± 1	24.3 ± 1.3	93.3 ± 7.1	6.4 ± 0.5*	0.13 ± 0.02
N	6	4:2	23 ± 4		23.3 ± 1.8	90.0 ± 4.8	5.3 ± 0.3*	0.09 ± 0.01
HNF4 ^{+/+}	24	12:12	35 ± 2		24.6 ± 0.7	124.8 ± 10.3	5.9 ± 0.4	0.40 ± 0.10
D (MODY-X)	12	6:6	38 ± 3	21 ± 3	25.2 ± 1.2	160.3 ± 14.0	6.9 ± 0.7	0.64 ± 0.17
N (unrelated individuals)	6	2:4	38 ± 2		24.1 ± 0.8	91.3 ± 3.5	4.5 ± 0.1	0.20 ± 0.01
N (RW family members)	6	4:2	28 ± 5		24.2 ± 1.6	87.2 ± 4.8	4.8 ± 0.2*	0.11 ± 0.01

Data are means ± SE. *Total HbA was measured in members of the RW family and was adjusted for HbA_{1c}. D, diabetic; N, normal glucose tolerance.

differentiation, and metabolism and is essential for the normal functioning of visceral endoderm (VE), liver, intestine, kidney, and pancreatic β -cells (8–10). HNF-4 α also regulates the expression of transcription factor HNF-1 α , thereby defining a transcriptional hierarchy responsible for these phenotypically indistinguishable forms of early-onset type 2 diabetes (11–13).

The first HNF-4 α /MODY1 mutation was found to be a non-sense mutation in codon 268, Q268X (3). This mutation generates a truncated protein that contained an intact DNA binding domain but lacked part of the AF2 region. Functional studies of this mutation have shown that the cause of diabetes is a loss-of-function mutation rather than a dominant-negative or gain-of-function mechanism (9).

Clinical studies have shown that HNF-4 α mutations are associated with impaired pancreatic β - and α -cell function characterized by abnormal insulin and glucagon secretion (12–15). Because HNF-4 α also plays major regulatory roles in other tissues including the liver, mutations in this gene could result in a pleiotropic phenotype such as impairment in cholesterol and lipoprotein metabolism in the liver. Recently, a mutation in the HNF-4 α gene has been shown to affect triglyceride metabolism (16), which further points to extra-pancreatic abnormalities in MODY1 patients.

In this study, we used a genetic screening to identify liver-secreted target genes of HNF-4 α in genetically manipulated embryonic stem (ES) cells. We then measured the serum levels of these proteins in subjects with HNF-4 α haploinsufficiency and in control groups. Our results indicate that a reduction in HNF-4 α activity in humans manifests as a unique gene expression profile that affects apolipoprotein (apo) and triglyceride concentrations.

RESEARCH DESIGN AND METHODS

Subjects. HNF4^{+/-} (Q268X) and nonaffected individuals of the RW pedigree were previously identified with sequencing analysis (3). A total of 12 diabetic patients with HNF-4 α mutations (D-HNF4^{+/-}), 6 nondiabetic subjects with HNF-4 α mutations (N-HNF4^{+/-}), 12 normal matched control subjects (N-HNF4^{+/+}), and 12 matched early-onset diabetic (MODY-X) patients (D-HNF4^{+/+}) were included in the study. The N-HNF4^{+/-} control group consisted of 6 N-HNF4^{+/+} RW relatives and 6 nonrelated N-HNF4^{+/-} matched control subjects. MODY-X patients and unaffected controls were of European descent, and most of these subjects were referred to the Rockefeller University General Clinical Research Center. Clinical diabetes was defined according to the new guidelines of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (17). Individuals were classified as MODY-X when their pedigrees were consistent with an autosomal dominant form of inheritance, when at least 2 subjects in a given pedigree were diagnosed at ≤ 35 years of age, in the absence of overt obesity, and in the absence of mutations in the genes encoding MODY1–5. Study groups were matched for sex, age, and BMI. Fast-

ing blood glucose and insulin levels in MODY-X subjects (D-HNF4^{+/-}) were significantly higher than in the other groups. This is most likely because of the different ascertainment procedure of these subjects and continued insulin treatment. The clinical characteristics of the 4 study groups are shown in Table 1.

Mutation analysis. MODY-X patients were screened for mutations in the HNF-4 α , GCK, HNF-1 α , PDX-1, and HNF-1 β genes by single-strand conformation polymorphism (SSCP) analysis as described (18). In addition, haplotype analysis was performed in most families using markers in the MODY1–5 gene locations. Abnormal SSCP conformers were reamplified and sequenced using a DNA sequencer (model 373A; Applied Biosystem, La Jolla, CA), a dideoxynucleotide cycle-sequencing protocol, and fluorescent-labeled dideoxynucleotide terminators as described by the manufacturer.

Growth and differentiation of ES cells in vitro. The generation of HNF4 α ^{+/-} and HNF4 α ^{-/-} ES cells and embryoid bodies (EBs) have been described previously (8). ES cells were maintained in ES cell medium supplemented with 1,000 U of leukemia inhibitory factor (LIF) on a primary embryonic fibroblast feeder layer as described (19). ES cells were induced to differentiate and to form EBs in vitro by growing them in bacterial petri dishes in the absence of primary embryonic fibroblasts and LIF for 3 weeks.

RT-PCR. Total RNA was extracted from ES cells using the TRIzol reagent according to the manufacturer's instructions (Gibco-BRL, Grand Island, NY). Contaminating genomic DNA was removed using 1 μ l RNase-free DNaseI (Boehringer Mannheim, Mannheim, Germany)/10 μ g total RNA. cDNA was synthesized using Moloney murine leukemia virus reverse transcriptase with dNTPs and random hexamer primers (Stratagene, La Jolla, CA). The cDNAs provided templates for polymerase chain reactions (PCRs) using specific primers at annealing temperatures ranging between 60 and 65°C in the presence of dNTPs, [α -³²P]dCTP, and Taq DNA polymerase. PCR synthesis for each primer pair was quantified at 15, 20, 25, and 30 cycles in a test reaction to ensure that the quantitative PCR amplification was in the linear range.

Selective amplification via biotin- and restriction-mediated enrichment. Selective amplification via biotin- and restriction-mediated enrichment (SABRE) subtraction was performed as described previously (20). Double-stranded cDNA was produced from oligo-purified mRNA and digested with *Sau3A*I. Linkers were annealed to the digested overhangs containing a *Bam*HI restriction site. Tester cDNA, in which differential messages were to be detected, was PCR reamplified with a biotinylated primer containing the *Bam*HI site. Driver or reference cDNA was amplified with a nonbiotinylated primer containing a mutant *Bam*HI site. A 33-fold excess of driver was hybridized to biotin-labeled tester, after which tester–tester homohybrids (representing differentially expressed genes) were purified with paramagnetic beads and digestion with *Bam*HI (21). A total of 3 rounds of subtraction were completed to enrich differentially expressed cDNAs.

Clinical biochemistry assays. Concentrations of Lp(a), apo(AI), and apo(B) were determined with immunoturbidimetric assays from Roche Diagnostics (Montclair, NJ) using a Hitachi 917 autoanalyzer (San Jose, CA). Apo(CIII) was quantified with a commercially available electroimmunoassay (Hydragel LpA-I; Sebia, Paris). The coefficients of variation (CVs) of Lp(a) and apo(B) were <6.4 and 2.7%, respectively.

Apo(AII) was determined independently with an immunoturbidimetric test from Immuno (Vienna, Austria) with a Cobas Bio analyzer (Roche Diagnostics) and an enzyme-linked immunosorbent assay (ELISA) test. For the latter test, mouse anti-human apo(AII) monoclonal antibody (PerImmune, Rockville, MD) diluted 1:100 in bicarbonate buffer was applied to wells of Nunc Maxisorb ELISA plates (Roskilde, Denmark) and was incubated overnight at

4°C. All subsequent incubations were performed at room temperature with gentle agitation. Wells were washed with phosphate-buffered saline (PBS) and were blocked with casein blocker (Pierce, Rockford, IL) for 1 h. Serum standards (Kamiya Biomedical, Tokyo) and serum samples diluted 1:30,000 in buffer A (casein blocker/0.5% Tween 20), were applied to the wells for 1 h. Plates were washed with buffer B (PBS/0.5% Tween 20), and a biotinylated goat anti-human apo(AII) polyclonal antibody (Biodesign, Saco, ME) diluted 1:1,000 in PBS/1% BSA was applied for 1 h. After washing, streptavidin-horse-radish peroxidase (HRP) (Pierce) diluted 1:1,000 was applied for 1 h. HRP enzyme was detected using incubation with TurboTMB substrate (Pierce). The reaction was terminated with 1 mol/l sulfuric acid, and the absorbance was measured at 450 nm on a SpectraMax plate reader (Molecular Devices, Sunnyvale, CA). The CV of this test was <7%.

Serum concentrations of α 1-antitrypsin, ceruloplasmin, and transferrin were determined using rate nephelometry on a Beckman Array 360 (Beckman-Coulter, Brea, CA) with specific antibodies to each analyte. The system measures the intensity of light as it is scattered by particles in suspension when a beam of light passes through a flow cell. The resulting antigen-antibody reaction is converted to a peak rate signal and is proportional to sample antigen (analyte) concentrations. The CVs were 4.2, 3.4, and 2.7%, respectively. Serum triglyceride concentrations were determined with an enzymatic colorimetric assay (GPO-Trinder, Sigma, St. Louis, MO). The CV of this test was <5%. Serum IGF binding protein 1 (IGFBP-1) concentrations were measured with an immunoradiometric assay and antibodies specific for human IGFBP-1 (DSL, Webster, TX).

Statistical analysis. Data are means \pm SE. The statistical analysis was performed using the Microsoft Excel 98 statistical package (Redmond, WA). Student's *t* test for 2-tailed analysis and unequal variance as parameters was used to assess statistical significance, and the null hypothesis was rejected at the 0.05 level.

RESULTS

Identification of liver-enriched HNF-4 α target genes. To gain a better understanding of a possible extrapancreatic defect in HNF-4 α haploinsufficient (MODY1) patients, we used a genetic screening to identify HNF-4 α target genes in the liver. HNF-4 α null mice die during development before formation of the liver and cannot be used to identify HNF-4 α -regulated genes. Heterozygous HNF-4 α mutant mice have normal glucose tolerance and do not mirror the human MODY1 disease. We therefore studied the loss of HNF-4 α function on target gene expression in EBs that were derived from ES cells in vitro (22). EBs contain VE, a tissue that displays many characteristics of the liver, including the expression of HNFs and their target genes (9,22).

We used 2 methods to identify liver-enriched genes that are regulated by HNF-4 α . The first method is a candidate gene approach in which genes that are primarily synthesized and secreted by hepatocytes were analyzed in EBs containing 2, 1, or no functional HNF-4 α allele. With this approach, we identified α 1-antitrypsin, α 1-antichymotrypsin, ceruloplasmin, transferrin, apo(AI), apo(AII), apo(B), and apo(CIII) (Fig. 1). We also subtracted cDNAs of HNF4 α ^{-/-} EBs from HNF4 α ^{+/+} EBs using SABRE, which is a PCR-based subtractive hybridization technique, to identify HNF-4 α -regulated liver-specific genes (20). Wild-type tester homohybrids (representing candidate HNF-4 α target genes) after the third round of subtraction were cloned into pGEM-5Z plasmids. A total of 60 clones were sequenced, and 29 were found to be unique. These clones were checked for differential expression by reverse transcription (RT)-PCR on EBs with 2, 1, or no functional HNF-4 α alleles. Of the clones, 2 (α -fetal protein [AFP] and IGFBP-1) were found to be strongly regulated by HNF-4 α (Fig. 1).

HNF-4 α haploinsufficiency is associated with reduced serum apo(AII), apo(CIII), Lp(a), and triglyceride levels. HNF-4 α is a key regulator of hepatocyte-specific gene expression. Therefore, we anticipated that loss of function by

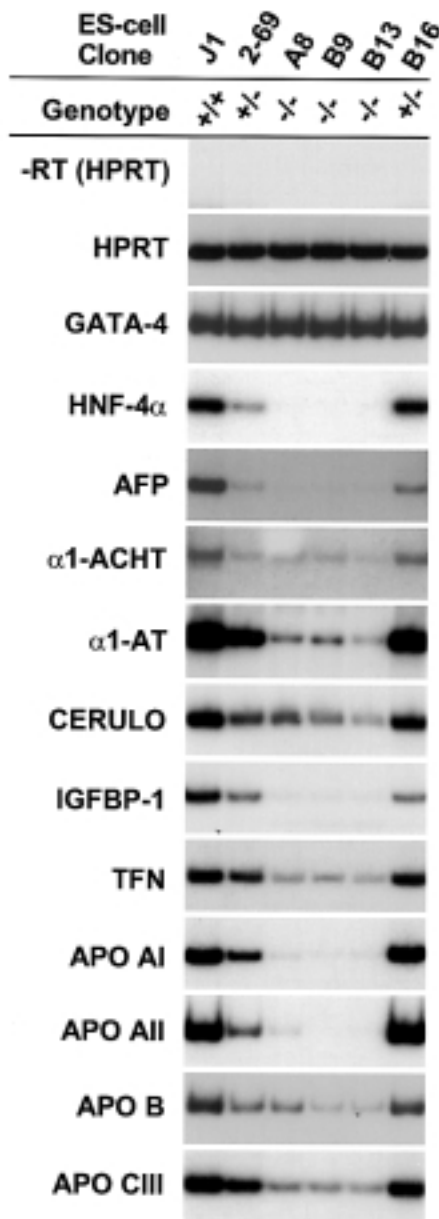


FIG. 1. Regulation of liver-specific genes by HNF-4 α in EBs. HNF-4 α target genes were identified using genetically manipulated EBs. The target genes included α 1-antitrypsin (α 1-AT), α 1-antichymotrypsin (α 1-ACHT), AFP, ceruloplasmin (CERULO), IGFBP-1, transferrin (TFN), apo(AI), apo(AII), apo(B), and apo(CIII). RT-PCR analysis of HNF-4 α -regulated genes in HNF4 α ^{+/+}, HNF4 α ^{+/-}, and HNF4 α ^{-/-} EBs shows that steady-state mRNA levels of these genes are either reduced or absent in HNF-4 α null EBs (22). -RT (HPRT) indicates that samples were not contaminated with genomic DNA. HPRT, hypoxanthine phosphoribosyltransferase; RT, reverse transcriptase.

HNF-4 α mutations could manifest in a pleiotropic MODY1 phenotype that results not only in pancreatic islets but also in liver dysfunction. To test this hypothesis, we obtained sera from the RW/MODY1 gene family. MODY1 in RW diabetic individuals is caused by a Q268X mutation in the HNF-4 α gene (3). This mutation results in a truncated HNF-4 α protein with no transcriptional activity (9). Our study groups consisted of 12 diabetic patients with an HNF-4 α mutation (D-HNF4 α ^{+/-}), 6 non-diabetic subjects with an HNF-4 α mutation (N-HNF4 α ^{+/-}), 12

TABLE 2
Serum concentrations of liver-specific proteins in subjects with or without the HNF4 α (Q268X)/MODY1 mutation

	Phenotype/genotype							P			
	HNF4 ^{+/-}	HNF4 ^{+/-} D	HNF4 ^{+/-} N	HNF4 ^{+/+}	HNF4 ^{+/+} D	HNF4 ^{+/+} N (unrl)	HNF4 ^{+/+} N (fam)	+/- vs. +/+	D vs. N	D vs. N _{unrl + fam}	N _{unrl} vs. N _{fam}
<i>n</i>	10	12	6	24	12	6	6	—	—	—	—
α 1-AT (mg/dl)	157.8 \pm 8.8	170.9 \pm 10.0	131.7 \pm 11.5	129.0 \pm 7.1	140.1 \pm 10.6	111.7 \pm 9.1	124.3 \pm 15.6	0.015	0.024	0.120	0.494
Cerulo (mg/dl)	35.2 \pm 1.9	37.1 \pm 2.4	31.5 \pm 2.8	45.5 \pm 3.2	51.0 \pm 5.6	43.3 \pm 5.1	58.7 \pm 9.5	0.009	0.150	0.089	0.188
TFN (mg/dl)	290 \pm 11.6	281 \pm 13.8	310 \pm 20.0	265 \pm 10.6	255 \pm 16.0	274 \pm 18.6	274 \pm 23.2	0.104	0.261	0.380	0.991
IGFBP-1 (ng/ml)	70.4 \pm 6.1	76.3 \pm 6.2	59.6 \pm 12.3	47.3 \pm 6.5	54.5 \pm 11.4	47.3 \pm 12.1	34.1 \pm 6.9	0.013	0.262	0.287	0.368
Apo(AI) (mg/dl)	115.2 \pm 6.9	119.3 \pm 7.9	105.6 \pm 14.1	119.1 \pm 7.0	126.8 \pm 10.9	131.5 \pm 11.6	91.2 \pm 5.6	0.697	0.430	0.276	0.014
Apo(AII) (mg/dl)	26.9 \pm 1.4	26.5 \pm 1.6	27.8 \pm 2.7	37.4 \pm 1.6	37.8 \pm 2.8	39.7 \pm 3.2	34.3 \pm 1.3	0.00001	0.683	0.803	0.156
Apo(B) (mg/dl)	79.4 \pm 5.8	82.3 \pm 8.0	72.4 \pm 3.0	97.3 \pm 6.0	104.2 \pm 9.0	102.3 \pm 13.2	78.3 \pm 6.4	0.037	0.268	0.256	0.138
Apo(CIII) (mg/dl)	19.8 \pm 1.5	18.9 \pm 1.6	19.6 \pm 1.0	26.5 \pm 2.0	29.3 \pm 0.7	25.5 \pm 2.2	22.2 \pm 2.7	0.010	0.730	0.188	0.345
Lp(a) (mg/dl)	12.1 \pm 3.5	13.5 \pm 4.8	8.6 \pm 3.9	45.2 \pm 6.3	57.7 \pm 8.6	29.5 \pm 9.6	35.8 \pm 14.2	0.00006	0.441	0.045	0.715
Triglyceride (mg/dl)	72.1 \pm 6.3	73.8 \pm 8.4	68.7 \pm 9.1	124.2 \pm 7.3	136.8 \pm 9.1	108.3 \pm 9.2	114.9 \pm 20.6	0.000003	0.686	0.084	0.775

Data are means \pm SE. α 1-AT, α 1-antitrypsin; D, diabetic; fam, RW family members; N, normal glucose tolerance; TFN, transferrin; unrl, unrelated individuals.

normal matched control subjects (N-HNF4^{+/+}), and 12 matched diabetic (MODY-X) patients (D-HNF4^{+/+}). These study subjects were matched regarding ethnicity, sex, age, and BMI. The clinical characteristics of the different study groups are shown in Table 1.

We measured the protein levels of HNF-4 α -regulated genes in the study subjects' sera, including α 1-antitrypsin, ceruloplasmin, transferrin, IGFBP-1, apo(AI), apo(AII), apo(B), apo(CIII), and Lp(a). In addition, we also measured total triglyceride serum concentrations. The results of our measurements are shown in Table 2. Serum concentrations of α 1-antitrypsin, ceruloplasmin, transferrin, apo(AI), and apo(B) showed no significant or consistent differences among the various study groups. However, we found that subjects who carry an HNF-4 α mutation have a significant reduction in serum apo(AII) (26.9 \pm 1.4 vs. 37.4 \pm 1.6 mg/dl; P = 0.000013), apo(CIII) (19.8 \pm 1.5 vs. 26.5 \pm 2.0 mg/dl; P = 0.01), Lp(a) (12.1 \pm 3.5 vs. 45.2 \pm 6.3 mg/dl; P = 0.000059), and triglyceride (72.1 \pm 6.3 vs. 124.2 \pm 7.3 mg/dl; P = 0.000031) levels when compared with individuals without the HNF-4 α mutation (Table 2). (In 1989, the mean serum concentration of triglycerides for 14 diabetic subjects of the RW pedigree [D-HNF4^{+/+}] was 77.7 \pm 7.3 mg/dl.) Moreover, no significant differences were observed when diabetic and nondiabetic subjects with or without the MODY1 mutation were compared (D-HNF4^{+/-} vs. N-HNF4^{+/-} and D-HNF4^{+/+} vs. N-HNF4^{+/+}, respectively) (Table 2, Fig. 2). This indicates that HNF-4 α haploinsufficiency rather than secondary effects resulting from impaired glucose homeostasis is the primary cause of decreased serum apo(AII), apo(CIII), Lp(a), and triglyceride concentrations. To exclude the possibility that the reductions we observed could be because of genetic (other than HNF-4 α) or environmental factors, we compared serum factors of HNF4^{+/+} individuals from the RW family (fam) and unrelated control subjects (unrl) with normal glucose tolerance (N) (N_{fam} vs. N_{unrl}). No significant differences between these 2 groups were observed, which indicates that familial aggregation of modifiers does not contribute to reductions in these serum proteins (Table 2).

DISCUSSION

HNF-4 α is a transcription factor of the steroid hormone receptor superfamily that has an important role as a key regulator of pancreatic islet and hepatic gene expression.

Genetic studies have shown that mutations in HNF-4 α result in an autosomal dominant form of type 2 diabetes characterized by defects in pancreatic β -cell function and glucose- and arginine-stimulated insulin release. Liver function has not been thoroughly assessed in patients with HNF-4 α mutations.

We have performed a genetic screening to identify hepatocyte-enriched genes that are regulated by HNF-4 α . We used the VE of EBs that were derived from wild-type and HNF-4 α null ES cells as a model system to identify HNF-4 α target genes. The VE is functionally related to the liver and shares many molecular characteristics, including the components of the HNF network and their targets (9,10,22). This model also allows the analysis of transcriptional regulation of HNF-4 α target genes on a genome-wide scale and in the context of native chromatin. A total of 9 proteins that are encoded by genes that are predominantly expressed in the liver and secreted into the blood were strongly dependent on the expression levels of HNF-4 α in the VE (Fig. 1) (9,22). We therefore tested whether the serum concentrations of these proteins were reduced in subjects with HNF-4 α haploinsufficiency compared with nondiabetic control subjects and thereby may serve as serum markers for MODY1. To differentiate between changes in gene expression that are caused by the genotype and effects that result from the phenotype of the study participants, we included 4 groups in our study design: diabetic patients with HNF-4 α mutations (D-HNF4^{+/-}), nondiabetic subjects with HNF-4 α mutations (N-HNF4^{+/-}), normal matched control subjects (N-HNF4^{+/+}), and matched early-onset diabetic subjects who do not have defects in the MODY1-5 genes (D-HNF4^{+/+}/MODY-X).

We found that subjects who carry an HNF-4 α mutation have a significant reduction in serum apo(AII), apo(CIII), Lp(a), and triglyceride levels compared with nondiabetic control subjects. We also observed that this reduction still holds when various genotypic and phenotypic comparisons were made, which indicates that HNF-4 α haploinsufficiency rather than confounding effects resulting from family aggregation or diabetes is the primary cause of the defect in lipoprotein metabolism. Our findings agree with a recent study (16) that observed a significant reduction of triglyceride plasma levels in individuals carrying a MODY1 mutation. In contrast with 2 other reports (23,24), we found that the serum levels of apo(AII) and Lp(a) were significantly decreased in individuals with a heterozygous HNF-4 α mutation. We believe that 1 of these discrepancies can be explained by the fact that the

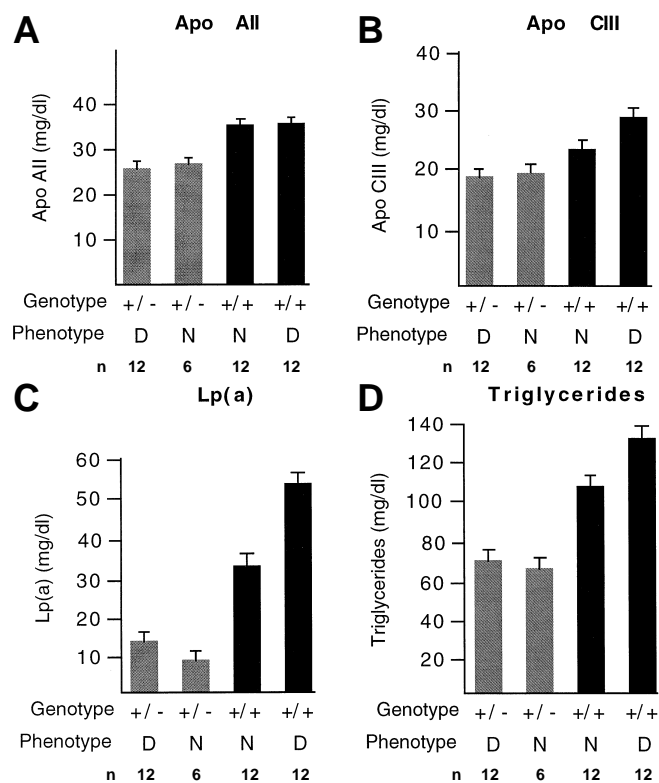


FIG. 2. Serum concentrations of apo(AII) (A), apo(CIII) (B), Lp(a) (C), and triglycerides (D) in nondiabetic individuals and subjects with HNF-4 α mutations. D, diabetic individuals; N, nondiabetic individuals.

HNF-4(R127W) mutation in these individuals is not a functional mutation but rather is a rare polymorphism and therefore does not lead to altered HNF-4 α -dependent gene expression (23,25). The other study, which reports a family with a HNF-4(R154X) mutation, found elevated Lp(a) levels and no significant differences in apo(AII), apo(CIII), and triglyceride levels (24). However, this study used a small sample size, including only 2 nondiabetic control subjects who were not well matched regarding age and BMI. Another explanation for the difference in Lp(a) levels may be confounding factors that were not ruled out. For example, Lp(a) shows wide quantitative variation among individuals, the variations in concentration are heritable, and the LPA gene itself accounts for almost all genetic variability in plasma Lp(a) levels (26,27). Differences in Lp(a) concentrations between HNF4^{+/+} and HNF4^{+/-} individuals may therefore also result from genetic variation in the LPA gene itself.

The serum levels of some HNF-4 α -regulated genes, including transferrin, α 1-antitrypsin, IGFBP-1, apo(AI), and apo(B), were not decreased in subjects with HNF-4 α mutations. Several reasons may account for this finding. Insulin and glucose levels per se can alter gene expression, and this effect may be strong and may mask differences between genotypes. For example, IGFBP-1 gene expression is known to be markedly upregulated in diabetic subjects, and this may account for the elevated IGFBP-1 serum levels in D-HNF4^{+/-} subjects (28). The lack of reduction in other HNF-4 α -regulated serum factors may also be explained by compensatory posttranscriptional mechanisms. Finally, differences between humans and mice may exist in gene expression of HNF-4 α target genes.

The mechanisms by which HNF-4 α regulates expression of the above genes are unknown but are likely to be both direct and indirect. Some of these genes, including apo(AII) and apo(CIII), are known to contain an HNF-4 α binding site and therefore may be directly regulated by HNF-4 α (7,29). HNF-4 α may mediate its regulation of these target genes through a transcriptional cascade involving HNF-1 α (10,11,30). However, a subset of genes may exist that is regulated by HNF-4 α but not by HNF-1 α because the expression of the glycolytic gene aldolase B is decreased in the absence of HNF-4 α but not in the absence of HNF-1 α (9,31). Such a subset of genes could, in theory, serve as a molecular marker to be used to differentiate between MODY1 and MODY3, which are 2 clinically indistinguishable forms of early-onset type 2 diabetes.

Our data suggest that the decrease in apo(AII), apo(CIII), and Lp(a) levels is caused by impaired transcriptional activation of these genes resulting from HNF-4 α haploinsufficiency. These transcriptional changes in the liver lead to a decrease in the serum levels of these factors. Lowered triglyceride concentrations may result from increased lipoprotein lipase (LPL) activity because apo(CIII) is an inhibitor of LPL activity in vitro (32), and transgenic mice overexpressing human apo(CIII) have elevated triglyceride levels in plasma because of the presence of enlarged triglyceride-rich lipoproteins with increased apo(CIII) levels (33). Low apo(AII) levels may also contribute to decreased triglyceride levels because overexpression of apo(AII) in transgenic mice leads to hypertriglyceridemia (34), and apo(AII) deficiency in knockout mice is associated with low free fatty acid levels (35). However, a 25% decrease in apo(AII) levels is not likely to have a major effect on lipid metabolism because human deficiency of apo(AII) has little influence on either lipid and lipoprotein profiles or the occurrence of cardiovascular disease (36). Subjects with HNF-4 α mutations may have a slightly lower risk of developing cardiovascular complications because Lp(a) and triglycerides, which are 2 independent risk factors for cardiovascular diseases, are reduced.

Our data suggest that HNF-4 α is a central regulator of glucose and lipid metabolism and that the development of HNF-4 α agonists and antagonists may result in powerful drugs for the treatment of insulin secretion defects and dyslipoproteinemias, respectively. Ideally, any future therapeutic drug development that targets HNF-4 α to improve β -cell function should increase HNF-4 α activity and exhibit pancreatic β -cell tissue specificity, whereas selective improvement of lipoprotein metabolism may be achieved with HNF-4 α antagonists in the liver and intestines.

In summary, genotype/phenotype relationship studies can dissect primary and secondary defects of pancreatic β -cell dysfunction and lipid/lipoprotein metabolism. Altered hepatocyte gene expression contributes to the phenotype of HNF-4 α /MODY1 diabetes. HNF-4 α haploinsufficiency causes primary defects in lipoprotein metabolism and is independent of altered glucose metabolism. HNF-4 α -dependent serum protein profiles may in the future serve as molecular markers that can be used to distinguish MODY1 diabetes from type 2 diabetes or other forms of MODY.

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