The Zinc Finger Protein 202 (ZNF202) Is a Transcriptional Repressor of ATP Binding Cassette Transporter A1 (ABCA1) and ABCG1 Gene Expression and a Modulator of Cellular Lipid Efflux*

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The zinc finger gene 202 (ZNF202) located within a hypoalphalipoproteinemia susceptibility locus on chromosome 11q23 is a transcriptional repressor of various genes involved in lipid metabolism. To provide further evidence for a functional linkage between ZNF202 and hypoalphalipoproteinemia, we investigated the effect of ZNF202 expression on ATP binding cassette transporter A1 (ABCA1) and ABCG1. ABCA1 is a key regulator of the plasma high density lipoprotein pool size, whereas ABCG1 is another mediator of cellular cholesterol and phospholipid efflux in human macrophage. We demonstrate here that the full-length ZNF202m1 isoform binds to Gnt repeats within the promoters of ABCA1 (−229/−210) and ABCG1 (−572/−552). ZNF202m1 expression in HepG2 cells dose-dependently repressed the promoter activities of ABCA1 and ABCG1. This transcriptional effect required the presence of the SCAN domain in ZNF202 and the functional integrity of a TATA box at position −24 of ABCA1, whereas the presence of Gnt binding motifs was nonessential. The state of ZNF202 SCAN domain oligomerization affected the ability of the adjacent ZNF202 Krüppel-associated box domain to recruit the transcriptional corepressor KAP1. Overexpression of ZNF202m1 in RAW264.7 macrophages prevented the induction of ABCA1 gene expression by 20(S)-OH-cholesterol and 9-cis-retinoic acid, further substantiating the interference of ZNF202 in critical elements of transcriptional activation. Finally, HDL and apoAI-mediated lipid efflux was significantly reduced in RAW264.7 cells stably expressing ZNF202m1. In conclusion, we have identified ABCA1 and ABCG1 as target genes for ZNF202-mediated repression and thus, provide evidence for a functional linkage between ZNF202 and hypoalphalipoproteinemia.

Molecular factors that determine plasma high density lipoprotein (HDL)^5 cholesterol levels include several known genes with defined roles in reverse cholesterol transport (1) and a variety of susceptibility loci with to date poorly characterized candidate genes. Mutations in the ATP binding cassette transporter A1 (ABCA1) gene have recently been causatively linked to familial HDL-deficiency syndromes (2–4). The transporter ABCA1 is regulated by cholesterol flux and facilitates the apoAI-dependent cellular export of cholesterol and phospholipids, thereby acting as a key regulator of plasma HDL (2, 3, 5). The half-size transporter ABCG1 is another member of the group of cholesterol-responsive ABC transporters. ABCG1, like ABCA1, has been shown to regulate cellular cholesterol and phospholipid efflux (2, 3, 6).

In past years, evidence has accumulated to suggest that a number of transcription factors play critical roles in the coordinate transcriptional regulation of genes involved in lipid metabolism (7, 8). Linkage analysis in large Utah pedigrees led to the identification of a low HDL-cholesterol locus on chromosome 11q23 that is distinct from the apoAI/C-III/AIV gene cluster (9). This novel familial susceptibility locus for hypoalphalipoproteinemia contains the zinc finger protein 202 (ZNF202) originally described as a testis-specific transcription factor (9, 10). ZNF202 is expressed in two common splice variants. The m1 splice form encodes a full-length protein of 648 amino acids with an amino-terminal SCAN domain, a central KRAB repression domain, and 8 carboxyl-terminal Cys2-His2 zinc finger motifs. The m3 splice form encodes a carboxyl-terminal-truncated protein of 133 amino acids that contains only the SCAN domain. The SCAn domain of ZNF202 has been shown to mediate selective protein oligomerization and the zinc finger motifs to bind to specific DNA elements (9, 11). Intriguingly, the ZNF202 DNA binding elements are present in promoters of various genes involved in lipid metabolism including apolipoproteins and lipid-processing enzymes. ZNF202 has been therefore proposed to function as a transcriptional regulator of lipid metabolism (9).

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§ The abbreviations used are: HDL, high density lipoprotein; ABC, ATP binding cassette; apo, apolipoprotein; ZNF, zinc finger protein; KRAB, Krüppel-associated box; KAP1, KrAB-associated protein 1; GST, glutathione S-transferase.

EXPERIMENTAL PROCEDURES

Cell Culture—HepG2 and RAW264.7 cells (American Type Culture Collection) were cultured in Dulbecco’s modified Eagle’s medium (Bio-Whittaker) supplemented with 10% fetal calf serum (Sigma) in a 5% CO2 atmosphere at 37 °C. Cells (1 × 106 cells/2 ml medium) were...

PAGE, polyacrylamide gel electrophoresis; SDP1, SCAN domain protein 1; GST, glutathione S-transferase.
seeded in 6-well plates overnight before transfection. In some transfection experiments HepG2 cells were washed 4 h after transfection and subsequently incubated in Dulbecco's modified Eagle's medium containing 5% lipoprotein-deficient serum, 10 μM 2053-OH-cholesterol, and 10 μM 9-cis-retinoic acid (Sigma). In all experiments cells were harvested after 24 h to measure luciferase activity or to prepare RNA and nuclear extracts.

**Cloning of Expression Constructs**—cDNAs encoding either the open reading frame of the ZNF202m1 isoform (GenBank™ accession number af207219, nucleotide positions 8–1960) (2, 3, 10) or a truncated product lacking the SCN domain (nucleotide positions 920–1960) were cloned into A3/V5/His-Topo plasmid (Invitrogen), and the sequence was confirmed by using an automated fluorescence DNA sequencer and the ALFexpress AutoRead sequencing kit (Amersham Pharmacia Biotech). The pcDNA3.1 ZNF202 (DV > AA) construct with a double amino acid substitution (DV > AA) in the ZNF202 KRAB domain was generated by using a site-directed mutagenesis kit (CLONTECH). GST fusions constructs with the ZNF202 SCAN or KRAB domain were generated as previously described (11). A cDNA encoding KAP1 was obtained from Edge Bio Systems and directionally subcloned into the pcDNA3.1/His expression vector (Invitrogen). Sequence fidelity was assessed by using an ABI PRISM 377 DNA sequence (PerkinElmer Life Sciences).

**In Vitro Protein Expression**—KAP1 and ZNF202 cDNA templates were expressed in vitro in rabbit reticulocyte lysates in the presence of [35S]methionine (Amersham Pharmacia Biotech). Rabbits were injected with 10 μg of the KAP1 cDNA template expressing rabbit reticulocyte lysate (1–2 μl) in a buffer containing 50 mM HEPES/HC1, pH 7.9, 6 mM MgCl2, 50 mM diithiothreitol, 100 μg/ml bovine serum albumin, 0.01% Nonidet P-40, and 2 μg of poly(dI-dC) (Amersham Pharmacia Biotech) and incubated for 20 min at room temperature. The respective promoter sequences encoded by the oligonucleotides are described in Fig. 1. Supershift experiments were performed with the addition of KAP1 antibodies (N84) in competition experiments, nuclear extracts were precleared for 10 min with a 50-fold molar excess of unlabeled oligonucleotides before the addition of radiolabeled probe. DNA-protein complexes were finally resolved on a native 8% polyacrylamide gel and analyzed by autoradiography.

**Cloning of Reporter Gene Constructs**—Reporter constructs for the ABCA1 and ABCG1 promoters, or as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14).

**Northern Blot Analysis**—HepG2 cells were transfected with increasing amounts of ZNF202 expression vectors using Fugene® reagent (Roche Molecular Biochemicals). The transfection efficiency ranged between 50 and 70%. Total RNA was isolated 48 h after transfection for Northern blot analysis using the QiAamp RNA isolation kit (Qiagen). Aliquots of 10 μg of total RNA were separated on denatured agarose gels and transferred to nylon membranes (Amersham Pharmacia Biotech). The membranes were hybridized with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14). The membranes were rehybridized with a ubiquitin-specific probe to confirm equal RNA loading. The tissue-specific expression of ZNF202 was assessed by hybridization with a random-prime radiolabeled ABCA1-specific probe that was derived from the 5′ end of the coding sequence as described elsewhere (2, 3, 14).

**Efflux Experiments**—RAW264.7 cells were transfected with a ZNF202m1 encoding pcDNA3.1/V5/His-Topo vector or an empty control vector. Cells were incubated in Dulbecco's modified Eagle's medium containing 500 μM/ml neomycin according to standard protocols. ZNF202 protein expression was confirmed by Western blotting. Efflux assays were performed as recently described with minor modifications (2, 3, 15). Briefly, stably transfected RAW264.7 cells were radiolabeled with 1.5 μCi/ml [3H]cholesterol and 10 μCi/ml [14C]choline and loaded with 40 μg/ml enzymatically modified low density lipoprotein that was prepared as described elsewhere (10). Cells were incubated in 6-well plates for 24 h in Dulbecco’s modified Eagle’s medium supplemented with 5% lipoprotein-deficient serum and 10 μM 2053-OH-cholesterol, 10 μM 9-cis-retinoic acid (Sigma), or 0.02% v/v ethanol. The cells were then washed and chased for 17 h with either 100 μg/ml HDLm protein, 10 μg/ml purified apoAI (Sigma), or 0.2% bovine serum albumin in the absence of radiolabeled lipids and enzymatically modified low density lipoprotein. Lipids were finally extracted as previously described (17). Radioactivity was determined by liquid scintillation counting. Lipid efflux is expressed as the ratio of counts in medium to total counts. Specific efflux rates were calculated by subtracting efflux rates in the presence of control bovine serum albumin from the efflux rates in the presence of the lipid acceptors apoAI or HDL.

RESULTS AND DISCUSSION

**ZNF202Binds Specific ABCA1 and ABCG1 Promotor Sequences**—It has been proposed that ZNF202 functions as a transcriptional repressor of various genes related to lipid trans-
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**Fig. 1. ZNF202 binds to GnT motifs within the ABCA1 and ABCG1 gene promotors.** A, domain organization of the ZNF202m1 protein (648 amino acids). Amino acid positions and described functions of SCAN, KRAB, and zinc finger domains are indicated. B, potential ZNF202 binding sites (GnT motifs) within the promotor sequences of ABCA1 and ABCG1 were reproduced as oligonucleotides. The GnT consensus motif was derived from the apolipoprotein AIV promotor (9). Sequences and positions within the respective promotor regions are shown. C, electrophoretic mobility shift assays were performed with in vitro transcribed and translated ZNF202m1 protein. Complex formation was inhibited by the addition of polyclonal antibodies against the KRAB domain of ZNF202 (lane 5). Specific DNA/ZNF202m1 complexes are indicated with brackets symbols, and the free probe is indicated with the letter P. Lane 1, free probe; lane 2, 1 μl of ZNF202m1: lane 3, 2 μl of ZNF202m1; lane 4, 1 μl of ZNF202m1 + 1 μl of N84 KRAB antibody; lane 5, 1 μl of ZNF202m1 + 1 μl (50-fold) of unlabeled oligonucleotides (wild-type competitor).

As depicted in Fig. 1, we found GnT motifs within the promotor region of ABCA1 at positions –210/–229 and ABCG1 at positions –552/–572. To examine the ability of ZNF202 to bind these DNA sequences, gel shift assays were performed with rabbit reticulocyte lysates expressing in vitro full-length ZNF202m1 protein and radiolabeled oligonucleotides encoding specific promotor fragments (Fig. 1, A and B). The published consensus ZNF202 DNA binding sequence, as found in the apoAIV promotor at positions –265/–243 and represented by an oligonucleotide, led to a specific and dose-dependent band shift after incubation with the ZNF202m1 lysate (Fig. 1C, lane 1–3) (9). Oligonucleotides reflecting the ABCG1 and ABCA1 ZNF202 DNA binding motifs revealed similar migratory gel properties after incubation with the ZNF202m1 lysate. The distinct intensities of the detected DNA–protein complexes may reflect individual affinities of these promotor fragments for ZNF202m1. The addition of KRAB polyclonal antibody that recognizes epitopes between amino acid position 177 and 329 of ZNF202m1 to the incubation mixture affected the affinity of ZNF202 for the apoAIV, ABCG1, or ABCA1 promotor fragment (Fig. 1C, lane 4). The addition of unlabeled oligonucleotides in a 50-fold excess competitively abolished the band shift (Fig. 1, lane 5). The addition of unlabeled oligonucleotides in a 50-fold excess competitively abolished the band shift (Fig. 1, lane 5), providing further evidence for the specific interaction of ZNF202m1 with its target sequences.

**Fig. 2. ZNF202 inhibits ABCA1 and ABCG1 promotor activity.** Luciferase assays performed with ABCA1 (–919/+244), ABCG1 (–2912/+50), apolipoprotein AIV (–718 to +30), and promotorless pGL3 reporter gene constructs. HepG2 cells were co-transfected with 2 μg of reporter gene construct, 1 μg of pSV β-galactosidase vector, and ZNF202m1 expression vector in various amounts as indicated (black bars) or SCAN domain-truncated ZNF202 vector (crossed bars). Cells were analyzed for promoter activity 24 h post-transfection. Results are presented as inhibition of luciferase activity (mean ± S.D. of triplicate measurements of three independent experiments) in comparison to cells transfected with empty control vector. Luciferase activity was normalized for β-galactosidase activity and protein concentrations. Absolute luciferase levels were 119-fold for ABCA1, 31-fold for apolipoprotein AIV, and 6-fold for ABCG1 above control vector level.

To resolve the discrepancy between the relatively low binding affinity of ZNF202 for the ABCA1 promotor sequence (Fig. 1) and the high capacity to repress the promotor activity (Fig. 2), we further investigated the interaction of ZNF202 with the ABCA1 promotor. Besides its affinity for GnT motifs, ZNF202 shares with other zinc finger proteins the ability to bind to GC boxes (9). The ABCA1 promotor harbors two GC boxes at positions –91 and –157 that bind the zinc finger proteins Sp1 and Sp3 (M. Porsch-Ozciurumaz, data not shown). We generated reporter gene constructs with truncated ABCA1 promotor inserts and mutated putative ZNF202 binding sites (Fig. 3). Surprisingly, we did not observe any loss in ZNF202-mediated repression with a reporter construct containing only the –79/+224 ABCA1 region and, thus, lacking all potential ZNF202 binding sites. ZNF202 may therefore interact with other elements of the transcriptional complex.

**KRAB domain-mediated transcriptional repression has been reported to result from interference with the TATA box-dependent basal transcription machinery (18, 19).** The –79/+224 ABCA1 reporter gene construct with a mutated TATA box at...
position –24 markedly decreased promotor activity but also abolished ZNF202-mediated repression (Fig. 3). Identical results were obtained with the +12/+24 construct containing only exon 1 of ABCA1. These data indicate that (i) the TATA box is of functional importance for ABCA1 expression and (ii) transcriptional repression by ZNF202 is likely mediated by a similar mechanism as reported for other Krüppel-type zinc finger proteins.

ZNF202 Binding to the Transcriptional Corepressor KAP1—ZNF202 shares a number of similar amino acids with the proposed consensus sequence for the KRAB homology domain, which was derived from the KOX1 gene (12). Within the KRAB domain, the amino acid identity between ZNF202 and KOX1 is 42%, and the similarity is 64%, as aligned with the BLAST algorithm (20). KOX1 has been shown to associate via its KRAB domain with KAP1, a 97-kDa nuclear phosphoprotein with all the hallmarks of a universal corepressor. The RING finger, B boxes (β1 and β2) and a coiled coil region at the amino terminus collectively constitute the KRAB interaction, or RBCC domain (12). We therefore analyzed the ability of ZNF202 to bind KAP1. Indeed, in vitro radiosynthesized KAP1 protein associated with the ZNF202 KRAB domain, as shown by affinity purification studies with GST-tagged ZNF202 fusion proteins (Fig. 4A). To evaluate the specificity of the ZNF202-KAP1 interaction, a double amino acid substitution (DV).AA) that has been previously shown to functionally inactivate the KOX1 protein was reproduced in the ZNF202 KRAB domain (12, 21). The wild type and mutated ZNF202m1 cDNA sequences were radiosynthesized in vitro and analyzed for their ability to bind KAP1. Resin-coupled KAP1 immunocomplexes were isolated from HeLa cells and, after incubation with ZNF202-expressing rabbit reticulocyte lysates, was analyzed by SDS-PAGE and fluorography (Fig. 4B). KAP1 associated readily with wild type ZNF202, whereas the DV > AA amino acid substitution within the KRAB domain abolished, as anticipated, the association of ZNF202 with KAP1.

The apolipoprotein AIV and ABCA1 genes both contain a TATA box that may be targeted by KRAB domain-mediated signaling (22). The underlying mechanism of ZNF202-induced transcriptional repression of the ABCG1 gene, which lacks a TATA box, needs to be further elucidated. The relatively low constitutive activity of the ABCG1 promotor may explain the possibility that minor ZNF202 alterations affect the pyrimidine-rich initiator element of ABCG1 (13). It also remains unclear how specificity of ZNF202 repression is defined. Pengue and Lania (19) provide evidence that KRAB domain-mediated transcriptional repression is not caused by a general
and unspecific inhibition of the RNA polymerase II machinery but strongly depends on the specific arrangement of basal promotor elements.

The amino-terminal SCAN domain of ZNF202 has been shown to readily oligomerize with SDP1 (11). We investigated therefore the possibility that SCAN domain-mediated protein oligomerization may affect the ability of the juxtaposed KRAB domain to interact with KAP1. ZNF202m1, SDP1, and mFPM315, a SCAN domain-encoding protein without detectable affinity for ZNF202 and, therefore, used as negative control (11), were expressed in vitro in rabbit reticulocyte lysates in the presence of a sulfur radiolabel. A HeLa cell-isolated KAP1 immunocomplex readily purified ZNF202 in the presence or absence of mFPM315 (Fig. 4C). However, in the presence of SDP1, ZNF202 lost its ability to bind the KAP1 immunocomplex. The state of SCAN domain oligomerization may therefore modulate the ability of ZNF202 to recruit the corepressor KAP1 and to mediate transcriptional repression. Although zinc finger proteins of the Cys2-His2 class are reported to bind DNA in monomeric form, our results suggest that SCAN domain-containing zinc finger proteins require specific homo- or heterodimerization for DNA binding and transcriptional modulation. The existence of SDP1 as a gene encoding an isolated SCAN domain as well as the truncated ZNF202m3 splice variant, which also contains only a SCAN domain, further highlights the modulating impact of SCAN domain interactions (11, 23).

ZNF202 Affects Induction of ABCA1 and Cellular Lipid Efflux—To further confirm the role of ZNF202 as a transcriptional repressor of ABCA1, we determined the ABCA1 mRNA abundance by Northern blot analysis using RNA preparations from HepG2 human hepatoma cells transfected with increasing amounts of expression vector encoding either ZNF202m1 (Fig. 5A, upper panel) or a ZNF202 fragment lacking the SCAN domain (Fig. 5A, lower panel). In accordance with the data from the promotor assays, the endogenous expression of ABCA1 mRNA was significantly decreased in ZNF202m1-transfected HepG2 cells, whereas mRNA levels were unaffected upon transfection with a ZNF202-truncated protein.

Because KRAB domain proteins were shown to repress both basal and activated promotor activity, we investigated the effect of ZNF202 under conditions that induce ABCA1 expression (Fig. 5B). Oxysterols such as 20(S)-OH-cholesterol in combination with 9-cis-retinoic acid have been shown to strongly induce ABCA1 expression by interaction with LXR/RXR heterodimers via a DR4 element (5); the physiological relevance of these compounds still remains unclear. Incubation with 10 μM 20(S)-OH-cholesterol and 10 μM 9-cis-retinoic acid led to a 3.5-fold induction of ABCA1 promotor activity in RAW264.7 rat macrophages (Fig. 5B). Transfection of these cells with 4 μg of ZNF202m1 expression vector almost completely abolished oxysterol-dependent induction of ABCA1. These data underscore the strong inhibitory capacity of ZNF202, which is likely driven by its SCAN domain.
HDL3-mediated cholesterol efflux. Since recent studies demonstrated that apoAI and not HDL3 is the lipid acceptor for the specific phospholipid efflux (100% or 5 dots, respectively).

Interestingly, the strongest suppression was observed in lipid efflux (Table I). Since ABCA1 and ABCG1 are induced in human tissues such as uterus, brain, intestine, fetal tissues, bone marrow, and leukocytes, which support a potential role for ZNF202 in the transcriptional regulation of ABCA1 and ABCG1 (26). Additional genes with selective cholesterol transport activity (Table I) will help to understand the role of ZNF202 in modulating the expression of ABCA1 and ABCG1 in vivo. It will be of special interest to identify genotypic sequence variations in regulatory motifs and functional domains of the ZNF202 gene. Abnormalities in ZNF202-oligomerizing proteins may also contribute to genetically based hypoalphalipoproteinemia and, thereby, further confirm the role of chromosome 11q23 as a HDL susceptibility locus.

The original description of ZNF202 as a testis-specific zinc finger protein (10) was based on expression studies with limited tissue samples. Using an RNA master blot derived from 75 distinct human tissues, we observed that ZNF202 expression is down-regulated during monocyte differentiation and repressed by cholesterol loading, thus demonstrating inverse regulation of ABCA1/ABCG1 and ZNF202 (C. Schumacher; data not shown).

The identification of additional ZNF202-interacting proteins and the elucidation of the transcriptional regulation of ZNF202 will help to understand the role of ZNF202 in modulating the expression of ABCA1 and ABCG1 in vivo. It will be of special interest to identify genotypic sequence variations in regulatory motifs and functional domains of the ZNF202 gene. Abnormalities in ZNF202-oligomerizing proteins may also contribute to genetically based hypoalphalipoproteinemia and, thereby, further confirm the role of chromosome 11q23 as a HDL susceptibility locus.

REFERENCES
1. Funke, H. (1997) Curr. Opin. Lipidol. 8, 189–196
2. Bodzioch, M., Orso, E., Klucken, J., Langmann, T., Bottcher, A., Diederich, W., Dohmen, W., Barlage, S., Buchler, C., Porsch-Greiner, M., Kaminiski, W. E., Hahnman, H. W., Oette, K., Roth, G., Aslanidis, C., Lackner, K. J., Rothe, G., Aslanidis, C., Lackner, K. J., and Schmitz, G. (1999) Nat. Genet. 22, 347–351
3. Brooks, W. A., Marcil, M., Clee, S. M., Zhang, L. H., Roomp, K., van-Dam, M., Yu, L., Brewer, C., Collins, J. A., Molhuizen, H. O., Loubser, O., Ouelette, B. F., Fichter, K., Ashbourne, E. K., Sensen, C. W., Scherer, S., Moti, S., Denis, M., Martindale, D., Frohlich, J., Morgan, K., Koop, B., Pimstone, S., Rastelten, J. J., and Hayden, M. R. (1999) Nat. Genet. 22, 336–345
4. Rust, S., Rosier, M., Funke, H., Reul, J., Amoura, Z., Piette, J. C., Deleuze, J. F., Brewer, H. B., Duverger, N., Denefle, P., and Assmann, G. (1999) Nat. Genet. 22, 352–355

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by a mechanism that targets crucial elements of transcriptional activation.

Finally, to demonstrate the functional relevance of ZNF202 on cellular phospholipid and cholesterol efflux, RAW264.7 cells that stably overexpress ZNF202m1 were generated. Because ABCA1 and ABCG1 expression are strongly induced by oxysterols (24, 25), we compared efflux rates under basal conditions and during incubation with 20(S)-OH-cholesterol and 9-cis-retinoic acid. As shown in Fig. 5C, apoAI and HDL3-mediated lipid efflux markedly depend on the stimulation with oxysterols. ZNF202-overexpressing cells revealed significantly reduced specific phospholipid efflux (upper panel) and cholesterol efflux (lower panel) rates as compared with mock-transfected cells. Interestingly, the strongest suppression was observed in HDL3-mediated cholesterol efflux. Since recent studies demonstrated that apoAI and not HDL3 is the lipid acceptor for the ABCA1 transporter, one may conclude that ZNF202 modulates additional genes with selective cholesterol transport activity independent of the ABCA1 efflux pathway (26).

ZNF202 Expression Profiling in Various Human Tissues—

The densitometrically determined spot intensities are expressed as relative mRNA abundance in comparison with expression in testis tissue (set to 100% or 5 dots, respectively). Densitometrically determined spot intensities are expressed as relative mRNA abundance in comparison with expression in testis tissue (set to 100% or 5 dots, respectively).

| Tissue                  | Expression | Tissue                  | Expression |
|------------------------|------------|------------------------|------------|
| Whole brain            | **         | Rectum                 | **         |
| Cerebral cortex        | **         | Kidney                 | **         |
| Frontal lobe           | **         | Skeletal muscle        | **         |
| Parietal lobe          | **         | Spleen                 | **         |
| Occipital lobe         | **         | Thymus                 | **         |
| Temporal lobe          | **         | Peripheral blood leukocyte | **      |
| Paracentral gyrus of cerebral cortex | ** | Lymp node | ** |
| Pons                   | **         | Bone marrow            | **         |
| Cerebellum, right      | ***        | Trachea                | **         |
| Corpus callosum        | **         | Lung                   | **         |
| Amygdala               | **         | Placentia              | **         |
| Caudate nucleus        | **         | Bladder                | **         |
| Hippocampus            | **         | Uterus                 | ****       |
| Medulla oblongata      | **         | Prostate               | **         |
| Putamen                | Not expressed | Testis            | **         |
| Substantia nigra       | **         | Ovary                  | Not expressed |
| Nucleus accumbens      | **         | Liver                  | **         |
| Thalamus               | **         | Pancreas               | **         |
| Pituitary gland        | **         | Adrenal gland          | **         |
| Spinal cord            | **         | Thyroid gland          | **         |
| Heart                  | **         | Saliwry gland          | **         |
| Aorta                  | ***        | Leukemia, HL-60        | **         |
| Atrium, left           | ***        | HeLa, S3               | Not expressed |
| Atrium, right          | ***        | Leukemia, K-562        | **         |
| Ventricle, left        | **         | Leukemia, MOLT-4       | **         |
| Ventricle, right       | **         | Burkitt’s lymphoma, Raji |   |
| Interventricular septum| ***        | Burkitt’s lymphoma, Daudi |   |
| Apex of the heart      | **         | Colorectal adenocarcinoma, SW480 | Not expressed |
| Esophagus              | **         | Lung carcinoma A549    | Not expressed |
| Stomach                | **         | Fetal brain            | **         |
| Duodenum               | **         | Fetal heart            | **         |
| Jejunum                | **         | Fetal kidney           | **         |
| Ileum                  | **         | Fetal liver            | **         |
| Ileocecum              | **         | Fetal thymus           | **         |
| Appendix               | **         | Fetal spleen           | **         |
| Colon ascending        | **         | Fetal lung             | **         |
| Colon transverse       | **         |                      | **         |
| Colon descending       | **         |                      | **         |

Monocytes during phagocytic differentiation and subsequent lipid loading using modified low density lipoprotein (6, 14), we tested under these conditions the expression of ZNF202 in human monocytes. Preliminary results indicate that ZNF202 expression is down-regulated during monocyte differentiation and repressed by cholesterol loading, thus demonstrating inverse regulation of ABCA1/ABCG1 and ZNF202 (C. Schumacher; data not shown).
ZNF202 Is a Transcriptional Repressor of ABCA1 and ABCG1

5. Schwartz, K., Lawn, R. M., and Wade, D. P. (2000) Biochem. Biophys. Res. Commun. 274, 794–802
6. Klucken, J., Buchler, C., Orso, E., Kaminski, W. E., Pursch-Özçürümmez, M., Liebsch, G., Kapinskiy, M., Diederich, W., Drobnik, W., Dean, M., Allikmets, R., and Schmitz, G. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 817–822
7. Kardassis, D., Lacetopir, M., Talianidis, I., and Zannis, V. (1996) Hypertension 27, 980–1008
8. Repa, J. J., and Mangelsdorf, D. J. (1999) Curr. Opin. Biotechnol. 10, 557–563
9. Wagner, S., Hess, M. A., Ormonde, H. P., Malandro, J., Hu, H., Chen, M., Kehrer, R., Frodsham, M., Schumacher, C., Beluch, M., Honer, C., Skolnick, M., Ballinger, D., and Bowen, B. R. (2000) J. Biol. Chem. 275, 15685–15690
10. Monaco, C., Helmer, C. M., Caprini, E., Vorechovsky, I., Russo, G., Croce, C. M., Barbanti, B. G., and Negrini, M. (1998) Genomics 52, 358–362
11. Schumacher, C., Wang, H., Honer, C., Ding, W., Koehn, J., Lawrence, Q., Couls, C. M., Wang, L. L., Ballinger, D., Bowen, B. R., and Wagner, S. (2000) J. Biol. Chem. 275, 17173–17179
12. Friedman, J. R., Fredericks, W. J., Jensen, D. E., Speicher, D. W., Huang, X. P., Neilson, E. G., and Rauscher, F. J. I. (1996) Genes Dev. 10, 2067–2078
13. Langmann, T., Porsch-Özçürümmez, M., Unkelbach, U., Klucken, J., and Schmitz, G. (2000) Biochim Biophys Acta 1494, 175–180
14. Langmann, T., Klucken, J., Reil, M., Liebsch, G., Luciani, M. F., Chimini, G., Kaminski, W. E., and Schmitz, G. (1999) Biochem. Biophys. Res. Commun. 257, 29–33
15. Orso, E., Broccardo, C., Kaminski, W. E., Bottcher, A., Liebsch, G., Drobnik, W., Gotz, A., Chambenoit, O., Diederich, W., Langmann, T., Spruss, T., Luciani, M. F., Rotte, G., Lackner, K. J., Chimini, G., and Schmitz, G. (2000) Nat. Genet. 24, 192–196
16. Bhakdi, S., Dorweiler, B., Kirchmann, R., Torzewski, J., Weise, E., Tranum, J. J., Walev, I., and Wieland, E. (1995) J. Exp. Med. 182, 1959–1971
17. Röger, G., Trumbach, B., Klums, B., Lackner, K. J., and Schmitz, G. (1995) Arterioscler. Thromb. Vasc. Biol. 15, 685–690
18. Pengue, G., Calabro, V., Bartoli, P. C., Pagliuca, A., and Lania, L. (1994) Nucleic Acids Res. 22, 2908–2914
19. Pengue, G., and Lania, L. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1015–1020
20. Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W., and Lipman, D. J. (2000) Nucleic Acids Res. 25, 3389–3402
21. Ryan, R. F., Schultz, D. C., Ayyanathan, K., Singh, P. B., Friedman, J. R., Fredericks, W. J., and Rauscher, F. J. I. (1999) Mol. Cell. Biol. 19, 4366–4378
22. Williams, S. C., Bruckheimer, S. M., Luiss, A. J., LeBoeuf, R. C., and Kinniburgh, A. J. (1986) Mol. Cell. Biol. 6, 3807–3814
23. Honer, C., Chen, P., Toth, M. J., and Schumacher, C. (2001) Biochim Biophys Acta 1517, 441–448
24. Costet, P., Luo, Y., Wang, N., and Tall, A. R. (2000) J. Biol. Chem. 275, 28240–28245
25. Venkateswaran, A., Repa, J. J., Lobacaro, J. M., Bronson, A., Mangelsdorf, D. J., and Edwards, P. A. (2000) J. Biol. Chem. 275, 14700–14707
26. Wang, N., Silver, D. L., Costet, P., and Tall, A. R. (2000) J. Biol. Chem. 275, 33053–33058.