Supplemental Method descriptions

Genotyping

DNA was extracted from blood samples of the low-HDL and FCHL family members and used for genotyping of SNPs using an in-house developed array technology [1]. To cover known common variation in the three *OSBPL* genes, tagSNPs were selected based on interrogation of HapMap data. Three SNPs for *OSBPL2*, 5 for *OSBPL9* (these two genes were each contained within a tight LD block) and 15 for *OSBPL10* were genotyped. Detailed information on the SNPs analyzed is displayed in Supplemental Table 1 and in Fig. 1. Haplotype structure of the genes was evaluated and Hardy-Weinberg equilibrium of all genotyped SNPs confirmed using Haploview 3.2 [2]. The Mendelian consistency of the genotype data was evaluated prior to any analyses using the Pseudomarker software [3]. Linkage and association with the low-HDL and high TG traits were evaluated using Pseudomarker under a dominant model. Age, BMI, and gender were used as covariates in both the dichotomized and the quantitative analyses.

The Genmets study sample (2,173 subjects) has been genotyped on Illumina 610K arrays having 598,203 SNPs successfully called with Illuminus software. Of these, 102 SNPs were found located in *OSBPL10* or its flanking regions (here defined as 31677-32 000 Kb of chromosome 3) having a success rate >95%, minor allele frequency >2% and Fisher's exact P-value for Hardy-Weinberg disequilibrium >1 x 10^{-4}. Individuals with low genotyping frequency (<95%) and of non-European ancestry (as checked with multidimensional scaling of the IBS-sharing) were excluded from the analysis, leading to 2,138 individuals being tested in the association analysis. For the association analysis individuals with the TG levels above the 95th population, sex, and age specific percentile (>3.4 mmol/l) were coded as cases and the rest as controls. The same coding was done for the individuals with the high density lipoprotein cholesterol (HDL) values below the 5th percentile (<0.8 mmol/l). For these two dichotomous phenotypes logistic regression was applied with age and sex as covariates.

Adipose tissue transcriptome analysis

Expression data of human adipose tissue was obtained from a study of 47 individual samples analyzed using the Affymetrix U133 Plus 2.0 arrays. The biopsies were collected from members of the same

low-HDL and FCHL families studied here, by a needle aspiration biopsy of periumbilical subcutaneous fat under local anesthesia. All samples were collected according to the Helsinki declaration and the ethics committees of the participating centers approved the study design.

Analysis of cholesterol and triglyceride biosynthesis

Huh7 cells on 6-well plates, treated with ORP10-specific or non-targeting control siRNAs as specified above, were washed with serum-free medium and pulse-labeled for 30 min at +37°C with [^{3}H]acetic acid (7.00 Ci/mmol, 50 μCi/well; GE Healthcare), followed by a 90-min chase with 25 mM mevalonate (Sigma-Aldrich). After washing with PBS, the cells were scraped into 900 μl of ice-cold 2% (w/v) NaCl. [^{14}C]cholesterol was added to the cell lysate to correct the results for material loss. After withdrawing an aliquot for protein analysis, cellular lipids were extracted with Methanol/$CHCl_3$ (1:1) as described previously [4]. The extracted lipids were separated by thin-layer chromatography (TLC) on silica-gel plates by using petroleum ether/diethyl ether/acetic acid (60:40:1) as the solvent. The plates were dried and stained with iodine vapor. The cholesterol bands identified based on co-migration with an unlabelled standard were scraped, and the [^{3}H] and [^{14}C] radioactivity was measured by liquid-scintillation counting. Labeling with [^{3}H]oleic acid was carried out on 6-well plates using 10 mCi/ml of the radioactive precursor (7.00 Ci/mmol, GE Healthcare), for 30 min, followed by chase in EMEM for 90 min, and lipid extraction. [^{3}H] radioactivity in triglycerides identified by co-migration with a triolein standard was determined as above. Protein amounts were measured using the DC Protein Assay (Bio-Rad, Hercules, CA). The results were normalized for protein to account for difference in cell number and for [^{14}C] radioactivity to account for material loss during lipid extraction.

mRNA quantification

Total RNA (2 μg) isolated from Huh7 cells treated with ORP10 or control siRNAs was treated with DNase I (Promega, Madison, WI) in the presence of RNase Inhibitor (Promega) and reverse-transcribed using Superscript II (Invitrogen) and random hexamer primers (Applied Biosystems, Foster City, CA). Each RNA sample was amplified in duplicate for the genes of interest and the housekeeping marker β-actin on a 7000 Sequence Detection System (Applied Biosystems) by using a SYBR-green kit (Applied Biosystems). Sequences of the primers used are listed in Supplemental Table 2. The

threshold was set in the linear range of fluorescence, and a threshold cycle (Ct) was measured for each well. The data was analyzed according to [5].

Assay for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity

Huh7 cells treated with ORP10-specific or control siRNAs as specified above were harvested, and HMG-CoA reductase activity in total membranes of the cells was assayed as previously described [6,7]. For each assay, 100 µg of membrane protein and 0.20 µCi of [3-^{14}C]HMG-CoA (57 mCi/mmol; GE Healthcare) were used. The reactions were carried out for 90 min at 37^{o}C, and the [^{14}C]mevalonic acid lactone generated was resolved by TLC and quantified by liquid scintillation counting.

Analysis of the subcellular localization of ORP10

ORP10 expressed from cDNA in different cell lines (Huh7, Caco-2, PANC-1) was detected by EGFP fluorescence or stained using the Xpress monoclonal antibody (Invitrogen), and double stainings were carried out with monoclonal anti-β-tubulin (TUB 2.1, Sigma-Aldrich, St. Louis, MO), anti-cytokeratin 8, 18, 19 (2A4), or anti-vimentin (24BA6)[8]. In some experiments, PANC-1 cells were treated for 1 h with demecolcine (10 µg/ml) or 2 h with 10 µg/ml vinblastine sulphate (Sigma-Aldrich). The specimens were viewed and images recorded with a Leica (Mannheim, Germany) TCS SP1 confocal microscope. In some experiments transfected Huh7 cells were before fluorescence microscopy incubated for 48 h in medium containing 5% lipoprotein deficient serum. For some specimens this was combined with a 24-h treatment with 50 µM lovastatin to potentiate the lipid depletion. For a comparison, human LDL (100 µg/ml protein) was included in the lipoprotein deficient medium for 48 h. Also the effect of a 4-h treatment with 5 µM 25-hydroxycholesterol was monitored.

Fluorescence recovery after photobleaching (FRAP)

Huh7 cells transfected for 20 h with EGFP-ORP10 were subjected to FRAP on an upright Leica TCS SP5 confocal microscope at 37 C with 5% CO_2 using a 63X NA0.9 dipping water objective. The laser used was a Coherent Sapphire 270 mW 488 nm solid state laser, for imaging the laser was used at 0.3% power and for bleaching at 100%. The image acquisition settings were selected so that bleaching rate

during image acquisition was negligible. One 1.4 sec scan was used to bleach a 6 x 6 µm area. Microtubules from this area were selected for the analysis. Exponential decay function with two half times was fitted to the data with Origin 7.5.

References

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