Supplemental online experimental procedure 1

Cloning of ABCA17 cDNA

A total of 7.2×10^5 plaques of a mouse genomic DNA library (Stratagene) were screened using a ^{32}P-nick-translated partial human ABCA3 cDNA fragment (corresponding to nucleotides +974 to +2428 relative to the putative translation start of the human ABCA3 cDNA) as a probe under high-stringency hybridization conditions, as described previously [24], and two positive clones encoding the *ABCA17* gene were isolated. Based on the putative exon–intron structure of the *ABCA17* gene, oligonucleotide primers were designed, and partial cDNA fragments corresponding to nucleotides +459 to +1410 and +1880 to +2227 relative to the putative translation start of the mouse ABCA17 cDNA were amplified by RT-PCR using total RNA extracted from mouse testis. To obtain the full-length mouse ABCA17 cDNA, a mouse testis cDNA library (Clontech) was screened using these partial ABCA17 cDNA fragments as probes. DNA sequencing was performed on an ABI 310 automated DNA sequencer (Applied Biosystems) using a Bigdye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) after subcloning of appropriate DNA fragments into pGEM vector (Promega). Both strands were sequenced. To obtain the 3′-end of mouse ABCA17 cDNA, RACE was carried out by the 3′-RACE system (Invitrogen) according to the manufacturer's instructions. Briefly, the first-strand DNA was synthesized from mouse testis total RNA using the antisense dT-primer 5′-GGCCACGCGTCGACTAGTAC$(T)_{17}$VN-3′ (V = A, C or G; N = A, C, G or T). The original mRNA template was removed by treatment with RNase H, and the 3′-end of the cDNA was first amplified by PCR using the second antisense primer (AP1) 5′-GGCCACGCGTCGACTAGTAC-3′ and the ABCA17-specific sense primer 5′-TCATGGTACAGGGCCAGTTC-3′ (corresponding to nucleotides +4712 to +4731). The 3′-end of the cDNA was then amplified from the PCR products using the AP1 primer and the second ABCA17-specific sense primer 5′-AAGAGCAAGTTTGGCATCGG-3′

(corresponding to nucleotides +4759 to +4778), and the amplified DNA was inserted into pGEM T-easy vector (Promega) followed by sequencing. To obtain the full-length cDNA for rat ABCA17, the following 7 pairs of sense and antisense primers were used for RT-PCR using total RNAs extracted from rat testis: F1: 5′-ATGGCCCCGTTCAAGAAGTTAAAAC-3′ and R1: 5′-AGGGTGTCCCCCATCGGCGTATG-3′ (corresponding to nucleotides +1 to +609); F2: 5′-TACCCTCCCAATCTAAGCCAAG-3′ and R2: 5′-CCATGTCTTTCGAGATCTCACAG-3′ (corresponding to nucleotides +550 to +1789); F3: 5′-TACTGTCTGCTCCGTTCTGACAGG-3′ and R3: 5′-CTGAGGCTTAACATAATGATCACCAG-3′ (corresponding to nucleotides +1704 to +2789); F4: 5′-TTCTCGGCGCAACTGGATGCTGGTG-3′ and R4: 5′-AAAGGGATGATAGCCCAGCCGTACAG-3′ (corresponding to nucleotides +2715 to +3548); F5: 5′-TCAGCATGGCTGCGTTCTGGCTCTCCG-3′ and R5: 5′-CCTGGTCAGCATGTTGAAAATG-3′ (corresponding to nucleotides +3371 to +4257); F6: 5′-TGTTGGCTGTGAACAAGGTGTCC-3′ and R6: 5′-TCTTGGCTTGTTCCATGATGCC-3′ (corresponding to nucleotides +4151 to +4975); and F7: 5′-GACGAGCACCAAAGCATGGTTCAG-3′ and AP1 primer 5′-GGCCACGCGTCGACTAGTAC-3′ (corresponding to nucleotides +4888 to +5322). RT-PCR products were subcloned into the pGEM-T easy vector and sequenced.

Supplemental online experimental procedure 2

Confocal microscopy

Cy3 and FITC fluorescence and DAPI were observed by confocal microscopy (Zeiss LSM510 META confocal microscope) using a silicon intensified target camera with a 560 nm long-pass filter (excitation wavelength 543 nm; He/Ne laser), with a 505–530 nm band-pass filter (excitation wavelength 490 nm; argon laser) and a 420–480 nm band-pass filter (excitation wavelength 405 nm; blue-diode laser) respectively. DsRed2 fluorescence

proteins were also observed by confocal microscopy using a silicon intensified target camera with a 560 nm long-pass filter (excitation wavelength 543 nm; He/Ne laser).

Supplemental online data

Supplemental Figure 1. Immunofluorescence labelling of ABCA1 and ABCG1 proteins in mouse testis. (**A--D**) Confocal microscopic image of mouse testis. Frozen mouse testis section immunolabelled with 1:1000-diluted anti-ABCA1 mouse monoclonal antibody (Kyowa Hakko Kogyo) was visualized with Cy3 (**A**; red). Nuclei were counterstained by DAPI (**B**; blue). DIC (differential interference contrast) image is shown in (**C**). Merged picture of (**A**), (**B**), and (**C**) is shown in (**D**). (**E--H**) Confocal microscopic image of mouse testis. Frozen mouse testis section immunolabelled with 1:1000-diluted anti-ABCG1 rabbit polyclonal antibody (Santa Cruz) was visualized with Cy3 (**E**; red). Nuclei were counterstained by DAPI (**F**; blue). DIC image is shown in (**G**). Merged picture of (**E**), (**F**) and (**G**) is shown in (**H**). Scale bar represents 50 μm (**D** and **H**).

Supplemental Figure 1