Functional Evidence for the Homodimerization of the Human Bile Acid Transporter ASBT (SLC10A2)
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Purpose
ASBT is the second member of the SLC10A family and it is primarily involved in the active reabsorption of bile acids from the distal ileum. Due to its pivotal role in bile acid and cholesterol homeostasis it has become a promising target for the treatment of hypercholesterolemia. Based on earlier studies we have developed a seven transmembrane domain model to understand ASBT topology. Previously ASBT has been reported to function as a monomer and dimer however; there is no direct biochemical evidence in support of ASBT homodimerization.

Methods
WT and cysteineless ASBT were transiently expressed in COS-1 cells. HA and Flag tags were inserted by inverted PCR mutagenesis at C-terminal of hASBT. DSP and DTSSP, two thiol-cleavable and bifunctional cross-linkers, were used for chemical cross-linking of hASBT. The physical interaction between two differentially tagged (HA and Flag) WT and cysteineless ASBT were examined by immunoprecipitation reaction. Functional dimerization of ASBT was analyzed by co-expressing WT and cysteineless ASBT in COS-1 cells followed by [3H]-TCA uptake and surface biotinylation. Saturation studies were performed to determine the effect of nonfunctional cysteineless ASBT on kinetic parameters ($K_m$ and $V_{max}$) of WT ASBT.

Results
ASBT was found to be in monomeric and dimeric forms after cross-linking with DSP and DTSSP. Immunoprecipitation experiments revealed direct interaction between differentially tagged WT and cysteineless ASBT. Furthermore, co-expression studies demonstrated dimerization of WT and cysteineless ASBT in the membrane and non-functional cysteineless ASBT showed a dominant negative effect on WT ASBT function which is further corroborated by kinetic analyses.

Conclusion
ASBT adapts a functional dimeric structure. These data suggest that disrupting the protein-protein interaction between ASBT monomers may provide a novel pharmacological target for the treatment of hypercholesterolemia.