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Biochim Biophys Acta. Author manuscript; available in PMC 2008 December 1.

Published in final edited form as:
Biochim Biophys Acta. 2007 December ; 1771(12): 1464–1468.

Normal binding of lipoprotein lipase, chylomicrons, and apo-AV to GPIHBP1 containing a G56R amino acid substitution

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Summary

GPIHBP1 is an endothelial cell protein that serves as a platform for lipoprotein lipase–mediated processing of triglyceride-rich lipoproteins within the capillaries of heart, adipose tissue, and skeletal muscle. The absence of GPIHBP1 causes severe chylomicronemia. A hallmark of GPIHBP1 is the ability to bind lipoprotein lipase, chylomicrons, and apolipoprotein (apo-) AV. A homozygous G56R mutation in GPIHBP1 was recently identified in two brothers with chylomicronemia, and the authors of that study suggested that the G56R substitution was responsible for the hyperlipidemia. In this study, we created a human GPIHBP1 expression vector, introduced the G56R mutation, and tested the ability of the mutant GPIHBP1 to reach the cell surface and bind lipoprotein lipase, chylomicrons, and apo-AV. Our studies revealed that the G56R substitution did not affect the ability of GPIHBP1 to reach the cell surface, nor did the amino acid substitution have any discernible effect on the binding of lipoprotein lipase, chylomicrons, or apo-AV.

Keywords

chylomicronemia; GPIHBP1; hypertriglyceridemia; apolipoprotein AV; lipoprotein lipase

We recently reported that glycosylphosphatidylinositol-anchored high density lipoprotein–binding protein 1 (GPIHBP1) plays a critical role in the lipolytic processing of triglycerides within chylomicrons [1]. The sole phenotype of chow-fed *Gpihbp1*-deficient (*Gpihbp1*$^{-/-}$) mice is severe chylomicronemia, with plasma triglyceride levels of 2000–5000 mg/dl [1,2]. The tissue pattern of *Gpihbp1* expression is similar to that of lipoprotein lipase (LPL), with high levels of expression in heart and adipose tissue, and moderate levels of expression in skeletal muscle. In each of these tissues, GPIHBP1 is found exclusively on the luminal face of endothelial cells. Expression of a mouse *Gpihbp1* cDNA confers upon CHO cells the ability

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to bind LPL and chylomicrons, as well as apo-AV-phospholipid disks [1]. Because GPIHBP1 binds LPL and chylomicrons avidly and because *Gpihbp1*$^{-/-}$ mice manifest severe chylomicronemia, we proposed that GPIHBP1 is a platform for triglyceride hydrolysis in capillaries [1,2].

Very recently, Wang and Hegele [3] screened 160 human subjects with chylomicronemia for mutations in GPIHBP1. They identified two brothers with severe chylomicronemia who were homozygous for a missense mutation in GPIHBP1 (G56R). Because three G56R heterozygotes in the family had milder hypertriglyceridemia, they suggested that the mutation was linked to the chylomicronemia phenotype. However, no experiments were performed to assess the functional relevance of the G56R substitution. The glycine at residue 56 is conserved in many mammalian species [2,3], but not all. For example, G56 is absent in hedgehog GPIHBP1.

Mature human GPIHBP1 contains a strongly acidic amino-terminal domain (residues 25–50) followed by a highly conserved Ly-6 motif (residues 65–136) that contains a putative N-linked glycosylation site (amino acid 78) [2,3]. We previously suggested that the acidic domain might be responsible for binding plasma proteins containing positively charged heparin-binding domains (e.g., lipoprotein lipase, apo-AV, apo-B) [1]. Residue 56 is located within a linker segment between the acidic domain and the Ly-6 motif. We hypothesized that the G56R mutation might interfere with the ability of GPIHBP1 to reach the cell surface or its ability to bind lipoprotein lipase, chylomicrons, or heparin-binding apolipoproteins such as apo-AV. Documenting that the G56R amino acid substitution interferes with GPIHBP1 function would represent an important finding, as it would support the conclusion that this mutation could cause chylomicronemia.

To address this hypothesis, we obtained a human GPIHBP1 cDNA (IMAGE ID 5754421, American Type Culture Collection, Manassas, VA) and cloned it into pTriEx-4 Neo (Novagen, Madison, WI), a mammalian expression vector containing a CMV promoter. An amino-terminal S-protein tag was introduced with PCR-based cloning techniques. The single-nucleotide substitution responsible for the G56R substitution was introduced by site-directed mutagenesis with the Quikchange kit (Stratagene, La Jolla, CA) and oligonucleotide primers 5′-CCAACAGGCTCCCTCGTGGCAGGAGC-3′ and 5′-GCTCCTGCCACGAGGGAGCCTGTTGG-3′.

We first tested whether GPIHBP1 harboring the G56R substitution reached the cell surface. Both wild-type GPIHBP1 and mutant (GPIHBP1-G56R) expression vectors were transiently transfected into a mutant CHO cell line (pgsA-745) that lacks the ability to synthesize HSPGs [4]. Confocal immunofluorescence microscopy revealed no difference in the localization of wild-type and mutant GPIHBP1; both were located at the cell surface and both yielded signals of similar intensity (Fig. 1).

To determine if LPL binds to GPIHBP1-G56R, we took advantage of a western blot LPL-binding assay described earlier [1]. The wild-type and mutant GPIHBP1 expression vectors were transfected into pgsA-745 CHO cells, alone or in combination with an expression vector encoding a V5-tagged human LPL [1]. Normally, the human LPL that is secreted into the cell culture medium is bound by GPIHBP1, but this LPL can be readily released into the medium by treating the cells with a phosphatidylinositol-specific phospholipase C (PIPLC). Western blots showed that wild-type and mutant GPIHBP1 bind roughly equal amounts of LPL; in both cases, the LPL was released into the medium with PIPLC treatment, indicating the wild-type and the mutant GPIHBP1 molecules are located on the cell surface, tethered by a glycosylphosphatidylinositol anchor (Fig. 2A). In an independent experiment, we tested the ability of heparin to release LPL from the surface of cells. Again, both wild-type GPIHBP1

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and GPIHBP1-G56R bound LPL that was secreted into the medium, and similar amounts of LPL were released into the medium with heparin (Fig. 2B).

We next assessed the ability of GPIHBP1-G56R to bind lipoprotein and apolipoprotein ligands. None of these experiments revealed any differences in the properties of GPIHBP1 and GPIHBP1-G56R. In transient transfection experiments, DiI-labeled chylomicrons (d < 1.006 g/ml lipoproteins from *Gpihbp1*$^{-/-}$ mice) bound exclusively to the subset of cells that had been transfected with GPIHBP1 or GPIHBP1-G56R (Fig. 3A); there was no binding to nontransfected cells. Cells that had been stably transfected with GPIHBP1-G56R also bound chylomicrons avidly (Fig. 3B). Also, dimyristoylphosphatidylcholine (DMPC) disks containing apo-AV bound to both GPIHBP1 and GPIHBP1-G56R, as judged by epifluorescence (Fig. 3C) and confocal microscopy (Fig. 3D).

These studies show that the G56R mutation in GPIHBP1 has no apparent impact on the ability of GPIHBP1 to reach the cell surface or its ability to bind LPL, triglyceride-rich lipoproteins, or apo-AV. These studies cast doubt on the proposition that the GPIHBP1-G56R mutant is dysfunctional, and raise significant doubt regarding whether the chylomicronemia in the family identified by Wang and Hegele [3] is due to that mutation. Nevertheless, the paper by Wang and Hegele [3] is important, as it clearly showed that *GPIHBP1* mutations are going to be extremely rare in patients with unexplained chylomicronemia. Also, their study alerted human geneticists to the existence of the G56R mutation; it will be interesting to determine if this mutation will be identified in normolipidemic humans or in other families with hyperlipidemia.

Our conclusion that the G56R mutation not alter the function of GPIHBP1 is subject to a few caveats. This study addressed all of the known properties of GPIHBP1—the ability to reach the cell surface and the ability to bind LPL, triglyceride-rich lipoproteins, and apo-AV-disks. However, it is possible that there are other, as-yet-undiscovered properties of GPIHBP1 that are relevant to the lipolytic processing of triglyceride-rich lipoproteins, and that those properties are significantly perturbed by the G56R mutation. Also, it is conceivable that incredibly minor changes in GPIHBP1 function could cause severe chylomicronemia *in vivo*, but that these minor changes are beyond the limits of detection in our *in vitro*experiments in CHO cells.

If future studies uncover the G56R mutation in other kindreds with chylomicronemia, and if this mutation is strongly linked to the hyperlipidemia phenotype, it will be important to create a gene-targeted mouse model with the G56R mutation. The analysis of such a mouse model would provide the most definitive insights regarding the effect of the G56R substitution on GPIHBP1 function.

Acknowledgments

We thank Ms. Jennifer Beckstead for preparing the apo-AV-phospholipid disks. Supported by NIH grants R01 HL087228-01 and U01 HL66621, a postdoctoral fellowship award from the NIH (5F32HD054159), and a Scientist Development Grant from the American Heart Association.

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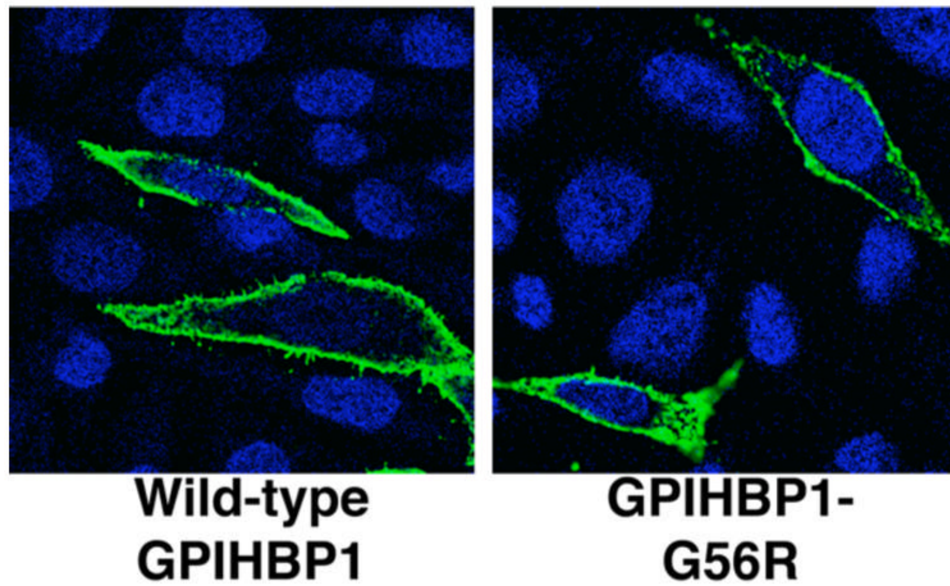

Fig. 1.

Transient transfection studies illustrating that both wild-type GPIHBP1 and GPIHBP1-G56R are located at the surface of pgsA-745 CHO cells [4]. GPIHBP1 was detected with a FITC-labeled antibody against the S-protein tag (green), and images were recorded with a Leica TCS-SP MP confocal inverted microscope (Heidelberg, Germany). Cell nuclei are stained with DAPI (blue). The pgsA-745 CHO cells were a generous gift from Dr. Jeffrey Esko.

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Fig. 2.

Wild-type GPIHBP1 and GPIHBP1-G56R both have the capacity to bind human lipoprotein lipase (LPL), and this LPL can be released by a phosphatidylinositol-specific phospholipase C (PIPLC) or by heparin. (A) Western blot analysis of cell extracts and cell culture media from pgsA-745 CHO cells transfected with a wild-type human GPIHBP1 expression vector or a GPIHBP1-G56R expression vector, alone or in combination with a human *LPL* expression vector (with a C-terminal V5 tag), before and after treatment of the cells with PIPLC (10 U/ml of PIPLC at 37°C for 1 h). LPL binds both GPIHBP1 and GPIHBP1-G56R; in both cases, the LPL is readily released from the surface of cells by PIPLC. (B) Western blot analysis of cell extracts and cell culture media from pgsA-745 CHO cells transfected with vector alone, a wild-type human GPIHBP1 expression vector, or a GPIHBP1-G56R expression vector, alone or in combination with a human *LPL*expression vector (with a C-terminal V5 tag), before and after treatment of the cells with heparin (1 U/ml for 1 h at 37°C). GPIHBP1 expressed on the

surface of cells binds the secreted LPL, so little appears in the medium; however, the GPIHBP1-bound LPL is readily released by heparin.

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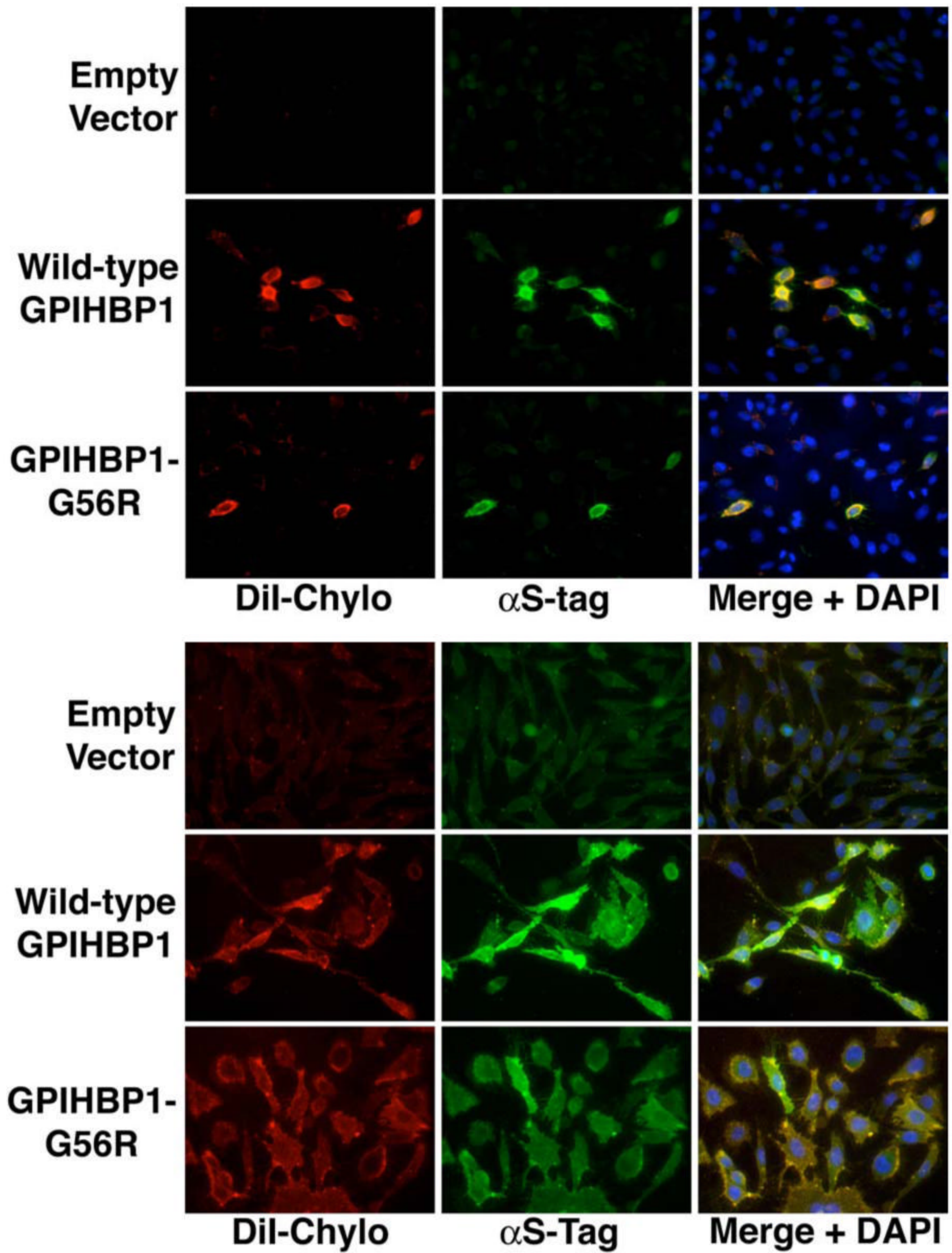

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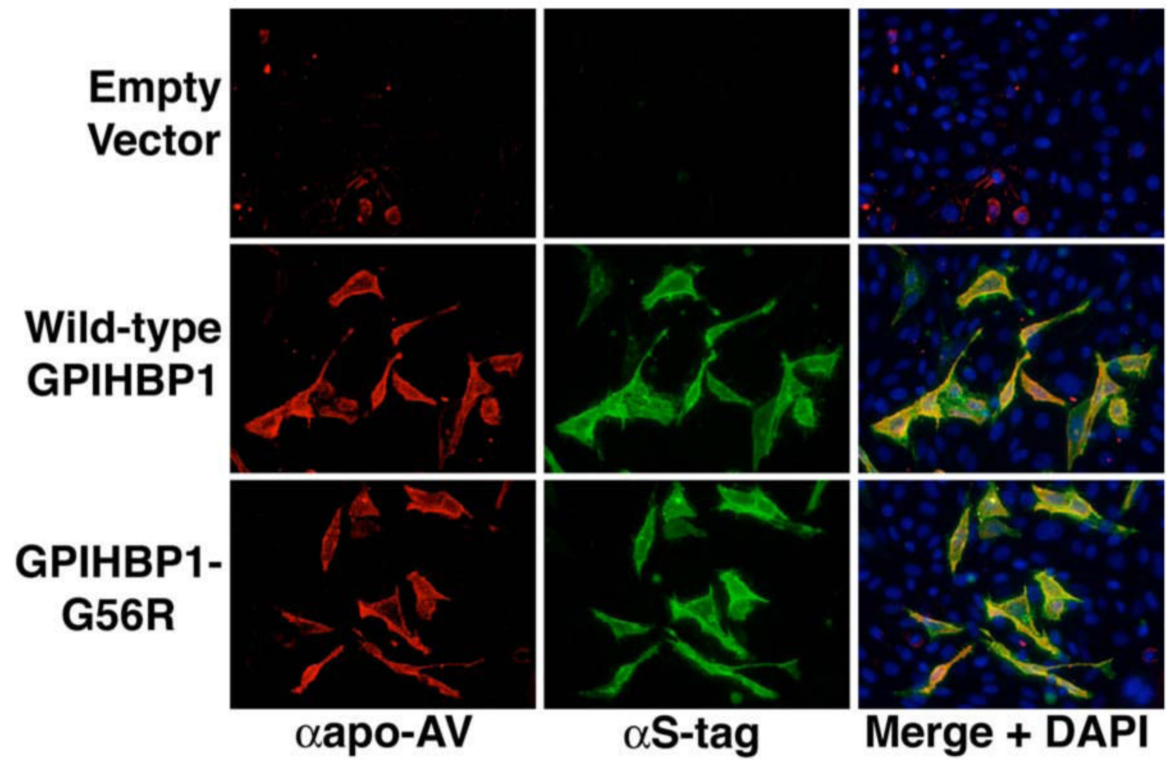

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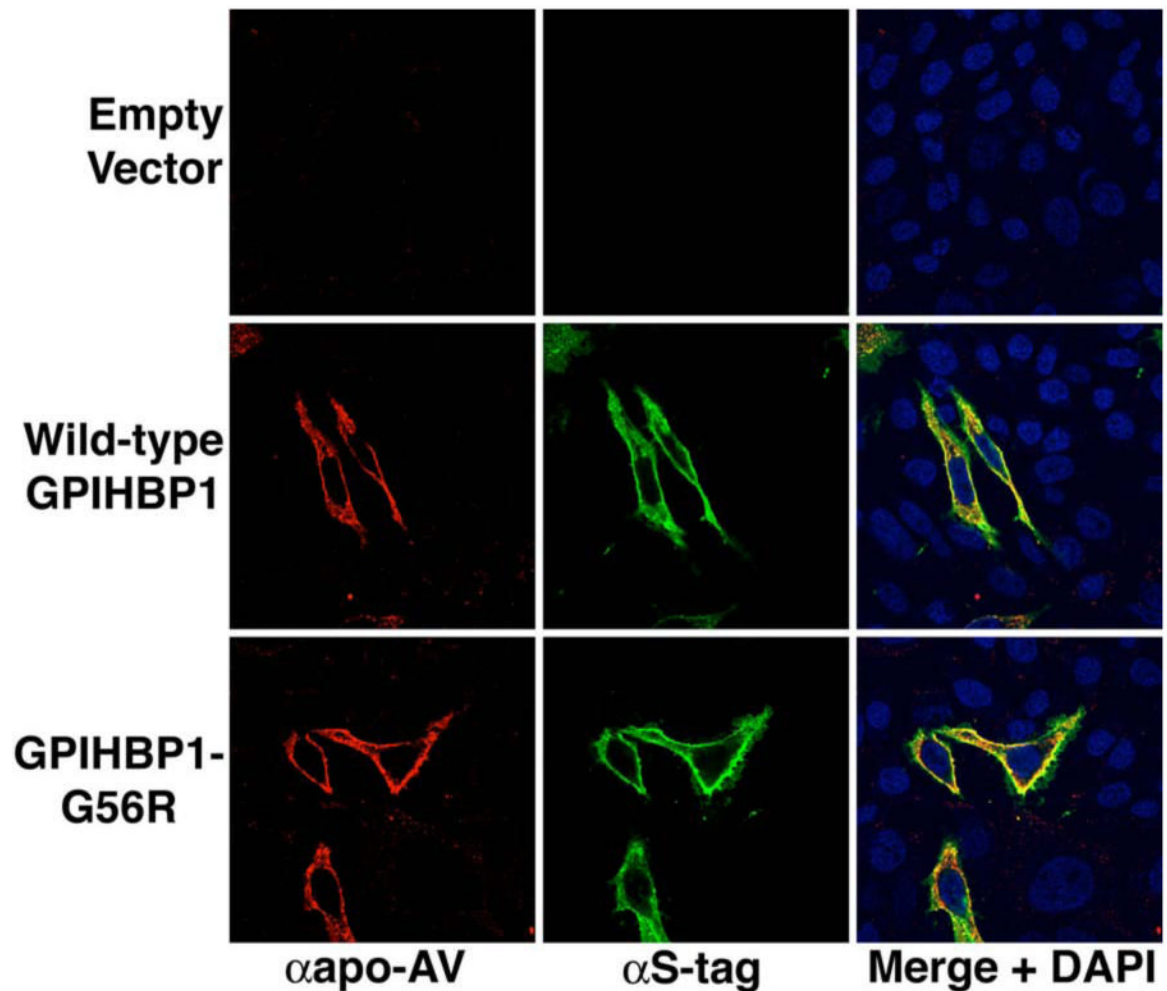

Figure 3.

Immunofluorescence microscopy experiments showing that chylomicrons and human apo-AV-phospholipid disks bind to both wild-type GPIHBP1 and GPIHBP1-G56R. (A,B) Binding of DiI-labeled chylomicrons (red) to ldlA7-CHO cells (a generous gift from Dr. Monty Krieger) [5] that had been transiently (A) or stably (B) transfected with wild-type GPIHBP1 or GPIHBP1-G56R expression vectors. GPIHBP1 was detected with a FITC-labeled antibody against the S-protein tag (green). Cell nuclei are stained with DAPI (blue). (C) Binding of human apo-AV-DMPC disks to pgsA-745 CHO cells that had been transiently transfected with either wild-type GPIHBP1 or GPIHBP1-G56R expression vectors. Apo-AV was detected with an apo-AV–specific mouse monoclonal antibody (Zymed, Carlsbad, CA). (D) Confocal microscopy images of the same experiments shown in panel D, revealing that both GPIHBP1 and apo-AV are located on the surface of transfected cells.