Brief Review

Genetics, Clinical Phenotype, and Molecular Cell Biology of Autosomal Recessive Hypercholesterolemia

Anne K. Soutar, Rossitza P. Naoumova, Linton M. Traub

Abstract—The recent characterization of a rare genetic defect causing autosomal recessive hypercholesterolemia (ARH) has provided new insights into the underlying mechanism of clathrin-mediated internalization of the LDL receptor. Mutations in ARH on chromosome 1p35-36.1 prevent normal internalization of the LDL receptor by cultured lymphocytes and monocyte-derived macrophages but not by skin fibroblasts. In affected cells, LDL receptor protein accumulates at the cell surface; this also occurs in the livers of recombinant mice lacking ARH, thereby providing an explanation for the failure of clearance of LDL from the plasma in subjects lacking ARH. The 50 known affected individuals are mostly of Sardinian or Middle Eastern origin. The clinical phenotype of ARH is similar to that of classic homozygous familial hypercholesterolemia caused by defects in the LDL receptor gene, but it is more variable, generally less severe, and more responsive to lipid-lowering therapy. Structural features of the ARH protein and its capacity to interact simultaneously with the internalization sequence of the LDL receptor, plasma membrane phospholipids, and the clathrin endocytic machinery suggest how ARH can play a pivotal role in gathering the LDL receptor into forming endocytic carrier vesicles. (Arterioscler Thromb Vasc Biol. 2003;23:1963-1970.)

Key Words: lipids ■ adaptor ■ clathrin ■ clinical phenotype ■ endocytosis ■ LDL receptor

Familial hypercholesterolemia (FH) is characterized by increased levels of plasma LDL cholesterol, which cause cholesterol deposition in tissues in the form of tendon xanthomas and atheroma, leading in turn to premature atherosclerosis and coronary heart disease. In most cases, FH is an autosomal dominant disorder in which mutations in the LDL receptor gene result in defective clearance of plasma LDL by the liver. Heterozygous FH is one of the most common inborn errors of metabolism, with a frequency of 1/500 in most populations. However, there is a strong gene dosage effect, and the much rarer homozygous FH patients exhibit a severe and highly characteristic clinical phenotype. A similar but rather milder phenotype is seen in individuals with a mutation in the gene for apolipoprotein B (apoB), the ligand for the LDL receptor. However, it has become clear that defects in other genes can also result in hypercholesterolemia that is typical of FH.

Despite the remarkable progress made in recent years in methods for rapid and unambiguous detection of mutations, several groups have reported being unable to find a mutation in the genes for the LDL receptor or apoB in patients with a typical heterozygous FH. Indeed, in some cases, the families were informative enough to allow exclusion of inheritance of an allele of either of these genes as the underlying cause of the phenotype. However, it is sometimes difficult to be certain that the hypercholesterolemia in all patients with the clinical signs of heterozygous FH is caused by an inherited defect. Thus, mapping studies to find novel genes that result in autosomal dominant hypercholesterolemia are not easy, but some progress has been made. A locus for dominant FH has been mapped to a region on chromosome 1, and another has been mapped to a region on chromosome 16. A candidate gene on chromosome 1 encoding a protease named NARC-1 has recently been proposed, but as yet, there is no firm experimental evidence indicating that the mutations observed are the underlying cause of hypercholesterolemia. A hypercholesterolemic phenotype similar to that in homozygous FH has also been reported in a family with homozygous mutations in the gene for 7α-hydroxylase, the first enzyme in the pathway of bile acid synthesis in the liver, but further families with this disorder remain to be identified.

On the other hand, over the years, a number of rare cases of what appeared to be an autosomal recessive, rather than dominant, form of homozygous FH have been described. In the families of these probands, all of whom had a clinical phenotype typically seen in homozygous FH, most of the parents and unaffected siblings had plasma LDL cholesterol levels within the normal range. Initially, defective LDL receptor function was excluded because cultured skin fibroblasts from the patients were able to take up and degrade 125I-labeled LDL normally. Mutations in the LDL receptor or apoB genes were subsequently excluded, either by se-
Figure 1. Genetic variation in ARH. The ARH gene lies on chromosome 1p35-36.1 and comprises 9 exons, indicated by numbered shaded boxes on the diagram; asterisks mark the position of the ATG (start) and TGA (stop) codons. The positions of known mutations are indicated below the gene (described in detail in the Table), and known polymorphisms that change the amino acid sequence are indicated above the diagram. All known mutations are predicted to introduce a premature termination codon or result in failure to produce mRNA. The relative sizes of exons are drawn to scale, as are the introns, but on a different scale. Shown below is a diagram of ARH mRNA.

Identification of the Genetic Defect

We mapped the defect in our two families to a region of ~10 cM on chromosome 1p36-35.13 but identification of candidate genes was hampered because the genome sequence of this region was incomplete. Analysis of mRNA content of normal and mutant cells on commercially available microarrays failed to reveal any differences in the expression of genes that might suggest candidates (authors’ unpublished data, 2002). In the meantime, the region was shown to harbor a novel gene (ARH, Figure 1), mutations in which were found to cosegregate in a recessive pattern with severe hypercholesterolemia in several families of Sardinian origin.16 The gene encoded a protein (currently named ARH) that contains a phosphotyrosine-binding (PTB) domain similar to that found in many adaptor proteins that are known bind NPXY motifs in the cytoplasmic domain of signaling receptors.17 This immediately suggested that it might play a role in LDL receptor internalization, even though there was no direct evidence at this stage.

Our original patients, as well as two affected brothers in a third kindred of English origin, were also found to be homozygous or compound heterozygous for novel mutations in ARH that are all predicted to result in the synthesis of severely truncated forms of the protein. This showed that the defect in LDL receptor internalization in EBV-lymphocytes in our patients was likely to be caused by defects in ARH, which we confirmed by demonstrating that retroviral expression of normal ARH cDNA in the EBV-lymphocytes can restore LDL receptor internalization (Figure 2).18

Mutations in ARH have now been found in all reported patients with autosomal recessive hypercholesterolemia, a disorder now referred to as ARH,16,18–22 and to date, some 50 patients have been described worldwide. The majority of these are of Italian and especially of Sardinian origin,11,23–26 but families of Lebanese,9,16,18 American,16 Iranian,16 Japanese,10,22 Asian Indian,14 English,18 Turkish,12 and Syrian19 origin have also been described. Not surprisingly, for a recessive disorder, most of the patients are homozygous for the same allele inherited from consanguineous or related parents, but the parents in the English family were unrelated, and the affected offspring were heterozygous for two different mutant alleles.18 Because ARH is a very rare disorder, it is not clear why it should be so relatively common in Sardinians and Southern Italians (Table). As indicated in the Table, there were presumably two different founder mutations in this population, but a recombination event between these two alleles resulted in a third allele that carries both mutations.25 So far, all the described mutations in ARH are predicted to introduce premature stop codons, either as a result of a point mutation or a frameshift (Figure 1 and Table). In view of this, it is surprising that ARH mRNA levels
in some mutant cells are almost normal\textsuperscript{16,18,20}; nonetheless, no ARH protein can be detected by immunoblotting cell extracts with anti-ARH antibodies in any patient’s cells that have been tested\textsuperscript{20} (authors’ unpublished data, 2002). A number of common single amino acid polymorphisms in ARH have been identified,\textsuperscript{18–20} but as expected from the recessive nature of the disorder, these do not appear to influence plasma cholesterol levels in the general population.\textsuperscript{19}

**Characterization of the Cellular Phenotype**

As was found with other patients with defects in ARH, skin fibroblasts from our patients in whom LDL receptor function in EBV-lymphocytes was defective show normal LDL uptake. Like lymphocytes, blood monocyte-derived macrophages from the same patients also exhibit defective internalization of the LDL receptor, suggesting that ARH is required for normal LDL receptor function in these cells and demonstrating that the phenotype in EBV-lymphocytes is not the result of EBV immortalization (Figure 2). Interestingly, we found that monocyte-derived macrophages from one of the two affected English brothers showed a total defect in LDL uptake and degradation, whereas a small proportion of cells in the culture from the other brother retained some high-affinity uptake.

At present, as discussed further below, there is no explanation for the difference in phenotype between cultured skin fibroblasts and EBV-lymphocytes or monocyte-derived macrophages or that within monocyte-derived macrophage cultures from one of the individuals. ARH is clearly required for normal LDL receptor–mediated endocytosis in hepatocytes, inasmuch as these cells are the major site of LDL receptor–mediated clearance of LDL in vivo. Failure of the liver to take up and degrade plasma LDL provides the only plausible explanation for the severe accumulation of LDL in the circulation observed in ARH patients. Recent studies have shown that recombinant mice, in which ARH has been obliterated, develop hypercholesterolemia when they are fed a high fat diet and that they also show abnormal accumulation of LDL receptor protein on the plasma membrane of liver cells.\textsuperscript{27}

**Clinical Phenotype of Patients**

The largest series of ARH patients described so far includes 28 Sardinians from 17 unrelated families and 4 Italians.\textsuperscript{25} Our 4 ARH families, each of which includes two affected siblings of the same sex,\textsuperscript{18} have been followed up between 9 and 23 years (authors’ unpublished data, 2002). The age at which individuals from different kindred have been diagnosed varies from \( \frac{1}{2} \) year to 46 years. The total serum cholesterol at assessment also ranges widely, from 9.6 to 27.1 mmol/L, with LDL cholesterol from 8.6 to 22.9 mmol/L. Serum triglyceride concentration was usually normal. At least one third of the probands had serum HDL cholesterol levels \(<1\text{ mmol/L.} \) The 3 affected siblings from a recently described

### Known Mutations in ARH in Patients With Autosomal Recessive Hypercholesterolemia

<table>
<thead>
<tr>
<th>Ethnic Origin</th>
<th>No. of Families</th>
<th>Exon</th>
<th>Mutation(s)*</th>
<th>Predicted Protein†</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pakistani</td>
<td>1</td>
<td>1</td>
<td>del GG86</td>
<td>Fs, G24 + 31X</td>
<td>18</td>
</tr>
<tr>
<td>Iranian</td>
<td>1</td>
<td>1</td>
<td>ins G65</td>
<td>Fs, G24 + 7X</td>
<td>16</td>
</tr>
<tr>
<td>American</td>
<td>1</td>
<td>1</td>
<td>del G65</td>
<td>Fs, G23 + 31X</td>
<td>16</td>
</tr>
<tr>
<td>Sardinian/Italian</td>
<td>&gt;9§</td>
<td>1</td>
<td>G86A</td>
<td>W22X</td>
<td>16,25</td>
</tr>
<tr>
<td>Sardinian/Sicilian</td>
<td>5</td>
<td>4</td>
<td>ins 432A</td>
<td>Fs, C143 + 27X</td>
<td>16,25,26</td>
</tr>
<tr>
<td>Sardinian</td>
<td>Allele 1</td>
<td>5</td>
<td>G86A</td>
<td>W22X</td>
<td>25</td>
</tr>
<tr>
<td>Sardinian</td>
<td>Allele 2</td>
<td>4</td>
<td>ins 432A</td>
<td>Fs, C143 + 27X</td>
<td>25</td>
</tr>
<tr>
<td>Sardinian</td>
<td>Allele 1</td>
<td>1</td>
<td>G86A + ins 432A</td>
<td>W22X</td>
<td>25</td>
</tr>
<tr>
<td>Lebanese</td>
<td>1</td>
<td>IVS1</td>
<td>ins 2.6kb</td>
<td>Null†</td>
<td>20</td>
</tr>
<tr>
<td>Syrian Druze</td>
<td>1</td>
<td>2 (IVS1)</td>
<td>G, C</td>
<td>Not known</td>
<td>19</td>
</tr>
<tr>
<td>Turkish/Lebanese</td>
<td>3</td>
<td>4</td>
<td>C481†</td>
<td>G136X†</td>
<td>16,18,21</td>
</tr>
<tr>
<td>English</td>
<td>Allele 1</td>
<td>1</td>
<td>ins C599</td>
<td>L203 + 16X†</td>
<td>18</td>
</tr>
<tr>
<td>English</td>
<td>Allele 2</td>
<td>~1–7</td>
<td>del exons ~1–7</td>
<td>Null†</td>
<td>18</td>
</tr>
<tr>
<td>Japanese</td>
<td>1</td>
<td>6</td>
<td>ins C599</td>
<td>L203 + 16X</td>
<td>22</td>
</tr>
<tr>
<td>Italian</td>
<td>1</td>
<td>IVS7</td>
<td>G, 77A</td>
<td>W249 + 63X</td>
<td>20</td>
</tr>
</tbody>
</table>

* Nucleotide numbering based on ATG = 1.
† No ARH protein is detectable in cells\textsuperscript{20} (and unpublished data).
‡ G24 + 31X indicates 31 additional amino acids are predicted to be encoded after G24 before a stop codon occurs.
§ Two brothers of Sardinian origin resident in the UK are homozygous for this mutation (unpublished data).
Syrian family were characterized by very low serum levels of HDL cholesterol, ranging from 0.59 to 0.69 mmol/L. Although the serum cholesterol levels of patients with ARH have been described as intermediate between FH heterozygotes and FH homozygotes, clearly, there is large phenotypic variability in ARH. Some patients have 3-fold higher serum cholesterol levels than others, and their levels are not very different from those of classic FH homozygotes. ARH appears to be a fully recessive disorder, and in the majority of the cases, the parents of affected individuals have normal serum cholesterol concentrations. However, at least 25% to 30% of the parents had cholesterol levels >6.2 mmol/L, and in some families described by us, the parents of probands had xanthelasmas.18

Another interesting feature of patients with ARH is the large, bulky xanthomas that are present from early childhood. These are usually tuberous, tendinous, or planar and are in most circumstances accompanied by corneal arcus and xanthelasmas. In 2 of our 4 families, the probands were referred to a lipid clinic by orthopedic or cosmetic surgeons because of bulky and troublesome xanthomas. Only 3 patients described so far lack xanthomas: an English patient, brother of the proband who was diagnosed at the age of 8 years and started on treatment with cholestyramine18; a patient of Sardinian origin, diagnosed before the age of 1 year, in whom treatment with bile acid sequestrants was begun at age 2 years; and the affected sister of a proband from Sardinia, who had xanthelasmas at the age of 22 years but no evident tendinous xanthomata. In some cases, joint pain has been noted, as in patients with classic FH, and the pain seems to subside when serum cholesterol is lowered substantially (authors’ unpublished data, 2002). Interestingly, in one patient, the joint pain subsided, never to appear again after serum total cholesterol was reduced from 27.1 mmol/L to 7 to 9 mmol/L on treatment. The pathophysiology of formation of the large xanthomas in ARH patients is not clear. It has been suggested that maintenance of LDL receptor activity in the macrophages of patients with ARH could promote increased cellular uptake and accumulation of cholesterol, resulting in accelerated xanthoma formation. However, we found that their monocyte-derived macrophages are similar to transformed lymphocytes, being unable to internalize and degrade LDL, and the question of what promotes the formation of these bulky xanthomas in ARH remains unanswered.

Like patients with homozygous FH, individuals with ARH are prone to premature atherosclerosis, and nearly half of the ARH patients reported in the literature have evidence for coronary artery disease. However, it seems that aortic valve stenosis is more rarely seen in ARH compared with FH homozygotes, and it appears later in life. Of the 28 Sardinians and 4 Italians, only 2 were reported to have aortic stenosis at the ages of 47 and 33 years. Four of our 8 individuals with ARH have documented aortic stenosis, and 3 had aortic valve replacement in their third to fourth decade. It is interesting that the characteristics of the aortic lesions in one of our ARH patients were very similar to those typically seen in homozygous FH and were characterized by supravalvar deposition of atheroma, together with fibrosis and inflammatory changes. Aortic root disease is the most common cardiac manifestation of homozygous FH, and by puberty, all patients have some degree of atheroma of the ascending aorta. This process seems to be delayed in patients with ARH. In addition to coronary artery disease and aortic stenosis, it seems that the carotid arteries are also frequently diseased. Three of our 8 ARH patients had severe carotid atherosclerotic disease, and 1 of them required carotid endarterectomy in his fourth decade (authors’ unpublished data, 2002). It is not yet clear whether the fatty liver observed in 2 ARH siblings of Japanese origin is a typical feature of the disease or is merely a coincidence in this family.

### Response to Lipid-Lowering Therapy

Patients with ARH seem to respond to lipid-lowering medication with significant reduction in serum cholesterol. This observation is in contrast to FH homozygotes previously described, in whom high doses of long half-life 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) reduce LDL cholesterol by ~30%. However, it has been suggested that maintenance of LDL receptor activity in the macrophages of patients with ARH could promote increased cellular uptake and accumulation of cholesterol, resulting in accelerated xanthoma formation. However, we found that their monocyte-derived macrophages are similar to transformed lymphocytes, being unable to internalize and degrade LDL, and the question of what promotes the formation of these bulky xanthomas in ARH remains unanswered.

Like patients with homozygous FH, individuals with ARH are prone to premature atherosclerosis, and nearly half of the ARH patients reported in the literature have evidence for coronary artery disease. However, it seems that aortic valve stenosis is more rarely seen in ARH compared with FH homozygotes, and it appears later in life. Of the 28 Sardinians and 4 Italians, only 2 were reported to have aortic stenosis at the ages of 47 and 33 years. Four of our 8 individuals with ARH have documented aortic stenosis, and 3 had aortic valve replacement in their third to fourth decade. It is interesting that the characteristics of the aortic lesions in one of our ARH patients were very similar to those typically seen in homozygous FH and were characterized by supravalvar deposition of atheroma, together with fibrosis and inflammatory changes. Aortic root disease is the most common cardiac manifestation of homozygous FH, and by puberty, all patients have some degree of atheroma of the ascending aorta. This process seems to be delayed in patients with ARH. In addition to coronary artery disease and aortic stenosis, it seems that the carotid arteries are also frequently diseased. Three of our 8 ARH patients had severe carotid atherosclerotic disease, and 1 of them required carotid endarterectomy in his fourth decade (authors’ unpublished data, 2002). It is not yet clear whether the fatty liver observed in 2 ARH siblings of Japanese origin is a typical feature of the disease or is merely a coincidence in this family.
lovastatin and 24 g cholestyramine was observed in a Turkish patient in whom total serum cholesterol decreased by >70% (from 27.1 mmol/L to 7 to 8 mmol/L). In contrast, in one Sicilian patient, high doses of statins were reported to be ineffective.

Overall, ARH patients respond to statins, bile acid sequestrants, and the combination of the two with sometimes remarkable reduction in serum cholesterol. In addition, in 1 ARH patient, partial ileal bypass led to 30% decrease in serum LDL cholesterol. In contrast, partial ileal bypass has proven disappointingly ineffective in controlling the hypercholesterolemia in patients with homozygous FH, and in some cases, serum cholesterol levels actually rose. Those patients with ARH who do not reach optimal cholesterol concentrations on lipid-lowering drugs have been treated in addition with apheresis at weekly or biweekly intervals with very good results.

The pathophysiological basis of the observation that ARH patients are more responsive to treatment with lipid-lowering drugs and partial ileal bypass than are classic FH patients is unclear. One can speculate that preserved LDL receptor activity in fibroblasts and possibly other cells, tissues, and organs may play a role. Increased level of expression of the LDL receptor and receptor-mediated catabolism during treatment with statins and/or bile acid sequestrants and partial ileal bypass, coupled with a reduced rate of endogenous cholesterol synthesis by high doses of statins as shown in patients with familial hypercholesterolemia, may be involved in providing a good response to treatment.

Physical Interaction of ARH With the LDL Receptor

Before the characterization of ARH, it was known that LDL receptors promote endocytosis of LDL by clustering within clathrin-coated regions on the plasma membrane. Characterized by a polygonal lattice composed principally of assembled clathrin trimers and the adaptor protein-2 (AP-2) complex, these coated regions invaginate to form an intracellular transport intermediate, the clathrin-coated vesicle. Sorting of receptor proteins into clathrin-coated vesicles is selective, and the minimal sequence necessary for efficient uptake of the LDL receptor is 802FDNPVY, located within its cytoplasmic domain. The importance of the FXNPXY internalization motif first emerged when a point mutation in the tyrosine residue (Y807C) was identified in an FH patient whose cells in culture exhibited impaired internalization of the LDL receptor. The transferrin receptor is efficiently sorted into clathrin-coated vesicles by binding directly to AP-2, but its internalization sequence (YXX0, where 0 represents a bulky hydrophobic residue) differs from that of the LDL receptor, and there is good evidence that these two receptors use separate sorting mechanisms to enter clathrin coats.

Thus, the identification of ARH immediately suggested that it was a likely candidate for mediating specific interaction of the LDL receptor with the endocytosis machinery.

The most striking feature of the 308–amino acid ARH protein is an amino-terminal PTB domain (Figure 4). The presence of a PTB module immediately suggests that ARH interfaces directly with the LDL receptor because the usual binding partner of this domain is an FXNPXY consensus sequence, although, commonly, the terminal tyrosine is a phosphotyrosine. Secondary structure algorithms predict 7 & strands flanked by amino- and carboxy-terminal &-helices for residues 60 to 180 of ARH. These correspond to the core structural elements of the canonical PTB domain: two orthogonally oriented antiparallel & sheets capped at each end by an extended &-helix. The PTB domain region of ARH displays significant identity to the PTB domains of numb (31%), Disabled-1 (Dab1, 29%), Disabled-2 (Dab2, 22%), and GULP (31%), each of which has been implicated in protein traffic or signaling from the plasma membrane.

Of particular interest is Dab1, which plays a central role in cortical lamination of the developing brain by binding to the FXNPXY motif of the VLDL receptor or apolipoprotein E receptor-2 via the PTB domain. The Dab2 PTB domain is 65% identical to Dab1. Dab2 colocalizes with the LDL receptor at the cell surface and binds physically to the FDNPVY internalization sequence of the LDL receptor, and the isolated PTB domain can selectively prevent LDL uptake when it is overexpressed in COS-7 cells. Together with ARH, these molecules clearly form a distinct subfamily of PTB domain proteins because they have an overall related architecture (Figure 4) and a substantially higher selectivity for the nonphosphorylated FXNPXY sequence and because they also bind directly to the acidic phospholipid phosphatidylinositol 4,5-bisphosphate [PtdIns(4,5)P2].

The structural basis for these binding preferences is revealed by the recent crystal structures of the Dab1 and Dab2 PTB domains. The FXNPXY motif adopts a type I &–& tight-turn conformation on binding PTB domains and the tyrosine is positioned abutting 2 loops joining & strands 4-5 and 6-7. In Dab1 and Dab2 (and ARH), these loops are short (4 to 6 residues), physically occluding placement of a bulky charged phosphotyrosine. Additional binding en-

![Image](http://atvb.ahajournals.org/)

**Figure 4.** PTB domain containing endocytic proteins. A schematic illustration of the domain organization of human ARH, Dab2, Dab1, and numb is shown. The location of AP-2 &-subunit appendage–binding sequences is indicated in blue; clathrin-binding sequences, in red; and the AP-2 &-appendage-binding sequence in ARH, in green. Secondary structure predictions suggest that for all 4 proteins depicted, polypeptide outside of the PTB domain is likely to be largely unstructured.
subset of PTB domains allows ARH, Dab1, and Dab2 to bind to the LDL receptor internalization sequence. The (F165V/A) prevents binding to the LDL receptor internalization sequence.

Structure predictions suggest that the carboxy-terminal domain of the protein complex, but the different proteins are not drawn to scale.

On the opposite side of the β sandwich, Dab1 has 6 basic side chains clustered in 3D space to coordinate the interaction between ARH and Dab2, and numb PTB domain of ARH. Indeed, mutation of this phenylalanine in ARH (F165V/A) prevents binding to the LDL receptor internalization sequence.

On the opposite side of the β sandwich, Dab1 has 6 basic side chains clustered in 3D space to coordinate the interaction between ARH and Dab2, and numb PTB domain of ARH. Indeed, mutation of this phenylalanine in ARH (F165V/A) prevents binding to the LDL receptor internalization sequence.

The related activities of this subset of intermediate endocytic adaptors could explain the clear but curious fact that fibroblasts derived from ARH patients internalize LDL apparently normally. Dab2 is highly expressed in fibroblasts and colocalizes with clathrin and AP-2, both of which bind to the LDL receptor. This suggests the capacity of ARH to bind clathrin suggests that this intermediate adaptor protein could couple FXNPXY-bearing cargo selection and PtdIns(4,5)P2 binding to clathrin-mediated endocytosis at the cell surface. Indeed, at steady state, some ARH is found in plasma membrane clathrin-containing structures in both HeLa cells and fibroblasts, although in fibroblasts, the protein is found only in a subpopulation of surface clathrin-coated structures.

The obvious accumulation of the LDL receptor on the sinusoidal surface of hepatocytes in the liver of ARH-null mice also supports a role for ARH in clathrin-mediated endocytosis of the LDL receptor.

Localization of ARH to clathrin-coated structures at the cell surface may not be due solely to clathrin engagement. In addition to clathrin, bacterially expressed ARH also pulls down the AP-2 adapt complex. Strikingly, like ARH, AP-2 can bind to select cargo molecules, clathrin, and PtdIns(4,5)P2 synchronously (Figure 5). The association with clathrin is also via a type I clathrin binding sequence, LLDLE, located within the β2 subunit of the adaptor. Nonetheless, AP-2 is not recovered together with ARH because of its association with ARH-bound clathrin and because disruption of the ARH LLDLE clathrin box does not affect AP-2 binding. Instead, an authentic AP-2 binding sequence is located between residue 250 and 280.

The clathrin and AP-2 binding determinants are both located within tracts of phylogenetically conserved residues and represent 13 of 36 invariant residues between the zebra fish and human ARH carboxy-terminal segments. The AP-2 binding region of ARH interacts physically with the β2 appendage, a folded globular domain that projects off the heterotetrameric AP-2 adaptor core via a flexible proline-rich hinge of the β2 subunit (Figure 5). ARH binds to a site on the platform subdomain of the β2 appendage, a surface also engaged by several other endocytic factors, including the β-arrestins.

In fact, the functional similarity of ARH to β-arrestin is striking. The β-arrestins oversee the internalization of activated heptahelical G-protein–coupled receptors by meshing phosphorylated receptors with the clathrin machinery. This activity is dependent on the capacity of the β-arrestins to bind PtdIns(4,5)P2, AP-2, and clathrin via a type I clathrin box. Given the similar functional attributes of ARH and β-arrestin (Figure 5), it seems probable that these proteins are both members of an emerging group of intermediate adaptors that expand the sorting repertoire of the endocytic clathrin coats while contributing to lattice assembly. Numb plays a similar role in sorting of the notch receptor and driving the intracellular distribution of AP-2.
Dab2 physically. The activity of Dab2 (or a related molecule) in these cells could be sufficient to facilitate rapid internalization of LDL receptors. It has been proposed that the FXNPXY internalization sequence binds to the μ2 subunit of the AP-2 adaptor, at a site separate from the YXXφ sequence-binding surface. However, this interaction is extremely weak, and it is known that LDL and transferrin receptors do not compete with each other for incorporation into coated pits and that each saturates the endocytic machinery at different receptor densities. This argues for a dedicated FXNPXY sorting component(s). Additional evidence for Dab2 playing an important role in LDL receptor family sorting is the proteinuria seen in Dab2−/− mice, suggesting that Dab2 is important for megalin endocytosis in the kidney. However, although the available evidence points toward ARH and Dab2 being functionally redundant, it still remains to be shown experimentally that the capacity of ARH to restore LDL uptake requires the endocytic interaction sequences.

Conclusions
Identification of this rare genetic defect in ARH has provided fascinating new insights into the functioning of the LDL receptor pathway, reminding us that there is still much to learn about the other proteins involved in the intracellular trafficking of this familiar and well-studied protein. Whether the role of ARH extends beyond sorting of the LDL receptor into clathrin vesicles and its subsequent internalization remains unresolved, but clearly, it or other proteins must be required for escorting the receptor on its complex recycling route through the cell to the basolateral surface of hepatocytes.

As yet, ARH has been demonstrated to be involved only in the endocytosis of the LDL receptor, and patients with defects in ARH do not appear to have any disorders unrelated to LDL receptor dysfunction, but its apparently widespread expression suggests that it may have other roles. A recent report suggests that ARH may bind to the Aβ peptide formed after cleavage of the amyloid β precursor protein (AβPP), but the physiological significance of the proposed interaction remains obscure. As far as inherited hypercholesterolemia is concerned, ARH seems likely to remain a rare recessive cause of the disorder, although the possibility that some mutations may result in a dominant phenotype should not be discarded out of hand, nor should the possibility that gene-gene or gene-environment interactions with some variants could influence LDL receptor function and, thus, plasma LDL levels in some cases.

References
22. Harada-Shiba M, Takagi A, Miyamoto Y, Tsushima M, Ikeda Y, Yokoyama S, Yamamoto A. Clinical features and genetic analysis of


Genetics, Clinical Phenotype, and Molecular Cell Biology of Autosomal Recessive Hypercholesterolemia
Anne K. Soutar, Rossitza P. Naoumova and Linton M. Traub

doi: 10.1161/01.ATV.0000094410.66558.9A
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/23/11/1963

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Arteriosclerosis, Thrombosis, and Vascular Biology can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/