Identification and functional analysis of a naturally occurring E89K mutation in the ABCA1 gene of the WHAM chicken

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Abstract The Wisconsin hypoalpha mutant (WHAM) chicken has a >90% reduction in plasma HDL due to hypercatabolism by the kidney of lipid-poor apoA-I. The WHAM chickens have a recessive white skin phenotype caused by a single-gene mutation that maps to the chicken Z-chromosome. This corresponds to human 9q31.1, a chromosomal segment that contains the ATP-binding cassette protein-1 (ABCA1) gene, which is mutated in Tangier Disease and familial hypoalphalipoproteinemia. Complete sequencing of the WHAM ABCA1 cDNA identified a missense mutation near the N-terminus of the protein (E89K). The substitution of this evolutionary conserved glutamate residue for lysine in the mouse ABCA1 transporter leads to complete loss of function, resulting principally from defective intracellular trafficking and very little ABCA1 reaching the plasma membrane. The WHAM chicken is a naturally occurring animal model for Tangier Disease.—Attie, A. D., Y. Hamon, A. R. Brooks-Wilson, M. P. Gray-Keller, M. L. E. MacDonald, V. Rigot, A. Tebon, L.-H. Zhang, J. D. Mulligan, R. R. Singaraja, J. J. Bitgood, M. E. Cook, J. J. P. Kastelein, G. Chimini, and M. R. Hayden. **Identification and functional analysis of a naturally occurring E89K mutation in the ABCA1 gene of the WHAM chicken. *J. Lipid Res.* 2002.** 43: **1610–1617.**

Supplementary key words HDL • hypoalphalipoproteinemia • Tangier Disease

The Wisconsin hypoalpha mutant (WHAM) chicken was discovered in 1981 in a flock of chickens maintained at the University of Wisconsin-Madison since 1948 (1). In contrast to normal chickens, the WHAM chickens have white skin and white beaks due to a deficiency of carotenoids, such as xanthophyll. The white skin phenotype is inherited as a recessive sex-linked mutation (originally designated "*y*" for "recessive yellow") on the Z-chromosome (1).

A decade later, Poernama et al. discovered that the WHAM chickens have a severe deficiency of HDL (2). Unlike conditions leading to defective VLDL production, however, this syndrome involves normal synthesis and secretion of apolipoproteins. Most notably, the rate of synthesis of apoA-I, the principal protein of HDL, is normal (3). Moreover, the apoA-I gene locus is excluded as a candidate gene for this mutant phenotype because the WHAM mutation is sex-linked in the chicken (1), whereas apoA-I is autosomal in chickens (3) as it is in mammals (4, 5). Since HDL production is drastically reduced while apoA-I synthesis is normal, this animal model exposed a post-secretory step that is rate-limiting for HDL production (3).

Metabolic studies in WHAM chickens provided the key to understanding their defect in HDL metabolism. When ^{125}I-labeled HDL particles were injected into WHAM chickens, their disappearance from the circulation was only moderately increased relative to normal chickens. However, when lipid-free ^{125}I-apoA-I was injected, it was

Manuscript received 6 June 2002 and in revised form 1 July 2002.
DOI 10.1194/jlr.M200223-JLR200

Abbreviations: ABCA1, ATP-binding cassette protein-1; FHA, familial hypoalphalipoproteinemia; PC, phosphatidylcholine; SM, sphingomyelin; WHAM, Wisconsin hypoalpha mutant.

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removed by the kidneys from the circulation 4-fold more rapidly in WHAM than in normal chickens (3). Because apoA-I synthesis and secretion are normal in WHAM chickens, we reasoned that another factor affecting the stability of apoA-I was limiting. Further analysis of serum lipids revealed a 70% reduction in phospholipids, implying that the primary defect is in phospholipid efflux (3). The dissociation between the metabolism of ^{125}I-labeled HDL compared with the rapid catabolism of ^{125}I-apoA-I, together with the defect in efflux, suggested that the primary defect in the WHAM chicken relates to lipidation of the lipid-depleted apoA-I particle.

Tangier Disease and familial hypoalphalipoproteinemia (FHA) are HDL deficiency disorders that are also characterized by hypercatabolism of apoA-I (6). Studies in fibroblasts from Tangier Disease and FHA patients reveal defects in phospholipid and cholesterol efflux (7–9). Consequently, Tangier Disease manifests as a cholesterol ester storage disorder (10, 11). Mutations in the ATP-binding cassette protein-1 (ABCA1) gene are responsible for both Tangier Disease and FHA (12–15), implying that this protein functions as a phospholipid and/or cholesterol transporter.

The phenotypes of WHAM chickens and of Tangier Disease patients share key common features. First, in both instances apoA-I was ruled out as a candidate gene. Second, apoA-I synthesis is normal. Finally, the in vitro studies in Tangier fibroblasts suggested a defect in lipid efflux (12–16), analogous to the in vivo findings in the WHAM chicken (3). Mapping of the Tangier Disease mutation to human chromosome 9 and its synteny with the region of the chicken Z-chromosome harboring the WHAM mutation provided genetic evidence that individuals with Tangier Disease and WHAM chickens may have mutations in the same gene. We have cloned and sequenced the chicken ABCA1 gene and show that a single missense mutation (E89K) in the amino terminus of ABCA1 results in altered trafficking of ABCA1 with its retention in the endoplasmic reticulum and loss of function at the plasma membrane. The WHAM chicken thus represents a naturally occurring animal model for Tangier disease.

MATERIAL AND METHODS

Measurement of carotenoids in plasma

One milliliter aliquots of plasma from normal and WHAM chickens were used to determine the absorption spectra utilizing a Cary 50 Bio UV-Visible spectrophotometer. Water was used as the blank. For the xanthophyll absorption spectrum, 0.2 mg of xanthophyll (Sigma No. X-6250) was dissolved in 1 ml chloroform for the spectrum shown. Chloroform was used as the blank.

Phospholipid analysis

Plasma lipoproteins were fractionated on a Superose 6HR 10/30 FPLC column (Pharmacia). The equivalent of 100 μl of plasma was injected onto the column. Five-hundred microliter fractions were collected and used for total cholesterol measurements (Sigma kit #352-50). Values represent total cholesterol mass per fraction. The identities of the lipoproteins have been confirmed by utilizing anti-apoB immunoreactivity for LDL and anti-apoA-I immunoreactivity for HDL (not shown). Triglyceride profiles were used to identify VLDL. Lipids were extracted from a 200 μl aliquot of whole plasma. Lipids were extracted (17), dried down under nitrogen, resuspended in 35 μl of $CHCl_3$ and spotted onto an activated TLC plate (Silica Gel 60, Aldrich No. Z29297-4), developed in the first dimension consisting of $CHCl_3$-MeOH-28% NH_3 (65:25:5, v/v/v) allowed to dry overnight and then developed in the second dimension consisting of $CHCl_3$-acetone-MeOH-glacial acetate-H_2O (6:8:2:2:1, v/v/v/v/v). To visualize lipids, plates were sprayed with 5% sulfuric acid, 5% glacial acetate and 0.5 mg/ml $FeCl_3$, followed by baking at 100°C for 30 min.

DNA sequencing, RT-PCR amplification, and sequence analysis

Total RNA was isolated from control and WHAM chicken liver and reverse transcribed with oligo-(dT)18 primer using SuperScript II reverse transcriptase (Life Technologies). cDNA was amplified using *Taq* DNA polymerase and primers derived from the published human and mouse ABCA1 cDNA sequences (15, 18), and primers derived from initial chicken sequence. Fifteen sets of primer pairs were used to amplify WHAM and control chicken cDNA samples, generating 15 overlapping DNA fragments covering 6,773 bp. To determine the 5′ untranslated portion of the mRNA, we performed 5′ RACE using the Marathon cDNA amplification kit (Clontech). DNA sequencing was performed directly on PCR products using an ABI 373 automated DNA sequencer (Applied Biosystems).

Detection of the WHAM mutation

The G265A mutation was detected by comparison of the cDNA sequence of normal and WHAM male chickens. Genotyping of the variant in normal and WHAM White Leghorn and normal Rhode Island Red chicken genomic DNA was performed by PCR amplification with primers in exon 4 (Forward: 5′-GTCACTTCCCAAACAAAGCTA-3′, Reverse: 5′-ATGGACGCATTGAAGTTTCC-3′). PCR product (15 μl) was incubated with *Hinf*I (10 units) in total volume (25 μl) for 1 h at 37°C, and products separated on 2% agarose gels. The presence of the G265A mutation destroys a *Hinf*I site in the PCR product.

Sequence alignment

Clustal W 1.8 with modifications, accessed through the Baylor College of Medicine search launcher (http://dot.imgen.bcm.tmc.edu:9331/multi-align/Options/clustalw.html), was used for multiple sequence alignments, with Boxshade for graphical enhancement (http://www.ch.embnet.org/software/BOX_form.html).

Generation and analysis of ABCA1 harbouring the WHAM mutation

The E to K mutation at position 89, corresponding to an A to G shift of nucleotide 348 in GB X75926, was introduced on the mouse ABCA1 backbone by fusion PCR with the following oligonucleotides: a-TATAAGCAGAGAGCTCGTTTA corresponding to the sequence at bp 94–118 in pBI vector, d- GATGCTTGATCTGCCGTA-bp 478–495 of GB X75926); b- and c-TCCCGGC**A**AGGCTCCC and GGGAGCCT**T**GCCGGGA, complementary oligonucleotides spanning the mutated nucleotide. The amplified fragment was reinserted into the ABCA1/(enhanced green fluorescent protein) backbone in pBI vector (19) by restriction digestion with *Not*I/*Bsr*GI. Introduction of the point mutation was confirmed by sequencing with the Dynamic ET terminator Cycle sequencing kit (Amersham Pharmacia Biotech, Uppsala Sweden).

HeLa cells were transiently transfected for 16 h with EXGEN

500 (Euromedex, Mundolsheim, France) accordingly to manufacturer's instruction and immediately seeded for immunofluorescence, biochemical and functional analysis.

Transfection efficiency, assessed by flow cytometric evaluation of (green flourescent protein) RFI (relative fluorescence intensity) in the whole cell population, was consistently higher than 30%. Intracellular trafficking was monitored by both immunofluorescence analysis and surface biotinilation at 60 h after transfection.

Immunofluorescence was carried out by standard protocols on slides seeded with 3–5 $\times$ 10^4 cells and analyzed in X-Y dimensions by a Leica TS100 confocal microscope.

Surface biotinylation was carried out on 3–5 $\times$ 10^6 cells with 1 mg/ml NHS-LC-biotin (Pierce) in ice cold PBS for 30 min, followed by lysis in RIPA buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1 mM EDTA and 1% Triton X-100) for 30 min at 4°C. Similar amounts of ABCA1, normalised with respect to both protein concentration in the samples and transfection efficiency, were immunoprecipitated with an anti-GFP antibody (clone7.1/13.1, Boehringer Indianapolis, IN), accordingly to standard protocols. The immunoprecipitated samples were fractionated by SDS-PAGE and blotted for 20 h onto nitrocellulose paper (Schleicher & Schuell, Dassel, Germany). The biotinylated protein was then revealed by ECL (Amersham Pharmacia Biotech, Uppsala, Sweden) after hybridization to streptavidin HRP (Amersham Pharmacia Biotech, Uppsala Sweden).

For functional analysis, fluorescence based assays for surface binding of cyanilated apoA-I or annexin V were carried out as described (20) at 60 h after transfection on 0.5–1 $\times$ 10^6 cells. Results, averaged from a minimum of four individual experiments, are expressed as percent of the binding elicited by wild-type ABCA1/EGFP chimera transfected in parallel.

RESULTS

Carotenoids in serum

Unlike normal chickens, the WHAM chickens have colorless rather than yellow fasting serum (**Fig. 1A**). A major contributor to the yellow color of chicken serum is dietary carotenoids, many of which are derived from corn. The different spectrum of WHAM versus normal serum closely matches that of the common corn-derived carotenoid, xanthophyll, indicating that the lack of color is due to the absence of carotenoids in the serum (Fig. 1B).

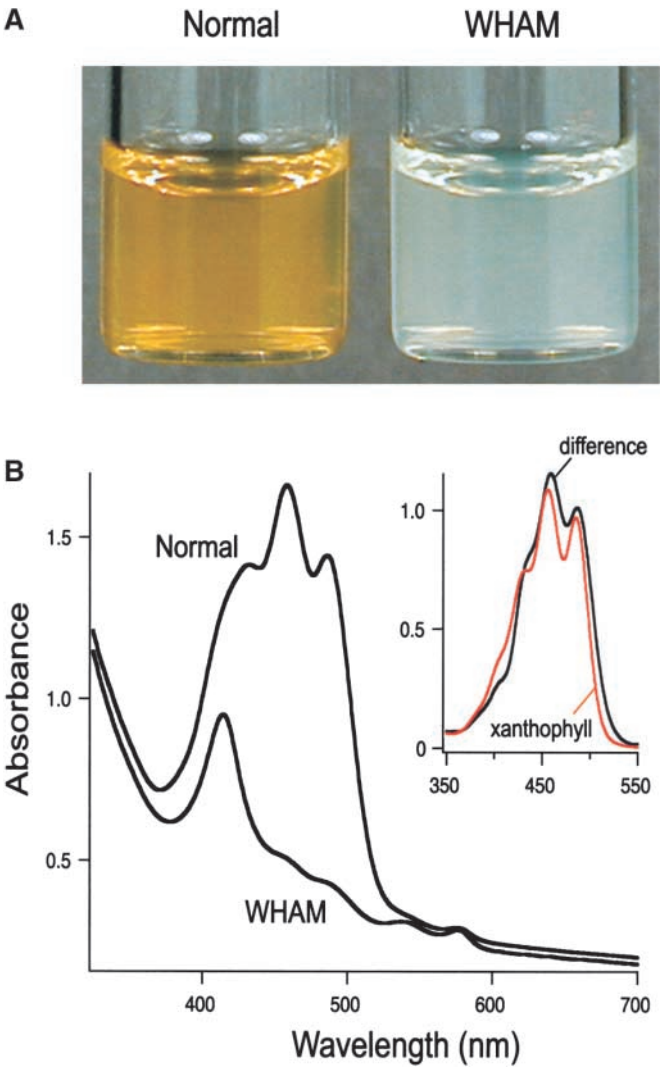

Fig. 1. A: Blood plasma from a Wisconsin hypoalpha mutant (WHAM) chicken has greatly reduced levels of carotene. Photograph of whole plasma revealing the absence of yellow coloration in WHAM chickens. B: Absorbance spectra of whole plasma from normal and WHAM chickens. The difference spectrum (black in the inset graph) was determined by subtracting the WHAM spectrum from the normal spectrum in B. This is compared with the absorbance spectrum for 5 μg/ml of xanthophyll, a naturally occurring carotenoid alcohol, in $CHCl_3$. The superposition of the difference and the xanthophyll spectra indicate that WHAM chickens lack carotenoids in their plasma.

Plasma lipoprotein and phospholipid phenotypes

The lipoprotein profiles of WHAM and Tangier plasma both show a similarly pronounced loss of HDL (**Fig. 2A**). In addition, the low HDL phenotype in the WHAM chicken is accompanied by a 40–50% reduction in LDL cholesterol, similar to that seen in Tangier patients. Prior work on the WHAM chicken suggested that phospholipids are limiting for HDL production (3). Plasma from WHAM chickens shows a substantial decrease in plasma phospholipid levels (Fig. 2B) identical to that seen in Tangier Disease. Two-dimensional TLC shows that in both the Tangier plasma and in the WHAM plasma, the most pronounced phospholipid deficiency is in phosphatidylcholine (PC) and sphingomyelin (SM) (Fig. 2B).

Identification of the WHAM mutation

The mutation in the WHAM chicken maps approximately 55 cM and 40 cM proximal to the chicken B and ID loci, respectively (21, 22) (**Fig. 3A**). This region contains the ALDOB (aldolase B) and MUSK genes, which map to chromosome Zq1.5–1.6 in chicken and is syntenic to 9q22.3–q32 in human (23, 24). The WHAM locus maps near these genes (21, 22) and is close to the map location of the ABCA1 gene on human chromosome 9 (12–15). Despite 300 million years of vertebrate evolution between chicken and human, the organization of the human genome is closer to that of the chicken than the mouse, a more closely-related species (25).

In view of the metabolic similarities between the WHAM chicken and patients with Tangier Disease and the

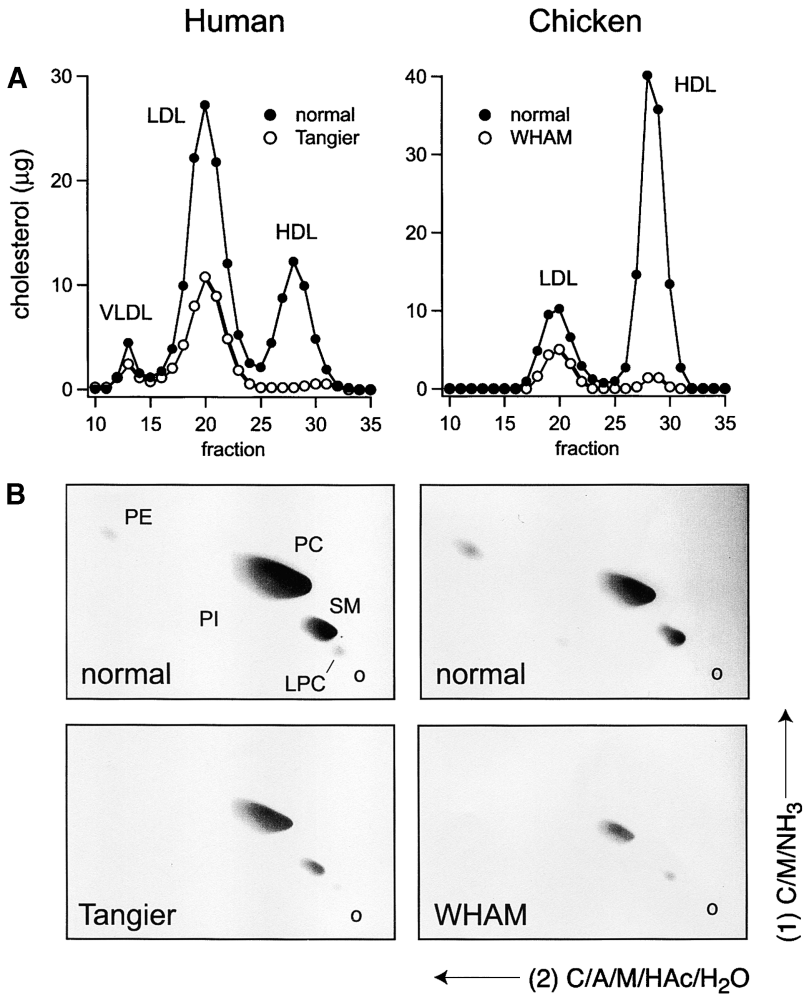

Fig. 2. The lipoprotein and phospholipid phenotypes of the WHAM chicken closely resembles that of a human patient with Tangier Disease. A: Plasma lipoprotein cholesterol profiles for human (left) and chicken (right) contrasting normal versus mutant profiles. The chickens have undetectable levels of cholesterol in VLDL. B: Two-dimensional phospholipid TLC analysis of equal aliquots of plasma from human (left) and chicken (right) comparing normal versus mutant. PE, phosphatidylethanolamine; PC, phosphatidylcholine; PI, phosphatidylinositol; SM, sphingomyelin; LPC, lysophosphatidylcholine; O, origin.

syntenic localization of the WHAM mutation and the human ABCA1 gene, we hypothesized that the chicken ABCA1 gene may be responsible for the WHAM phenotype. To investigate this hypothesis, we sequenced the coding region of ABCA1 from both WHAM and normal chickens. The human and chicken ABCA1 sequences are 78% identical at the nucleotide level and 85% identical (and show 92% homology) at the amino acid level. The chicken gene is less similar to the human gene than is the mouse gene, which has 88% nucleotide identity and 95% amino acid identity (97% homology) to the human gene (Genbank Submission #AF362377).

The sequences of the normal and mutant chickens ABCA1 CDNAs were identical with the exception of a G to A transition in WHAM DNA at nucleotide 265, corresponding to a glutamic acid to lysine substitution at amino acid 89 (E89K) (Fig. 3B). The G265A mutation eliminates a *Hinf*I restriction site present in the normal chicken sequence, and facilitated development of a PCR-based *Hinf*I RFLP assay to confirm the mutation in chicken genomic DNA (Fig. 3C). This alteration is a non-conservative amino acid substitution at a residue that is conserved in the ABCA1 gene between human, mouse, chicken, and Takifugu *rubripes* ("fugu" (Fig. 3D). The mutation segregates with the phenotype of HDL deficiency in the WHAM chickens and is not seen in wild-type White Leghorn chickens or in another strain of chicken that was investigated, New Hampshire (not shown).

Functional analysis of the E89K mutation in the ABCA1 gene

In order to establish the impact of the WHAM mutation on the function of the ABCA1 transporter, we engineered a construct harbouring the E89K mutation on a murine ABCA1/EGFP chimeric backbone. The effects of this mutation on the intracellular trafficking and function of the

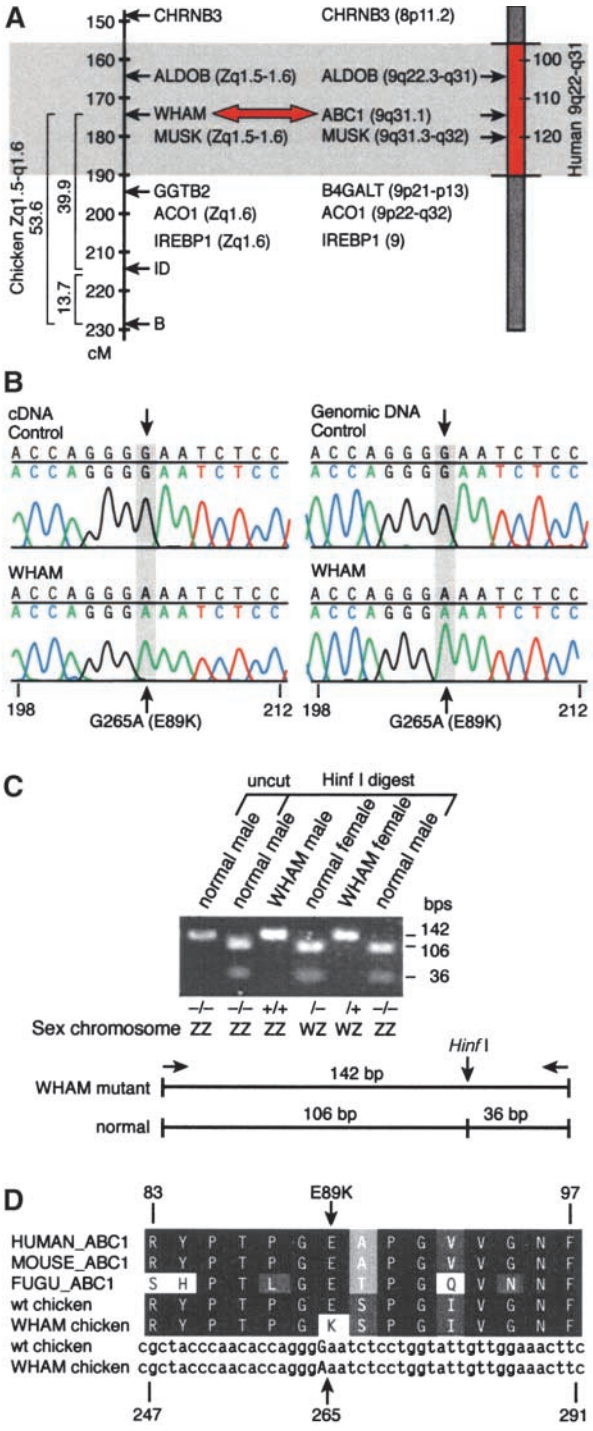

Fig. 3. A: The WHAM mutation maps to a Z chromosome region syntenic to the 9q31.1 location of human ATP-binding cassette protein-1 (ABCA1). To the left is the chicken Z chromosome combined genetic and cytogenetic map. To the right is a combined human genetic and cytogenetic map. Positions of markers mapped genetically or physically are indicated by dashed arrows. Genes mapped only cytogenetically are positioned relative to other markers with the cytogenetic location in brackets. WHAM was genetically mapped relative to ID and B (the relative distances and the calculated WHAM-B distance are indicated) (21, 22). B: The WHAM chicken ABCA1 gene has a single amino acid substitution (E89K) relative to normal White Leghorn chicken. Total liver RNA from WHAM and normal male chickens was subjected to standard RT-PCR and sequencing methods (left panel) using primers corresponding to the cDNA sequences most conserved between human and mouse ABCA1 (not shown). The open reading frame (corresponding to amino acids 27 to 2261) was sequenced, revealed a single homozygous G to A transition in WHAM cDNA at position 265. (Numbering of nucleotides and amino acids is according to the new, longer open reading frame of human ABCA1 (30). The same alteration was observed in PCR product of chicken genomic DNA (right panel). C: RFLP analysis confirms the presence of the WHAM mutation in genomic DNA. The WHAM alteration destroys a *Hinf* I site, resulting in a 142 bp uncut fragment rather than the 106 bp and 36 bp fragments of normal chickens. The chicken sex chromosomes of each bird tested are indicated below the photo; male chickens are ZZ, female chickens are ZW. Genbank Accession number: AF362377. D: The glutamate residue at the position of the non-conservative E89K substitution is conserved between human (CAA10005), mouse (CAA53530), Takifugu *rubripes* ("fugu"), and chicken. The WHAM mutation is thus predicted to have a deleterious effect on activity of the ABCA1 protein. The fugu amino acid sequence was predicted from nucleotide sequence of a cosmid containing the fugu ABCA1 gene (data not published).

transporter were then analyzed in transiently transfected HeLa cells. Morphological analysis highlighted a dramatic retention of the protein in the endoplasmic reticulum at 48 h and 60 h after transfection (**Fig. 4A**, **B**). At these time points, the wild-type product is predominantly located at the plasma membrane (Fig. 4A). This mistargeting was further supported by the virtual absence of transporter accessible to cell surface biotinylation (Fig. 4C). A minor amount of WHAM ABCA1, however, does reach the plasma membrane, but does not exceed 10–20% of the wild-type ABCA1 detected in similar conditions.

Although the inability to reach the membrane provides in itself an explanation for the lack of ABCA1 associated functions, we tested whether the WHAM ABCA1 transporter when present at the plasma membrane was able to specifically bind to apoA-I or to annexin V. These assays address two functions associated with the expression of the ABCA1 transporter, namely the exposure of a specific binding site for apolipoproteins on the cell surface and the lipid transport activity.

In both assays, flow cytometric evaluation of the GFP expressing transfected cell failed to detect any significant

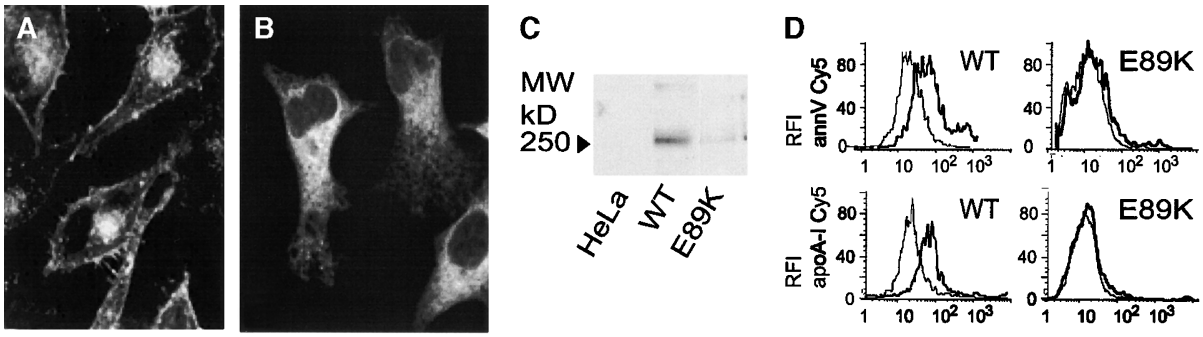

Fig. 4. The loss of ABCA1 function in WHAM mutations originates from a defect in intracellular trafficking. A: Confocal microscopic analysis of wild-type ABCA1/GFP chimera transfected HeLa cells shows that ABCA1 in its natural state accumulates mainly at the plasma membrane in discrete vesicles in the cytoplasm. B: Confocal microscopic analysis of HeLa cells transfected with the WHAM/EGFP chimera show massive retention in the endoplasmic reticulum. The mistargeting of the mutated transporter is confirmed by the virtual absence of protein accessible to surface biotinylation. C: The migration of the 250 kDa protein corresponding to the ABCA1/EGFP chimeric product (WT) is indicated. The expression of WHAM/EGFP in transfected cells fails to reconstitute the ABCA1-elicited (WT) surface binding of annexin-V (Ann V Cy^5) and apoA-I (apoA-I Cy^5). D: A representative FACS profile is shown. Thick and thin lines correspond to cells gated positive or negative for GFP fluorescence. Values of WHAM elicited binding (expressed as percent of wild-type ABCA1 /EGFP) are 13.8 ± 5. (n = 6) for annexin V and 20 ± 11 for apoA-I (n = 4). RFI, relative flourescence intensity.

binding (Fig. 4D) (values expressed as percent of the binding elicited by wild-type ABCA1/EGFP are 13.8% ± 5; n = 6 for annexin V and 20% ± 11 for apoA-I; n = 4.). The WHAM mutant therefore acts as an essentially complete-loss-of-function mutation.

DISCUSSION

In this study, we present the first animal model with a naturally-occurring mutation in the ABCA1 gene. Our prior studies of the metabolic abnormalities in the WHAM chicken indicated that there is a normal rate of apoA-I secretion, yet there is hypercatabolism of apoA-I. We proposed that the defect is in the availability of phospholipid for HDL production in the bloodstream (2, 3). The similarity of the metabolic phenotypes between Tangier patients and WHAM chickens, together with the synteny between the Z-linked region where the WHAM mutation maps and that of ABCA1 suggested that the WHAM mutation resides in the ABCA1 gene.

Comparative gene sequencing is an effective tool for the study of the functional importance of specific amino acid residues. The functional conservation of glutamic acid-89 over 400 million years in the ABCA1 gene of the fugu, chicken, mouse, and human genomes provides evidence for this glutamic acid to lysine change having significant effects on ABCA1 function in the chicken. The dramatically reduced HDL levels in the WHAM chicken provides further compelling evidence for the rate-limiting role of the ABCA1 gene in HDL synthesis and conclusively demonstrates that ABCA1 is absolutely required for maintenance of HDL levels in different species.

The non-conservative E89K mutation described here makes a strong case that this is indeed the WHAM mutation. The mutation is in an N-terminal segment of ABCA1 whose topology has been controversial. The original descriptions of ABCA1 proposed that the first 640 amino acids are cytoplasmic and precede the first transmembrane domain. However, recent studies of Fitzgerald et al. (26) have shown that several of the N-glycosylation sites within this segment are in fact glycosylated, indicating that this segment had to be translocated across the ER and must therefore have an exofacial orientation. Thus, it is proposed that the first transmembrane domain is in a type 2 orientation, followed by amino acids 44–640. The WHAM mutation would therefore be exposed to the endoplasmic reticulum lumen and be in a position to disrupt the folding of the protein. It is also important to note that this segment also includes a 60-amino acid N-terminal region that was initially excluded from the presumed open reading frame of ABCA1 and was subsequently shown to be essential to its function (26).

The strict conservation of this glutamate (E89) residue (from fugu to chicken) suggests a functional importance that we have confirmed by the in vitro analysis of the WHAM transporter in transfected cells. The presence of the WHAM mutation impairs physiological intracellular trafficking since most of the transporter appears to be retained in the endoplasmic reticulum. Naturally occurring mutations in the ABC transporters also have been shown to affect intracellular trafficking. Indeed, the most frequent mutation causal for cystic fibrosis, (the ΔF508 mutation) leads to a temperature sensitive defect in protein folding and impaired trafficking along the secretory pathway (27).

As a result of the WHAM mutation, only limited amounts of ABCA1 reach the plasma membrane, and fail to elicit the functional effects associated with the expression of wild-type ABCA1. In particular, the complete absence of apoA-I binding upon expression of WHAM transporter is sufficient to impair cellular release of PL to lipid poor HDL particles.

The serum from WHAM chickens is colorless, a conse-

quence of greatly reduced levels of carotenoids. Tangier serum has the same carotenoid content as normal human serum. In the WHAM chicken, carotenoids are absorbed normally (unpublished observations) and are cleared from the circulation. Unlike the human patients, the WHAM chicken is deficient in virtually all lipoproteins, thus the reduced carrying capacity for carotenoids is the likely explanation for the colorless serum.

The phenotype of Tangier Disease and of WHAM chickens establishes a critical role for ABCA1 in the supply of lipids to the lipid-poor HDL particle. A large body of literature proposes a role for HDL in transport of cholesterol from extrahepatic tissues to the liver, a process termed "reverse cholesterol transport." Implicit in this model is that the bulk of the lipids that end up in HDL particles originate in extraheptic tissues. The drastic effect that the ABCA1 mutation has on HDL levels in Tangier Disease and in the WHAM chickens supports a major role for ABCA1 in the supply of lipids for HDL. The fact that ABCA1 functions to export lipids from cells is consistent with HDL assembly as an extracellular event in which the first step is the binding of apoA-I to phospholipids. Indeed, apoA-I spontaneously forms HDL precursor particles when exposed to phospholipids (28).

The WHAM chicken supplied the first genetic evidence that vertebrates, like invertebrates, have an extracellular lipoprotein assembly pathway. The key observation was that despite normal apoA-I synthesis and secretion, the chickens are unable to produce stable HDL particles. The mutant chickens have a deficiency in plasma phospholipid which suggested that the dearth of phospholipid in the bloodstream was likely the primary defect responsible for the HDL deficiency syndrome (3). Subsequent studies in Tangier fibroblasts established that a lipid transport defect underlies this disease (7–9). It remains to be established which tissue makes the largest contribution to HDL lipids in an ABCA1-dependent fashion. Recent studies by Haghpassand et al. (29) show that macrophages, despite being highly enriched in ABCA1, do not make a significant contribution to HDL lipids. In the WHAM chicken, there is substantial cholesterol ester accumulation in the liver and intestine (unpublished observations), suggesting that ABCA1 is most active in lipid transport out of these tissues. If this is the case, then it will be important to make a distinction between the role of ABCA1 in the contribution of lipids to HDL and the role of ABCA1 in macrophages relative to atherosclerosis susceptibility. If these are indeed two separate roles, then the traditional view of reverse cholesterol transport would still be valid vis-à-vis atherosclerosis, but not necessarily be relevant to the bulk of HDL lipid transport.

We thank Susan Pope and Widya Paramita for their excellent assistance in the maintenance of the chickens. Albert Lee and Agripina Saurez have provided superb technical support. This work was funded by Xenon Genetics, Inc. of Vancouver BC, Canada, and by grants from: the Heart & Stroke Foundation of Canada (M.R.H.); the Canadian Network of Centers of Excellence (M.R.H.); and the Canadian Institute for Health Research (M.R.H.).

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