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Chapter 1

Apolipoprotein E Isoforms and AMD

Kimberly A Toops, Li Xuan Tan and Aparna Lakkaraju

Abstract The cholesterol transporting protein apolipoprotein E (ApoE) occurs in three allelic variants in humans unlike in other species. The resulting protein isoforms E2, E3 and E4 exhibit differences in lipid binding, integrating into lipoprotein particles and affinity for lipoprotein receptors. ApoE isoforms confer genetic risk for several diseases of aging including atherosclerosis, Alzheimer's disease, and age-related macular degeneration (AMD). A single E4 allele increases the risk of developing Alzheimer's disease, whereas the E2 allele is protective. Intriguingly, the E4 allele is protective in AMD. Current thinking about different functions of ApoE isoforms comes largely from studies on Alzheimer's disease. These data cannot be directly extrapolated to AMD since the primary cells affected in these diseases (neurons vs. retinal pigment epithelium) are so different. Here, we propose that ApoE serves a fundamentally different purpose in regulating cholesterol homeostasis in the retinal pigment epithelium and this could explain why allelic risk factors are flipped for AMD compared to Alzheimer's disease.

Keywords Apolipoprotein E · ApoE isoforms · Age-related macular degeneration · Retinal pigment epithelium · Cholesterol

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1.1 Introduction

Age-related macular degeneration (AMD), like other multifactorial diseases of aging, has no simple genetic underpinning. A complex mixture of environmental factors, lifestyle choices, and genes influence whether AMD will develop, how rapidly it will advance, and how severe the resulting visual dysfunction will be (Fritsche et al. 2014). Vision loss in AMD results from death of the photoreceptors, particularly in the macula. Photoreceptor loss reflects the terminal step in a cascading pathology whose genesis is in the posterior-most portion of the retina: the RPE, Bruch's membrane (BM) and choroid complex.

The tissue that is the initial site of damage in AMD, the RPE, forms the outer blood-retinal barrier and is responsible for the health and maintenance of the photoreceptors and the choriocapillaris (Toops et al. 2014). One of the many functions of the RPE is to act as the central organizing hub for cholesterol homeostasis for the outer retina (Fliesler and Bretillon 2010; Pikuleva and Curcio 2014). Several independent lines of evidence indicate that cholesterol homeostasis in the RPE and adjacent Bruch's membrane is dysregulated in AMD: one, cholesterol-rich lesions with material at least partly derived from the RPE are found in both sub-retinal and sub-RPE deposits (Bowes Rickman et al. 2013; Pikuleva and Curcio 2014). Two, several critical members of the cholesterol homeostasis pathway including hepatic lipase (LIPC), cholesteryl ester transfer protein (CETP), ATP-binding cassette sub-family A member 1 (ABCA1), and apolipoprotein E (ApoE) have been implicated in modulating AMD susceptibility (Katta et al. 2009; Liu et al. 2012; Fritsche et al. 2014). Of these, how ApoE gene variants alter AMD risk is especially intriguing because of the opposite allele-risk associations between AMD and Alzheimer's disease (AD) (Thakkinstian et al. 2006; McKay et al. 2011; Sivak 2013).

1.2 ApoE Isoforms Structure and Function

The human ApoE gene occurs in three allelic variants E2, E3 and E4 that vary by just two nucleotides resulting in three protein isoforms with amino acid variations at positions 112 and 158. These single amino acid changes profoundly effect protein function because they modify salt bridges within different helices of ApoE leading to altered receptor binding and lipid binding (Mahley and Rall 2000; Huang 2010). Key differences between the three ApoE isoforms are summarized in Table 1.1. The E2 isoform binds poorly to the low-density lipoprotein receptor (LDL-R) compared to E3 or E4 (<2%). E4 associates preferentially with very low-density lipoproteins (VLDL) whereas E2 and E3 associate with high-density lipoproteins (HDL) (Mahley and Rall 2000; Huang 2010). Humans are the only known species that express multiple ApoE isoforms. ApoE expressed by non-human primates and mice is structurally homologous to human ApoE4 with Arg at positions 112 and 158; however, these sequences have Thr at position 61 instead of Arg. This single amino acid switch prevents the formation of an N- and C- terminal domain interaction and results in non-human ApoE functioning more like human ApoE3 (Mahley and Rall 2000; Raffai et al. 2001).

Table 1.1 General properties of the three different human ApoE isoforms are summarized. ^aPopulation frequency is reported for having at least one allele of a given isoform; total estimated frequencies of the six possible ApoE phenotypes are 55 % E3/E3, 25 % E3/E4, 15 % E3/E2, with E4/E4, E2/2, and E4/E2 being rare phenotypes with 1–2 % occurrence (Mahley and Rall 2000).

^bSingle polymorphisms lead to alternate amino acids at positions 112 and 158 in the human ApoE isoforms protein primary sequence. ^c ApoE2 has been reported to have less than 2 % of the binding capability to LDL-R compared to E3 or E4 (Mahley and Rall 2000)

Properties of human ApoE isoforms				
Isoform	Population frequency (%) ^a	Sequence ^b 112 158	LDL-R affinity	Lipoprotein binding
ApoE2	7	Cys Cys	Very low ^c	HDL
ApoE3	78	Cys Arg	High	HDL
ApoE4	15	Arg Arg	High	VLDL, HDL

1.3 Evidence for ApoE in Human Diseases

1.3.1 Hyperlipidemia

ApoE was first implicated in regulating the balance of serum cholesterol and triglyceride levels (Huang 2010). In this context, ApoE, a component of lipoproteins (primarily chylomicrons, VLDL, and a subset of HDL particles), facilitates entry into cells by acting as a ligand for the low-density lipoprotein receptor (LDL-R), LDL-R like protein (LRP), heparan sulfate proteoglycans, and additional non-canonical receptors (Mahley and Rall 2000; Carlo et al. 2013). E4 is highly enriched in VLDL particles due to its altered lipid-binding region that shows a preference for binding triglyceride-enriched particles. E2 and E3 are more common in HDL particles due to a preference in their lipid-binding regions for phospholipids (Huang 2010). Both E2 and E4 alleles are associated with the development of hyperlipidemia and downstream atherosclerotic lesions, but for different reasons (Mahley and Rall 2000; Huang 2010). Because E2 is a much poorer ligand than E4 for LDL-R, effective uptake of HDL particles is prevented, leading to hyperlipidemia type III in E2 homozygotes. The preferential binding of E4 to VLDL particles leads to a feedback loop of decreased cellular uptake of LDL particles, which can result in hyperlipidemia.

1.3.2 Alzheimer's Disease

In contrast to the above scenario, in individuals with either one or two copies of E4 the risk of developing AD increases by 4- or 12-fold respectively compared to E3 homozygotes (Huang 2010). ApoE4 is the best-characterized risk factor for early-onset familial AD and an estimated 65–80 % of AD patients have at least one E4 allele (Carter 2007). Conversely, ApoE2 has been proposed to be mildly protective for AD, although this remains a weak association without a clear mechanism (Maezawa et al. 2004). ApoE4 is thought to contribute to AD mainly by altering how neurons

process the amyloid precursor protein (APP) through a cholesterol-mediated pathway. This pathway results in the accumulation of intra- and extra- neuronal toxic amyloid beta ($A\beta$) fragments, which eventually kill hippocampal neurons (Carter 2007; de Chaves and Narayanaswami 2008; Huang 2010; Leduc et al. 2010). The mechanism for this is complex and depends on interactions between ApoE, ApoE cell surface receptors, cholesterol, APP and $A\beta$, within neurons and in the surrounding astrocytes and extracellular space. E4 appears to stabilize toxic $A\beta$ oligomers, which renders them resistant to lysosomal degradation (Cerf et al. 2011). E4 contributes to AD via other mechanisms that are independent of $A\beta$: one, E4 is a poor supplier of cholesterol for membrane repair in damaged neurons (Rapp et al. 2006; de Chaves and Narayanaswami 2008; Leduc et al. 2010); and two, E4 acts as a pro-inflammatory molecule to exacerbate neuronal damage (Guo et al. 2004).

1.3.3 Age-Related Macular Degeneration

Epidemiological studies suggest that ApoE2 confers risk in AMD, whereas ApoE4 appears to be protective, although the association of E4 with protection is stronger than E2 with risk (McKay et al. 2011). ApoE and its cargo, cholesterol, are abundant components of drusen, the protein- and lipid-rich lesions in the Bruch's membrane characteristic of AMD (Anderson et al. 2001; Curcio et al. 2011; Bowes Rickman et al. 2013; Pikuleva and Curcio 2014). ApoE in drusen could originate from either the retina or the choroidal circulation (or both, since these sources are not mutually exclusive). However, mounting evidence indicates that the material that forms drusen, including ApoE, is secreted from the RPE (even if it is initially transported into the retina from the circulation, as may be the case for certain lipids) (Pikuleva and Curcio 2014). Thus, the retina is an active cholesterol producing and processing tissue and cholesterol efflux mechanisms are critical for maintaining retinal cholesterol homeostasis (Fliesler and Bretillon 2010; Pikuleva and Curcio 2014).

1.4 Cellular Identity and Differential ApoE Function Contributing to Risk

How ApoE4 can be detrimental to neuronal health has been studied extensively in AD. Little is currently known regarding isoform-specific functions of ApoE in the RPE and how these could contribute to AMD. Local sources of ApoE within the retina are the RPE and the Muller glia, indicating that ApoE is a major cholesterol transport in the retina (Anderson et al. 2001; Li et al. 2006; Johnson et al. 2011). RPE cells express the uptake receptors for ApoE (LDL-R and LRP) as well as the machinery for cholesterol efflux (ABCA1 and ABCG1) (Ebrahimi and Handa 2011; Pikuleva and Curcio 2014). Since cholesterol (free, esterified, and oxidized) is a core component of drusen (Curcio et al. 2005), dysregulation of cholesterol

homeostasis seems to be a key player in AMD pathology (Curcio et al. 2011; Ebrahimi and Handa 2011; Pikuleva and Curcio 2014). And it is in this characteristic that hippocampal neurons and RPE cells most likely diverge.

First, whereas RPE have the capacity to synthesize and take up ApoE-containing lipoproteins, neurons are largely at the mercy of the astrocytes for ApoE production and lipid transport (Leduc et al. 2010). This is a critical distinction since very little cholesterol enters the CNS from the circulation and neurons rely on local synthesis and transport of cholesterol to generate and maintain their long membrane-rich axons. As a reflection of this, neuronal plasma membrane has high levels of lipoprotein receptors particularly LRP, which has a strong preference for ApoE2 and E3 (Rapp et al. 2006). On the other hand, although RPE cells express ApoE receptors, they seem to be spatially discreet (i.e., apical vs. basolateral distributions) and with a different abundance (Tserentsoodol et al. 2006a; Tserentsoodol et al. 2006b; Zheng et al. 2012). A comprehensive analysis of this expression remains to be done.

The RPE therefore acts as a hub for ingress and egress of ApoE-cholesterol, while neurons are largely a terminal acceptor. This implies that as far as ApoE is concerned, RPE may be more similar to astrocytes than neurons. Astrocytes are also active producers of ApoE-cholesterol particles and like the RPE, express ABCA1 and ABCG1, which participate in efflux of ApoE rich pseudo-HDL particles (Wu et al. 2010; Johnson et al. 2011; Ito et al. 2014). Astrocytes express LDL-R and LRP but appear to preferentially bind and uptake ApoE4 and E3 containing lipoproteins (Rapp et al. 2006). Astrocytes exposed to ApoE2-, E3- or E4-loaded cholesterol exhibited ApoE isoform-dependent uptake ($E4 = E3 > E2$) that was exactly opposite to that seen in neurons ($E2 = E3 > E4$). Further, astrocytes internalized their cholesterol efficiently, whereas in neurons, the cholesterol was retained on the plasma membrane.

1.5 Implications

If the RPE is functionally similar to astrocytes with regard to cholesterol handling, rather than neurons, then the reversed risk alleles for AD and AMD may not be such a puzzle after all. The RPE and astrocytes can preferentially efflux ApoE containing pseudo-HDL particles for efficient intercellular cholesterol transport. In the brain, this becomes problematic for neurons in ApoE4 expressors because poor cholesterol efflux both increases A β generation and decreases its degradation. In the retina, a different balance is struck because the RPE is capable of both efflux and re-uptake. This will be more efficient for E4 than E2 due to the presence of LDL-R in RPE, which avidly binds E3 and E4 but has almost no affinity for E2. Experiments aimed at testing how efficiently different ApoE isoforms traffic cholesterol in and out of the RPE will help establish a cellular, mechanistic basis for puzzling epidemiological data.

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