Review

Impact of apoE genotype on oxidative stress, inflammation and disease risk

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Although in developing countries an apolipoprotein E4 (apoE4) genotype may offer an evolutionary advantage, as it has been shown to offer protection against certain infectious disease, in Westernised societies it is associated with increased morbidity and mortality, and represents a significant risk factor for cardiovascular disease, late-onset Alzheimer's disease and other chronic disorders. ApoE is an important modulator of many stages of lipoprotein metabolism and traditionally the increased risk was attributed to higher lipid levels in E4 carriers. However, more recent evidence demonstrates the multifunctional nature of the apoE protein and the fact that the impact of genotype on disease risk may be in large part due to an impact on oxidative status or the immunomodulatory/anti-inflammatory properties of apoE. An increasing number of studies in cell lines, targeted replacement rodents and human volunteers indicate higher oxidative stress and a more pro-inflammatory state associated with the ε4 allele. The impact of genotype on the antioxidant and immunomodulatory/anti-inflammatory properties of apoE is the focus of the current review. Furthermore, current information on the impact of environment (diet, exercise, smoking status, alcohol) on apoE genotype-phenotype associations are discussed with a view to identifying particular lifestyle strategies that could be adapted to counteract the 'at-risk' E4 genotype.

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1 Introduction

Cardiovascular disease (CVD) is the leading cause of mortality worldwide, with latest statistics suggesting that it is responsible for 17.5 million deaths annually [1] with several fold higher numbers thought to suffer from CVD-related morbidity. Furthermore, it is estimated that there are currently about 18 million people worldwide with Alzheimer's disease (AD) [2], with aging populations demographics associated with an ever-increasing incidence.

Since the characterisation of the almost complete human genome was first published in 2001 and the subsequent description of gene variations, which are available in public databases, there has been a large research focus on the association between common single nucleotide polymorphisms (SNPs) in critical genes and risk of diseases. This has not only led to a better understanding of the patho-physiological mechanisms of such diseases, but also to the identification of genotypic biomarkers that could potentially be used as predictors of future disease risk.

One of the most studied gene has been the apolipoprotein E (apoE) gene, with 3891 PubMed papers and 54 individual SNPs (www.ncbi.nlm.nih.gov) published at the time of writing this review. The most widely described SNPs, which are the focus of the current review, are undoubtedly those that give rise to the apoE2, E3 and E4 protein isoforms. The E4 allele, which is present in approximately 25% of the Caucasian population, has been associated with increased risk of CVD, and it is the major known genetic risk factor for maturity onset AD. In addition, apoE4 has been shown to modulate the risk of many other disorders (see below).

The mechanisms by which apoE genotype has an impact on these diseases is not fully understood. Traditionally, the 40–50% higher risk of CVD associated with the apoE4 allele [3] was attributed to small increments in circulating cholesterol and triglycerides (TAG) levels observed in

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Abbreviations: **Aβ**, amyloid-β; **AD**, Alzheimer's disease; **apoE**, apolipoprotein E; **CHD**, coronary heart disease; **CVD**, cardiovascular disease; **GSH**, glutathione; **SNP**, single nucleotide polymorphisms; **TBARS**, thiobarbituric acid reactive substances

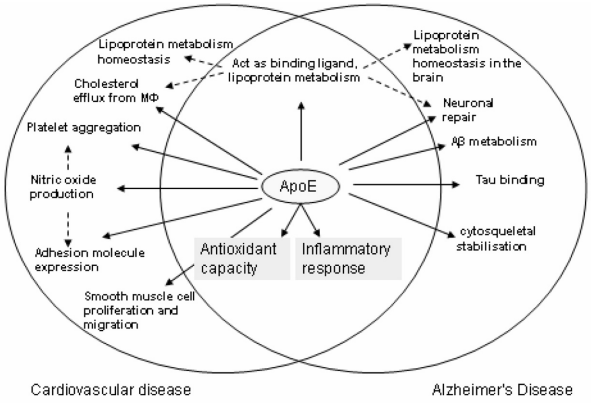

Figure 1. The multifunctional role of ApoE; MΦ Macrophage, Aβ Amyloid beta

apoE4 carriers. However, it is recognised that the relatively moderate effects on the lipid profile (see review [4]) cannot be the sole explanation for the genotype-mediated disease differential and, in addition, it does not explain the associations with AD and other conditions. Recently, two novel key mechanisms by which apoE could affect many biological processes have been recognised. These are its antioxidant and inflammation modulatory properties, which have been shown to be altered by genotype, and could therefore help explain many of the apoE isoform-disease associations (Fig. 1). The aim of this review is to summarise the existing evidence of these two aspects of apoE function in the context of the associations of apoE4 and disease risk.

2 ApoE and the impact of genotype on apoE structure and function

The first identified role of apoE was as a regulator of lipoprotein metabolism: it is involved in hepatic lipoprotein secretion, lipoprotein metabolism in the circulation, and serves as a high-affinity ligand for cellular lipoprotein uptake. However, apoE has been shown to exert multiple functions, independent of its role in lipid metabolism. ApoE is mainly synthesised by the liver but is also produced in tissues such as the brain and macrophages. It is thought that around 20–40% of apoE stems from extra-hepatic tissues [5–7]. In the brain, apoE has been shown to protect Tau from phosphorylation [8], to influence the metabolism of amyloid-β (Aβ) [9] and participate in neuronal repair [10]. Macrophage-derived apoE is abundant in the lesion site of atherosclerotic plaques, where it has been shown to influence many processes such as platelet aggregation [11], macrophage cholesterol efflux [12, 13], the expression of adhesion molecules by the endothelial cells [14] and inhibition of smooth muscle cell proliferation and migration [15].

ApoE is an arginine-rich protein containing 299 amino acid residues (34 kDa) and results from the cleavage of a 317 amino acid primary translation product [16]. Its gene has been mapped to chromosome 19 in a cluster with APOC1 and APOC2. Three common alleles ε2 (rs 7412), ε3, and ε4 (rs 429358), result in apoE2, apoE3 and apoE4 isoforms, respectively. ApoE isoforms differ in amino acid residues at positions 112 and 158 [17]. ApoE3 is the most common isoform and contains cysteine and arginine at these sites, whereas apoE2 has two cysteines and apoE4 two arginines (Table 1). The amino acid exchanges lead to structural differences that have an impact on the protein functionality. On the one hand, the substitution of an arginine by a cysteine at position 158 in apoE2, results in a 50–100-fold weaker binding affinity of the protein for cell surface LDL receptors [18, 19]. As a result, homozygosity for apoE2 is associated with type III hyperlipoproteinaemia, a condition characterised by high circulating TAG levels.

On the other hand, substitution of cysteine by arginine at position 112 in apoE4, does not affect the binding affinity to the LDL receptors, but changes the conformation of the side chain of Arg61. This is thought to impact on the chemical and thermal stability of the protein and in the formation of folding intermediates, with apoE2 showing the greatest stability and least formation of intermediates, while apoE4 shows the opposite properties, forming a typical molten globule configuration [20]. In addition to stability, it is thought that these differences in apoE4 protein folding explain the differential lipoprotein binding preferences [20, 21], with apoE4 binding preferably to larger liquid-rich lipoproteins (VLDL and LDL) and apoE2 and apoE3 preferring smaller lipoproteins such as HDL. This in turn is thought to be largely responsible for the moderate increments (~8%) in LDLC observed in apoE4 carriers [4].

Additionally, this impact of apoE genotype on protein structure is being increasingly shown to modify the effects of apoE on the above-mentioned processes unrelated to lipid metabolism. Of importance, apoE has been demonstrated to possess antioxidant properties in a genotype-dependent manner (apoE2 > E3 > E4), and has been also shown to influence the inflammatory response, two common pathological hallmarks of CVD and AD (Fig. 1).

3 ApoE genotype and disease risk

Extensive epidemiological data are available which demonstrates an association between apoE genotype and risk of CVD and AD. The meta-analysis of Song *et al*. [3], a review of 48 studies, demonstrated that compared to the wild-type E3/E3 genotype, carriers of the e4 allele had a 42% higher risk of coronary heart disease (CHD). However, the association between apoE polymorphism and stroke is still controversial [22, 23]. Case-control studies of apoE genotype and longevity reveal that, in elderly populations, there is a deficit in apoE4 in comparison to younger populations [24], which is mainly attributed to the higher CVD incidence.

Table 1. Structure and action pf apoE isoforms

Isoform	SNP ID	Residues		LDL receptor binding affinity	Lipoprotein affinity	Associated lipid changes
		112	158			
apoE2	rs7412	Cys	Cys	<2%	HDL	Type III hyperlipoproteinaemia (increased triglyceride levels)
apoE3		Cys	Arg	High	HDL	
apoE4	rs429358	Arg	Cys	High	VLDL, CM	Moderate ~8% increased LDL-cholesterol

Furthermore, the ε4 allele is a firmly established genetic risk factor for AD. In a meta-analysis conducted by Bertram *et al.* [25], odds ratios of 4.3 and 15.6 were noted for E3/E4 and E4/E4 individuals relative to E3/E3, individuals.

Although not so widely studied, apoE genotype has also been associated with several other diseases/disorders (Table 2). For instance, apoE4 is associated with poorer outcome following traumatic brain injury [26, 27] and with increased post-operative cognitive dysfunction [28, 29]. Although apoE genotype is not associated with an increased risk of developing multiple sclerosis, apoE4 is found to be a predisposing factor to a faster disease progression [30]. Additionally, it has also been related to increased risk of HIV-associated dementia [31]. Altogether, this highlights that apoE plays an essential role in neurobiology. ApoE4 has also been observed to be associated with psoriasis, a chronic inflammatory skin disease [32] and has been suggested to be a genetic risk factor for left ventricular failure in β-thalassemia. This disease is characterised by haemolytic anaemia, with consequent iron overload and chronic tissue damage [33]. Therefore, in general, an apoE4 genotype has been associated with increased risk of diseases characterised by oxidative stress and a pro-inflammatory status. However, the strength of the association varies widely between different populations, indicating an impact of other genetic variants and environmental variables on apoE-disease association.

In contrast, apoE4 has been shown to confer protection against age-related macular degeneration [34]. Although the mechanism remains unknown, it is hypothesised that apoE genotype-mediated differences in membrane dynamics due to differences in apoE conformations could lead to altered transport of lipids and cholesterol in apoE4 carriers, avoiding the formation of drusen, characteristic structures formed in age-related macular degeneration [35]. In addition, apoE4 has been shown to have a role in protecting against some infectious diseases [36]. For instance, apoE4 is associated with less severe Giardia infections in Brazilian shantytown children [37] and with protection of children against the outcomes of early childhood diarrhoea [36]. ApoE4 has also been shown to be protective against liver damage caused by the hepatitis C virus [38]. Therefore, apoE4 may have provided initial evolutionary advantages in pathogen resistance, particularly in situations of malnourishments. The higher incidence of the ε4 allele in pre-industrialised countries is consistent with this theory [4]. For these reasons, the ε4 allele is considered a "thrifty" genotype, providing advantages in ancestral or pre-industrialised populations. However, in Westernised populations, where chronic non-infectious disorders are the major killers, the apoE4 genotype is likely to be non-advantageous.

4 ApoE genotype and oxidative stress

The antioxidant properties of apoE were first identified in the middle of the last decade [39 – 42]. Soon afterwards Miyata and Smith [43] postulated that the apoE genotype could influence the antioxidative properties of the protein and thereby impact on both CVD and AD (Table 3, which details studies examining the association between apoE genotype and oxidative status). They showed an antioxidant activity in the order apoE2 > E3 > E4, tested using various techniques including the chemiluminescence antioxidant assay, a copper-mediated lipoprotein oxidation assay measured by thiobarbituric acid reactive substances (TBARS) and a copper-mediated lipoprotein assay measured by diene formation. Since then, there have been several additional studies that have demonstrated that apoE4 is associated with increased oxidative stress (see below).

Due to the strong association of apoE4 with AD, most work has been done in relation to the pathophysiology of this condition. On the one hand, studies with AD patients have revealed that apoE4 is associated with increased lipid peroxidation in post-mortem brains [44 – 46] and with elevated hydroxyl radical levels in blood [47]. However, no impact of genotype on blood antioxidant enzymes were evident [47, 48]. In mice, it has been shown that Aβ induction of oxidation in isolated synaptosomes leads to increased reactive oxygen species formation in mice expressing apoE4 relative to apoE3 [49]. Furthermore, Yao *et al*. [50] found increased levels of F_2-isoprostanes in brains of apoE4 male mice, but found no genotype differences in female mice.

Table 2. ApoE4 associations to disease risk

ApoE4 increased risk	ApoE4 decreased risk
Alzheimer's disease [101, 102]	Hepatitis C [38]
Traumatic brain injury outcome [26, 27]	Diarrhoea in children [36, 37]
Postoperative cognitive dysfunction [28, 29]	Age-related macular degeneration [34, 35]
HIV-associated dementia [31]	
Cardiovascular disease [103]	
Left ventricular failure in β-thalassemia [33]	
Psoriasis [32]	

More recently a new body of evidence has emerged, from both human observational and cell culture studies, indicating a role of apoE genotype on oxidative status measures, relevant to the progression of CVD. In a study aimed at assessing the impact of genotype on risk of CHD, Humphries and co-workers [51] elucidated a highly significant genotype – smoking interaction, with apoE4 carriers being more susceptible to smoking damage (a major source of oxidative stress) than the non-apoE4 individuals. In 'never' smokers, there was no genotype effect on risk of CVD. However, in smokers, the risk was 1.68 (95% CI 1.01 – 2.83) and 3.17 (1.82 – 5.5) in men homozygous for e3 and for ε4 carriers, respectively. This apoE genotype – smoking-CHD risk interaction appears to be robust as it has since been confirmed in several additional studies [53 – 54]. It has been hypothesised that the lower antioxidant capacity of apoE4 relative to other isoforms is responsible for the exacerbation of the detrimental effects of tobacco smoking.

Furthermore, a number of studies have examined an impact of apoE genotype on oxidative status-dependent mediators or biomarkers of oxidative stress. It was shown that in smokers, but not in non-smokers, apoE4 subjects exhibited ~30% increased oxidised LDL (ox-LDL), while apoE2 had ~30% higher total antioxidant status, measured as the capacity to inhibit the peroxidase-mediated formation of the 2,2-azino-bis-3-ethylbensthiazoline-6-sulfonic acid ($ABTS^{+}$) radical [53]. Consistent with these findings, in a mixed group of smokers and non-smokers with total cholesterol >200 mg/100 mL, apoE4 carriers showed an ~30% increase in F_2-isoprostanes [55]. Furthermore, in a study with post-menopausal women, serum concentration of malondialdehyde-modified LDL (MDA-LDL) in the following order E4 > E3 > E2 [56] were observed. In contrast, in transgenic mice expressing either apoE3 or apoE4, we found no differences in the antioxidant defence system in different tissues and only a tendency towards increased F_2-isoprostanes was observed in apoE4 mice (Jofre-Monseny, unpublished data). Although these results may seem contradictory, they are in accordance with the hypothesis of Talmud *et al*. [53], who suggested that an additional source of oxidative stress is needed to observe an apoE genotype-mediated impact on oxidative status. To date, human observational studies have largely focused on smoking status as a source of oxidative stress. Although there is no evidence currently available, it is likely that apoE genotype differences will also be apparent in other subgroups with a risk of a compromised oxidative status, such as trained athletes or individuals with a low fruit and vegetable intake.

With the purpose of gaining a deeper understanding of the possible role of apoE antioxidant properties in the process of atherogenesis, we have recently investigated the impact of apoE genotype on macrophage oxidative status. Macrophages are not only of interest because they are key cells in the development of atherosclerosis, but also because they are the cells that produce and secrete apoE in the vascular wall. Although apoE has been shown to have multiple functions within the macrophage itself and adjacent cells found in the vascular wall [11, 12], the contribution of the antioxidant capacity of apoE or the impact of genotype on antioxidant function on these processes is relatively unknown. We found that apoE genotype had no influence on protection against hydrogen peroxide-induced cytotoxicity or in the intracellular levels of GSH. However, cells secreting apoE4 showed higher membrane oxidation and produced more nitric oxide (NO) and superoxide anion radicals ($O_2^{\bullet -}$) upon stimulation with LPS and with phorbol myristate acetate (PMA) [57], respectively.

In addition, there are a limited number of *in vitro* studies that have focused on the impact of apoE genotype on LDL oxidation. It has been shown, in a copper-mediated and in a enhanced chemiluminescence reaction dependent on horseradish peroxidase model that apoE inhibits lipoprotein oxidation in an isoform-dependent manner (E2 > E3 > E4) [43, 58, 59]. In contrast, in a model, independent of metal induction, no impact of isoform was evident with all forms of apoE inhibiting LDL oxidation [58]. Furthermore, Pham and co-workers [59] identified the receptor-binding domain (residues 141 – 155) as responsible for the LDL oxidation inhibitory properties of apoE.

The molecular mechanisms responsible for the antioxidant capacity of apoE have not as yet been fully elucidated. The *in vitro* models of copper-catalysed LDL oxidation aim to mimic the *in vivo* oxidation process, and measure a combination of radical scavenging and transition metal chelation. Miyata and Smith [43] hypothesised that apoE exerted its antioxidant properties by metal sequestration, and demonstrated that apoE was retained by metal columns, with maximal retention by the cupric column; however, differen-

Table 3. Summary of the studies on apoE and oxidative stress

Author, year	Model	Species	Parameter/biomarker	Outcome
Miyata and Smith 1996 [43]	Neuronal cells and *in vitro assays*	CC	– B12 neurones protection from hydrogen peroxide – Antioxidant mediated quenching of an enhanced chemiluminescence – Inhibition of copper mediated lipoprotein oxidation – Copper mediated lipoprotein oxidation ($T_{1/2}$) – Metal binding (cupric, ferrous, ferric and zinc)	– E2 > E3 > E4 (conditioned media) – E2 > E3 > E4 – E2 > E3 > E4 – E2 > E3 > E4 – E2=E3=E4
Fernandes *et al.* 1999 [48]	AD patients and controls	H	– MDA in plasma and erythrocytes, enzymatic and non-enzymatic defence in plasma, erythrocytes, platelets and leukocytes – Uric acid and catechol-O-methyltransferase	– E2 = E3 = E4 – E4 < E3 = E2
Ramassamy *et al.* 1999 [44]	AD patients Frontal cortex	H	– Lipid oxidation (TBARS) – Catalase, Glutathione peroxidase – SOD	– E4 > E3 – E4 < E3 – E3 = E4
Ramassamy *et al.* 2000 [45]	AD patients Hippocampus	H	– TBARS in hippocampus – Catalase, GPx, GSH	– E4 > non-E4 – E4 < E3
Ihara Y *et al.* 2000 [47]	AD patients	H	– Hydroxyl radical levels – Superoxide dismutase activity and protein levels	– E4 > non-E4 – E3 = E4
Jolivalt *et al.* 2000 [104]	*In vitro* assay		– Susceptibility to oxidation of the protein	– E4 > E3 > E2
Pedersen *et al.* 2000 [60]	*In vitro* assay		– Interaction of pure apoE with 4-hydroxynonenal	– E2 > E3 > E4
Tamoka *et al.* 2000 [46]	Post-mortem brain AD	H	– TBARS generated de novo after oxidative stimuli	– E4/E4 > E3/E4 > E3/E3 > E3/E2
Humpries *et al.* 2001 [51]	Prospective cardiovascular surveillance (Second Northwich Park Heart Study) (male)	H	– Gene-environment interactions	– Smoking increases risk of coronary artery disease particularly in E4 – End points: fatal coronary heart disease, non-fatal myocardial infarction, coronary artery surgery and silent myocardial infarction
Lauderback *et al.* 2002 [49]	Mice apoE3 and apoE4 brain	M	– Reactive oxygen species formation and protein and lipid oxidation in isolated synaptosomes	– E4 > E3 = E2
Mabile *et al.* 2003 [58]	Metal induced and macrophage LDL oxidation	CC	– Oxidation of LDL by AAPH (free radical scavenging activity) – LDL oxidation by copper (TBARS and LDL electrophoretic mobility) – Cell mediated oxidation model- measurement in LDL containing medium of LA and AA consumption, TBARS, LDL electrophoretic pattern	– E2 = E3 = E4 – E2 > E3 > E4 – E3 = E4 > E2 (Results due to increased cholesterol efflux in E2; more substrate in medium susceptible to oxidation of LDL)

Table 3. Continued

Author, year	Model	Species	Parameter/biomarker	Outcome
Yao *et al.* 2004 [50]	Mice apoE3 and apoE4 brain	M	– F_2-Isoprostances in brain	– Male: E4 > E3 – Female:E4 = E3
Tsuda *et al.* 2004 [105]	Postmenopausal women	H	– Serum concentration of malondialdehyde-modified low-density lipoprotein (MDA-LDL)	– E4 > E3 > E2
Talmud *et al.* 2005 [53]	Framingham Offspring Study (men)	H	– CVD risk	– In non smokers: E2 = E3 = E4 – In smokers: E4 > E2 > E3 → Genotype – smoking interaction
	Caucasian patients with diabetes (males and females)	H	– Ox-LDL	– In non smokers: E2 = E3 = E4 – In smokers: E2 = E3 < E4
			– Total antioxidant status	– In non smokers: E2 = E3 = E4 – In smokers: E2 = E3 > E4
Dietrich *et al.* 2005 [55]	274 subjects (males and females)	H	– F_2-isoprostanes	E4 > non E4 (in subjects with total cholesterol > 200 mg/100 mL)
Pham *et al.* 2005 [59]	*In vitro*		– Inhibition of copper mediated lipoprotein oxidation (conjugated diene formation)	– E3 > E4
Jofre-Monseny *et al.* 2007 [57]	Macrophages	CC (M)	– Membrane oxidation – Nitric oxide and superoxide anion radical production – glutathione, α-tocopherol	– E4 > E3 – E4 > E3 – E3 = E4

H, human; M, mouse; R, rat; CC, cell culture.

ces between isoforms were not apparent in this assay. Alternatively, Pham *et al*. [59] argued that the peptide that is responsible for the inhibition of LDL oxidation is a region rich in positively charged amino acids, so that a direct interaction with the positively charged copper ion is unlikely, and support, therefore, a free radical scavenging activity of this region. Pedersen *et al.* [60] suggest that apoE may have a role in binding to 4-hydroxynonenal (HNE), and thereby have a detoxifying role. In these *in vitro* studies, it was shown that E2 > E3 > E4 are able to bind HNE.

Altogether, it appears evident that apoE genotype influences the antioxidative capacity of the lipoprotein. However, further studies are needed to gain insight into the molecular basis of the association, and also to establish the relative importance of apoE genotype-mediated differences in oxidative status to the pathogenesis of CVD and AD.

5 ApoE genotype and inflammation

The immunosuppressive properties of apoE were first described almost two decades ago, with the earliest indications originating from investigations on T cell proliferation [61 – 63] (Table 4, which details studies examining the association between apoE and immune function and inflammation). Later, it was shown that apoE deficiency impaired the immune response to *Listeria monocytogenes*, *Klebsiella pneumoniae* and LPS [64 – 66]. Almost in parallel to investigations of the differential antioxidant effects of apoE isoforms, the differential inflammation modulatory properties of the protein isoforms were examined [67 – 74]. These studies were also conducted mainly using models of AD and brain inflammation.

5.1 Inflammation in relation to AD

In 1998, Egensperger and co-workers [68] demonstrated that the microglial activation in frontal and temporal cortices in AD brains increased in an apoE4-allele-dose manner. However, most of our current evidence stems from trials with rodents, either engineered to express human apoE3 or apoE4, or used to create primary cell culture in which recombinant apoE3 or apoE4 protein is added. In mixed neuronal-glial cultures, addition of both recombinant

Table 4. Summary of the studies on apoE immune function and inflammation

Author, year	Model	Species	Parameter	Outcome
Pepe and Curtiss 1986 [61]	ApoE isolated from plasma	H	– Mitogen induced lymphocyte proliferation ([^{3}H]thymidine uptake)	– ApoE immunosuppressive
Kelly *et al.* 1994 [62]	T lymphocytes Mitogen: PHA, PMA + PHA, OKT3	H	– Mitogen induced lymphocyte proliferation ([^{3}H]thymidine uptake) – IL2	– ApoE suppresses T-cell proliferation by decrease of IL2 (activity levels, but not mRNA or protein)
Mistry *et al.* 1995 [63]	ApoE isolated from patients Peripheral blood mononuclear cells. Mouse spleen cells	H/M	– Cell cycle	– ApoE blocks growth factor-responsive T-cells in the $G1_A$ phase of the cell cycle.
Laskowitz *et al.* 1997 [67]	Mixed neuronal – glial cultures from apoE deficient mouse. Addition of recombinant apoE3 or E4. LPS administration	M	– TNF-α	– ApoE reduce TNFa secretion following LPS stimulation (E3 = E4) $\rightarrow$ need of preincubation to observe the effects $\rightarrow$ exogenous source of apoE may be insensitive to isoform differences??
Roselaar and Daugherty 1998 [64]	ApoE deficient mice	M	– Immune response to *L. monocytogenes* – Macrophage activation – TNFα	– ApoE deficiency causes impaired immune response – ApoE deficiency causes enhanced macrophage – ApoE deficiency causes inflammation
Stöhr *et al.* 1998 [106]	Monocytes in peripheral blood	H	– Differentiation of mononuclear phagocytes. CD16a (indicates Fc-receptor-dependent phagocytic activity)	– (E4/E4 > E3/E3)
Egensperger *et al.* 1998 [68]	AD brains	H	– Microglial activation in frontal and temporal cortices	– E4/E4 > E4/E3 > E3/E3
de Bont *et al.* 1999 [65]	ApoE deficient mice Intravenous LPS administration	M	– Immune response to LPS and *K. pneumoniae* – TNFα, IL1α, IL1β, IL6	– ApoE protects against LPS and *K.pneumoniae* – ApoE decreased TNFα but IL1α, IL1β and IL6 did not differ
Lynch *et al.* 2001 [69]	Mixed glial culture of apoE deficient mice. LPS exposure Mice vs apoE deficient mice. Intravenous administration of LPS	M	– mRNA TNF-α and IL6	– ApoE deficient > wild type

Table 4. Continued

Author, year	Model	Species	Parameter	Outcome
Van Oosten *et al.* 2001 [66]	Rats and mice Intravenous LPS administration	R M	– apoE serum levels – Cytokine production	– i.v. LPS administration increases apoE serum levels – ApoE prevents LPS-induced production of cytokines and subsequent death → physiological role of apoE in protecting against sepsis
Drabe *et al. 2001 [82]*	Patients undergoing cardiopulmonary bypass	H	– IL8 and TNF-α release by monocytes	– E4 > E3 → Increased systemic inflammatory response in ApoE4 carriers
Tenger and Zhou 2003 [107]	T cells and macrophages isolated from apoE deficient mice and wild type Stimulation with INF_{γ}	M	– Expression of CD40 and CD80 – Major histocompatibility complex class II molecules I-A.	– ApoE deficiency causes higher expression of CD40 and CD80 and also o f the major histocompatibility complex class II molecules I-A
Lynch *et al.* 2003 [70]	Targeted replacement mice apoE3 and apoE4. Intravenous LPS administration	M	– Systemic and brain TNF-α and IL6	– E4 > E3 apoE (133-149) (receptor binding region) → suppression of inflammation
Guo *et al.* 2004 [71]	Mixed glia (95% astrocytes, 5% microglia) from rats Stimulated with LPS and Aβ. Exogenous apoE administration	M	– Aβ induced NO synthase, COX-2	– When stimulated with LPS and Aβ: anti-inflammatory role of apoE – But exogenous apoE alone → induces IL1b (E4 > E3) → overproduction of apoE may exacerbate inflammation
März *et al.* 2004 [85]	739 subjects with stable angiographic coronary artery disease (CAD), 570 control	H	– CRP – Fibrinogen and white cell count	– E4 < E3 <E2 – E2 = E3 = E4
Ophir 2005 [72]	Mice E3 E4 brain LPS injection	M	– Expression of inflammation related genes (mRNA and protein levels) – NFκB activation	– E4 higher and more prolonged than in E3 (Changed genes enriched in NFkB ; E4 > E3) – E4 > E3
Maezawa *et al.* 2006 [73]	Targeted replacement mice E2, E3, E4 Microglia (LPS activation)	M	– Microglia activation induced neurone cytotoxicity – Microglia p38-MAPK-dependent cytokine activation	– E4 > E3 > E2 – E4 > E3 > E2
Maezawa *et al.* 2006 [74]	Targeted replacement mice E2, E3, E4 Astroglia (LPS activation)	M	– Primary astrocyte cytokine secretion – NFκB	– E2 > E3 > E4 – E2 > E3 > E4

Table 4. Continued

Author, year	Model	Species	Parameter	Outcome
Tziakas *et al.* 2006 [83]	Acute coronary syndrome and chronic stable angina patients	H	– IL10 (anti-inflammatory cytokine) – CRP	– E3/E4 < E3/E3 < E2/E3 – E3/E4 > E3/E3
Tsoi *et al.* 2007 [78]	Mouse J774A.1 peritoneal macrophages expressing apoE2, apoE3, apoE4	CC (M)	– TNF-α, IL6 expression – ERK1/2 activity	– E3 < E2=E4 – E3 < E2 = E4
Jofre-Monseny *et al.* 2007 [77]	Mouse RAW 264.7 macrophages expressing apoE3 and apoE4	CC (M)	– TNF-α – IL10 – NFkB transactivation – HO-1	– E4 > E3 – E3 > E4 – E4 > E3 – E4 > E3

H, human; M, mouse; R, rat; CC, cell culture.

apoE3 and apoE4 reduced LPS-induced TNFα secretion, with no differences between isoforms observed [67]. In contrast, a later study with transgenic mice showed that systemic and brain TNFα and IL6 secretion were higher in apoE4 than in apoE3 mice [70].

Enhanced inflammation was also observed in apoE4 mice brain and in mice microglia following LPS stimulation relative to apoE3 [72, 73] with the opposite effects shown for astroglia, indicating cell-specific effects. Furthermore, an isoform-specific difference in microglial NO production has been reported, in which apoE4 produce greater NO amounts than apoE3 mice [75, 76].

The exact molecular mechanism by which apoE modulates LPS-induced brain inflammation brain remains to be elucidated. However, it was shown that a peptide containing the receptor-binding region (residues 133 – 149) was enough to suppress inflammation [70]. Of note, is the fact that this peptide is almost the same peptide that Pham *et al*. [59] demonstrated to be responsible for the antioxidant properties.

ApoE has been postulated to influence different signalling pathways. Ophir *et al*. [72] demonstrated that the genes that were most differentially expressed in apoE4 compared to apoE3 were significantly enriched in nuclear factor κB (NFκB) response elements. In addition, it was shown that microglial NFκB activation was greater in apoE4 mice. On the other hand, Maezawa *et al*. [73, 74] support the hypothesis that apoE genotype-mediated effects in microglia are p38MAPK dependent.

5.2 Inflammation in relation to CVD

The macrophage innate immune response is a key feature of atherosclerosis. We tested the hypothesis that apoE4 was associated with enhanced inflammation by using a murine monocyte-macrophage cell line (RAW 264.7) stably transfected to express equal amounts of apoE3 or apoE4. Indeed, we found cytokine disequilibrium between the pro- and anti-inflammatory cytokines. LPS-stimulated macrophages secreting apoE4 showed increased TNFα, but decreased IL10 in comparison to apoE3 macrophages [77]. In addition, increased NFκB transactivation in apoE4-cells was evident, suggesting a prominent role of this transcription factor pathway in mediating apoE genotype differences in response. We also demonstrated [77] (Table 4) that the expression of heme oxygenase-1 (HO-1), a stress response anti-inflammatory protein, was increased in apoE4 macrophages, suggesting that its increased synthesis in apoE4 cells could be a response to a pro-inflammatory status produced to counteract in part the detrimental effects associated with increased cytokine production. At the same time, Tsoi and co-workers [78] published a study with a similar cell culture model (J774A.1) and showed that apoE4 and apoE2 macrophages produced higher amounts of TNFa and IL6. In addition, it was shown that these effects were partly mediated by modulation of the ERK1/2 MAP-kinase signalling pathway, suggesting that apoE isoforms differentially modulates the activation of parallel signalling cascades triggered by LPS.

The precise mechanisms by which apoE isoforms alter the innate immune response remain undefined. However, as oxidative stress is a known modulator of this response it is likely that the differential antioxidant capacity of the apoE isoforms could be in part responsible for the differential modulation of the redox-sensitive transcription pathways such as NFκB and MAP kinases. Alternatively, apoE could act through binding to cell surface receptors. It has been postulated that apoE immunomodulatory properties could be mediated by the LDL receptor-related protein (LRP), with consequent mobilisation of intracellular calcium [79]. However, the affinity to this receptor has not been proven to differ among the apoE isoforms [80].

A weakness of all of this evidence is that in almost all the studies, LPS was used as an inflammation inducer. Although this is a commonly used approach to investigate innate immune response, and there are data that support a

role of LPS and its receptor, Toll-like receptor 4 (TLR4), in the process of atherogenesis [81]; the relevance of these models for the pathogenesis of AD and CVD needs to be verified. It would be of great interest to reveal whether these effects are also observed with more physiological inflammation inducers such as ox-LDL.

Nevertheless, limited data in humans show an effect of apoE genotype on pro- and anti-inflammatory cytokines independently of any exogenous source of experimental inflammation inducer. In a study of patients undergoing cardiopulmonary bypass surgery, a process that induces a transient rise in pro-inflammatory cytokines mainly released by activated monocytes, it was shown that apoE4 was associated with increased IL8 and TNFa [82]. In patients with acute coronary syndrome, significant lower levels of the anti-inflammatory IL10 were observed in ε4 carriers, with the same trend evident in chronic stable angina patients [83]. This is in accordance with our findings in macrophages, supporting the hypothesis of an inflammatory imbalance between the pro- and anti-inflammatory cytokines in apoE4 carriers. The secretion of other mediators of inflammation that participate in the adhesion of inflammatory cells to the vascular surface, such as vascular cell adhesion molecule-1 (VCAM-1), were found to be modified by the apoE genotype (E4 > E3 > E2) (Minihane *et al*., unpublished results). Conversely, VCAM-1, intracellular adhesion molecule-1 and E-selectin were not altered by the apoE genotype in a study with low-HDL and normolipidemic subjects [84]. Other established indicators of systemic inflammation, fibrinogen and white cell count were not related to the apoE genotype [85].

5.3 ApoE and C-reactive protein

The levels of C-reactive protein (CRP) have been robustly associated with apoE genotype with apoE4 individuals presenting with lower and E2 carriers with higher CRP levels than E3/E3 individuals [84 – 87]. In addition, we observed the same association in targeted replacement mice expressing apoE3 and apoE4 (Jofre-Monseny, unpublished data). At present the mechanisms responsible for this association remain unclear. CRP is a product of hepatic stimulation by "messenger cytokines" such as IL6. Currently, it is considered the most robust inflammatory marker of CVD risk. [88]. At present, it seems contradictory that apoE4 is associated with increased brain and macrophage inflammation, and increased CVD, while at the same time is related to low levels of CRP. März and associates [85] suggest that the metabolism of CRP could be related to the mevalonate/cholesterol synthetic pathway, which may be down-regulated in apoE4 carriers in response to altered lipoprotein metabolism and hepatic uptake. Whatever the mechanism, it may be necessary to re-evaluate the meaning of CRP as a predictor marker according to the apoE genotype. If apoE genotype modulates CRP synthesis by a cytokine-independent mechanism, the CVD risk could be underestimated if CRP was used as a prediction factor in apoE4 carriers. However, if CRP is not just a surrogate marker, but also a causal factor and exerts direct functions in the development of atherosclerosis [89], the detrimental effects of apoE4 might be partly counteracted by lower levels of CRP. In addition, it is possible that the increased CRP levels observed in apoE2-carriers partly counteract the observed beneficial effects associated to apoE2 isoform (such as lower cholesterol levels [4] and better antioxidant properties [43]), which may explain the observation of no CVD-risk reduction consistently reported in ε2 carriers [3].

6 Is it meaningful to genotype for apoE?

The ε4 allele, which is carried by 25% of Caucasian populations, is associated with a 40 – 50% increased risk of CVD [3]. As CVD remains the main source of morbidity and mortality in Westernised societies, a reduction in the CVD burden associated with an apoE4 genotype would be of wide public health benefit. Although not fully resolved, numerous studies have reported on the impact of apoE genotype on the responsiveness of CVD biomarkers to environmental (diet, smoking status, alcohol intake, exercise) change, with ε4 carriers being particularly responsive. This indicates the potential of altered lifestyle as a means of reducing or negating the increased risk of CVD in those identified as having an apoE4 genotype.

6.1 Smoking status

As mentioned before, apoE4 carriers are more sensitive to the detrimental effects of tobacco smoking [51, 53, 90]

6.2 Alcohol drinking

There are limited data showing that the effects of alcohol on plasma lipids are modulated by the apoE genotype, yet inconsistent alcohol-apoE genotype-CVD phenotype associations have been reported, *e.g*., it was found [91] that in male non-drinkers, no effects of apoE genotype on LDL-cholesterol (LDL-C) levels could be observed, while in drinkers, the apoE genotype was associated with different LDL-C levels with apoE2 < apoE3 < apoE4. In contrast, it was reported [92] that in apoE2, alcohol consumption increased LDL-C, whereas in apoE4, it decreased LDL-C. Furthermore, the increase in HDL associated with alcohol appears to be stronger in subjects without the apoE4 allele than in those with the apoE4 [93]. However, no interaction between apoE4 and drinking was found on the prevalence of carotid atherosclerosis [90]. In addition, a prospective population-based study concluded that the risk of dementia increased with increasing alcohol consumption only in apoE4 carriers [94].

6.3 Physical activity

Beneficial effects of physical activity on HDL-C levels were observed in apoE4 men but not in women. Men carrying the ε4 allele had lower HDL-C levels if sedentary and higher HDL-C levels if physically active than apoE3 individuals, with the opposite effects evident in apoE2 carriers [92]. A recent study with older women suggested that aerobic physical activity could have a beneficial impact on cognitive performance particularly in apoE4 homozygotes [95].

6.4 Saturated fat, total fat and cholesterol intake

In general apoE4 individuals have been shown to be the most responsive to reduced saturated and total fat, and cholesterol intake (for review see [4])

6.5 Antioxidant supply

Given that apoE4 is associated with increased oxidative stress, it has been hypothesised that apoE4 carriers could potentially benefit from antioxidant supplementation [96]. Vitamin C supplementation (60 mg/day) was able to down-regulate monocyte-derived pro-inflammatory mediators in apoE4 individuals who smoked. In this study, apoE4-non-smokers were much less responsive than apoE4-smokers [97]. However, this study only included apoE4 carriers. The impact of apoE genotype on the responsiveness of oxidative status and inflammatory markers to antioxidant supplementation in E3 *versus* E4 in a non-smoking group has never been investigated. We investigated the effects of α-tocopherol supplementation in apoE3 and apoE4 transgenic mice. Our data suggest that the transport of α-tocopherol into the tissues may be altered by the apoE genotype, in which apoE4 show decreased α-tocopherol levels in tissues and increased levels in plasma. These data are supported by other reports showing increased plasma concentrations of α-tocopherol in apoE4 carriers [98, 99].

6.6 Caloric restriction

Caloric restriction has been shown to be beneficial in diseases associated with oxidative stress. In apoE null mice, caloric restriction could retard atherosclerosis through a mechanism that was independent of plasma cholesterol levels [100]. We propose that this could be a further potential measure by which apoE4 individuals could reduce the increased CVD burden.

6.7 ApoE genotype, environment interactions

To date, there is not sufficient consistent information to advocate specific dietary recommendations or pharmaceutical therapies to help negate the impact of an apoE4 genotype [92]. Although these data are highly suggestive of diet-genotype interactions, with strong indication that in apoE4 carriers such lifestyle approaches as of avoiding smoking, increasing physical activity and antioxidant intake and reducing alcohol, total fat and saturated fat intake could in part negate the apoE4-mediated increases in CVD risk, the data are inconsistent. This may be in large part attributable to the fact that most of the evidence considered, to date, is derived from observational or intervention trials in which inaccurate assessment of lifestyle, in particular dietary intake, and small group number in the rare allele groups associated with retrospective genotyping has led to inconsistent findings.

Furthermore, although less information is currently available, it is likely that apoE4-AD risk associations are also sensitive to environmental factors. However, research in this area is in its relative infancy.

7 Conclusions

ApoE polymorphism is associated with many diseases that apparently have different origins. However, two important hallmarks of these diseases are oxidative stress and inflammation. There is increasing evidence demonstrating that apoE4 is associated with increased oxidative stress and inflammation, which is likely to in part mediate the effect of genotype on AD and CVD burden. At present our understanding of the strength of association between apoE genotype and AD and CVD is relatively well developed, with growing evidence that it is modifiable by lifestyle changes. However, before a more widespread use of apoE gene profiling can be used as a predictive tool to help identify individuals at above-average risk for these two conditions, clear guidelines regarding which lifestyle changes can be adopted to help negate the increased risk in E4 carriers need to be available.

Although at present a developing body of evidence is becoming available regarding lifestyle-genotyping interactions in the area of CVD, no such information is available for AD. Larger intervention trials, using prospective recruitment of study participants are needed to gain a fuller understanding of apoE genotype-lifestyle-CVD/AD risk associations and to gain an understanding of the mechanisms underlying these interactions.

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