Revisiting the genetics of APOE

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Abstract
Apolipoprotein E (APOE) is a lipid-transport protein expressed in almost all tissues, including the brain. In addition to lipid delivery, brain APOE also regulates amyloid beta clearance and aggregation. In humans, there are three main isoforms, APOE2, APOE3 and APOE4, with structural differences that influence protein function. APOE4 is the most important genetic risk factor for Alzheimer's disease and Dementia with Lewy bodies.
In this review, we will focus on the genetic variability of *APOE* and its association with different diseases (mainly neurodegenerative, psychiatric and lipid-related). Despite the increasing number of studies, the association of *APOE* genetic variants with other neurological conditions beyond Alzheimer's disease and Dementia with Lewy bodies is still far from clear.
We will also discuss the association of different structural and functional aspects of APOE with different diseases, particularly the amyloid beta-dependent and -independent mechanisms, such as tau-mediated neurodegeneration, associated with Alzheimer's disease pathogenesis.
As the most significant genetic risk factor for Alzheimer's disease, *APOE* has a central role in the risk assessment of this disease. Consequently, a better understanding of the impact of common and rare *APOE* variants will not only contribute to a more accurate risk management of these patients, but it will also clarify the potential of APOE as a therapeutic target.

Keywords: APOE, genetics, variants, isoform, disease, brain

Heading title: Genetics of APOE

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Revisitando a genética da APOE

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Resumo
A apolipoproteína E, usualmente denominada como APOE, é uma proteína essencial no transporte de lípidos com expressão na maioria dos tecidos. No cérebro, para além do seu envolvimento no metabolismo dos lípidos, contribui também para a eliminação e agregação da proteína beta amilóide. No organismo humano existem várias isoformas da APOE, sendo as isoformas APOE2, APOE3 e APOE4 as mais frequentes. As diversas isoformas apresentam diferenças estruturais com consequência na função proteica. A isoforma APOE4 tem sido identificada por consecutivos estudos como o principal factor de risco genético para a doença de Alzheimer e para a demência com corpos de Lewy.
Neste estudo iremos focar-nos na variabilidade genética do gene *APOE* e na sua associação com diferentes doenças: doenças neurodegenerativas, psiquiátricas e associadas ao metabolismo lipídico. Apesar do crescente número de estudos realizados, a influência das variantes genéticas do gene *APOE* na maioria destas doenças ainda não é totalmente conhecida, com excepção da doença de Alzheimer e da demência com corpos de Lewy.
Será também destacada a relação entre as diferenças estruturais e os aspectos funcionais da APOE em diferentes patologias, em particular nos mecanismos dependentes e independentes de beta amilóide, como a neurodegeneração associada à proteína tau, envolvidos na patogénese da doença de Alzheimer.
Como factor de risco genético mais significativo para a doença de Alzheimer, o *APOE* tem potencialmente um papel na avaliação do risco desta doença. Consequentemente, uma melhor compreensão do impacto das variantes neste gene não só contribuirá para uma avaliação de risco de doença mais assertiva, como também ajudará a esclarecer o potencial da APOE como alvo terapêutico.

Palavras-chave: APOE, genética, variantes, doença, cérebro

Título de cabeçalho: Genética do APOE

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Introduction

Apolipoprotein E (APOE) was first described in 1973 by Shore and Shore (1), but it was only in 1975 that Utermann and collaborators decided to denominate this glycoprotein as APOE (2). APOE is an amphipathic protein that belongs to the family of apolipoproteins (3). In humans, three major APOE isoforms exist with different properties (4). In the early 90s, the association between the APOE4 isoform and Alzheimer's disease (AD) was discovered (5) and since then multiple studies have been performed to understand the impact of APOE in AD pathogenesis. In addition, the impact of APOE genotype has also been shown in other neurological conditions (6). In this article we review the genetics of APOE, its relation with protein structure and function and association with diseases. We focus essentially on neurological conditions, particularly in AD and how the information obtained from the genetic study of *APOE* can improve the risk assessment of these patients.

Gene and locus

The *APOE* gene is located on chromosome 19q13.32, it includes 4 exons separated by three introns and comprises 3646 bp (7). Exon 1 and the beginning of exon 2 correspond to the 5' untranslated region (UTR), while the last portion of exon 4 encodes the 3'-UTR (**Figure 1**). The *APOE* gene is in close proximity to other apolipoproteins genes, such as *APOC1*, *APOC4*, and *APOC2*. Strong linkage disequilibrium (LD) was observed between variants located in *APOE* and those in surrounding genes spanning 50 Kb (8). Using 21 *APOE* single nucleotide polymorphisms (SNPs), Yu and collaborators identified 35 different haplotypes in Caucasian individuals, with five haplotypes corresponding to over 75% of the haplotypic distribution, and 13 haplotypes corresponding to over 95%. Furthermore, different ethnic groups showed distinct LD patterns (8). Regarding methylation status, *APOE* has a CpG island located in the 3' coding region (exon 4) and CpG sites are hypo or hypermethylated according to the genomic location (9). CpG sites in the promoter and in exon 4 were shown to be hypermethylated, while CpG sites in the first two exons and introns exhibited hypomethylation. The genotype of specific variants influences the methylation level. The allele A of the promoter variant -219T/G (rs405509) increases the methylation in some CpG sites reducing gene expression and the three main APOE alleles (*APOE* ε2, *APOE* ε3 and *APOE* ε4) have different methylation levels with the presence of CpG sites in *APOE* ε4 allele (10).

Expression

APOE production and secretion occurs in most human tissues. Plasmatic APOE is mainly synthesised by hepatocytes (up to 75%). Moreover, other cells and tissues such as macrophages, adipocytes, spleen, and kidney are also important sources of APOE (11). In the brain, APOE is mainly produced by astrocytes , but also by neurons and microglia in stress situations (12), and cerebrovascular pericytes (13).

Proximal and distal regulatory binding sites are involved in the complex process of regulation of *APOE* transcription that takes place in a cell-specific manner. Several transcription factors bind to *APOE* promoter, such as AP2, LXRα/RXRα and LXRβ/RXRα (14). In hepatic cells two enhancers were identified, HCR.1 and HCR.2, that control the apoE/apoCI/apoCIV/apoCII gene cluster expression (15); while in macrophages and adipose tissue two multi-enhancer regions have been identified: ME.1 and ME.2 (16).

Protein

The translated APOE product is a 36.2 KDa protein composed of 317 amino acids. This precursor protein has a signal peptide of 18 amino acids on the N-terminal that is removed cotranslationally. Subsequently, in the Golgi apparatus, APOE suffers O-linked glycosylation and sialylation and finally the 34 KDa glycoprotein is secreted (17).

In 2011, Chen and collaborators revealed, for the first time, the full structure of APOE. Using nuclear magnetic resonance (NMR), the authors reported an helix-bundle structure with three domains: an N-terminal domain (residues 1-167) containing antiparallel four-helix-bundle, a hinge domain (residues 168-205) with two helices that regulates the interaction between N- and C-terminals, and a C-terminal domain

(residues 206-299) composed by three helices. A salt-bridge between Lys95 and Glu255 and an H-bond between Arg61 and Thr194 promote the interaction of both terminals (18).

Functions

APOE is a glycoprotein mainly involved in the transport of lipids and cholesterol throughout the body. APOE is an important constituent of lipoproteins such as very low density lipoproteins (VLDL) synthesized by the liver and chylomicrons generated in the intestine from dietary fat and cholesterol (11). The lipid ligation occurs through the lipid binding domain located in the C-terminal (residues 244-272). Moreover, APOE also has a receptor binding region located in the N-terminal (residues 136-150), allowing this protein to function as a ligand in receptor-mediated endocytosis of lipoprotein particles (**Figure 1**). APOE binds to cell surface receptors culminating in the internalization of transported lipids by hepatic and extrahepatic cells. Low density lipoprotein receptor (LDLR), LDLR-related protein 1 (LRP1), VLDL receptor (VLDLR), and APOEE receptor 2 (APOER2) are the major APOE receptors. The binding affinity to these receptors depends on APOE lipidation status and isoform. In addition, APOE can also bind to cell surface heparan sulfate proteoglycan (HSPG) (19). APOE secreted by macrophages and present in HDL particles participates in reverse transport of cholesterol, redirecting excess cholesterol produced by peripheral tissues to the liver for elimination (20).

In the brain, APOE has an important role in neuroplasticity. It is the predominant apolipoprotein of HDL in the central nervous system (21). Lipidated APOE binds to LDL receptor family members and is endocytosed. The released cholesterol is used in synaptogenesis and maintenance of synaptic connections, while APOE can be recycled back to cell surface or be degraded (22). APOE also acts as a chaperone protein required in amyloid β (Aβ) clearance (23). According to the classical view, when lipidated, APOE binds to Aβ and the Aβ-APOE complex is internalized by LRP1 in the blood brain barrier (BBB) and brain cells (22). It also influences the aggregation and deposition of Aβ (22). However, Verghese and collaborators argue that the physical interaction between soluble Aβ and APOE observed in previous studies just occurred due an overload of soluble Aβ compared to APOE lipoprotein. The authors concluded that in physiological ratios soluble Aβ does not bind to lipidated APOE. The alternative model proposes that soluble Aβ and APOE compete for the receptor LRP1, and consequently APOE impairs soluble Aβ clearance (24). Supporting this theory, it was observed an increase in Aβ clearance in the presence of reduced APOE levels (25) and a direct clearance of Aβ through LRP1 (26). Despite these evidences, the direct binding of APOE to Aβ can not be completely ruled out, since APOE is present in plaques (27). Furthermore, APOE was also shown to be involved in the regulation of inflammation, tau phosphorylation, actin polymerization and long-term potentiation (LTP), as described later in this review.

Isoforms and genetic variants

Three major isoforms are described for APOE: APOE2, APOE3 and APOE4. The three isoforms differ at positions 112 and 158 of the protein. APOE4 is the ancestral isoform and has the amino acid arginine in positions 112 and 158 of the protein. The APOE3 isoform is derived from APOE4 and presents a cysteine in position 112 and an arginine in residue 158 of the protein. APOE2 contains cysteines in both positions (4). At the gene level, these isoforms correspond to three alleles: *APOE* ε2, *APOE* ε3 and *APOE* ε4 that are associated with two SNPs, rs429358 and rs7412, corresponding to the previously described amino acid changes at positions 112 and 158, respectively.

APOE ε3 is the most frequent allele (77.9% in Caucasians) followed by *APOE* ε4 (13.7%) and *APOE* ε2 (8.4%) with slight differences between distinct ethnic groups (28). In Portugal, the allele frequencies fit within the range of values obtained for other European populations: 83.6%-88.2% for *APOE* ε3, 7.4%-10.0% for *APOE* ε4 and 4.4%-6.4% for *APOE* ε2 (29–31). A study performed with 126 healthy unrelated individuals born in the Azores also obtained similar allele frequencies: 83.7%, 9.5% and 6.8% for alleles *APOE* ε3, *APOE* ε4 and *APOE* ε2, respectively (32). Eisenberg and collaborators found a lower *APOE* ε4 frequency in populations living in regions with moderate latitude and temperatures compared to populations that live in extreme environments. This difference may be related to higher metabolic rates in the individuals living in

extreme environments, which requires higher cholesterol levels. In accordance, *APOE* ε4 carriers were found to have higher cholesterol levels (33).
The two polymorphisms confer different properties to the three isoforms. A higher molecular stability was found for APOE2, while APOE4 is the isoform with the lowest stability (34). Consequently APOE2 is the most abundant isoform in plasma and CSF (35,36). Also, due to the presence of two cytosine nucleotides in the variants rs429358 and rs7412, *APOE* ε4 has more CpG sites and was found to be hypermethylated comparatively to *APOE* ε2 (10). APOE4 has more affinity to VLDL particles, but APOE2 and APOE3 preferentially associate with small HDL particles (37). Affinity to the receptors is also influenced by the isoform: both APOE3 and APOE4 have similar affinity to the LDL receptor, but the affinity to this receptor is less than 2% for APOE2 (38,39). These variations are associated with structural differences between the isoforms. In APOE2 the cysteine residue at position 158 alters the conformation of the receptor binding region, between residues 136 and 150, leading to a defective binding to the LDL receptor. APOE4 has a closed conformation (a molten globule state) due to Arg112 residue. This arginine leads to the formation of a salt bridge between Arg61 and Glu255 residues, culminating in a different C-terminal with an increased affinity to lipids (40–42).
There are other genetic variants located in the promoter, exons and introns, but almost all of these are rare (minor allele frequency (MAF) <1%). In fact, in the large gnomAD database (http://gnomad.broadinstitute.org/) containing variants from over 123,000 exomes and 15,000 genomes there are only 6 SNPs reported with a MAF > 1%: the two SNPs associated with alleles ε2, ε3 and ε4, and four intronic variants (**Table I**).

APOE and disease

APOE and Alzheimer's disease

Alzheimer's disease is the most common form of dementia and is neuropathologically defined by the combined presence of extracellular amyloid-beta (Aβ) plaques and intracellular neurofibrillary tangles of phosphorylated tau protein (43) in the brain of patients. *APOE* ε4 is the main genetic risk factor for AD, being associated with a semi-dominant inheritance of late onset AD (LOAD) (44,45). The impact of *APOE* ε4 in AD was reported for the first time in 1993 (5) and since then *APOE* has been a constant hit in genome wide association studies (GWAS) when studying AD samples from different populations (44–49). In the Portuguese population, Fernandes and collaborators (50) as well as Rocha and collaborators (51) also demonstrated that *APOE* ε4 is more frequent in AD patients when compared to controls. The last study obtained an odds ratio of 5.93 for the association of *APOE* ε4 with the risk of developing AD (51).
The risk for LOAD is dose related: it is 2-3-fold higher in individuals carrying one *APOE* ε4 allele and increases to 12-fold if carrying two copies of *APOE* ε4 (52). *APOE* ε4 also reduces the age of onset in a dose dependent manner (53). Contrary to *APOE* ε4, the *APOE* ε2 allele has been shown to be associated with a reduced risk and increased age at onset of AD (28,54,55).
The exact mechanism through which *APOE* ε4 influences AD pathogenesis is still not fully known. APOE and Aβ were found co-localised in senile plaques, in amyloid deposits found in vessel walls and in neurofibrillary tangles of AD patients (56). *APOE* ε4 carriers have a higher amyloid load in their brains than non-carriers (57). Several studies have associated APOE with Aβ metabolism, aggregation and deposition. Recently, Huang and collaborators showed that APOE binding to APOE receptors activates the DLK-MKK7-ERK1/2 cascade, followed by cFos phosphorylation and stimulation of transcription factor AP-1, culminating in *APP* transcription and Aβ production (**Figure 2**). Activation of this pathway was stronger for APOE4 than for APOE3 or APOE2 (58). It has also been shown that lipidated APOE binds to soluble Aβ in an isoform-dependent manner (APOE2 > APOE3 >> APOE4) (59). It also promotes Aβ clearance by different mechanisms, such as uptake and degradation by astrocyte, microglia and neurons (60), clearance through the BBB (61) and extracellular proteolytic degradation (62), in the same isoform-dependent manner, which leads to a reduced clearance in the presence of APOE4 isoform (63). APOE is known to promote Aβ fibrillization, aggregation and deposition in an opposite isoform-dependent manner (APOE4 >> APOE3 > APOE2) to that mentioned for Aβ clearance (63–65).
APOE4 also contributes to AD pathogenesis independently of Aβ **(Figure 2)**. It has been shown to: increase tau phosphorylation and neurofibrillary tangle formation (66,67); disrupt mitochondrial function due to lower

levels and activity of mitochondrial respiratory enzymes (68); reduce cerebral glucose metabolism (69,70); be associated with a less efficient transport of lipids and cholesterol, required for membrane repair and synaptic plasticity (71); increase the levels of iron in the brain (72); reduce the anti-inflammatory properties of APOE (73) and compromise vascular integrity and function, related with the accelerated pericyte loss (74).

More specifically, APOE4 delays the recycling after endocytosis of APOE receptors back to the membrane. These receptors interact with PSD95 and NMDAR leading to Ca^{2+} influx increasing long-term potentiation (LTP) (75,76). The Ca^{2+} influx also leads to ERK1/2 phosphorylation that activates CREB. CREB promotes transcription of *AID* and *BDNF*, which provide broad-spectrum neuroprotective effects (77). In addition, APOER2 and VLDLR are two reelin and APOE receptors also involved in tau phosphorylation and actin polymerization regulation. Reelin inhibits tau hyperphosphorylation through the DAB1-PI3K-AKT pathway that inactivates GSK3β, required for tau phosphorylation and LTP increase. PI3K activated by reelin also activates LIMK1 that inhibits cofilin, reducing cofilin actin-depolymerizing activity, which leads to actin polymerization and dendritic spine growth increase (78–80). However, Aβ oligomers have the opposite effect of reelin activation leading to GSK3β activation and LIMK1 blockage (81,82). As a consequence of APOER2 and VLDLR retention due to APOE4, the reelin pathway is blunted and loses its capacity to inhibit tau phosphorylation, to promote LTP and actin polymerization and dendritic spine growth. These receptors also regulate JNK activation (83,84), through JIP1/2 (85). JIP1/2 inhibits JNK signalling, a protein that contributes to inflammation (85). In the presence of APOE4 this pathway is impaired leading to a lower APOE-mediated anti-inflammatory effect (73).

More recently, it was also shown that APOE contributes to the changes in microglia phenotypes observed in neurodegenerative diseases. APOE present in lipoproteins or bound to apoptotic neurons binds to TREM2 leading to their phagocytosis. After this, APOE mediates a switch from a homeostatic to a neurodegenerative microglia phenotype (86,87). No APOE isoform-dependent differences in binding affinity between TREM2 and APOE were found (88,89) and further studies will be required to understand the impact of the different APOE isoforms in the changes of microglia phenotypes. However, TREM2 expression was found to be reduced in the presence of APOE4 comparatively to APOE3 (90).

In addition to the role of APOE2, APOE3 and APOE4 isoforms in AD, the effect of other variants in the gene has also been studied. Three promoter polymorphisms, -491A/T (rs449647), -427T/C (rs769446), and -219T/G (rs405509), have been extensively studied but the results regarding an association with AD risk have not been consistent. In 1998, Lambert and collaborators after studying 49 LOAD patients and 45 controls reported an increased risk of occurrence of AD associated with the T allele of -219T/G polymorphism, a decreased risk associated with T allele of -491A/T polymorphism and no association with AD for the -427T/C polymorphism (91). Limon-Sztencel and collaborators also found a protective effect of the G allele of -219T/G polymorphism (82). However, in a more recent meta-analysis carried out by Xiao and collaborators the C allele of -427T/C was associated with an increased risk of AD, while the other two polymorphisms did not show association with the disease (92). The intronic polymorphism +113G/C (rs440446) was found in linkage with *APOE* ε4 allele (93), and further studies did not find an independent association with AD (82,94). The intronic SNP rs769449 was found associated with a reduction in $A\beta_{42}$ levels and an increase in tau and $ptau_{181}$ levels in CSF (95,96).

In 2014, Medway and collaborators reported the impact of p.Leu28Pro, p.Arg145Cys and p.Val236Glu in LOAD risk. The authors concluded that p.Leu28Pro was in complete linkage disequilibrium with *APOE* ε4, not representing an independent association with LOAD risk; the p.Arg145Cys was too rare to be analysed, but p.Val236Glu was associated with a decreased risk of LOAD (OR = 0.10) independently of *APOE* ε2, *APOE* ε3 and *APOE* ε4 haplotypes (97).

APOE and other neurological diseases

The association between *APOE* and Dementia with Lewy bodies (DLB) has been repeatedly demonstrated. In fact, *APOE* is the strongest genetic risk factor for DLB. *APOE* ε4 is associated with increased risk for DLB with an *APOE* ε4 allele frequency established within 24% and 32% in DLB patients in comparison to 7%-15% in controls (98–102). Recent studies revealed that the APOE4 isoform confers a shorter disease duration and earlier age of death (99,103,104). Similarly to AD, the *APOE* ε2 allele reduces the risk for the development of DLB and delays the onset of disease (105).

In a recent study with patients with Parkinson's disease (PD), Mengel and collaborators did not find *APOE* ε4 affecting cognitive performance (106), contradicting previous results where this allele was associated with worse cognitive performance (107,108). In 2004, a meta-analysis showed a positive association between *APOE* ε2 and sporadic PD (109), which was not replicated in recent genome-wide association studies.
The role of APOE in frontotemporal dementia (FTD) remains unclear too. Like for AD, APOE2 has been suggested to have a protective effect while APOE4 has been associated with an increase in risk (110–112). However, other studies presented APOE2 as a risk factor for FTD (113). Authors argued that the presence of an APOE association with clinical FTD cases is most likely due to the inclusion of cases misdiagnosed as FTD that are in fact AD cases (114). Small studies found APOE4 carriers to show a more severe brain atrophy in specific regions (115).
Cerebrovascular disorders are influenced by APOE isoforms as APOE4 is known to disrupt the BBB by reducing the blood flow, increasing its leakiness and incorporating neurotoxic proteins (74,116). APOE polymorphisms are significantly associated with susceptibility to vascular dementia (117). For ischemic stroke, APOE4 is a risk factor as well, especially in Asian populations, but APOE2 does not seem to be protective (118–120). In an association study of hemorrhagic stroke cases, strong independent hits were found for both APOE2 and APOE4 (121).
The role of APOE in schizophrenia is not completely clear. In 1995, Harrington and collaborators found an increased frequency of *APOE* ε4 allele in schizophrenic patients, considering this allele as a risk factor for schizophrenia (122). Another study demonstrated that female patients carriers of *APOE* ε4 alleles presented an earlier age of onset and a higher risk of suffering from the negative syndrome subtype of disease when compared to schizophrenic women non-carriers of *APOE* ε4 (123). However, subsequent studies did not replicate this association (124–127). The *APOE* ε2 allele and genotype ε3/ε2 were less frequent in patients with this disease, suggesting that the ε2 allele might have a protective effect (128), but other studies showed contradictory results. A recent study by Al-Asmary and collaborators found higher frequencies of *APOE* ε2 allele and genotypes ε2/ε3 and ε2/ε4 in patients when compared to controls. Interestingly, the authors also found that the frequency of the ε4 allele was significantly higher in patients with positive symptoms. In this study lower frequencies were also obtained for *APOE* ε3 allele and ε3/ε3 genotype (129), which was found by others too (130). A study performed in 60 Mexican families found an increase of female carriers with *APOE* ε3, whereas *APOE*-219G was preferentially transmitted in males (131). These conflicting results may be associated with the number of samples, ethnicity of the studied cohorts or environmental factors. Increased APOE levels were also found in cerebral regions implicated in schizophrenia (132,133).
The impact of *APOE* genotypes has also been studied in bipolar disorder. Early onset bipolar patients presented a higher *APOE* ε4 allele frequency compared to late onset patients or controls (134) and this allele was also associated with worse performance in executive tasks performed by young non-treated patients (135). Similar to schizophrenia conflicting results have also been seen for bipolar disease, with other authors not finding differences in *APOE* allele frequencies between bipolar disorder cases and controls (136,137). In this disease a decreased plasmatic expression of *APOE* was reported (138) and *APOE* expression in the brain was also found to be region specific in patients with this disease (139).
The *APOE* ε2 allele was reported to have a protective effect in major depressive disorder in Taiwanese patients (140). A meta-analysis performed in 2008 confirmed the same result in Caucasians (141). However, no association between *APOE* and major depressive disorder was found in 17,507 British adults (142) and in Russian patients (143). Again, some studies have shown an association between late-life depression and *APOE* ε4 allele (144), while others did not show a significant association between *APOE* genotype and this disease (145).
Several studies have also been carried out to understand the impact of *APOE* in multiple sclerosis (MS) (146), but the conclusions remain controversial. Some studies have reported *APOE* ε4 as a risk factor for MS or associated with progression of cognitive deficits (147,148). However, absence of association was also found (149,150). Studies in the Portuguese population did not identify any correlation between *APOE* genotype and MS (151,152).

APOE and other diseases

The impact of APOE in non-neurological conditions has also been recognised decades ago. Due to APOE2 reduced capacity to bind to LDL receptor, the presence of two *APOE* ε2 alleles is associated with the

recessive form of type III hyperlipoproteinemia. However, this allele does not have a complete penetrance: its presence is necessary but not always sufficient to induce the disease. Furthermore, several rare *APOE* mutations have been described as causative of a dominant form of type III hyperlipoproteinemia and the majority of these mutations involve substitutions of arginine or lysine residues located in the receptor-binding region (153,154). Other *APOE* mutations are causative of lipoprotein glomerulopathy, a dominant disorder with incomplete penetrance involving the kidney. In this disease, the most common *APOE* mutations are located in the LDL-receptor binding domain (154,155). *APOE* ε4 has been associated with increased LDL cholesterol levels and consequent increased cardiovascular risk, including in Portuguese individuals (31).
APOE genotypes have also been associated with viral infections. Carriers of the *APOE* ε4 allele were shown to develop more recurrent cold sores caused by HSV-1 (156) and higher rate of oral herpetic lesions (157). The offspring of APOE4 mice female progenitors were found to have higher HSV-1 levels in the brain comparatively to those of APOE3 female progenitors (158). *APOE* ε4/ε4 genotype was also found to be associated with an accelerated HIV infection and progression to death when compared with the *APOE* ε3/ε3 genotype (159). Other studies did not confirm the association between *APOE* genotype and time of death caused by HIV (160). APOE is also known to be necessary for HCV assembly and release (161). In this case and contrary to HSV-1 and HIV, studies suggest that *APOE* ε4 allele has a protective role in HCV infection (162,163).

Clinical implications of *APOE* genotype in AD

The genetic risk prediction of complex LOAD is not straightforward. Although *APOE* ε4 is the main LOAD genetic risk factor, it is neither necessary nor sufficient to cause LOAD and its testing is largely not recommended in a clinical setting due to the absence of current effective therapies or preventive options. Additionally, different studies have shown that different factors such as sex, ethnic group, environmental exposure and genetic modifier variants may influence *APOE* ε4 risk and complicate the interpretation of results (28,164). More recently it has been shown that the combination of non-*APOE* alleles significantly improves LOAD risk prediction over *APOE* alone. These different genetic variants can be combined into a polygenic risk score to improve predictive ability. The results also improve when considering other characteristics such as family history of disease, age at onset and biomarkers. However, so far, this has not yet achieved the values of sensitivity and specificity required for clinical use (165–167).
Clinical trials have been conducted to reduce Aβ production or aggregation, or to facilitate Aβ clearance. The genetic study of individuals included in these trials may contribute to better results. In the TOMORROW trial the risk prediction for LOAD includes *APOE* and *TOMM40* genotypes (168). Furthermore, a clinical trial using an anti-Aβ antibody did not reveal a significant efficacy, but potential differences were found between *APOE* ε4-negative and *APOE* ε4-positive individuals, suggesting that individuals without the *APOE* ε4 allele had a better response to the antibody and, consequently, that this drug could be more useful for these patients (169). Together these data indicate that genetic information of cohorts included in clinical trials should be taken into account and suggest the utility of genetic stratification.
Therapeutic options based on APOE have also been explored. Some examples are the modulation of the structure of APOE4 in order to make it similar to that of APOE3; regulation of APOE levels; inhibition of APOE aggregation and proteolysis; use of APOE-mimetic peptides; gene therapy directed towards APOE; blockage of the APOE/Aβ interaction; and modulation of the APOE lipidation state (19,63).

Conclusions

Two common polymorphism in *APOE* produce three isoforms with structural differences. As a consequence the three isoforms have different functions in lipid transport, in brain homeostasis and neuronal plasticity. It is clear that the ε4 allele of *APOE* is the major genetic risk factor for AD and DLB. However, the exact mechanisms involved in the pathogenesis of these diseases are not known yet, but seem to include Aβ-dependent and -independent pathways. Other neurological conditions have also been associated, in some studies, with *APOE* genotype. However, in these cases, the results have been inconsistent over the years and the role of APOE in these conditions remains largely inconclusive. The *APOE* ε2 allele and rare variants are associated with lipidic disorders, sometimes, with cardiovascular consequences.

With the increase in the number of sequencing studies being performed, novel variants are being identified in known and new diseases. This will allow for a better understanding of the role of *APOE* in disease as well as the impact of the different variants in risk prediction and penetrance.
In AD, the genetic study of APOE already allows for the identification of individuals with high risk for the development of the disease and can, in the future, permit early-life interventions. Given the important genetic role of APOE in this disease, it should not only be considered in clinical trials, but should also be the focus of new therapeutical strategies.

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Tables

Table I - *APOE* variants with alternative allele frequency > 1% present in the gnomAD database. Positions are given according to GRCh37/hg19. Protein positions are relative to the translated protein containing the peptide signal of 18 amino acids. Global AAF represents alternative allele frequencies for the global population and were obtained from the gnomAD database (http://gnomad.broadinstitute.org/). Chr: Chromosome; AAF: alternative allele frequencies.

Figures

Figure 1 - APOE locus, gene and protein.
In the middle panel (gene) the light blue areas represent untranslated regions 5' and 3', while the dark blue areas correspond to the coding region of the exons. The protein (bottom panel) is divided in three regions: N-terminal region (blue) containing the receptor-binding domain (red), the hinge region (between amino acids 168 and 205), and the C-terminal region (green) with the lipid-binding domain (yellow). The two polymorphisms, in positions 112 and 158 of the protein, that distinguish the three more common APOE isoforms (APOE2, APOE3 and APOE4) are located in the N-terminal region. Adapted from ([6,11]).

Figure 2 - Signaling pathways affected by APOE4.
APOE binds to different receptors leading to the activation of several pathways and receptor endocytosis. The presence of APOE4 leads to lower levels of APOE receptors and NMDAR in the membrane. This results in more inflammation, tau hyperphosphorylation, actin depolymerization, increased LTP and reduced levels of neuroprotective molecules. In addition, APOE4 stimulates the production of APP, leading to higher levels of Aβ in the brain. All these factors contribute for the development of AD.

Table I

Chr	Position	rsID	Reference	Alternative	Transcript Consequence	Protein Consequence	Type	Global AAF	European (Non-Finnish) AAF
19	45409579	rs769448	C	T			intron	0.02034	0.03342
19	45412079	rs7412	C	T	c.526C>T	p.Arg176Cys	missense	0.06538	0.07669
19	45410002	rs769449	G	A			intron	0.09179	0.1145
19	45411941	rs429358	T	C	c.388T>C	p.Cys130Arg	missense	0.14254	0.14893
19	45410444	rs769450	G	A			intron	0.39341	0.40434
19	45409167	rs440446	C	G			intron	0.62118	0.63565

Figure 1

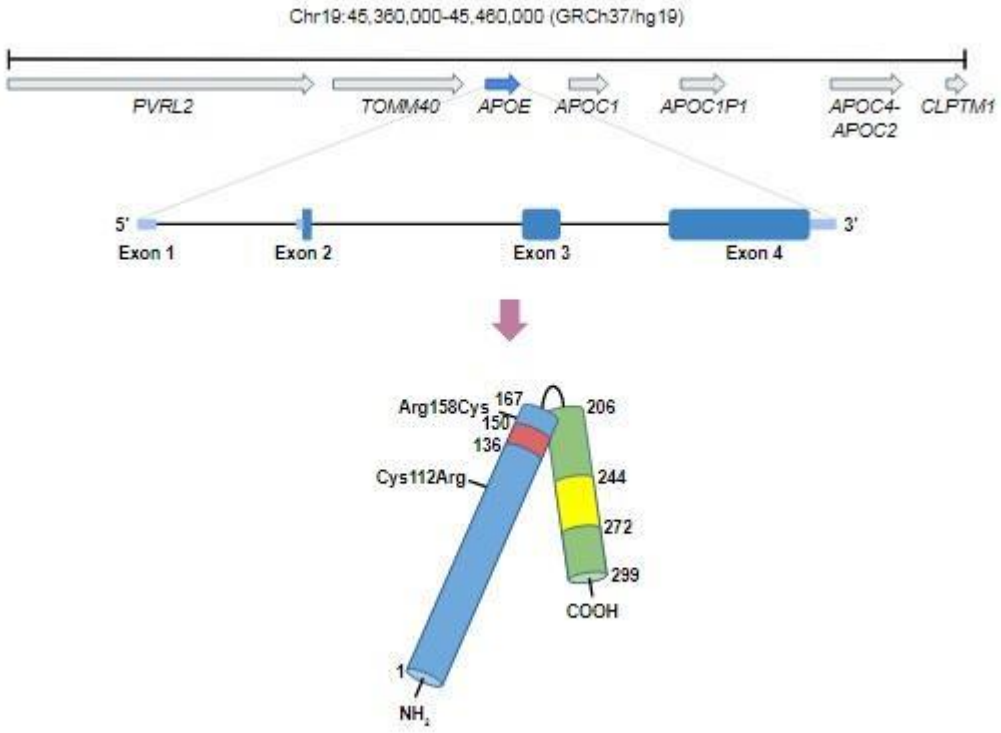
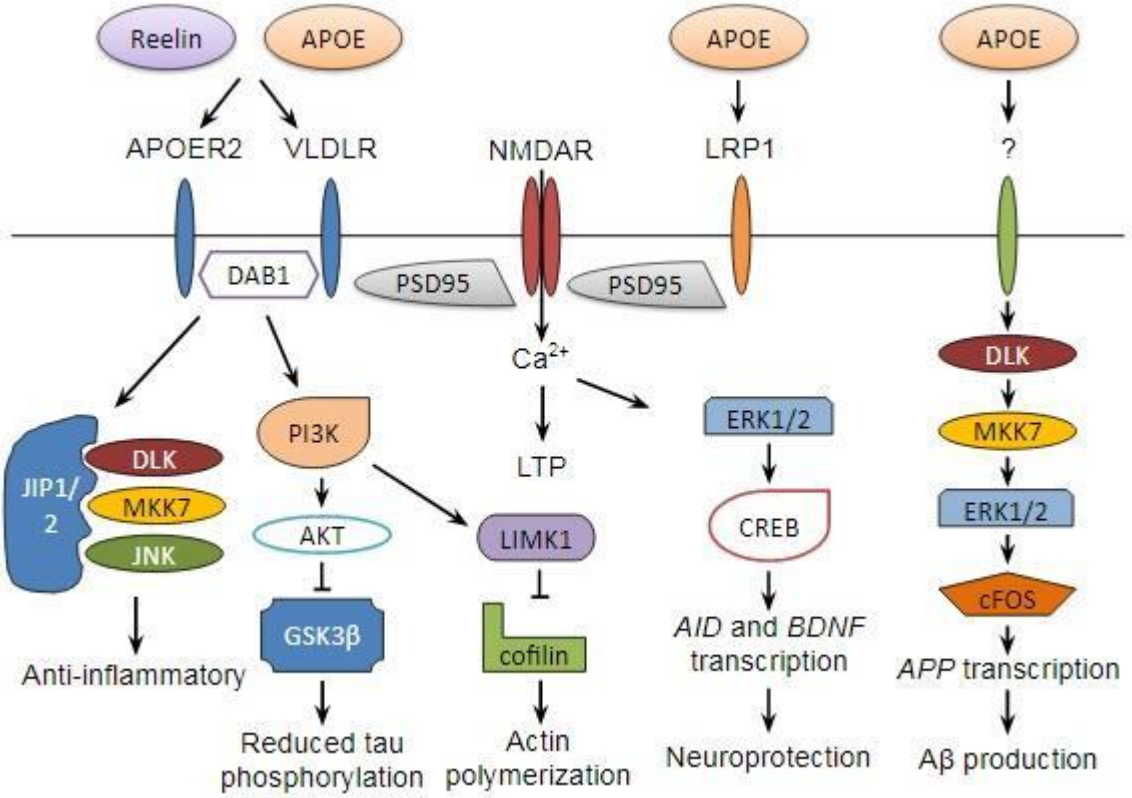

Chr19:45,360,000-45,460,000 (GRCh37/hg19)
PVRL2
TOMM40
APOE
APOC1
APOC1P1
APOC4-
APOC2
CLPTM1
5'
3'
Exon 1
Exon 2
Exon 3
Exon 4
Arg158Cys
167
150
136
206
Cys112Arg
244
272
299
COOH
1
NH2

Figure 2


Reelin
APOE
APOE
APOE
APOER2
VLDLR
NMDAR
LRP1
?
DAB1
PSD95
PSD95
Ca2+
DLK
MKK7
JNK
JIP1/
2
PI3K
AKT
GSK3β
LTP
LIMK1
cofilin
ERK1/2
CREB
AID and BDNF
transcription
DLK
MKK7
ERK1/2
cFOS
APP transcription
Anti-inflammatory
Reduced tau
phosphorylation
Actin
polymerization
Neuroprotection
Aβ production