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Evolution of human apolipoprotein E (APOE) isoforms: Gene structure, protein function and interaction with dietary factors

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ABSTRACT

Apolipoprotein E (APOE) is a member of the vertebrate protein family of exchangeable apolipoproteins that is characterized by amphipathic α-helices encoded by multiple nucleotide tandem repeats. Its equivalent in flying insects – apolipophorin-III – shares structural and functional commonalities with APOE, suggesting the possibility of an evolutionary relationship between the proteins. In contrast to all other known species, human APOE is functionally polymorphic and possesses three major allelic variants (*ε4*, *ε3* and *ε2*). The present review examines the current knowledge on *APOE* gene structure, phylogeny and APOE protein topology as well as its human isoforms. The *ε4* allele is associated with an increased age-related disease risk but is also the ancestral form. Despite increased mortality in the elderly, *ε4* has not become extinct and is the second-most common allele worldwide after *ε3*. *APOE ε4*, moreover, shows a non-random geographical distribution, and similarly, the *ε2* allele is not homogenously distributed among ethnic populations. This likely suggests the existence of selective forces that are driving the evolution of human APOE isoforms, which may include differential interactions with dietary factors. To that effect, micronutrients such as vitamin D and carotenoids or dietary macronutrient composition are elucidated with respect to APOE evolution.

Contents

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1. Introduction

Apolipoprotein E (APOE) is an amphipathic plasma protein that is found in terrestrial and marine vertebrates, including mammals, reptiles and fish. The tertiary APOE structure is dominated by the N- and C-terminal domains (NT and CT) and their physical interaction, the so-called domain interaction. Although APOE protein size and amino acid sequences differ between vertebrate classes and species, the general structure and function are conserved. APOE belongs to the family of exchangeable apolipoproteins, where its major role is to mediate lipid transfer between circulating lipoproteins and tissues by binding to membrane receptors. In addition, APOE nonspecifically binds to lipophilic inflammatory components with high affinity, including amyloid beta, lipopolysaccharides, lipoteichoic acids and beta-glucans, which leads to the clearance of pathogenic agents and contributes to the first-line innate immunity response (de Bont et al., 1999; Deroo et al., 2015; Nankar and Pande, 2013; Palusinska-Szysz et al., 2015; Rossignol et al., 2011; Van Oosten et al., 2001).

Importantly, three major APOE isoforms exist in humans, and this functional *APOE* polymorphism is peerless in the animal kingdom. The human isoforms APOE4, APOE3 and APOE2 arise from the three allelic variants *APOE ε4*, *ε3* and *ε2*. While most publications focus on the increased risk of chronic age-related diseases, such as cardiovascular and Alzheimer's disease in *APOE ε4* carriers, fundamental research on the reasons and mechanisms of evolution and the varying worldwide distribution of the human *APOE* alleles is often neglected. The knowledge on functional differences among APOE4, E3 and E2 is still limited and may have been hampered by the delay in the structural elucidation of the full-length protein, which was achieved in 2011 (Chen et al., 2011), several decades after its discovery in 1975 (Utermann, 1975). The following review will, therefore, also focus on the physiological and metabolic implications associated with the differences in the protein structure and topology of the two major human isoforms, APOE4 and APOE3. In addition, potential evolutionary scenarios such as interaction with dietary (micro)nutrients will be used to explain the biological role of APOE isoforms and their distribution among different populations.

2. APOE is part of a phylogenetically old protein family

APOE is part of a phylogenetically old protein family that contains the Pfam family domain PF01442 (Apolipoprotein A1/A4/E domain) that has been aligned to protein sequences of a few hundred species (http://pfam.xfam.org/). The corresponding proteins are widely expressed across the animal kingdom but are also found in bacteria, therefore dating the evolution of this domain family long before metazoan development to a time point before eukaryotic divergence from prokaryotes. In humans, the apolipoproteins E, A1, A4 and A5 can be aligned to the family. Together with APOC1-3, they also group as exchangeable apolipoproteins that distinguish by structure and biological function from the non-exchangeable apolipoproteins B-100 and B-48 (*APOB*). Non-exchangeable apolipoproteins and other lipid-exchange proteins are members of a multispecies, multigene super-family that is present in vertebrates and invertebrates (Babin et al., 1999). Prominent members, such as mammalian APOB or apolipophorin and vitellogenin present in invertebrates, share conserved amino acid motifs that suggest divergence from a common ancestral predecessor.

To investigate the hypothesis that exchangeable apolipoproteins likewise cluster in a multigene super-family that may be traced back very early in the metazoan linage, phylogenetic analyses using protein sequences from representative species at different steps in the eukaryotic evolution were performed (Fig. 1A). It appears that proteins similar to apolipoproteins are already present in *Lophotrochozoa, Cnidaria* (early *Eumetazoa*) and even choanoflagellates (closest living relative of animals, Fig. 1B) and cluster either with human APOE or APOB as equivalents of exchangeable or non-exchangeable apolipoproteins. These data suggest that the ancient predecessors of all apolipoproteins already existed in very early eukaryotes before the metazoan divergence occurred and that these common sequence motifs have spread throughout the animal kingdom and have individually evolved with increasing phyla, orders and species. For instance, insect apolipophorin-III (ApoLp-III) clusters in a branch with human APOE, and both are likewise distant to the equivalent sequences from sponges (Porifera) or choanoflagellates, which represent preceding stages of evolution. The similarity and evolutionary relationship of ApoLp-III and APOE will be discussed in more detail below.

2.1. The multigene family of exchangeable apolipoproteins

In the 1980s, the nucleotide sequences of rodent *APOC, APOA* and *APOE* were analyzed and summarized as the mammalian multigene family encoding exchangeable apolipoproteins (Allan et al., 1995; Boguski et al., 1986; Li et al., 1988). The similarity between the multigene family members reaches 40% at the nucleotide but only 15% at the amino acid level. Almost all human exchangeable apolipoprotein genes consist of four exons, whereby the mature proteins are encoded by exons 3 and 4. Several attempts have been made to explain sequence evolution and phylogenetic relationships between the family members. It has been discovered that members of the *APOC/APOA/APOE* family contain 11-nucleotide sequence repeats, suggesting the preexistence of a common ancient primitive minigene that by manifold duplication and modification gave rise to the different apolipoprotein genes (Luo et al., 1986; Rajavashisth et al., 1985). It was reported that *APOC1, C2, C3, A1, A4* and *APOE* contain a homologous block of 33 codons upstream from the exon 3/intron 3 junction. Their respective exons 4 appear to consist of more variable numbers of 11-codon-repeats, suggesting that the evolution of individual family members was caused by intraexonic modification in exon 4. In the case of *APOA4*, for instance, exons 3 and 4 seemed to have fused, resulting in a great increase in the length of exon 3 and the absence of a fourth exon. Based on the simple assumption that exon length and sequence complexity (as the sequence deviates from tandem repeats) reflect the extent of divergence and phylogenetic distance, a time plan for the evolution of the mammalian apolipoprotein genes was proposed. According to this hypothetical scheme, the multigene family originated from a primordial apolipoprotein gene that may have been very similar to *APOC1* (Luo et al., 1986), which is the shortest among the mammalian apolipoprotein genes (Table 1).

There were several attempts to estimate the emergence of the common sequence (the primordial apolipoprotein) with quite diverging results, dating the evolution of *APOC1* to 0.5 up to 1.2 billion years ago (Barker and Dayhoff, 1977; Luo et al., 1986). The latter estimation would comply with the existence of distantly related apolipoprotein-like proteins in choanoflagellates and early metazoan species that have been identified in our phylogenetic analysis (Fig. 1A). *APOE* is widely expressed among vertebrates, including fish, reptiles and mammals, but apparently not in birds (Babin et al., 1997; Duggan and Callard, 2001). The mammalian and the reptilian gene contains four exons, which is in contrast with the fish gene that contains five exons. However, the encoded protein increases in length from fish to mammals due to intronic deletion and intraexonic elongation during the course of evolution.

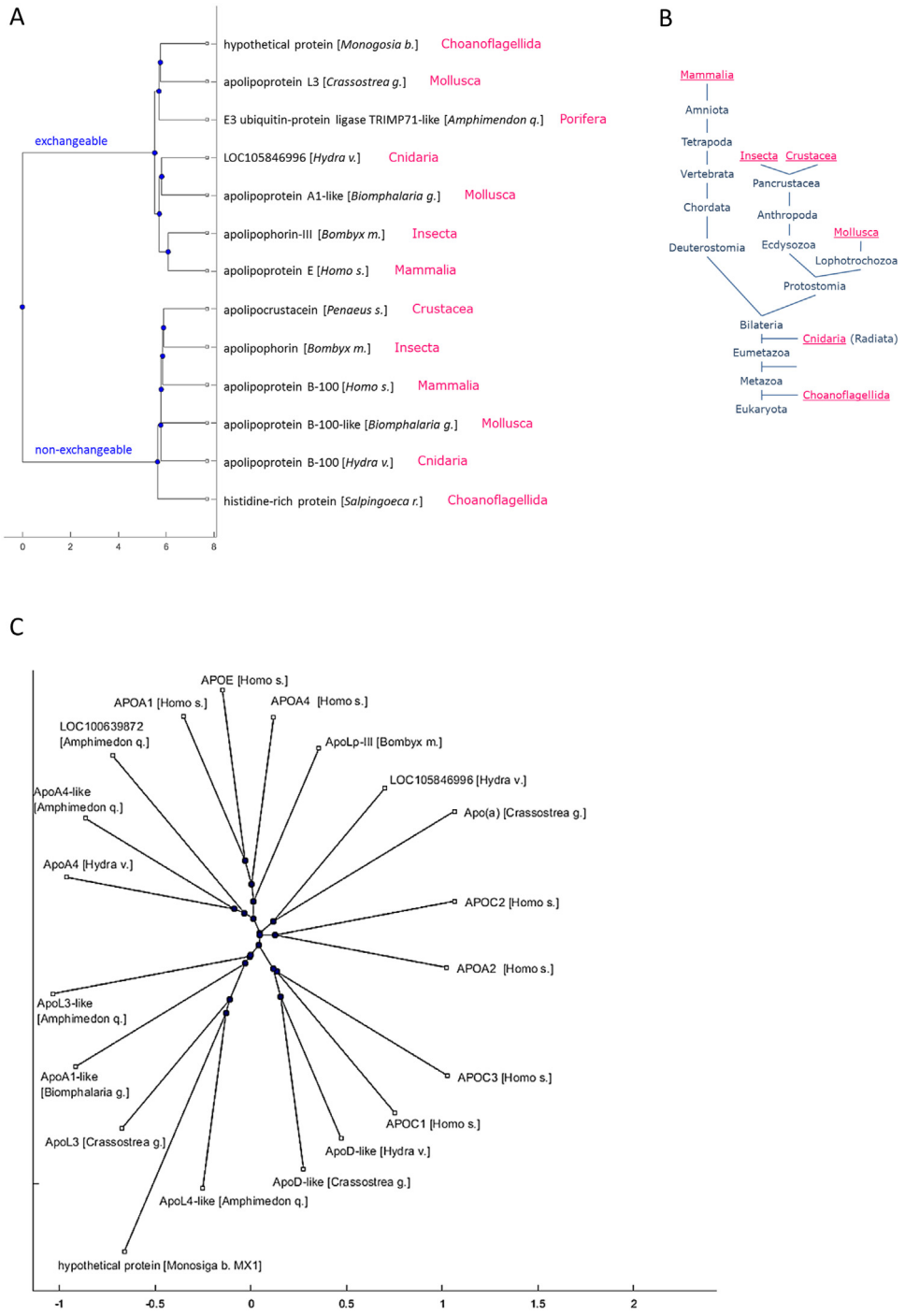

Fig. 1. Evaluation of a potential phylogenetic relationship of exchangeable and non-exchangeable apolipoproteins among eukaryotes based on protein sequence. A) Construction of a phylogenetic tree from pairwise distances (GONNET matrix) of representative species along the eukaryotic lineage (B) showing the separation into exchangeable and non-exchangeable apolipoproteins in all species. Neighbor-joining tree of numerous exchangeable apolipoproteins indicates a close relationship of human APOE and APOA with insect ApoLp-III (C).

2.2. Evaluation of phylogenetic relationships between human APOE and insect apoLp-III

Given the relatively high similarity of APOE and ApoLp-III among eukaryotic apolipoproteins (Fig. 1), we compared the nucleotide and protein sequences of ApoLp-III and the human *APOC/APOA/APOE* multigene family. We also included APOA5, which is a more recently identified exchangeable human apolipoprotein that was absent in the earlier alignments from 1986. According to the hypothesis that the mammalian multigene family diverged from a primordial apolipoprotein sequence (Luo et al., 1986), we compared the nucleotide sequences of exons 3 and 4, which code for the mature proteins. We calculated pairwise distances, including *ApoLp-III* (exons 2, 3, and 4) in our analysis (Fig. 2A). The analyzed sequences grouped into two close clusters, whereby *APOE*, *APOA1*, *APOA4* and *APOA5* are relatively distant from the others. This may at least partly be due to the differences in nucleotide counts of the protein coding sequences (Table 1). *APOC1* has been proposed to be the oldest gene, and in our analysis, exhibited the greatest distance from *APOA5,* suggesting that the latter

Table 1
The human multi-gene family of *APOC*, *APOE* and *APOA* and the insect *ApoLp-III*. Exons three and four (and exon two* of *ApoLp-III*) encode the mature proteins. Presence of amino acid patterns specific for insect ApoLp-III or mammalian APOE was examined using PattInProt (70% similarity threshold). ApoLp-III data refer to the *Bombyx mori* gene, except for results of pattern recognition that refer to the consensus sequence calculated from the protein sequence of the following species: *Bombyx mori, Manduca sexta, Nilaparvata lugens, Apis mellifera, Culex quinquefasciatus, Tenebrio mollitor, Locusta migratoria*.

Gene	Exon 3 [nt]	Exon 4 [nt]	Mature protein [aa]	Pfam domain	ApoLp-III motif	APOE motif
APOC1	136	177	57	04691	–	–
APOA2	133	230	77	04711	–	–
APOC2	160	407	79	05355	–	–
APOC3	124	308	79	05778	–	–
ApoLp-III	207*+159	176	164	07464	6 x	2 x
APOA1	157	659	243	01442	3 x	1 x
APOE	193	863	299	01442	3 x	3 x
APOA4	1180	–	376	01442, 06386	13 x	2 x
APOA5	112	1698	343	01442	2 x	1 x

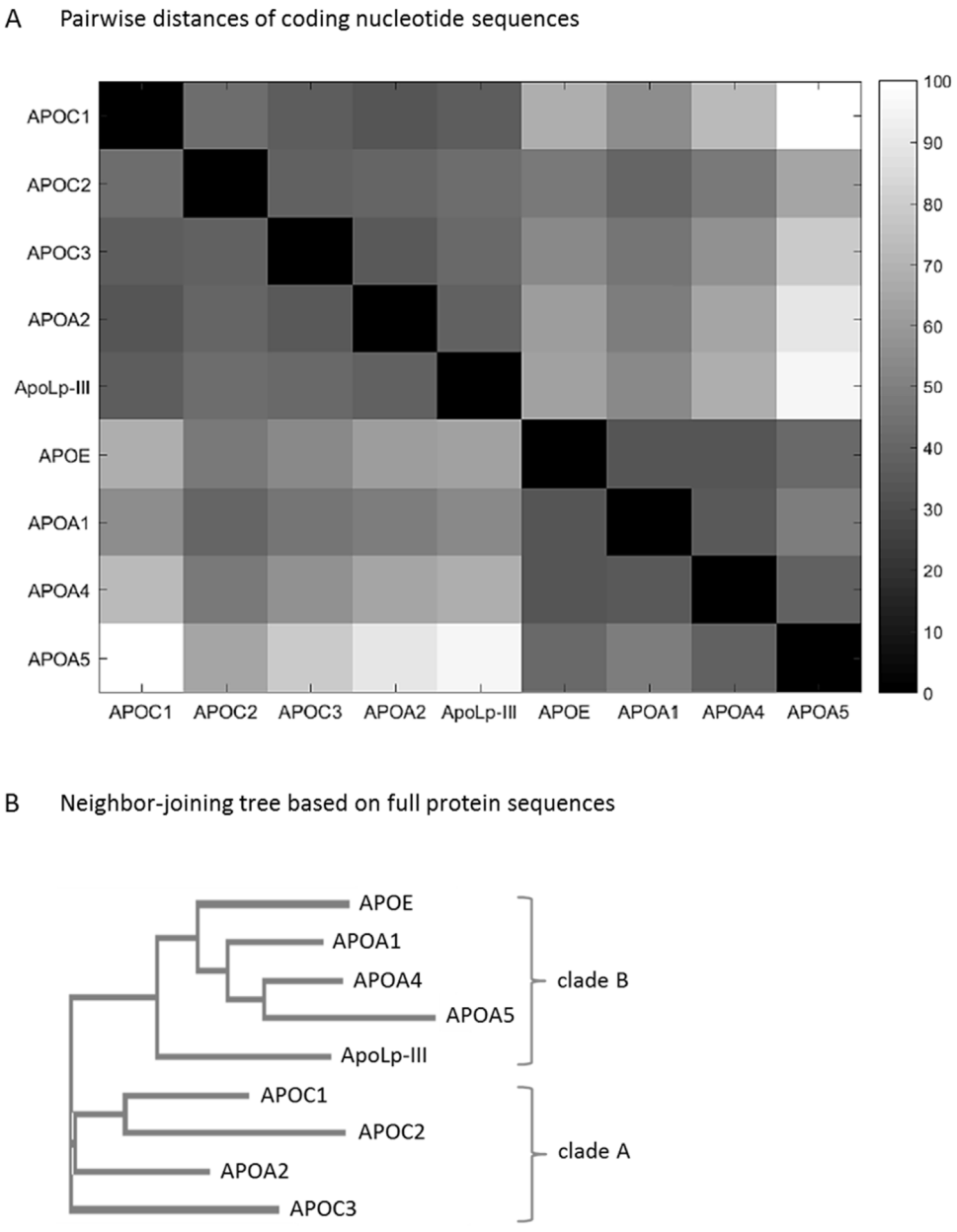

Fig. 2. Comparison of the nucleotide and protein sequences of human exchangeable apolipoproteins with insect ApoLp-III. A) Pairwise distance matrix comparing the coding nucleotide sequences: exons 3 and 4 of human *APOC1*, *C2*, *C3*, *A1*, *A2*, *A5* and *E* as well as exons 2–4 of *ApoLp-III* from *Manduca sexta*. Pairwise distance values [%] are associated with the maximum value. B) Phylogenetic tree from multiple-sequence global alignments using Fast Fourier Transform. The tree was built using the Neighbor-joining algorithm with distance correction using the web tool MAFFT (http://www.ebi.ac.uk/Tools/msa/mafft/). Branch length is proportional to the inferred evolutionary change.

may have evolved most recently. This may be true despite the great differences in exon length between *APOC1* and *APOA5*, considering the fact that *APOA4* is similar in length to *APOA5* but shows a lower distance value. *APOA1* and *APOC2* show the lowest overall distance to the others, implying that these two genes may be closest to the common predecessor and therefore are the least diverged. It is important to note that the sequence of *ApoLp-III* seems to fit into the human multigene family, exhibiting a relatively low distance to *APOC1-3* and *APOA2*. We then compared full protein sequences and constructed a phylogenetic tree using the webtool S*imple Phylogeny* (EMBL-EBI) (Goujon et al., 2010; Larkin et al., 2007). In brief, pairwise distances of global alignments were used for tree construction using the Neighbor-joining algorithm with distance correction via the MAFFT tool (Li et al., 2015). It appears that two major clades are formed with APOC1, C2 and APOA2 in one (clade A) and APOE, APOA1, A4 and A5 in the second (clade B) (Fig. 2B). ApoLp-III clusters together with APOE in the latter clade, while APOC3 is positioned separately but close to clade A.

Based on these novel results, the following evolutionary hypotheses may be deduced: i) (human) exchangeable apolipoproteins may be separated into two to three clades according to their protein sequence, ii) ApoLp-III is structurally similar to APOE and APOA1, 4 and 5, and iii) it may be possible that the divergence of the exchangeable apolipoproteins (into clade A and B) occurred at an early evolutionary time point before the bilateral lineage split. Divergence of vertebrate APOE and insect ApoLp-III would have subsequently occurred, which implies that these proteins are actually relatives that descended from a close common ancestor. The bilateral lineage split has been dated to a period 570–660 million years ago (Peterson et al., 2004). The dating of apolipoprotein gene evolution, however, remains a vague estimate, particularly given the high rate of nucleotide substitution that exceeds the average rate for mammalian genes (Luo et al., 1986). Evolution of *ApoLp-III* is supposed to coincide with the emergence of flying insects approximately 0.5 billion years ago (Smith et al., 1994).

2.3. Conserved amino acid patterns in APOE and ApoLp-III

The protein structure of APOE is dominated by the high proportion of amphipathic alpha-helices and internal sequence homology, which is common to all known vertebrate homologues. There is also an encoded signal peptide of approximately 20 amino acids in length that is absent in the mature protein. Despite the high species diversity, all known APOE homologues exhibit the LDL receptor binding domain, which consists of seven or eight basic amino acid residues. APOE from mammals, fish and reptiles shows up to 30% similarity at the amino acid level (Babin et al., 1997; Duggan and Callard, 2001; Durliat et al., 2000; Wang et al., 2014).

In mice, rats and humans, APOE is composed of internal repeats of 11 and 22 amino acid residues (encoded by the *APOC/APOA/APOE* family homologous block in exons 3 and 4) that build the amphipathic α-helical structure of the protein. These sequence repeats contain the conserved amino acid motif [DE]-[DE]-x-*R*-x-*R*-L-G that is inherent in mammalian APOE (Rajavashisth et al., 1985). Sequence homology between different mammalian species varies between 61 and 98% with 47 (out of 299) highly conserved residues and five conserved larger amino acid clusters (Frieden, 2015). These highly conserved cluster regions either include important binding sites (e.g., for lipids, receptors and thyroid hormones) or propagate structural conformation and spatial behavior of the mature protein (Benvenga et al., 1993; Frieden and Garai, 2013), indicating the high functional conservation of APOE (Table 2).

Similar to APOE, insect ApoLp-III contains multiple internal amino acid repeats, although with a higher degree in length variability. For example, Cole et al. identified twelve repeat units throughout the ApoLp-III sequence from *Manduca sexta,* ranging from 7 to 16 (but mostly 14 or 15) residues. In addition the authors observed a common amino acid pattern inherent in the first eight residues of each repeat based on their hydropathy and charge: hydrophobic – acidic/amide – acidic/amide – hydrophobic –hydrophilic – basic – basic – hydrophobic (Cole et al., 1987). This amino acid pattern appears to be quite similar to the APOE pattern described above, particularly the tandemly repeated appearance of acidic residues ([DE]-[DE]) and the repetition of arginine (R) as the basic amino acid followed by a small hydrophobic residue such as leucine (L).

Table 2
Functionally or structurally important residues of human APOE compared to those residues identified as highly conserved among diverse mammalian species.

Residues supposedly involved in biochemical functions		Highly conserved residues
Thyroid hormone binding:	26–40	26, 34–49
Domain interaction:	131–164	136, 142–151 &
Receptor binding:	130–155	155–165
Oligomerization site:	230–243 &	229, 235, 242, 246
	262–270 &	253–280
	271–287	
Lipid binding:	261–272 &	
	250–290	
Domain interaction:	270–290	

To substantiate the assumption of common evolution, the existence of APOE and ApoLp-III amino acid patterns was examined in the consensus sequences derived from representative species. For APOE, eight vertebrates, including fish (*Danio rerio, Oncorhynchus mykiss, Acipenser sinensis*) and amphibian (*Xenopus tropicalis)* as well as terrestrial reptile and mammalian species (*Gekko japonicas, Mus musculus, Pan troglodytes* and *Homo sapiens*) were chosen. The consensus sequence of ApoLp-III was calculated from seven sequences, which represented six different insect orders. Both consensus sequences contained multiple tandemly repeated sequence units of different lengths; in the case of APOE, there were units of 22 amino acids and a few with 12 or 11 amino acids, and in the case of ApoLp-III, there were units of approximately 14/15 and 33 amino acids (Fig. 3). The two consensus sequences were then screened for the specific amino acid patterns using the web pool PattInProt (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_pattinprot.html) with a similarity threshold value of 70%. The specific amino acid motifs were identified in the respective consensus sequences of APOE and ApoLp-III. Moreover, the ApoLp-III-specific amino acid pattern was also identified in the APOE consensus sequence, and the APOE-specific pattern was observed in the ApoLp-III consensus sequence. Although the APOE pattern in the ApoLp-III consensus sequence is not as complete as the ApoLp-III pattern in APOE, it is interesting as it points to common gene evolution. Local alignment (Clustal Omega, http://www.uniprot.org/align/) of the mature protein sequences may provide an additional indication of the divergent regions of the proteins. It appears that APOE contains additional sequences mainly at the N-terminus (residues 1–57) and at the end of the C-terminus (residues 229–280) compared to ApoLp-III (Fig. 3C). Overall, there is amino acid identity of 11% (32 residues) and similarity of 26% (79 residues) between human APOE and ApoLp-III (*Bombyx mori*).

To develop a scenario for common gene evolution based on the abovementioned data, we also searched for APOE and ApoLp-III patterns in the amino acid sequences of the other human exchangeable apolipoproteins (Table 1). The ApoLp-III pattern was also found in other human apolipoproteins but only in those that contain the APOE motif. This may be due to the high similarity between the patterns themselves and emphasizes the phylogenetic neighborhood of APOA1, APOA4, APOA5, APOE and ApoLp-III. Concerning the evolution of exchangeable apolipoproteins, it may be hypothesized

A Repeated amino acid motif:

APOE: x-[**DE**]-[**DE**]-x-**R**-x-**R**-**L**-G-x-x

ApoLp-III: [LAVIFYW]-[**DE**NQ]-[**DE**NQ]-[LAVIFYW]-[CNQSTMH]-[**R**K]-[**R**K]-[**L**AVIFYW]

B Internal repeats:

APOE consensus sequence

ApoLp-III consensus sequence

C

APOE consensus sequence

1 ARRTMKVLWIILALALLSGCHARALFQVETEPEPELHDQVEWEPRSRWEEAIDRFWDYIRWINTRADEIR

70 EELKGSQISRELDGLIDDTMGELKVYRDDLEDKLAPYAEDTRARFGKDLQALQGRLGTDM**EDVRGRL**VQ

140 YRGELRTMLGQNT**DDIRNRI**NTYLRKLRKRLNRDT**DDLRKRL**ATYMEDIREGAERG**IDAIRERL**GPYIS

210 QGRDRAQTIGSLAGQPLRDRAQAWGQRLGALQEAMGDQARDLRDQLKER**AEELRDKL**EETAEDIRTTLE

280 GFADRLRSWFEPYVEDLRRQWEGLMDKIKESVGTSPIIAPVPSDNH

ApoLp-III consensus sequence

1 MAAIFFIVFAIVLVACVMLAHAAMVRRDAPPDNNFFEDIEQHSQEFHNTFSEQFNSVLG

60 LANPKDDEDINKALKDGSDNFLNQLQEFTSSLQGEVKDHNGKVDEALEDVRQNLERT**AE**

120 **DLRK**AAHPDVERK**ANEFRDKL**QAGIQNVVQESQKLAKKISSNAEETNEKLAPKLKEAYD

150 DFVKHTEDIQNKIHEAAAEPEKNSKQ

D

```
ApoLp-III   1   ------------------------------------------------------DA    2
APOE        1   KVEQAVETEPEPELRQQTEWQSGQRWELALGRFWDYLRWVQTLSEQVQEELLSSQVTQEL   60
                                                                        :

ApoLp-III   3   PDFFKDIEHHTKEFHKTLEQQFNSLTKSKDAQDFSKA-------WKDGSESVLQQLNAFA   55
APOE       61   RALMDETMKELKAYKSELEEQLTPVAEETRA-RLSKELQAAQARLGADMEDVCGRLVQYR  119
                  ::.:  :. * ::. **:*:. :::.. *  :**           . *.*  :*  :

ApoLp-III  56   KSLQGALGDANGKAKEAL----EQSRQNIERTAEELRKA--------HPDVEKNATALRE  103
APOE      120   GEVQAMLGQSTEELRVRLASHLRKLRKRLLRDADDLQKRLAVYQAGAREGAERGLSAIRE  179
                 .:*. **::. : :  *    .: *:.: * *::*:*         : ..*:. :*:**

ApoLp-III 104   KLQAAVQNTVQESQKLAKKVSSNVQETNEKLAPKIKAAYDDFAKNTQEVI----------  153
APOE      180   RLGPLVEQGRVRAATVGSLAGQPLQERAQAWGERLRARMEEMGSRTRDRLDEVKEQVAEV  239
                :*   *::   .: .:.. ... :**  :  . :::*  :::...*:: :

ApoLp-III 154   ----------------------------------------KKIQEAANAKQ--------  164
APOE      240   RAKLEEQAQQIRLQAEAFQARLKSWFEPLVEDMQRQWAGLVEKVQAAVGTSAAPVPSDNH  299
                                                       :*:* *..:.
```

Fig. 3. Presence of internal repeats and similar amino acid motifs in the protein sequence of APOE and ApoLp-III. A) Proposed amino acid motifs in the sequence of human APOE (Rajavashisth et al., 1985) and insect ApoLp-III (*Manduca sexta*) (Cole et al., 1987). B) Schematic protein structure of the consensus sequence of vertebrate APOE and insect ApoLp-III. Internal repeats were de novo identified with the web tool TRUST (http://www.ibi.vu.nl/programs/trustwww/) (illustrated in gray). The consensus sequences were calculated using MATLAB (R2016b) from the protein sequences from the following species: *Danio rerio, Acipenser sinensis, Onchorhynchus mykiss, Xenopus tropicalis, Gekko japonicas, Mus musculus, Pan troglodytes, and Homo sapiens* for APOE; and *Manduca sexta, Bombyx mori, Nilaparvata lugens, Apis mellifera, Culex quinquefasciatus, Tenebrio mollitor* and *Locusta migratoria* for ApoLp-III. C) Localization of proposed amino acid motifs in the respective consensus sequences of APOE and ApoLp-III. Highlighted residues (underlined and in bold) indicate compliance with the amino acid motif of the respective protein homologue. D) Local alignment of mature APOE (*Homo sapiens*) and ApoLp-III (*Bombyx mori*) with Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). Identical residues are marked with an asterisk as are full stops and colons.

that the separation in less and more complex proteins, according to the absence or presence of APOE/ApoLp-III amino acid motifs, must have occurred very early in metazoan development. This assumption is based on the finding that the APOE/ApoLp-III motifs are present in insects as well as in mammals, which indicates that they must have evolved before the divergence of Deuterostomia and Protostomia. Our results argue against the parallel evolution of ApoLp-III and APOE (Cole et al., 1987).

2.4. Functional comparison of APOE and ApoLp-III

Along with sequence homology, the encoded proteins share common biochemical and functional characteristics (Table 3). APOE and ApoLp-III are both amphipathic proteins that exhibit an up-and-down alpha-helix bundle conformation that opens up upon lipid binding. APOE is mainly associated with triglyceride rich VLDL particles to mediate extra-hepatic lipid supply and with HDL particles for reverse cholesterol transport. These processes involve binding of APOE to specific membrane receptors including the members of the LDL receptor family. There is practically no lipid-free APOE in the mammalian body and in its lipid-free form, APOE would rapidly oligomerize. In contrast, ApoLp-III primary occurs lipid-free in hemolymph until it is recruited by lipoprotein particles called lipophorin (Weers and Ryan, 2006). Long-distance flight activity of insects requires sustained energy supply using diacylglycerol (DAG) as substrate. Particle density decreases upon DAG-load creating low density lipophorin (LDLp) and the increasing particle hydrophobicity is balanced by recruitment of ApoLp-III. After the release of DAG at the flight muscle, ApoLp-III likewise detaches from LDLp and returns to the lipid-free pool in the hemolymph. The lipid supply of flight muscles seems to operate without the involvement of receptor binding. Accordingly, there is no apparent receptor binding domain contained in the consensus sequence of ApoLp-III opposing the well conserved LDLR binding domain of APOE among vertebrates. It is remarkable that ApoLp-III naturally occurs lipid-free, while APOE would rapidly self-associate and form oligomers. The physical explanation for the poor self-association of ApoLp-III may lie in the shorter C-terminus compared to APOE (Fig. 3C) lacking those residues that were made responsible for the oligomerization of APOE. However, APOE and ApoLp-III carry the essential commonality of possessing additional physiological functions that lie beyond simple lipid transport. These include pathogen pattern recognition and interaction with microbial surface components, particularly binding to inflammatory lipids preventing their aggregation and enabling their clearance, as part of the innate humoral immune system (Table 3) (Azuma et al., 2000; Gupta et al., 2010; Kim et al., 2011; Nankar and Pande, 2013, Palusinska-Szysz et al., 2015; Pane et al., 2016; Rossignol et al., 2011; Wen et al., 2016).

3. Protein structure of human lineage-specific APOE

3.1. Structural differences of APOE4 and APOE3

Attempts to elucidate the structure of the full-length protein have been hampered by the rapid oligomerization of lipid-free APOE, which may also be the reason why there is practically no lipid-free APOE in the body. It was only recently that a targeted mutation of single residues in the CT allowed for the accumulation of a monomeric lipid-free protein at significant concentrations and enabled the first description of an NMR structure of full-length APOE3 (Chen et al., 2011). Based on this decisive data, a novel prediction of the topology of the two major isoforms (APOE3 and APOE4), with special regard to their structural differences, was then suggested (Frieden and Garai, 2012; Frieden and Garai, 2013). According to these observations, there is a strong domain interaction through salt bridges and hydrogen bonds between the second and third helix of the CT and the third and fourth helix of the NT. The domain interaction shields the charged residues of the receptor binding region of the NT and exposes a hydrophobic outer surface that readily interacts with lipids, heparan sulfate proteoglycans (HSPG) and amyloid beta (Aβ). Upon interaction with lipids, a conformational change is triggered, the helix bundle structure opens up, and the CT moves away from the NT, exposing the buried hydrophilic residues of the NT. Thereby, the amphipathic nature of APOE is fully developed, and in its lipid-bound form, APOE becomes a potent ligand for the LDL receptor family (Chen et al., 2011). However, this model may only be conditionally true for APOE4, as the authors used APOE3 as the template for the site-directed mutant model. The isoform-dependent amino acid exchange (Arg112Cys) is known to impact protein conformation (Dong and Weisgraber, 1996; Raffai et al., 2001); however, the initially predicted domain interaction of APOE4 is not accurate considering the recent findings. For instance, the domain interaction that had been formerly attributed uniquely to the APOE4 isoform was thought to be caused by a salt bridge between Arg61 and Glu255 (Dong and Weisgraber, 1996). This alleged salt bridge formation and the resultant domain interaction were responsible for the structural and functional differences between APOE4 and APOE3; therefore, the idea of small molecules interfering with this domain interaction was intensely pursued (Brodbeck et al., 2011). However, this alleged salt bridge was not confirmed elsewhere (Williams et al., 2015), and the recent NMR structure revealed that the NT and CT domains of APOE3 likewise interact by forming a hydrogen bond between Arg61 and Thr194 and a salt bridge between Lys95 and Glu255 (Frieden and Garai, 2012). Moreover, it appears that the domain interaction is actually less stable in APOE4 than APOE3, which fundamentally changes the former understanding of APOE topology (Hatters et al., 2006).

The amino acid exchange at residue 112 is supposed to be propagated to residues 5–21 and 271–279 that appear distant in the sequence but may indeed be very close in spatial proximity to the respective residues that distinguish APOE4 from APOE3 (Frieden and Garai, 2012; Nguyen et al., 2009). In that way, the larger arginine residue is responsible for the perturbation of noncovalent interactions, creating greater dynamic motion in different regions of the APOE4 isoform compared to APOE3. This may contribute to an easier dissociation of the domains and thereby enable binding to larger particles such as VLDL, which is characteristic of APOE4. The isoform-dependent difference in the spatial orientation of the region spanning residues 261–272 is supposed to stabilize the NT of APOE3 and its domain interaction, whereas APOE4 appears in a "less organized" conformation (Mizuguchi et al., 2014). More recently, a study using discrete molecular dynamics computed the heat capacity of the APOE isoforms and observed that APOE4 is least thermally stable even though it forms a misfolded intermediate that exerts the highest density of contacts between the N- and C-terminal domains among the isoforms. In this model, the hinge region also plays an important role in the domain–domain interaction (Williams et al., 2015). Finally, the isoform-dependent topological differences of the CT also affect the self-association behavior, with faster dissociation of APOE4 oligomers and thus faster initiation of binding to lipoprotein surfaces compared to APOE3 (Mizuguchi et al., 2014). Table 4 summarizes the isoform-specific structural differences and the functional implications.

3.2. Divergence from primate APOE

To identify human lineage-specific characteristics, the amino acid sequences of APOE4 were compared to three primate species *Pan troglodytes* (NP_001009007.1), *Gorilla gorilla* (AAG28579.1) and

Table 3
Structural, biochemical and functional characteristics of APOE and ApoLp-III.

	APOE (human)	ApoLp-III (flying insects)
Molecular mass and number of residues	Human: 34 kDa, 299 amino acid residues (mature protein)	18–20 kDa, ~ 161–166 amino acid residues (mature protein)
Appearance	mostly lipidated plasma, liver, numerous other tissues	hemolymph (lipid-free or associated with LDLp), larvae
Lipoprotein association	VLDL, HDL through opening of the four helix-bundle, 2 APOE molecules per HDL, no complete belt formed	up to 16 molecules per LDLp through helix opening, at the particle periphery in a belt-like manner
Domains/structure	two tertiary structured domains: NT and CT, connected by a hinged loop segment, arranged in a bundle of four amphipathic α-helices	no distinct domain appearance, arranged in a bundle of five amphipathic α-helices
Characteristics	domain interaction, rapid oligomerization of full protein induced by the CT, only monomeric in absence/mutation of certain CT residues	monomeric even at high concentrations
Cysteine residues	none in mature human APOE4 and non-human APOE one in APOE3, two in APOE2	none
Receptor binding	yes, highly conserved binding domain for the LDL receptor family	no, presumably alternative lipid shuttle without receptor involvement
Heparin binding	yes	no
hormone binding	Thyroid hormone (TH) binding motif APOE expression regulated by TH	unknown
Metal binding	copper, iron, zinc	unknown
Binding to pathogen membrane compounds	lipopolysaccharide, lipoteichoic acid, other inflammatory lipids, β-glucans	lipopolysaccharide, lipoteichoic acid, β-glucans
(Supportive) immune function	anti-inflammatory activity, isoform-dependent regulation of innate immune response (APOE4 > APOE3), broad spectrum antibacterial activity of derived peptides (residues 133–162)	stimulating phagocytosis, increasing antibacterial activity, NO production, supporting antiplasmoidal epithelial response in the gut, increase of antioxidative defense enzymes, increasing prophenoloxidase activity

CT, C-terminal domain of APOE; NT, N-terminal domain of APOE.

Table 4
Summary of structural functional differences of APOE4 and APOE3.

	APOE4	APOE3
Amino acid residue 112	Arg	Cys
Lipoprotein preference	VLDL	HDL
Aβ-binding	Similar binding to Aβ oligomers via Lys–Lys crosslinks, Aβ clearance may be APOE4 < APOE3	
Residues 5–30	Different nonconvalent interactions and spatial orientation, no effect on domain interaction, but different dynamic motion between the isoforms	
Residues 116–123	Different nonconvalent interactions and spatial orientation, greater dynamic motion in APOE4	
Residues 261–272	Involved in lipid binding, per se similar affinity, however different folding between isoforms and affected by oligomerization	
Residues 271–279	Different nonconvalent interactions and spatial orientation, greater dynamic motion in APOE4, possible reason for different lipid binding activity of the isoforms	
Domain interaction (NT-CT)	Greater motion dynamics	Relatively more stable
Thermal stability	APOE4 < APOE3	
Dissociation of oligomers	Rate-limiting for lipid binding initiation, faster in APOE4	
Spatial orientation	Less organized	Higher degree of organization

CT, C-terminal domain of APOE; NT, N-terminal domain of APOE.

Pongo pygmaeus (AAG28580.1) (Clustal Omega), and eleven non-synonymous variations were observed. Four of these substitutions (residues 32, 116, 198 and 201) are restricted to single primate species, while another three are shared by *H. sapiens* and either *P. pygmaeus* (residues 15, 287) or *G. gorilla* (residue 5). The remaining four amino acid exchanges are unique to humans, of which three are conservative, leading to the exchange of alanine to threonine (position 18) or valine (at position 135) and valine to leucine (at position 174). These substitutions do not change the hydrophobicity, charge or size of the residues. None of these mutations lie in a region that is clearly defined as a functional domain; however, the methionine-to-valine exchange at position 287 present in humans and gorillas may have a minor impact on APOE structure. The M-to-V exchange does not greatly impact on the charge or hydrophobicity, but it decreases the spatial size of the residue. Residues 271–287 are involved in the domain interaction of the CT with helices 3 and 4 of the NT. Mutation of 5 single residues of the CT, including position 287, prevented the rapid oligomerization of lipid-free APOE (Chen et al., 2011), emphasizing the key role of residue 287 in the formation of oligomers. Therefore, it is possible that the change of spatial size by M287 V substitution exerts an effect on the stability of lipid-free APOE oligomers. However, the most significant difference between human and non-human primate APOE lies in residue 61.

The threonine-to-arginine exchange not only increases spatial extent but also introduces a positive charge at position 61. This non-conservative substitution is due to a transversion from cytosine to guanine, which often occurs under oxidative conditions such as the photooxidation of DNA (Kino and Sugiyama, 2001). After the loss of body hair, the skin of ancient humans was directly exposed to UV radiation, presumably resulting in a high degree of oxidative damage to biological molecules. Assuming that the oxidative stress-induced DNA mutation led to the substitution of threonine by arginine in ancestral human APOE (APOE4), one may deduce that the subsequent C → T transition (rs429358) that gave rise to the younger *ε3* allele occurred as a corrective measure. This subsequent nucleotide exchange led to an arginine-to-cysteine exchange at position 112 in the mature protein, removing the positive charge and reducing the spatial size of the former residue. Both residues 61 and 112 are supposed to be in very close proxim-

ity to each other, and their charge and spatial size affect the domain interaction (Frieden and Garai, 2013). The change of the charge and size of either of them is directly propagated to the domain interaction. Looking at APOE4, there are two relatively large and positively charged residues (R61 and R112), whereas primate APOE and APOE3 possess at least one smaller and less-charged residue (T61 and C112, respectively). As a consequence, the strength of the domain interaction, helix bundle opening and lipoprotein preference is similar between primate APOE and APOE3 and different from APOE4 (Zannis et al., 1985).

Therefore, it is speculated in the present manuscript that due to the loss of body hair and increased UV-induced oxidative damage experienced by early hominins, a random mutation appeared in the human lineage (T61R), introducing a positive charge that impacted the domain interaction. This impact was corrected by removing a second positive charge (R112C) in close proximity, giving rise to the evolutionarily younger APOE3 isoform. This hypothesis is strengthened by the fact that the C → T transition (rs429358) that led to the R112C substitution lies within a CpG island, which is known as a mutation hot-spot that readily mutates in a naturally occurring process (Seripa et al., 2011). Therefore, the correction of the presence of two positive charges was enabled by the easily mutated region containing amino acid residue 112. Also, the second human polymorphism that led to the *APOE ε2* allele and encoded the arginine-to-cysteine substitution at position 158 is related to a similar hot-spot mutation site (C → T transition, rs7412) (Seripa et al., 2011). However, this hypothesis of human APOE evolution is highly speculative and other factors causing the T61R and R112C mutations are conceivable, including the exposure to pathogens and nutritional factors or a role in advancing brain development.

The analysis of Neanderthal and Denisovan nuclear DNA (Green et al., 2010; Reich et al., 2010) enabled the sequence comparison of present-day and archaic hominin APOE, which supported the notion that APOE4 is the ancestral isoform among present-day isoforms. In at least five important positions (R18, R61, R112, V135 and L174), Denisovan APOE perfectly matches APOE4 (McIntosh et al., 2012). Unfortunately, the APOE sequence from Neanderthals was not fully available, and therefore, only one of the respective positions could be identified, which was lysine at residue 61. This result is surprising because it neither matches the primate nor the hominin sequence. Furthermore, the responsible codon was supposed to be AAA, which differs from ACG or AGG in primates and humans and is, therefore, presumably a misread from high-throughput sequencing of AGG (McIntosh et al., 2012). In any case, the respective position would be filled with a positively charged amino acid (either lysine (AAA) or arginine (AGG)), which elicits similar consequences on protein topology and function. Therefore, the question arises as to whether the substitution of threonine by a bigger and positively charged amino acid at position 61 possibly occurred by chance (as proposed elsewhere (Enard, 2012)) or whether there were any functional advantages for early humans that are not yet revealed or not yet connected with APOE. Similarly, the corrective R112C substitution may have been a simple adjustment of the deleterious effects of the previous mutation (Enard, 2012) or the change caused by other factors including genetic or environmental changes that may have favored its correction and enabled the superiority of the novel APOE3 isoform.

4. Targeted questions on human APOE evolution

4.1. Why did human APOE evolve (T → R exchange at position 61)?

Dating the divergence of humans from primates back to at least 7.5 million years ago and the last common ancestor of Denisovan and anatomically modern humans to 1 million years ago (Arnason, 2016) renders a time window of at least 6 million years for the occurrence of the mutation that gives rise to the T → R exchange at position 61. The introduction of the larger positively charged arginine (compared to the former threonine) slightly reduces C-terminal domain stability (Nguyen et al., 2014) and may allosterically impact the interaction of helix 4 of the NT and the CT (Frieden and Garai, 2013). Unfortunately, little experimental data are available regarding residue 61. R61T-mutated APOE4 exhibits marginally higher surface exposure of hydrophobic residues than wild-type APOE4. However, neither lipid binding nor oligomerization was significantly affected by this single amino acid exchange at position 61 (Nguyen et al., 2014). This is quite surprising, as the exchange of the positive charge at 112 had such a major impact on protein function. This may emphasize a supportive role of the rather minor substitution at position 287 (methionine in some primates and valine in humans) that acts in concert with position 61. It may be conceivable that the simultaneous substitution at both sites is required to exert significant functional effects and hence alter lipid binding, helix bundle opening and the oligomerization of 'novel' human APOE compared to 'former' primate APOE; but this is rather speculative and warrants further investigation.

Support of this 'combination hypothesis' is provided by the notion that chimp APOE is considered to exhibit chemical properties that are comparable to human APOE3. Both proteins share common lipoprotein preferences and isoelectrical points (Zannis et al., 1985) even though they differ by 7 amino acids, including those at 61 and 287 as well as the prominent position 112. Similarly, mouse APOE carries threonine at position 61, which is why many authors refer to murine APOE as 'APOE3-like.' In contrast to primate APOE, murine APOE only shares 70% similarity with human APOE and bears additional differences, including a 6-amino acid shorter length. Therefore, data derived from mouse studies that focus on single amino acid mutations should be reviewed with caution. In this context, arginine-61-mutated murine APOE (resembling the 'novel' human mutation at 61) exhibited increased catabolism at lower plasma levels and markedly reduced HDL binding compared to murine wild-type APOE (Raffai et al., 2001). These results oppose the abovementioned findings of threonine-61-mutated human APOE4, suggesting that the interaction of APOE (especially position 61) with lipoproteins and lipoprotein receptors is different in mice compared to humans. The results of *in vitro* lipoprotein binding of R61-mutated murine APOE incubated with human plasma support this point. However, *in vitro* murine R61-APOE preferably bound to human VLDL, which is similar to human APOE4, but is in contrast to its wild-type murine counterpart that was enriched in human HDL (Raffai et al., 2001). Again, it must be emphasized that these results oppose the data of *in vivo* murine R61-APOE lipoprotein binding.

Further insight comes from NMR structure elucidation and modeling. As mentioned earlier, Frieden & Garai assumed that the substitution of residue 61 by arginine to create 'novel' human APOE has a similar effect on domain interaction as the substitution of residue 112 (Frieden and Garai, 2013). They supposed that by the introduction of positively charged residues, the strength of the domain interaction is attenuated, enabling easier opening of the APOE helix bundle in the presence of lipids, as is observed when comparing APOE4 with APOE3 (Nguyen et al., 2009). Protein stability against chemical or heating denaturation may be seen as measures of domain interaction strength, and it is, therefore, not surprising that protein stability is reduced in APOE4, which has positively charged residues at both positions (Mizuguchi et al., 2014). The substitution of the positive charge at 112 and 158 by smaller uncharged cysteine residues (giving rise to APOE3 and APOE2, respectively) increases protein stability (Clement-Collin et al., 2006). Furthermore, APOE4 was suggested to exhibit a smaller

degree of oligomerization and faster dissociation, thereby facilitating lipid binding (Mizuguchi et al., 2014). Taken together; these data suggest that evolution of the 'novel' human APOE may have given rise to a less stable but readily lipid binding apolipoprotein that favored large lipid-rich lipoproteins such as VLDL. Moreover, it may have exhibited less pronounced oligomerization and exposed less hydrophobic surface residues than its primordial precursor in non-human primates. However, this hypothesis needs experimental support focusing on the two human lineage-specific mutations at 61 and 287 that may possibly act in concert.

As long as the functional implications of human-specific APOE remain unclear, potential evolutionary reasoning is widely prevented. The evolution of modern humans is related to major hallmarks that can be roughly summarized as increased brain size, bipedal upright walking, increased life expectancy beyond reproductive age and increased dietary quality due to cooking and meat eating. Therefore, it seems obvious that APOE may be related to at least one of these matters. Finch and his co-workers repeatedly referred to APOE as a meat-adaptive gene (Finch, 2007; Finch and Sapolsky, 1999; Finch and Stanford, 2004) that is surely involved in the mediation of life expectancy and brain function. However, beyond increased energy and micronutrient density, meat eating offers other benefits. Herbivores, such as our early hominin ancestors, are supposed to rely primarily on large seeds and underground storage organs (Luca et al., 2010) that may also contain partly toxic secondary plant metabolites. During the evolution of meat eating approximately 2.5 million years ago (Luca et al., 2010), early humans encountered a new food-borne threat that shifted from plant toxins to meat-residing parasites and pathogens, especially in carrion. Secondary plant compounds are metabolized by xenobiotic metabolism, involving phase II enzymes that are under the transcriptional control of Nrf2. There is evidence of reduced Nrf2-mediated gene expression and hepatic cellular defense in the presence of APOE4 compared to APOE3, which is closer to primate APOE (Graeser et al., 2011). In response to the dietary shift from a purely plant-based diet to increased meat eating, the need for Nrf2-mediated detoxification of plant toxins would have been reduced. Lower Nrf2 activity could be seen as a reallocation of energy and cost saving from an evolutionary perspective, which would have been beneficial for APOE4. The concomitant relatively higher expression of phase I enzymes in response to APOE4 (Graeser et al., 2011) may moreover be associated with the increased hydroxylation capacity, including activation of vitamin D, which may have contributed to increased growth and cell differentiation, likewise favoring the 'novel' human APOE4 compared to primate APOE.

In regards to the adverse effects of increased meat eating, such as hypercholesterolemia and hypertriglyceridemia, APOE4 expressing humans are not different from chimpanzee or other great apes. The data from captive primates show elevated cholesterol levels that exceed human clinical reference values when fed diets containing animal-derived lipids (Finch and Stanford, 2004; Steinetz et al., 1996). Other hypotheses involving enhanced defense against food-borne pathogens (Azevedo et al., 2014), increased newborn health, survival and mental development (Becher et al., 2006; Wright et al., 2003) as well as differential interactions with micronutrients and adapting to changing dietary patterns (Huebbe et al., 2015; Huebbe et al., 2016; Huebbe et al., 2011) may be possible. These, however, are still crude theories derived from the functional comparison of APOE4 with APOE3 and may provide only limited insight into the potential evolutionary advantage of human APOE4 over primate APOE. It should also be taken into account that the evolution of human APOE4 with the introduction of R61 could have occurred by chance without providing direct advantages. The theory of random photooxidation-induced mutation as a consequence of body hair loss and increasingly UV-exposed skin would support this notion.

4.2. What is the origin of APOE3?

In worldwide present-day populations, APOE3 is the most common isoform, with frequencies of 85% in Asia, 79% in Europe, 69% in Africa, 82% in North America and 77% in South America (Singh et al., 2006). APOE4 is second-most common worldwide, with particular enrichment in indigenous populations of Central Africa (40%), Oceania (37%) and Australia (26%). The distribution across Europe and Asia follows an apparent North-to-South gradient, with low APOE4 abundance in the Mediterranean area or South China (< 10%) and increasing frequency in the North (up to 25%) (Egert et al., 2012; Hu et al., 2011). Overall, it appears that APOE3 is selected against APOE4, but the predominance may be mitigated in certain environmental niches, such as populations with an indigenous lifestyle or a particular geographical latitude. This would argue against selective neutrality (Fullerton et al., 2000), but it supports the spatially and temporally varying selection of the *APOE* locus. Time-depth analysis dated the divergence of the *ε3* and *ε4* haplotypes back to 150,000 to 220,000 years ago based on the assumption of neutral selectivity (Fullerton et al., 2000). However, this approximation underestimated the timing of the hominid-hominin lineage split by at least 2 million years, implying that the variation that gave rise to *APOE ε3* must have evolved much earlier than 220,000 years ago. In contrast, in case of non-neutral selection against the *ε4* allele, the rs429358 variation (*ε3*/*ε2*) would be much younger.

There is recent evidence that indicates an archaic hominin presence in Southeast Asia > 200,000 years ago (van den Bergh et al., 2016) and fully modern humans in China as early as 120,000 years ago (Liu et al., 2015). These fossil evidence argue against the proposed 'Out-of-Africa' theory of single or multiple migration waves of modern humans between 70,000 to 40,000 years ago that spread from Africa to Asia and Europe (Henn et al., 2012; Tassi et al., 2015). More reasonably, it was suggested that a Southern Asian subgroup migrated westwards, entering the Levant, Europe and Africa approximately 50,000 years ago (Arnason, 2016; Liu et al., 2015). Apart from the origin and migration route of modern humans at that time, it seems obvious that certain modern humans must have already carried the *ε3* allele, which enabled its geographical distribution. It is interesting to note that the *ε3* allele frequency is highest in Asia followed by Europe and Africa in descending order. It may suggest that the *ε3* allele originated and prevailed in Asia during the past more or less 200,000 years. Migration waves to the Levant, Europe and Africa as well as to North America and South America spread the allele worldwide, but the supremacy of APOE3 decreased with the increasing distance. This assumption would be substantiated, when spatially and temporally selectivity on APOE3 is assumed. Selective pressure acting on APOE3 may have been most dominant in Asia at a certain time period, perhaps up to 70–50,000 years ago before westward migration began. The existence of population-related differences of *APOE* genotype-disease risk-associations will be discussed in a subsequent section (see Section 4.4).

4.3. What is the evolutionary advantage of APOE3?

It is remarkable that the only difference that paved the way for the evolutionary success of APOE3 over the ancestral APOE4 seems to be a single amino acid exchange. The biochemical and functional consequences of the exchange of arginine to cysteine at position 112 led to a strengthening of the domain interaction and enabled APOE3 to be less prone to denaturation, helix opening and oligomer dissociation. The new isoform exerts a different lipoprotein preference (favoring HDL) and slightly changes the blood lipid profile towards lower LDL cholesterol. By the introduction of cysteine, the only sulfur-containing side chain of the mature APOE3 protein is gained, which possibly elicited functional changes such as metal

binding. The predominance of APOE3 over APOE4 has traditionally been attributed to the altered age-related disease risk, including cognitive decline, Alzheimer's disease and cardiovascular pathologies (Dose et al., 2016, Egert et al., 2012). APOE3 appears to express higher levels of antioxidative protective proteins (Graeser et al., 2011; Graeser et al., 2012), be less neuronally susceptible to oxidative insults (Huebbe et al., 2007) and exhibit better neuronal repair functions, improving the outcome of head injuries compared to APOE4 (Friedman et al., 1999). Furthermore, APOE3 compared to APOE4 appears to be associated with an energy-conserving phenotype (Huebbe et al., 2015) that would provide certain advantages in populations that suffer from recurring famine periods. In addition, it was observed that APOE3 is associated with higher beta-carotene levels (Huebbe et al., 2016), which may potentially contribute to improved brain function in the elderly. Despite the higher levels of oxidative damage and cardiovascular risk factors associated with the *ε4* genotype (Corella et al., 2011; Niu et al., 2009; Ramassamy et al., 2000), the responsiveness to potentially protective dietary factors such as bioactive plant compounds is lower in *ε4* allele carriers than in *ε3* allele carriers (Chin et al., 2014; Egert et al., 2010). These data may emphasize that APOE3 is more flexible and may be more adaptive to environmental changes, including (micro)nutrient intake. Adaptation is considered the main driver of evolution and underlies the worldwide success of APOE3.

Altogether, these notions may aid in explaining the predominance of the *ε3* allele. However, the better cognitive fitness at old age may be the most important underlying reason for APOE3′s supremacy. The so-called grandmothering effect has been debated for a long time because critics doubted the force of selective pressure beyond reproductive age. Only recently, the evolution of post-reproductive lifespan, which is almost unique to humans, has been attributed to positive selection of preventing age-related cognitive decline (Schwarz et al., 2016). The increased survival of younger kin was suggested as the main driver of post-reproductive maintenance of brain function. The authors referred to APOE *ε3* as a 'derived' allele that compensates for functional changes that occurred during human brain evolution and restores the former molecular condition of primate APOE. Therefore, the *ε3* allele would not exhibit direct advantageous effects on early life fitness but will exhibit benefits at post-reproductive stages (Schwarz et al., 2016). The APOE4 isoform, similarly, seems not only disadvantageous but also may be inferior in certain situations or at certain ages (Table 5), rendering the *APOE* gene locus a case of antagonistic pleiotropy. Moreover, due to different life expectancy and disease-risk associations, post-reproductive selection pressure likely contributes to the population-specific accumulation of APOE alleles. For example, in Africans, the APOE4-Alzheimer's disease risk association is much less pronounced and sometimes absent (Gureje et al., 2006; Hendrie et al., 2014), while the *ε4* allele frequency is among the highest worldwide (Singh et al., 2006), indicating that post-reproductive selection pressure against APOE4 is rather small. In an Australian cohort, elderly *ε4* allele carriers showed similar cognitive performance and even higher verbal fluency compared to *ε3* allele carriers (Alexander et al., 2007), which is interesting considering the relatively higher *ε4* allele frequency in Oceania (Singh et al., 2006).

4.4. *Allele effects in different populations – what counteracts the extinction of* APOE *ε4?*

APOE4 is an independent risk factor for age-dependent Alzheimer's disease, which is directly related to the number of *ε4* alleles (Corder et al., 1993). Age-related mortality due to cardiovascular pathology or cognitive decline and all-cause mortality are significantly higher in *ε4* than non-*ε4* allele carriers (Ewbank, 2004). The *ε4* allele frequency is depleted with increasing age, and survival to very old ages appears virtually impossible (Nebel et al., 2005; Schachter et al., 1994); this is observed in women more than men (Joshi et al., 2016; Kulminski et al., 2014) and is applicable to certain populations. However, taking a more in depth look, the disease risk association seems largely affected by ethnic origin. Hypertension and intracerebral hemorrhage risks are markedly increased in Asian and Caucasian *APOE ε4* carriers (Niu et al., 2009; Tzourio et al., 2008; Zhang et al., 2014). The association of the *ε4* allele with Alzheimer's disease is weak in Hispanics and African-descendant Americans (Farrer et al., 1997) and either absent (Gureje et al., 2006) or only observable at homozygosity (Hendrie et al., 2014) in Africans. Interestingly, African Americans and Hispanics exhibit an increased risk for Alzheimer's disease compared to non-Hispanic Caucasians even in the absence of APOE4 (Tang et al., 1998). This observation would support a post-reproductive selection pressure for the *ε3* allele, which would be negligible in a population facing an already higher risk of cognitive decline independently of APOE. Similarly, the higher disease risk associated with Caucasian *ε4* allele carriers may be a result of synergistic effects and interactions with other, possibly genetic, factors.

Both arguments are in congruence with the higher proportion of APOE4 in Africans and African-descendants (Singh et al., 2006; Tang et al., 1998), where it is supposed to be less detrimental. While the proportion of elderly cognitively normal African-Americans is higher among *ε4* than non-*ε4* allele carriers, cognitive decline (Beydoun et al., 2013) and age-related mortality are increased in non-Hispanic Caucasians carrying the *ε4* allele (Jacobsen et al., 2010). In contrast, in an indigenous population from South America, the *ε4* allele appears to accumulate with increasing age (+60) with no apparent adverse effects on age-related mortality (Vasunilashorn et al., 2011). Interestingly, the authors emphasized the high infection rate present in this population, which may be better tolerated by APOE4 compared to APOE3. This is supported by findings from APOE4-expressing Brazilian children who showed better outcome from heavy diarrhea (Oria et al., 2010; Oria et al., 2005).

When taken together, these data may explain why *APOE ε4* has not become extinct. The data suggest that on the one hand, it may not necessarily be an early life advantage that counteracts deleterious effects later in life, which is called antagonistic pleiotropy. It may likely be the dilution of age-related deleterious effects on ethnic backgrounds that hampers *ε4* allele decline and results in its relative accumulation in certain populations. On the other hand, the presented data illustrate that neither APOE3 nor APOE4 provides universal advantages that apply equally to any developmental phase in life, any environmental condition or any ethnic background. For instance, despite adverse effects on mortality and brain function in the elderly, APOE4 appears protective during early childhood development in Caucasians. Perinatal and infant mortality is decreased, while newborn health status, infant mental development and neuronal protection are improved by APOE4 compared to APOE3 (Becher et al., 2008; Becher et al., 2006; Wright et al., 2003). Given the strong interaction of the *APOE* genotype with elderly cognitive function in Asians and Caucasians, it is quite remarkable that intelligence levels in children and young adults are similar between *ε4* and *ε3* carriers, with at best a tendency towards higher levels in *ε4* compared to *ε3* carriers in Asians, Europeans and European-descendants in North America (Chen et al., 2015; Shaw et al., 2007; Taylor et al., 2011; Turic et al., 2001; Yu et al., 2000).

Taking into account the novel insight regarding modern human existence in East Asia as early as 120,000 years ago (Arnason, 2016; Liu et al., 2015), the evolution and worldwide distribution of the APOE isoforms may be reconsidered. Based on the assumption that modern humans of Asian origin migrated westwards, spreading to the Levant, Africa and Europe, and headed north, entering North

Table 5
Hypotheses on functional reasons and potential selective pressures contributing to the evolution and distribution of human APOE isoforms.

Isoform	Evolutionary effect	Potential explanation
E4	divergence from primates/human evolution	adaptation to meat eating (improved digestion of dietary lipids, better defense against parasites/pathogens) supporting cognitive development at young ages adaptation to up-right walking, long-distance running or migratory lifestyle
	north-to-south gradient	adaptation to vitamin D insufficiency due to falling UV-exposure
	U-shaped relation of allele frequency with absolute latitude	adaptation to climate extremes by better modulation of body temperature due to elevated cholesterol levels
	accumulation in indigenous populations	adaptation to severe infection load (lower severity of immune response) better newborn health status, lower perinatal mortality and abortion rate, higher fertility
	lower frequency in East Asians	E4-disease risk-associations much stronger than in other ethnicities
E3	fixation of mutation and worldwide predominance	energy-conserving phenotype improves adaptation to times of hunger/insecurity of food supply higher responsiveness to (micro)nutrients and plant bioactives reduced age-related mortality and disease risk, at least in certain populations better neuronal maintenance/repair also with increasing age
	accumulation in civilized/industrialized populations	adaptation to "modern lifestyle" including sedentary lifestyle, dietary specialization with high proportion of cereals and processed food, increased life expectancy (> 80 years of age)
E2	potential natural selection	decreased age-related disease risk protective effects on brain function

and South America, the distribution of the isoforms may be as follows. The impact of the *ε3* allele must have grown in a South Asian subpopulation, possibly through selective pressure against cognitive decline in the elderly. As we know, the association of the APOE genotype with cognitive decline in the elderly is most pronounced on the Asian ethnic background. During the time of migration, the selective pressure may have been diluted due to novel environmental factors such as fluctuating life expectancy. Such factors may include facing novel pathogens or changing climate conditions. It is interesting to note that fertility and fecundity may be higher in *ε4* allele carriers (Corbo et al., 2004; Jasienska et al., 2015; Meng et al., 2012), which additionally would counterbalance the benefits of *ε3* later in life. Furthermore, there is evidence indicating APOE isoform-dependent differences in skeletal muscle performance (Hagberg et al., 1999; Huebbe et al., 2015; Seip et al., 2011; Thompson et al., 2004; Yu et al., 2014), including increased utilization of fatty acids as the energy substrate in the presence of APOE4. This fact may have provided advantages by increasing endurance in locomotion and promoting the migration of *ε4* carriers.

Once people had settled in a new environment, mild climate conditions (such as that present in the Mediterranean area) and the development of agricultural economies may have again been supportive of APOE3. The benefit of an energy-conserving phenotype that curbs energy dissipation, which is characteristic of APOE3 compared to APOE4, has already been discussed above. In contrast, more extreme climates beyond the 45th latitude or near the equatorial level favored the accumulation of APOE4 (Eisenberg et al., 2010). In this context, the enhanced vitamin D and calcium status in the geographical regions subjected to low UV radiation may have accounted for the north-to-south gradient of the *ε4* allele that is evident in Europe (Huebbe et al., 2011). Greater allocation of energy levels for thermoregulation have also been thought to contribute to a better adaptation to extreme climates in the presence of APOE4 (Eisenberg et al., 2010).

The question of why *ε3* is most prevalent worldwide despite local accumulation of *ε4* in mainly indigenous populations may partly be answered by the levels of beneficial and adverse effects in different ethnic backgrounds. In addition, indigenous preindustrial populations often suffer from heavy infection burden in the absence of modern medical care, while modern Western civilizations face adverse lifestyle factors such as high dietary energy consumption and a sedentary lifestyle. APOE isoform-dependent modulation has been associated to both conditions (Azevedo et al., 2014; Fujioka et al., 2013; Jeenduang et al., 2015; Olivieri et al., 2007; Sima et al., 2007; Sun et al., 2016; Vasunilashorn et al., 2011; Wozniak et al., 2004).

4.5. What drives the evolution of the youngest isoform, APOE2?

The second main non-synonymous polymorphism in the *APOE* gene (rs7412) gave rise to the youngest major isoform, APOE2. The timing of its emergence is quite difficult to assess due to its patchy worldwide distribution and the uncertain knowledge of potential selective pressures. *APOE ε2* is the least common major allele with a general worldwide frequency of 7.3%. It is remarkable that there is an absence of *APOE ε2* in many Amerindian and certain African indigenous populations as well as Australian aborigines, despite the fact that it generally accumulates in Africa (9.9%) and Oceania (11.1%) (Singh et al., 2006). Based on the assumption of neutral selectivity, the age of the APOE2 variation was estimated to be 80,000 years old (Fullerton et al., 2000). This rough estimation is, however, difficult to reconcile with the above described migration scenario of the modern humans of East Asian origin and the colonization of America. Genomic and archaeological analyses revealed that North and South America were populated most likely by subsequent founder groups from South Central Siberia, Mongolia and Northern China approximately 14,500 – 11,500 years ago after the last glacial maximum (Dryomov et al., 2015; Schurr and Sherry, 2004; Tackney et al., 2015). The absence of APOE2 in many North and South American native societies and the low general allele frequency across the two continents (4.9 and 4.6%) suggest that there was most likely a very small number of *ε2* allele carriers among the founder groups that colonized the Americas. It may even be possible that the *ε2* allele was absent in the first migration waves. Because APOE2 may have played a minor role in the American ancestors residing in Central East and Northern Eurasia until at least 15,000 years ago, it is suggested that the novel APOE isoform must have evolved in Southern Asian regions without substantial geographical distribution. Based on the assumption that a subgroup of early modern humans residing in southern Asia migrated westwards to Africa approximately 50,000 years ago (Arnason, 2016), the allele must have already been present in this subpopulation in a considerable number. This hypothesis is supported by the relatively high *ε2* allele frequency in present-day Southeast Asia, Oceania and Africa

(e.g., 18% in Thailand, 15.4% in New Guinea, 19% in South African Zulu (Chikosi et al., 2000; Singh et al., 2006)). The *ε2* allele may have emerged 80,000 years ago in a south Asian subgroup that was geographically (or at least genetically) isolated until their migration to Africa, Europe and Oceania. Alternatively, the *ε2* allele may have evolved in a much shorter period but was under natural selection. In the latter case, a strong evolutionary advantage must have been provided by the novel isoform.

The *ε2* allele has been associated with a lower risk and delayed age of onset of Alzheimer's disease compared to other alleles and has been shown to protect against cognitive decline (Farrer et al., 1997, Konishi et al., 2016). Moreover, it contributes to the survival to advanced age and is associated with reduced mortality in the elderly (Kulminski et al., 2016; Schachter et al., 1994). The coronary artery disease risk is significantly reduced in *ε2* compared to non-*ε2* allele carriers of different ethnic backgrounds (Bennet et al., 2007; Kulminski et al., 2016; Yang et al., 2004). In contrast to the protection against age-related diseases, APOE2 has been associated with increased infection rates and increased severity of malaria in early childhood (Aucan et al., 2004; Rougeron et al., 2013; Wozniak et al., 2003). However, heterogeneous APOE genotypes, including *ε2/ε3,* appeared to have a better disease outcome, and therefore, it was suggested that early childhood infection may protect against disease complications later in life (Wozniak et al., 2004). Other factors such as fertility, pregnancy loss and perinatal mortality have been studied, but unequivocally beneficial effects could not be established for APOE2 (Ahmadi et al., 2012; Becher et al., 2006; Corbo et al., 2004; Gerdes et al., 1996; Jamalzei et al., 2013). Given the late-life benefits of APOE2 compared to the adverse effects of APOE4, it appears contradictory that both isoforms are relatively accumulated, for example, in indigenous populations of Africa, where advanced age is not very pronounced compared to modern civilizations. In addition, if late-life survival and health were a trade-off, then only the *ε2* allele would accumulate while the *ε4* allele would be depleted. This may suggest that the selection for *ε2* is against *ε3* rather than against *ε4*. However, as long as selective mechanisms are not fully elucidated, this hypothesis remains speculative. Furthermore, this hypothesis would be in contrast to the worldwide predominance of the ε3 allele.

5. Conclusions and future perspectives

The potential underlying mechanisms of APOE isoform evolution are not yet fully elucidated and are still under debate. In particular, the idiosyncratic distribution of APOE4 and APOE2 in indigenous populations raises challenging questions. Is it possible that there are functional interactions with the APOE isoforms that are yet overlooked? And second, is it possible that the worldwide *ε3* allele preference is a result of adaptation to the modern lifestyle? These and other remaining questions may at least partly be answered by considering the nutritional interactions with the APOE variation. A nutritional perspective may, in many ways, open up new avenues in understanding the evolutionary advantages and adaptation of the isoforms to certain lifestyles, especially given the apparent involvement of APOE in dietary lipid metabolism. Paradigmatic examples include the differential adaptation to impending vitamin D insufficiency, the contribution to the human lineage-specific evolution of increasing circulating beta-carotene, the development of an energy-conserving phenotype and the adaptation to varying levels of secondary plant compounds in the diet, all of which have been discussed in the present study.

Nonetheless, additional factors may drive APOE evolution, such as detoxification of inflammatory triggers or pathogen recognition. These possibilities have thus far received little attention but may gain importance in the future. In the present review, the structural and functional similarities of APOE and the insect ApoLp-III have been noted, and the comparison may help to deduce further insight into the biological role of APOE. Pathogen recognition and detoxification are particularly quite well studied for ApoLp-III, and this knowledge may support the development of novel approaches for APOE research. Furthermore, studying the effect of the human-specific mutation at position 61 (T → R) in combination with position 287 (M → V) on the interaction of APOE with dietary and pathogenic factors may be a fruitful way of understanding human APOE evolution.

In recent years, an interesting association of the APOE4 isoform with reduced neuronal expression of sirtuins and other neuroprotective factors has been established (Lattanzio et al., 2014; Theendakara et al., 2013). The most surprising is the evidence of the direct transcriptional control of genes related to trophic support, synaptic function or microtubule assembly by APOE, which has been shown to translocate to the nucleus and bind to DNA at approximately 1700 gene promoter regions, including *Sirt1* (Theendakara et al., 2016). In addition, a newly recognized *APOE* promoter polymorphism (rs405509) that has been shown to impact cognitive impairment in the elderly has been identified (Chang et al., 2016; Ma et al., 2016b), and this adverse effect may synergize with *APOE ε4* (Ma et al., 2016a). These novel findings may elucidate new research directions for APOE research in the future, leaving the beaten track of simple APOE-plasma cholesterol associations.

References

Ahmadi, R., Rahimi, Z., Vaisi-Raygani, A., Kiani, A., Jalilian, N., 2012. Apolipoprotein E genotypes, lipid peroxidation, and antioxidant status among mild and severe preeclamptic women from western Iran: protective role of apolipoprotein epsilon2 allele in severe preeclampsia. Hypertens. Pregnancy 31, 405–418.

Alexander, D.M., Williams, L.M., Gatt, J.M., Dobson-Stone, C., Kuan, S.A., Todd, E.G., Schofield, P.R., Cooper, N.J., Gordon, E., 2007. The contribution of apolipoprotein E alleles on cognitive performance and dynamic neural activity over six decades. Biol. Psychol. 75, 229–238.

Allan, C.M., Walker, D., Segrest, J.P., Taylor, J.M., 1995. Identification and characterization of a new human gene (APOC4) in the apolipoprotein E, C-I, and C-II gene locus. Genomics 28, 291–300.

Arnason, U., 2016. The Out of Africa hypothesis and the ancestry of recent humans: cherchez la femme (et l'homme). Gene 585, 9–12.

Aucan, C., Walley, A.J., Hill, A.V., 2004. Common apolipoprotein E polymorphisms and risk of clinical malaria in the Gambia. J. Med. Genet. 41, 21–24.

Azevedo, O.G., Bolick, D.T., Roche, J.K., Pinkerton, R.F., Lima, A.A., Vitek, M.P., Warren, C.A., Oria, R.B., Guerrant, R.L., 2014. Apolipoprotein E plays a key role against cryptosporidial infection in transgenic undernourished mice. PLoS One 9, e89562.

Azuma, M., Kojimab, T., Yokoyama, I., Tajiri, H., Yoshikawa, K., Saga, S., Del Carpio, C.A., 2000. A synthetic peptide of human apoprotein E with antibacterial activity. Peptides 21, 327–330.

Babin, P.J., Thisse, C., Durliat, M., Andre, M., Akimenko, M.A., Thisse, B., 1997. Both apolipoprotein E and A-I genes are present in a nonmammalian vertebrate and are highly expressed during embryonic development. Proc. Natl. Acad. Sci. U. S. A. 94, 8622–8627.

Babin, P.J., Bogerd, J., Kooiman, F.P., Van Marrewijk, W.J., Van der Horst, D.J., 1999. Apolipophorin II/I, apolipoprotein B, vitellogenin, and microsomal triglyceride transfer protein genes are derived from a common ancestor. J. Mol. Evol. 49, 150–160.

Barker, W.C., Dayhoff, M.O., 1977. Evolution of lipoproteins deduced from protein sequence data. Comp. Biochem. Physiol. B 57, 309–315.

Becher, J.C., Keeling, J.W., McIntosh, N., Wyatt, B., Bell, J., 2006. The distribution of apolipoprotein E alleles in Scottish perinatal deaths. J. Med. Genet. 43, 414–418.

Becher, J.C., Keeling, J.W., Bell, J., Wyatt, B., McIntosh, N., 2008. Apolipoprotein E e4 and its prevalence in early childhood death due to sudden infant death syndrome or to recognised causes. Early Hum. Dev. 84, 549–554.

Bennet, A.M., Di Angelantonio, E., Ye, Z., Wensley, F., Dahlin, A., Ahlbom, A., Keavney, B., Collins, R., Wiman, B., de Faire, U., Danesh, J., 2007. Association of apolipoprotein E genotypes with lipid levels and coronary risk. JAMA 298, 1300–1311.

Benvenga, S., Cahnmann, H.J., Robbins, J., 1993. Characterization of thyroid hormone binding to apolipoprotein-E: localization of the binding site in the exon 3-coded domain. Endocrinology 133, 1300–1305.

Beydoun, M.A., Beydoun, H.A., Kaufman, J.S., An, Y., Resnick, S.M., O'Brien, R., Ferrucci, L., Zonderman, A.B., 2013. Apolipoprotein E epsilon4 allele interacts with sex and cognitive status to influence all-cause and cause-specific mortality in US. older adults. J. Am. Geriatr. Soc. 61, 525–534.

Boguski, M.S., Birkenmeier, E.H., Elshourbagy, N.A., Taylor, J.M., Gordon, J.I., 1986. Evolution of the apolipoproteins. Structure of the rat apo-A-IV gene and its relationship to the human genes for apo-A-I C-III, and E. J. Biol. Chem. 261, 6398–6407.
Brodbeck, J., McGuire, J., Liu, Z., Meyer-Franke, A., Balestra, M.E., Jeong, D.E., Pleiss, M., McComas, C., Hess, F., Witter, D., Peterson, S., Childers, M., Goulet, M., Liverton, N., Hargreaves, R., Freedman, S., Weisgraber, K.H., Mahley, R.W., Huang, Y., 2011. Structure-dependent impairment of intracellular apolipoprotein E4 trafficking and its detrimental effects are rescued by small-molecule structure correctors. J. Biol. Chem. 286, 17217–17226.
Chang, P., Li, X., Ma, C., Zhang, S., Liu, Z., Chen, K., Ai, L., Chang, J., Zhang, Z., 2016. The effects of an APOE promoter polymorphism on human white matter connectivity during non-Demented aging. J. Alzheimers Dis. 55, 77–87.
Chen, J., Li, Q., Wang, J., 2011. Topology of human apolipoprotein E3 uniquely regulates its diverse biological functions. Proc. Natl. Acad. Sci. U. S. A. 108, 14813–14818.
Chen, X.F., Wei, Z., Wang, T., Zhang, Z.L., Wang, Y., Heckman, M.G., Diehl, N.N., Zhang, Y.W., Xu, H., Bu, G., 2015. Demographic and lifestyle characteristics, but not apolipoprotein e genotype, are associated with intelligence among young chinese college students. PLoS One 10, e0143157.
Chikosi, A.B., Moodley, J., Pegoraro, R.J., Lanning, P.A., Rom, L., 2000. Apolipoprotein e polymorphism in south african zulu women with preeclampsia. Hypertens. Pregnancy 19, 309–314.
Chin, D., Hagl, S., Hoehn, A., Huebbe, P., Pallauf, K., Grune, T., Frank, J., Eckert, G.P., Rimbach, G., 2014. Adenosine triphosphate concentrations are higher in the brain of APOE3- compared to APOE4-targeted replacement mice and can be modulated by curcumin. Genes Nutr. 9, 397.
Clement-Collin, V., Barbier, A., Dergunov, A.D., Visvikis, A., Siest, G., Desmadril, M., Takahashi, M., Aggerbeck, L.P., 2006. The structure of human apolipoprotein E2, E3 and E4 in solution. 2. Multidomain organization correlates with the stability of apoE structure. Biophys. Chem. 119, 170–185.
Cole, K.D., Fernando-Warnakulasuriya, G.P., Boguski, M.S., Freeman, M., Gordon, J.I., Clark, W.A., Law, J.H., Wells, M.A., 1987. Primary structure and comparative sequence analysis of an insect apolipoprotein Apolipophorin-III from Manduca sexta. J. Biol. Chem. 262, 11794–11800.
Corbo, R.M., Ulizzi, L., Scacchi, R., Martinez-Labarga, C., De Stefano, G.F., 2004. Apolipoprotein E polymorphism and fertility: a study in pre-industrial populations. Mol. Hum. Reprod. 10, 617–620.
Corder, E.H., Saunders, A.M., Strittmatter, W.J., Schmechel, D.E., Gaskell, P.C., Small, G.W., Roses, A.D., Haines, J.L., Pericak-Vance, M.A., 1993. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261, 921–923.
Corella, D., Portoles, O., Arriola, L., Chirlaque, M.D., Barrricarte, A., Frances, F., Huerta, J.M., Larranaga, N., Martinez, C., Martinez-Camblor, P., Molina, E., Navarro, C., Quiros, J.R., Rodriguez, L., Sanchez, M.J., Ros, E., Sala, N., Gonzalez, C.A., Moreno-Iribas, C., 2011. Saturated fat intake and alcohol consumption modulate the association between the APOE polymorphism and risk of future coronary heart disease: a nested case-control study in the Spanish EPIC cohort. J. Nutr. Biochem. 22, 487–494.
de Bont, N., Netea, M.G., Demacker, P.N., Verschueren, I., Kullberg, B.J., van Dijk, K.W., van der Meer, J.W., Stalenhoef, A.F., 1999. Apolipoprotein E knock-out mice are highly susceptible to endotoxemia and Klebsiella pneumoniae infection. J. Lipid Res. 40, 680–685.
Deroo, S., Stengel, F., Mohammadi, A., Henry, N., Hubin, E., Krammer, E.M., Aebersold, R., Raussens, V., 2015. Chemical cross-linking/mass spectrometry maps the amyloid beta peptide binding region on both apolipoprotein E domains. ACS Chem. Biol. 10, 1010–1016.
Dong, L.M., Weisgraber, K.H., 1996. Human apolipoprotein E4 domain interaction. Arginine 61 and glutamic acid 255 interact to direct the preference for very low density lipoproteins. J. Biol. Chem. 271, 19053–19057.
Dose, J., Huebbe, P., Nebel, A., Rimbach, G., 2016. APOE genotype and stress response – a mini review. Lipids Health Dis. 15, 121.
Dryomov, S.V., Nazhmidenova, A.M., Shalaurova, S.A., Morozov, I.V., Tabarev, A.V., Starikovskaya, E.B., Sukernik, R.I., 2015. Mitochondrial genome diversity at the Bering Strait area highlights prehistoric human migrations from Siberia to northern North America. Eur. J. Hum. Genet. 23, 1399–1404.
Duggan, A.E., Callard, I.P., 2001. Phylogenetic distribution of apolipoproteins A-I and E in vertebrates as determined by Western blot analysis. J. Exp. Zool. 290, 255–264.
Durliat, M., Andre, M., Babin, P.J., 2000. Conserved protein motifs and structural organization of a fish gene homologous to mammalian apolipoprotein E. Eur. J. Biochem. 267, 549–559.
Egert, S., Boesch-Saadatmandi, C., Wolffram, S., Rimbach, G., Muller, M.J., 2010. Serum lipid and blood pressure responses to quercetin vary in overweight patients by apolipoprotein E genotype. J. Nutr. 140, 278–284.
Egert, S., Rimbach, G., Huebbe, P., 2012. ApoE genotype: from geographic distribution to function and responsiveness to dietary factors. Proc. Nutr. Soc. 71, 410–424.
Eisenberg, D.T., Kuzawa, C.W., Hayes, M.G., 2010. Worldwide allele frequencies of the human apolipoprotein E gene: climate, local adaptations, and evolutionary history. Am. J. Phys. Anthropol. 143, 100–111.
Enard, W., 2012. Functional primate genomics?leveraging the medical potential. J. Mol. Med. (Berl) 90, 471–480.
Ewbank, D.C., 2004. The APOE gene and differences in life expectancy in Europe. J. Gerontol A Biol. Sci. Med. Sci. 59, 16–20.
Farrer, L.A., Cupples, L.A., Haines, J.L., Hyman, B., Kukull, W.A., Mayeux, R., Myers, R.H., Pericak-Vance, M.A., Risch, N., van Duijn, C.M., 1997. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. JAMA 278, 1349–1356.
Finch, C.E., Sapolsky, R.M., 1999. The evolution of Alzheimer disease, the reproductive schedule, and apoE isoforms. Neurobiol. Aging 20, 407–428.
Finch, C.E., Stanford, C.B., 2004. Meat-adaptive genes and the evolution of slower aging in humans. Q. Rev. Biol. 79, 3–50.
Finch, C.E., 2007. The biology of human longevity. In: Inflammation, Nutrition, and Aging in the Evolution of Lifespans. Academic Press, San Diego.
Frieden, C., Garai, K., 2012. Structural differences between apoE3 and apoE4 may be useful in developing therapeutic agents for Alzheimer's disease. Proc. Natl. Acad. Sci. U. S. A. 109, 8913–8918.
Frieden, C., Garai, K., 2013. Concerning the structure of apoE. Protein Sci. 22, 1820–1825.
Frieden, C., 2015. ApoE: the role of conserved residues in defining function. Protein Sci. 24, 138–144.
Friedman, G., Froom, P., Sazbon, L., Grinblatt, I., Shochina, M., Tsenter, J., Babaey, S., Yehuda, B., Groswasser, Z., 1999. Apolipoprotein E-epsilon4 genotype predicts a poor outcome in survivors of traumatic brain injury. Neurology 52, 244–248.
Fujioka, H., Phelix, C.F., Friedland, R.P., Zhu, X., Perry, E.A., Castellani, R.J., Perry, G., 2013. Apolipoprotein E4 prevents growth of malaria at the intraerythrocyte stage: implications for differences in racial susceptibility to Alzheimer's disease. J. Health Care Poor Underserved 24, 70–78.
Fullerton, S.M., Clark, A.G., Weiss, K.M., Nickerson, D.A., Taylor, S.L., Stengard, J.H., Salomaa, V., Vartiainen, E., Perola, M., Boerwinkle, E., Sing, C.F., 2000. Apolipoprotein E variation at the sequence haplotype level: implications for the origin and maintenance of a major human polymorphism. Am. J. Hum. Genet. 67, 881–900.
Gerdes, L.U., Gerdes, C., Hansen, P.S., Klausen, I.C., Faergeman, O., 1996. Are men carrying the apolipoprotein epsilon 4- or epsilon 2 allele less fertile than epsilon 3 epsilon 3 genotypes? Hum. Genet. 98, 239–242.
Goujon, M., McWilliam, H., Li, W., Valentin, F., Squizzato, S., Paern, J., Lopez, R., 2010. A new bioinformatics analysis tools framework at EMBL-EBI. Nucleic Acids Res. 38, W695–699.
Graeser, A.C., Boesch-Saadatmandi, C., Lippmann, J., Wagner, A.E., Huebbe, P., Storm, N., Hoppner, W., Wiswedel, I., Gardemann, A., Minihane, A.M., Doring, F., Rimbach, G., 2011. Nrf2-dependent gene expression is affected by the proatherogenic apoE4 genotype-studies in targeted gene replacement mice. J. Mol. Med. (Berl) 89, 1027–1035.
Graeser, A.C., Huebbe, P., Storm, N., Hoppner, W., Doring, F., Wagner, A.E., Rimbach, G., 2012. Apolipoprotein E genotype affects tissue metallothionein levels: studies in targeted gene replacement mice. Genes Nutr. 7, 247–255.
Green, R.E., Krause, J., Briggs, A.W., Maricic, T., Stenzel, U., Kircher, M., Patterson, N., Li, H., Zhai, W., Fritz, M.H., Hansen, N.F., Durand, E.Y., Malaspinas, A.S., Jensen, J.D., Marques-Bonet, T., Alkan, C., Prufer, K., Meyer, M., Burbano, H.A., Good, J.M., Schultz, R., Aximu-Petri, A., Butthof, A., Hober, B., Hoffner, B., Siegemund, M., Weihmann, A., Nusbaum, C., Lander, E.S., Russ, C., Novod, N., Affourtit, J., Egholm, M., Verna, C., Rudan, P., Brajkovic, D., Kucan, Z., Gusic, I., Doronichev, V.B., Golovanova, L.V., Lalueza-Fox, C., de la Rasilla, M., Fortea, J., Rosas, A., Schmitz, R.W., Johnson, P.L., Eichler, E.E., Falush, D., Birney, E., Mullikin, J.C., Slatkin, M., Nielsen, R., Kelso, J., Lachmann, M., Reich, D., Paabo, S., 2010. A draft sequence of the Neandertal genome. Science 328, 710–722.
Gupta, L., Noh, J.Y., Jo, Y.H., Oh, S.H., Kumar, S., Noh, M.Y., Lee, Y.S., Cha, S.J., Seo, S.J., Kim, I., Han, Y.S., Barillas-Mury, C., 2010. Apolipophorin-III mediates antiplasmodial epithelial responses in Anopheles gambiae (G3) mosquitoes. PLoS One 5, e15410.
Gureje, O., Ogunniyi, A., Baiyewu, O., Price, B., Unverzagt, F.W., Evans, R.M., Smith-Gamble, V., Lane, K.A., Gao, S., Hall, K.S., Hendrie, H.C., Murrell, J.R., 2006. APOE epsilon4 is not associated with Alzheimer's disease in elderly Nigerians. Ann. Neurol. 59, 182–185.
Hagberg, J.M., Ferrell, R.E., Katzel, L.I., Dengel, D.R., Sorkin, J.D., Goldberg, A.P., 1999. Apolipoprotein E genotype and exercise training-induced increases in plasma high-density lipoprotein (HDL)- and HDL2-cholesterol levels in overweight men. Metabolism 48, 943–945.
Hatters, D.M., Peters-Libeu, C.A., Weisgraber, K.H., 2006. Apolipoprotein E structure: insights into function. Trends Biochem. Sci. 31, 445–454.
Hendrie, H.C., Murrell, J., Baiyewu, O., Lane, K.A., Purnell, C., Ogunniyi, A., Unverzagt, F.W., Hall, K., Callahan, C.M., Saykin, A.J., Gureje, O., Hake, A., Foroud, T., Gao, S., 2014. APOE epsilon4 and the risk for Alzheimer disease and cognitive decline in African Americans and Yoruba. Int. Psychogeriatr. 26, 977–985.
Henn, B.M., Cavalli-Sforza, L.L., Feldman, M.W., 2012. The great human expansion. Proc. Natl. Acad. Sci. U. S. A. 109, 17758–17764.
Hu, P., Qin, Y.H., Jing, C.X., Lu, L., Hu, B., Du, P.F., 2011. Does the geographical gradient of ApoE4 allele exist in China?: A systemic comparison among multiple Chinese populations. Mol. Biol. Rep. 38, 489–494.
Huebbe, P., Jofre-Monseny, L., Boesch-Saadatmandi, C., Minihane, A.M., Rimbach, G., 2007. Effect of apoE genotype and vitamin E on biomarkers of oxidative stress in cultured neuronal cells and the brain of targeted replacement mice. J. Physiol. Pharmacol. 58, 683–698.
Huebbe, P., Nebel, A., Siegert, S., Moehring, J., Boesch-Saadatmandi, C., Most, E., Pallauf, J., Egert, S., Muller, M.J., Schreiber, S., Nothlings, U., Rimbach, G., 2011. APOE epsilon4 is associated with higher vitamin D levels in targeted replacement mice and humans. FASEB J. 25, 3262–3270.

Huebbe, P., Dose, J., Schloesser, A., Campbell, G., Gluer, C.C., Gupta, Y., Ibrahim, S., Minihane, A.M., Baines, J.F., Nebel, A., Rimbach, G., 2015. Apolipoprotein E (APOE) genotype regulates body weight and fatty acid utilization-Studies in gene-targeted replacement mice. Mol. Nutr. Food Res. 59, 334–343.
Huebbe, P., Lange, J., Lietz, G., Rimbach, G., 2016. Dietary beta-carotene and lutein metabolism is modulated by the APOE genotype. Biofactors 42, 388–396.
Jacobsen, R., Martinussen, T., Christiansen, L., Jeune, B., Andersen-Ranberg, K., Vaupel, J.W., Christensen, K., 2010. Increased effect of the ApoE gene on survival at advanced age in healthy and long-lived Danes: two nationwide cohort studies. Aging Cell 9, 1004–1009.
Jamalzei, B., Fallah, S., Kashanian, M., Seifi, M., 2013. Association of the apolipoprotein E variants with susceptibility to pregnancy with preeclampsia. Clin. Lab. 59, 563–570.
Jasienska, G., Ellison, P.T., Galbarczyk, A., Jasienski, M., Kalemba-Drozdz, M., Kapiszewska, M., Nenko, I., Thune, I., Ziomkiewicz, A., 2015. Apolipoprotein E (ApoE) polymorphism is related to differences in potential fertility in women: a case of antagonistic pleiotropy? Proc. Biol. Sci. 282, 20142395.
Jeenduang, N., Porntadavity, S., Nuinoon, M., Horpet, D., Thepkwan, N., Thaworn, P., Theanmontri, S., 2015. Studies of the CETP TaqIB and ApoE polymorphisms in southern thai subjects with the metabolic syndrome. Biochem. Genet. 53, 184–199.
Joshi, P.K., Fischer, K., Schraut, K.E., Campbell, H., Esko, T., Wilson, J.F., 2016. Variants near CHRNA3/5 and APOE have age- and sex-related effects on human lifespan. Nat. Commun. 7, 11174.
Kim, Y.I., Kim, H.J., Kwon, Y.M., Kang, Y.J., Lee, I.H., Jin, B.R., Han, Y.S., Kim, I., Cheon, H.M., Ha, N.G., Seo, S.J., 2011. RNA interference mediated knockdown of apolipophorin-III leads to knockdown of manganese superoxide dismutase in Hyphantria cunea. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 159, 303–312.
Kino, K., Sugiyama, H., 2001. Possible cause of G-C-> C-G transversion mutation by guanine oxidation product, imidazolone. Chem. Biol. 8, 369–378.
Konishi, K., Bhat, V., Banner, H., Poirier, J., Joober, R., Bohbot, V.D., 2016. APOE2 is associated with spatial navigational strategies and increased gray matter in the hippocampus. Front. Hum. Neurosci. 10, 349.
Kulminski, A.M., Arbeev, K.G., Culminskaya, I., Arbeeva, L., Ukraintseva, S.V., Stallard, E., Christensen, K., Schupf, N., Province, M.A., Yashin, A.I., 2014. Age, gender, and cancer but not neurodegenerative and cardiovascular diseases strongly modulate systemic effect of the Apolipoprotein E4 allele on lifespan. PLoS Genet. 10, e1004141.
Kulminski, A.M., Raghavachari, N., Arbeev, K.G., Culminskaya, I., Arbeeva, L., Wu, D., Ukraintseva, S.V., Christensen, K., Yashin, A.I., 2016. Protective role of the apolipoprotein E2 allele in age-related disease traits and survival: evidence from the Long Life Family Study. Biogerontology 17, 893–905.
Larkin, M.A., Blackshields, G., Brown, N.P., Chenna, R., McGettigan, P.A., McWilliam, H., Valentin, F., Wallace, I.M., Wilm, A., Lopez, R., Thompson, J.D., Gibson, T.J., Higgins, D.G., 2007. Clustal W and clustal x version 2.0. Bioinformatics 23, 2947–2948.
Lattanzio, F., Carboni, L., Carretta, D., Rimondini, R., Candeletti, S., Romualdi, P., 2014. Human apolipoprotein E4 modulates the expression of Pin1, Sirtuin 1, and Presenilin 1 in brain regions of targeted replacement apoE mice. Neuroscience 256, 360–369.
Li, W.H., Tanimura, M., Luo, C.C., Datta, S., Chan, L., 1988. The apolipoprotein multigene family: biosynthesis, structure, structure-function relationships, and evolution. J. Lipid Res. 29, 245–271.
Li, W., Cowley, A., Uludag, M., Gur, T., McWilliam, H., Squizzato, S., Park, Y.M., Buso, N., Lopez, R., 2015. The EMBL-EBI bioinformatics web and programmatic tools framework. Nucleic Acids Res. 43, W580–584.
Liu, W., Martinon-Torres, M., Cai, Y.J., Xing, S., Tong, H.W., Pei, S.W., Sier, M.J., Wu, X.H., Edwards, R.L., Cheng, H., Li, Y.Y., Yang, X.X., de Castro, J.M., Wu, X.J., 2015. The earliest unequivocally modern humans in southern China. Nature 526, 696–699.
Luca, F., Perry, G.H., Di Rienzo, A., 2010. Evolutionary adaptations to dietary changes. Annu. Rev. Nutr. 30, 291–314.
Luo, C.C., Li, W.H., Moore, M.N., Chan, L., 1986. Structure and evolution of the apolipoprotein multigene family. J. Mol. Biol. 187, 325–340.
Ma, C., Zhang, Y., Li, X., Chen, Y., Zhang, J., Liu, Z., Chen, K., Zhang, Z., 2016a. The TT allele of rs405509 synergizes with APOE epsilon4 in the impairment of cognition and its underlying default mode network in non-demented elderly. Curr. Alzheimer Res. 13, 708–717.
Ma, C., Zhang, Y., Li, X., Zhang, J., Chen, K., Liang, Y., Chen, Y., Liu, Z., Zhang, Z., 2016b. Is there a significant interaction effect between apolipoprotein E rs405509 T/T and epsilon4 genotypes on cognitive impairment and gray matter volume? Eur J. Neurol. 23, 1415–1425.
McIntosh, A.M., Bennett, C., Dickson, D., Anestis, S.F., Watts, D.P., Webster, T.H., Fontenot, M.B., Bradley, B.J., 2012. The apolipoprotein E (APOE) gene appears functionally monomorphic in chimpanzees (Pan troglodytes). PLoS One 7, e47760.
Meng, F.T., Wang, Y.L., Liu, J., Zhao, J., Liu, R.Y., Zhou, J.N., 2012. ApoE genotypes are associated with age at natural menopause in Chinese females. Age (Dordr) 34, 1023–1032.
Mizuguchi, C., Hata, M., Dhanasekaran, P., Nickel, M., Okuhira, K., Phillips, M.C., Lund-Katz, S., Saito, H., 2014. Fluorescence study of domain structure and lipid interaction of human apolipoproteins E3 and E4. Biochim. Biophys. Acta 1841, 1716–1724.
Nankar, S.A., Pande, A.H., 2013. Physicochemical properties of bacterial pro-inflammatory lipids influence their interaction with apolipoprotein-derived peptides. Biochim. Biophys. Acta 1831, 853–862.
Nebel, A., Croucher, P.J., Stiegeler, R., Nikolaus, S., Krawczak, M., Schreiber, S., 2005. No association between microsomal triglyceride transfer protein (MTP) haplotype and longevity in humans. Proc. Natl. Acad. Sci. U. S. A. 102, 7906–7909.
Nguyen, D., Dhanasekaran, P., Phillips, M.C., Lund-Katz, S., 2009. Molecular mechanism of apolipoprotein E binding to lipoprotein particles. Biochemistry (Mosc). 48, 3025–3032.
Nguyen, D., Dhanasekaran, P., Nickel, M., Mizuguchi, C., Watanabe, M., Saito, H., Phillips, M.C., Lund-Katz, S., 2014. Influence of domain stability on the properties of human apolipoprotein E3 and E4 and mouse apolipoprotein E. Biochemistry (Mosc). 53, 4025–4033.
Niu, W., Qi, Y., Qian, Y., Gao, P., Zhu, D., 2009. The relationship between apolipoprotein E epsilon2/epsilon3/epsilon4 polymorphisms and hypertension: a meta-analysis of six studies comprising 1812 cases and 1762 controls. Hypertens. Res. 32, 1060–1066.
Olivieri, O., Martinelli, N., Bassi, A., Trabetti, E., Girelli, D., Pizzolo, F., Friso, S., Pignatti, P.F., Corrocher, R., 2007. ApoE epsilon2/epsilon3/epsilon4 polymorphism, ApoC-III/ApoE ratio and metabolic syndrome. Clin. Exp. Med. 7, 164–172.
Oria, R.B., Patrick, P.D., Zhang, H., Lorntz, B., de Castro Costa, C.M., Brito, G.A., Barrett, L.J., Lima, A.A., Guerrant, R.L., 2005. APOE4 protects the cognitive development in children with heavy diarrhea burdens in Northeast Brazil. Pediatr. Res. 57, 310–316.
Oria, R.B., Patrick, P.D., Oria, M.O., Lorntz, B., Thompson, M.R., Azevedo, O.G., Lobo, R.N., Pinkerton, R.F., Guerrant, R.L., Lima, A.A., 2010. ApoE polymorphisms and diarrheal outcomes in Brazilian shanty town children. Braz. J. Med. Biol. Res. 43, 249–256.
Palusinska-Szysz, M., Zdybicka-Barabas, A., Cytrynska, M., Wdowiak-Wrobel, S., Chmiel, E., Gruszecki, W.I., 2015. Analysis of cell surface alterations in Legionella pneumophila cells treated with human apolipoprotein E. Pathog. Dis. 73, 1–8.
Pane, K., Sgambati, V., Zanfardino, A., Smaldone, G., Cafaro, V., Angrisano, T., Pedone, E., Di Gaetano, S., Capasso, D., Haney, E.F., Izzo, V., Varcamonti, M., Notomista, E., Hancock, R.E., Di Donato, A., Pizzo, E., 2016. A new cryptic cationic antimicrobial peptide from human apolipoprotein E with antibacterial activity and immunomodulatory effects on human cells. FEBS J. 283, 2115–2131.
Peterson, K.J., Lyons, J.B., Nowak, K.S., Takacs, C.M., Wargo, M.J., McPeek, M.A., 2004. Estimating metazoan divergence times with a molecular clock. Proc. Natl. Acad. Sci. U. S. A. 101, 6536–6541.
Raffai, R.L., Dong, L.M., Farese Jr., R.V., Weisgraber, K.H., 2001. Introduction of human apolipoprotein E4 domain interaction into mouse apolipoprotein E. Proc. Natl. Acad. Sci. U. S. A. 98, 11587–11591.
Rajavashisth, T.B., Kaptein, J.S., Reue, K.L., Lusis, A.J., 1985. Evolution of apolipoprotein E: mouse sequence and evidence for an 11-nucleotide ancestral unit. Proc. Natl. Acad. Sci. U. S. A. 82, 8085–8089.
Ramassamy, C., Averill, D., Beffert, U., Theroux, L., Lussier-Cacan, S., Cohn, J.S., Christen, Y., Schoofs, A., Davignon, J., Poirier, J., 2000. Oxidative insults are associated with apolipoprotein E genotype in Alzheimer's disease brain. Neurobiol. Dis. 7, 23–37.
Reich, D., Green, R.E., Kircher, M., Krause, J., Patterson, N., Durand, E.Y., Viola, B., Briggs, A.W., Stenzel, U., Johnson, P.L., Maricic, T., Good, J.M., Marques-Bonet, T., Alkan, C., Fu, Q., Mallick, S., Li, H., Meyer, M., Eichler, E.E., Stoneking, M., Richards, M., Talamo, S., Shunkov, M.V., Derevianko, A.P., Hublin, J.J., Kelso, J., Slatkin, M., Paabo, S., 2010. Genetic history of an archaic hominin group from Denisova Cave in Siberia. Nature 468, 1053–1060.
Rossignol, T., Kelly, B., Dobson, C., d'Enfert, C., 2011. Endocytosis-mediated vacuolar accumulation of the human ApoE apolipoprotein-derived ApoEdpL-W antimicrobial peptide contributes to its antifungal activity in Candida albicans. Antimicrob. Agents Chemother. 55, 4670–4681.
Rougeron, V., Woods, C.M., Tiedje, K.E., Bodeau-Livinec, F., Migot-Nabias, F., Deloron, P., Luty, A.J., Fowkes, F.J., Day, K.P., 2013. Epistatic Interactions between apolipoprotein E and hemoglobin S Genes in regulation of malaria parasitemia. PLoS One 8, e76924.
Schachter, F., Faure-Delanef, L., Guenot, F., Rouger, H., Froguel, P., Lesueur-Ginot, L., Cohen, D., 1994. Genetic associations with human longevity at the APOE and ACE loci. Nat. Genet. 6, 29–32.
Schurr, T.G., Sherry, S.T., 2004. Mitochondrial DNA and Y chromosome diversity and the peopling of the Americas: evolutionary and demographic evidence. Am. J. Hum. Biol.: Off. J. Hum. Biol. Counc. 16, 420–439.
Schwarz, F., Springer, S.A., Altheide, T.K., Varki, N.M., Gagneux, P., Varki, A., 2016. Human-specific derived alleles of CD33 and other genes protect against postreproductive cognitive decline. Proc. Natl. Acad. Sci. U. S. A. 113, 74–79.
Seip, R.L., Zoeller, R.F., Angelopoulos, T.J., Salonia, J., Bilbie, C., Moyna, N.M., Miles, M.P., Visich, P.S., Pescatello, L.S., Gordon, P.M., Tsongalis, G.J., Bausserman, L., Thompson, P.D., 2011. Interactive effects of APOE haplotype, sex, and exercise on postheparin plasma lipase activities. J. Appl. Physiol. (1985) 110, 1021–1028.
Seripa, D., D'Onofrio, G., Panza, F., Cascavilla, L., Masullo, C., Pilotto, A., 2011. The genetics of the human APOE polymorphism. Rejuvenation Res. 14, 491–500.
Shaw, P., Lerch, J.P., Pruessner, J.C., Taylor, K.N., Rose, A.B., Greenstein, D., Clasen, L., Evans, A., Rapoport, J.L., Giedd, J.N., 2007. Cortical morphology in children and

adolescents with different apolipoprotein E gene polymorphisms: an observational study. Lancet Neurol. 6, 494–500.
Sima, A., Iordan, A., Stancu, C., 2007. Apolipoprotein E polymorphism?a risk factor for metabolic syndrome. Clin. Chem. Lab. Med. 45, 1149–1153.
Singh, P.P., Singh, M., Mastana, S.S., 2006. APOE distribution in world populations with new data from India and the UK. Ann. Hum. Biol. 33, 279–308.
Smith, A.F., Owen, L.M., Strobel, L.M., Chen, H., Kanost, M.R., Hanneman, E., Wells, M.A., 1994. Exchangeable apolipoproteins of insects share a common structural motif. J. Lipid Res. 35, 1976–1984.
Steinetz, B.G., Randolph, C., Cohn, D., Mahoney, C.J., 1996. Lipoprotein profiles and glucose tolerance in lean and obese chimpanzees. J. Med. Primatol. 25, 17–25.
Sun, Y., Wei, R., Yan, D., Xu, F., Zhang, X., Zhang, B., Yimiti, D., Li, H., Sun, H., Hu, C., Luo, L., Yao, H., 2016. Association between APOE polymorphism and metabolic syndrome in Uyghur ethnic men. BMJ Open 6, e010049.
Tackney, J.C., Potter, B.A., Raff, J., Powers, M., Watkins, W.S., Warner, D., Reuther, J.D., Irish, J.D., O'Rourke, D.H., 2015. Two contemporaneous mitogenomes from terminal Pleistocene burials in eastern Beringia. Proc. Natl. Acad. Sci. U. S. A. 112, 13833–13838.
Tang, M.X., Stern, Y., Marder, K., Bell, K., Gurland, B., Lantigua, R., Andrews, H., Feng, L., Tycko, B., Mayeux, R., 1998. The APOE-epsilon4 allele and the risk of Alzheimer disease among African Americans, whites, and Hispanics. JAMA 279, 751–755.
Tassi, F., Ghirotto, S., Mezzavilla, M., Vilaca, S.T., De Santi, L., Barbujani, G., 2015. Early modern human dispersal from Africa: genomic evidence for multiple waves of migration. Invest. Genet. 6, 13.
Taylor, A.E., Guthrie, P.A., Smith, G.D., Golding, J., Sattar, N., Hingorani, A.D., Deanfield, J.E., Day, I.N., 2011. IQ, educational attainment, memory and plasma lipids: associations with apolipoprotein E genotype in 5995 children. Biol. Psychiatry 70, 152–158.
Theendakara, V., Patent, A., Libeu, C.A. Peters, Philpot, B., Flores, S., Descamps, O., Poksay, K.S., Zhang, Q., Cailing, G., Hart, M., John, V., Rao, R.V., Bredesen, D.E., 2013. Neuroprotective sirtuin ratio reversed by ApoE4. Proc. Natl. Acad. Sci. U. S. A. 110, 18303–18308.
Theendakara, V., Peters-Libeu, C.A., Spilman, P., Poksay, K.S., Bredesen, D.E., Rao, R.V., 2016. Direct transcriptional effects of apolipoprotein e. J. Neurosci. 36, 685–700.
Thompson, P.D., Tsongalis, G.J., Seip, R.L., Bilbie, C., Miles, M., Zoeller, R., Visich, P., Gordon, P., Angelopoulos, T.J., Pescatello, L., Bausserman, L., Moyna, N., 2004. Apolipoprotein E genotype and changes in serum lipids and maximal oxygen uptake with exercise training. Metabolism 53, 193–202.
Turic, D., Fisher, P.J., Plomin, R., Owen, M.J., 2001. No association between apolipoprotein E polymorphisms and general cognitive ability in children. Neurosci. Lett. 299, 97–100.
Tzourio, C., Arima, H., Harrap, S., Anderson, C., Godin, O., Woodward, M., Neal, B., Bousser, M.G., Chalmers, J., Cambien, F., MacMahon, S., 2008. APOE genotype, ethnicity, and the risk of cerebral hemorrhage. Neurology 70, 1322–1328.
Utermann, G., 1975. Isolation and partial characterization of an arginine-rich apolipoprotein from human plasma very-low-density lipoproteins: apolipoprotein E. Hoppe. Seylers Z. Physiol. Chem. 356, 1113–1121.
van den Bergh, G.D., Li, B., Brumm, A., Grun, R., Yurnaldi, D., Moore, M.W., Kurniawan, I., Setiawan, R., Aziz, F., Roberts, R.G., Suyono Storey, M., Setiabudi, E., Morwood, M.J., 2016. Earliest hominin occupation of Sulawesi Indonesia. Nature 529, 208–211.
Van Oosten, M., Rensen, P.C., Van Amersfoort, E.S., Van Eck, M., Van Dam, A.M., Breve, J.J., Vogel, T., Panet, A., Van Berkel, T.J., Kuiper, J., 2001. Apolipoprotein E protects against bacterial lipopolysaccharide-induced lethality: a new therapeutic approach to treat gram-negative sepsis. J. Biol. Chem. 276, 8820–8824.
Vasunilashorn, S., Finch, C.E., Crimmins, E.M., Vikman, S.A., Stieglitz, J., Gurven, M., Kaplan, H., Allayee, H., 2011. Inflammatory gene variants in the Tsimane, an indigenous Bolivian population with a high infectious load. Biodemography Soc. Biol. 57, 33–52.
Wang, X., Shang, X., Luan, J., Zhang, S., 2014. Identification, expression and function of apolipoprotein E in annual fish Nothobranchius guentheri: implication for an aging marker. Biogerontology 15, 233–243.
Weers, P.M., Ryan, R.O., 2006. Apolipophorin III: role model apolipoprotein. Insect Biochem. Mol. Biol. 36, 231–240.
Wen, D., Wang, X., Shang, L., Huang, Y., Li, T., Wu, C., Zhang, R., Zhang, J., 2016. Involvement of a versatile pattern recognition receptor, apolipophorin-III in prophenoloxidase activation and antibacterial defense of the Chinese oak silkworm. Antheraea pernyi. Dev. Comp. Immunol. 65, 124–131.
Williams, B., 2nd Convertino, M., Das, J., Dokholyan, N.V., 2015. ApoE4-specific misfolded intermediate identified by molecular dynamics simulations. PLoS Comput. Biol. 11, e1004359.
Wozniak, M.A., Faragher, E.B., Todd, J.A., Koram, K.A., Riley, E.M., Itzhaki, R.F., 2003. Does apolipoprotein E polymorphism influence susceptibility to malaria? J. Med. Genet. 40, 348–351.
Wozniak, M.A., Riley, E.M., Itzhaki, R.F., 2004. Apolipoprotein E polymorphisms and risk of malaria. J. Med. Genet. 41, 145–146.
Wright, R.O., Hu, H., Silverman, E.K., Tsaih, S.W., Schwartz, J., Bellinger, D., Palazuelos, E., Weiss, S.T., Hernandez-Avila, M., 2003. Apolipoprotein E genotype predicts 24-month bayley scales infant development score. Pediatr. Res. 54, 819–825.
Yang, S.L., He, B.X., Liu, H.L., He, Z.Y., Zhang, H., Luo, J.P., Hong, X.F., Zou, Y.C., 2004. Apolipoprotein E gene polymorphisms and risk for coronary artery disease in Chinese Xinjiang Uygur and Han population. Chin. Med. Sci. J. 19, 150–154.
Yu, Y.W., Lin, C.H., Chen, S.P., Hong, C.J., Tsai, S.J., 2000. Intelligence and event-related potentials for young female human volunteer apolipoprotein E epsilon4 and non-epsilon4 carriers. Neurosci. Lett. 294, 179–181.
Yu, B., Chen, W., Wang, R., Qi, Q., Li, K., Zhang, W., Wang, H., 2014. Association of apolipoprotein E polymorphism with maximal oxygen uptake after exercise training: a study of Chinese young adult. Lipids Health Dis. 13, 40.
Zannis, V.I., Nicolosi, R.J., Jensen, E., Breslow, J.L., Hayes, K.C., 1985. Plasma and hepatic apoE isoproteins of nonhuman primates. Differences in apoE among humans, apes, and New and Old World monkeys. J. Lipid Res. 26, 1421–1430.
Zhang, R., Wang, X., Tang, Z., Liu, J., Yang, S., Zhang, Y., Wei, Y., Luo, W., Wang, J., Li, J., Chen, B., Zhang, K., 2014. Apolipoprotein E gene polymorphism and the risk of intracerebral hemorrhage: a meta-analysis of epidemiologic studies. Lipids Health Dis. 13, 47.