Chapter 11

Normal Aging and Dementia

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Additional information is available at the end of the chapter

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Abstract

Normal aging begins after 60 years of age. According to Harman, the accumulation of free radicals, which results from weakening of repair and protective mechanisms, takes place in the aging brain. It is believed that especially in the population of the most elderly there is increased incidence of both dementia and depression. The causes of these central nervous system disorders in the aging human body are changes at the molecular level, such as changes in the biochemical parameters, the accumulation of mutations in nuclear and mitochondrial DNA, and epigenetic changes. Biomarkers associated with aging of the brain include accumulated deposits of β-amyloid (Aβ), disturbed cholesterol homeostasis, altered neuroimaging parameters, and impaired glucose metabolism. Genetic factors are also responsible for normal aging, for example, *SIRT1*, *AKT1*, and *CDKN1A*, and among them the longevity genes, such as *FOXO3A* and *CETP*. Dementia as well as cognitive decline may be modified by poly-T variants of *TOMM40* and *APOE* alleles via influencing the level of apolipoprotein E (apoE) in the brain and in the plasma as well as by its ability of Aβ clearance.

Identifying the molecular factors associated with aging and dementia may help introduce new approaches to preventing geriatric disorders, including depression and dementia.

Keywords: molecular factors, dementia, normal aging

1. Introduction

Currently, average life expectancy in the world is over 60 years. The world's longest life expectancy is in Japan, at 82.2 years, and in Australia, at 80.6 years. In Europe, the longest-

lived people are the French, at 80.6 years, the Swedes, at 80.6 years, the Italians, at 79.9 years, the Greeks, at 79.3 years, the Dutch, at 79.1 years, and the Germans, at 78.9 years. It is predicted that in Europe from 2005 to 2050, the number of people following into their 80th year of life will increase by 43 million [1].

In psychological studies on the elderly, three subperiods were stratified among people over 60 years old; these included *young olds* (65–75 years of age), *old olds* (75–85 years old), and *eldest olds* (over 85 years of age). Deterioration of cognitive functions was visible in the *eldest olds* age group, while asymptomatic structural changes in the brain, such as cortical atrophy, poli- and leukoaraiosis (deterioration of white matter, present in 8–90% of the elderly with no signs of dementia), or decreased glucose metabolism and deteriorated subcortical and cortical flow, could be detected by neuroimaging even among the *young olds* [2].

Progressive aging of the population is one of the factors determining the increasingly frequent occurrence of cognitive impairment and dementia syndromes. Dementia, due to its prevalence in the population (it occurs in approximately 10% of those 65 years of age and in approximately 30–40% of those 90 years of age), requires great concern and clinical care. It is estimated that by 2040, the number of elderly people with dementia in the world will exceed 80 million [3].

According to the classification of mental disorders in the American Psychiatric Association's DSM – IV (Diagnostic and Statistical Manual of Mental Disorders) [4], there is no isolated, separate diagnostic category for "dementia," but the criteria for this diagnosis are contained in the various types of dementia, for example, Alzheimer's disease (AD), vascular dementia (VD), or in other diseases. According to these criteria, a diagnosis of dementia is necessary to determine the presence of multiple cognitive deficits that cause significant disturbances in the functioning of social exclusion and mental illness (depression) and delirium.

Dementia is a progressive impairment of the functional status and significantly reduces the quality of life of older people in all its dimensions, since physical disability and the loss of sphincter control coexist along with dementia. The most common cause of dementia in the oldest patients is the degenerative process that is underway in the brain in the course of AD. In old age, an important process associated with the degeneration of neurons in AD is cerebral arteriosclerosis. Dementia, with dying neurons, is caused by both pathologies. A high percentage of the causes of dementia in the elderly may develop depression. In these patients, the following is observed especially often: loss of interest, sleep disturbances, psychomotor disturbances, and problems with concentration. In turn, the use of multiple drugs in dementia complicates the diagnosis of depression [4].

Depression is defined as an emotional distress syndrome (states of depressed mood, depression), which is often co-morbid with somatic diseases and/or with intensifying their symptoms. Depression is a common and serious problem among the elderly and increases mortality. Approximately 15% of people over the age of 65 have symptoms of depression, which impede daily functioning [2, 4].

It is believed that the functional and cognition changes observed in older persons are associated with disturbances at the molecular level in the aging body. Molecular changes in the aging

process may relate to genomic instability as a result of accumulation of mutations, telomere attrition and epigenetic alterations, and alteration in the level of brain biomarkers [2].

To select significant studies for this review, the authors conducted multiple searches through public databases, including PubMed and Scopus, by using the following search strategy: ("normal aging" or "aging") and ("dementia" or "cognitive decline") and ("biomarker" or "SNP" or "genetic polymorphism" or "mutation"). The last search was performed in February 2016. A subsequent data mining through review articles and references facilitated finding additional eligible studies.

2. Brain biomarkers and cognitive function in normal aging

Central changes leading to impaired brain and cognition functions have been reported in normal brain aging, but data are inconclusive [5, 6]. A study [5] using functional magnetic resonance imaging (MRI) with gadolinium contrast confirmed changes in the hippocampus associated with impairment of cognitive function in elderly people. Also, a study conducted on 564 cognitively normal individuals (average age was 78 years) using MRI and fluorodeoxyglucose positron emission tomography (FDG-PET) and Pittsburgh Compound B (PiB) PET indicated impairment of cognition and imaging biomarkers. The causes of these central changes in the brain of the aged subjects seem to have been increased β-amyloid (Aβ) levels [6]. In the senescent brain, accumulation of Aβ deposits is eminent, in the form of senile plaques as well as fibrillary tangles in the neurons. The lesions may develop in the human brain as late as in one's 80s (frequently with no signs of dementia). The slower the accumulation of lesions is, the longer the time period required to develop dementia [7, 8]. Cerebral amyloidosis has been associated not only with Aβ deposition but also with higher pulse pressure in the presence of neurodegeneration, which may lead to more rapid progression of dementia [9]. However, more recent data indicate that Aβ deposition may in time exceed brain structural changes, such as grey matter volume, as measured by MRI, and neuronal hypometabolism assessed using PET with 18F-fluorodeoxyglucose (FDG) [10]. Moreover, the cognitive decline in elderly patients is associated with brain infarcts [11]. Also dietary factors, such as ω-3 polyunsaturated fatty acids (PUFAs), were shown to be associated with normal brain function; the PUFA concentration remains in reverse correlation with brain atrophy in cognitively normal elders [10].

3. Molecular changes in normal aging and dementia

In the aging process, the epigenetic changes lead to expression alteration of genes associated with vital functions of cells, such as mitochondrial function, as well as protective and repair mechanisms, as shown in **Table 1**.

One of genetic hallmarks of aging is genomic instability which includes accumulation of genetic damage both in nuclear DNA (nDNA) and mitochondrial DNA (mtDNA). mtDNA is

more susceptible to DNA damaging factors than nDNA due to its oxidative environment. Also DNA repair mechanisms are less efficient in mitochondria than in nucleus [23], although mitochondria have most of the DNA repair pathways existing in the nucleus [24]. The mitochondrial reactive oxygen species (ROS) theory of aging (mitochondrial free radical theory of aging [MFRTA]) proposed by Harman assumes that free radicals generated in normal metabolism cause mtDNA mutations and ageing is a result of oxidative damage accumulation. According to MFRTA, maximum life span can be decreased by mtDNA damage caused by oxidative stress [25]. Mitochondrial reactive oxygen species (mtROS) may play signaling role in mitochondrial stress during ageing [26]. It is also suggested that mtDNA mutation accumulation and mitochondrial dysfunction during aging are a result of decreased activity of autophagy and mitophagy [27]. Moreover, a study performed on 18 three-generation families of women shows decline in mtDNA copy number, mitochondrial protein expression, and oxidative function with age [28]. Oxidative DNA damage and mitochondrial dysfunction lead to neuronal loss and may play a role in the development of dementia. The decreased level of antioxidants was observed among dementia individuals [29, 30]. It was suggested that high levels of ROS and decline in neuronal DNA damage response may be associated with neuronal dysfunction and cognitive impairment characterized by lower Mini-Mental State Examination (MMSE) score [31]. Additionally, it is known that oxidative damage leads to frontal-executive dysfunction [32].

Gene/encoded product	Locus	Role in aging	References
SIRT1 sirtulin 1	10q21.3	Age-related decreased level of SIRT1 is associated with impaired oxidative stress response and changes in glucose metabolism. Indirectly may be involved in age-related diseases, for example, retinal degeneration, hypertension, and cardiovascular diseases.	[12]
AKT1 protein Kinase B	14q32.32	Decreased level of AKT1 with age alters regulation of glucose metabolism, apoptosis, cell proliferation and cell migration, and PI3K/AKT/mTOR pathway.	[13]
CDKN1A Cyclin-dependent kinase inhibitor 1 (p21)	6p21.2	Possible promoter of aging due to pro-aging activity of p53.Oxidative stress increases expression of CDKN1A and overexpression of p21 may be involved in age-related diseases such as atherosclerosis, amyloidosis, AD, and arthritis.	[14] [15]
CETP Cholesterol ester transfer protein	16q21	Responsible for cholesterol homeostasis in central nervous system. Decreased level of CETP results in healthier aging, slower memory decline, less frequency of dementia, and lower AD risk.	[16] [17]
FOXO3A Transcriptional factor FOXO3A	6q21	Involved in insulin metabolism and insulin/IGF1 signaling pathway. Protection from oxidative stress and reduction of age-related diseases.	[18] [19]
IGF-1 Insulin-like growth factor 1	12q23.2	Decreased level of IGF-1 with age leads to cell senescence.	[20]
PON1 Paraoxonase 1	7q21.3	Decreased level of PON1 with age impairs oxidative stress response and is a risk factor for cardiovascular diseases due to LDL oxidation.	[21] [22]

Table 1. The role of genes and their encoded products in aging.

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Another mechanism involved in aging is epigenetic alterations. Epigenetics is defined as molecular traits that are "stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence." The epigenetic pathways include DNA methylation, histone modifications, and noncoding RNA [33].

The analyses of CpGs methylation changes show that genome-wide global levels of DNA methylation decrease during aging. Despite this, many promoters of genes which are unmethylated in young gain methylation in old individuals [34, 35]. Other authors [36] suggest that DNA methylation changes may result in age-associated immune deficiency. It is possible that hypermethylation is caused by programmed changes, while hypomethylation may be the result of environmental and stochastic processes. Multiple studies have identified genes undergoing hyper- and hypomethylation with age. The first group includes genes involved in process such as cell adhesion, cell-cell signaling, ion transport, neuron differentiation, and other genes associated with development. The hypermethylated genes are regulated by a common group of transcription factors, whereas hypomethylated genes are involved in metabolic process, RNA splicing, regulation of ligase activity and protein ubiquitination, transmission of nerve impulse, and many others. The hypomethylation in neurons may cause age-related memory deficits [36, 37]. The abnormal profile of methylation may contribute to dementia. It is shown that mutation in DNA methyltransferase 1 (*DNMT1*), gene encoding an enzyme crucial for methylation, leads to a condition called hereditary sensory and autonomic neuropathy (HSAN1) with early onset of dementia and hearing loss [38]. Other authors suggest [39] that changes in methylation may be involved in age-related cognitive functions decline.

Histone modification includes acetylation, methylation, phosphorylation, citrullination, ubiquitination, SUMOylation, adenosine diphosphate (ADP) ribosylation, deimination, and proline isomerization, in which the first three are the most-studied topics. Modifications can change the chromatin structure by histone-histone or histone-DNA interactions. The chromatin packaging affects many processes such as transcription, repair, replication, and chromosome condensation. Acetylation is associated with activation of transcription, while the result of lysine methylation can be either activation or repression [40]. Chromatin packaging changes during aging [41]. It is shown that higher level of histone acetylation facilitates memory and learning processes; therefore, acetylation decrement may lead to cognitive impairments and is associated with aging [42]. Also histone methylation may affect the life span; loss of H3K9 trimethylation which results in reduction of heterochromatin is the hallmark of aging [43]. The acetylation of H4K16 is necessary for maintaining the structure of chromatin and when impaired, the process of double-strand break repair is less efficient [44]. Subsequently, histone tail proteolytic cleavage, especially H3, may be involved in aging, but the exact mechanism remains unclear [45]. Additionally, the decline in histone chaperon levels is observed during aging and may be the answer for defective DNA repair mechanisms [46].

Both age-related changes in DNA methylation and histone modification alter the experience-dependent synaptic plasticity in hippocampus by changing the chromatin structure. Thus, it may be involved in memory loss and learning difficulties. Epigenetic changes may be possible brain biomarkers of cognitive aging [47].

4. Genes associated with age-dependent dementia

Several genes are involved in age-dependent dementia. Most of them, such as *ABCA7*, *APOE*, *APOC1*, *BIN1*, *CASS4*, *CELF1*, *CD33*, *CD2AP*, *CELF1*, *CLU*, *CR1*, *DSG2*, *EPHA1*, *FERMT2*, *HLA-DRB5/DBR1*, *IL1RAP*, *INPP5D*, *MEF2C*, *MS4A6A/MS4A4E*, *NME8*, *PCDH11X*, *PICALM*, *PLD3*, *PTK2B*, *TOMM40*, *TREM2*, *TRIP4*, *TRPC4AP*, *SLC24H4-RIN3*, *SORL1*, and *ZCWPW1*, are associated with AD, which is the most prevalent cause of dementia in elders [48]. AD affects 24 million people worldwide and accounts for 60–80% of dementia cases [49]. *APOE* cluster (*APOE, TOMM40*) and *CLU* gene of the genes mentioned above, as well as Aβ cascade genes (*APP, PSEN1, PSEN2*), have also been mentioned as linked to memory decline in cognitively normal adults.

4.1. *APP, PSEN1,* and *PSEN2*

Aβ is formed in a process called an amyloid cascade which involves the amyloid precursor protein (APP), encoded by the *APP* gene located on chromosome 21. APP is a transmembrane protein with high expression in developing human neurons. In normal conditions, APP is truncated by proteolytic enzymes such as α- and β-secretases. However, in individuals developing AD, APP is processed by an alternative pathway involving γ-secretase. This leads to the production of the 40–42 amino-acid Aβ peptide. The active subunits of γ-secretase presenilin 1 and/or 2 are encoded by genes *PSEN1* and *PSEN2*, respectively. So far, 230 clinically significant mutations have been described in *APP*, *PSEN1*, and *PSEN2* genes; however, these mutations are very rare and account for around 0.5% of dementia cases. Moreover, most of these mutations are considered to be associated with a familial form of AD. On the other hand, asymptomatic carriers of the mutations in Aβ cascade genes demonstrate significant changes in cognitive functions, advancing with age [48].

4.2. *TOMM40*, translocase of the outer mitochondrial membrane 40 homolog (TOM40)

TOMM40 is a gene encoding for translocase of the outer mitochondrial membrane 40 homolog (TOM40). The gene is located on chromosome 19 in a cluster with *APOE*. Both genes remain in linkage disequilibrium and are associated with dementia [50]. Most reports concerning the role of *TOMM40* in developing dementia have focused on a variable length poly-T sequence polymorphism (rs10524523) located in intron 6 [51–53]. The number of deoxythymidine residues in the rs10524523 polymorphism comprise the so-called "alleles" of the poly-T repeat as "short" (abbreviated here as S), "long" (L), or "very long" (VL). These remain in strong linkage with the *APOE* variants, as the *TOMM40* L allele is almost exclusively observed in the *APOE* E4 carriers, while *TOMM40* S and/or the VL variants are associated with *APOE* E3 [51–53]. The poly-T variation in *TOMM40* was shown to be significantly associated with the age of onset of dementia [52]. Moreover, as shown in [51], the *TOMM40* poly-T variant may facilitate the estimation of dementia progression in AD patients, independently from the status of other dementia loci. They also implied that the genome-wide association studies (GWAS) signals recorded in the *APOE* locus may indeed arise from *TOMM40*.

It has been suggested that Aβ may exert intracellular toxicity mediated by TOM40, for example, by affecting the function of cellular power plants—the mitochondria. According to [50], the mechanism underlying TOM40's role in dementia involves its ability to uptake Aβ to the mitochondrion, as Aβ has been found to be co-localized with cristae proteins in the mitochondria. Subsequently, after absorption the intracellular Aβ would cause increased production of ROS, thus leading to DNA damage and premature neuronal death.

Several studies have shown that poly-T *TOMM40* variation may influence cognitive performance in healthy elderly people. The authors of study [54] investigated a cohort of 1613 elderly volunteers whose cognitive decline was followed for a period of 14 years (range = 12–18 years). This study showed that the *TOMM40* S variant repressed the activity of luciferase assay. Correspondingly, expression of the S variant was half of that in the VL variant. Moreover, they observed that the S variant significantly reduced vocabulary ability, diminished age-dependent vocabulary decline, in subjects as compared to the VL variant carriers. Other authors [55] conducted longitudinal modeling of a cognitive aging study on a cohort of 639 subjects with no signs of dementia, aged 21 to 97 years with a known *TOMM40* and *APOE* status. The volunteers underwent neuropsychological testing every 2 years. This study [55] showed that the influence of *TOMM40* variation on memory decline was particularly visible in subjects before 60 years of age ($p = 0.009$), however only in *TOMM40* VL/VL carriers whose improvement after the test-retest was significantly less pronounced than in the S/S and S/VL carriers. Moreover, the authors performed a neuropsychological examination and testing using the human analog of the Morris water maze and brain MRI on 59 cognitively normal volunteers, stratified as S/S, S/VL, and VL/VL carriers. They found that the S/S group performed better on world-centered navigation ($p \leq 0.004$) and world-centered delayed recall ($p \leq 0.014$) but not on self-centered navigation tests. They also found that the *TOMM40* variants significantly influenced the brain structure. The S/S group had a thicker right entorhinal cortex ($p \leq 0.043$) than the S/VL and VL/VL groups, whereas significant thinning of the left entorhinal cortex and the left posterior cingulate cortex was present only in the VL/VL group ($p = 0.043$ and $p = 0.024$, respectively) as compared to the S/S group [55]. In another interesting study [56], the authors stratified 117 healthy adults (medium age: 55 years) with the *APOE* E3/E3 genotype according to the *TOMM40* status into three groups, S/S, S/VL, and VL/VL, and performed memory tests and structural brain imaging. They found that the asymptomatic carriers of the *TOMM40* VL/VL genotype performed worse on testing of episodic learning and had a smaller volume in the posterior cingulate as compared to the S/S and S/VL groups [56].

These studies suggest that the *TOMM40* role is not limited to decreasing age at the onset of dementia but may also influence the brain structure and hasten memory decline in cognitively normal, healthy individuals.

5. Cholesterol, lipoproteins, and dementia

Of the many lipids, cholesterol is believed to play a major role in brain function and development, as the brain contains as much as 23% of the total cholesterol deposits [57]. One of the

most pronounced groups of genes described as dementia risk factors are involved in the transport of cholesterol and may be accounted for as apolipoproteins [58]. A misbalanced lipid metabolism may be associated with memory loss [59]. According to another paper [60], patients with higher levels of high-density lipoproteins (HDLs) had a decreased risk of developing dementia at the time of the study and in the future. For patients from the upper quartile (with a plasma HDL concentration higher than 55 mg/dL), the dementia hazard was decreased by 60%. Studies on the subjects were continued by several other teams; however, the obtained results seem to be rather inconsistent [61–67].

Generally, the HDL level is believed to negatively correlate with the prevalence of dementia in elderly people; however, many studies have implied that HDL influence may be characteristic of the VD development [58, 68].

5.1. *CLU*, apolipoprotein J (apoJ)

Apolipoprotein J (apoJ, also known as clusterin), encoded by the *CLU* gene, has been shown to probably be associated with dementia. Genome-wide association studies (GWAS) performed by authors [69] identified a genetic variation (rs11136000) which was a significant risk factor of dementia. The role of apoJ in the pathomechanism of AD is not fully understood. It has recently been shown that apoJ plasma levels positively correlate with the risk of dementia, as elevated apoJ levels have been reported in the plasma of AD patients as well as in their brain and cerebrospinal fluid (CSF). The study [70] showed that apoJ plasma, but not CSF, levels are elevated in AD patients and were a risk factor of dementia (HR 18.6). This biomarker was also shown to be significantly correlated with cognitive decline in AD patients and reversely correlated in individuals with mild cognitive impairment (MCI). Moreover, increased plasma apoJ levels in MCI indicated an amplified risk of further cognitive decline. Furthermore, it was shown that genetic variation in the *CLU* region may amplify the influence of personality type on the performance of declarative memory in older, non-demented adults [71].

5.2. *APOE*, apolipoprotein E (apoE)

ApoE is encoded by the *APOE* gene, which is located on the long arm of chromosome 19. It comprises four exons. Two frequent polymorphisms were described on the last exon: rs7412 and rs429358. These variants encode for three common alleles of *APOE*: E2, E3, and E4, encoding for apoE ε2, ε3, and ε4, respectively.

ApoE in physiological conditions is a major cholesterol carrier and one of the most vital proteins responsible for maintaining cholesterol homeostasis in the brain. ApoE is mostly synthesized by astrocytes and probably does not cross the blood-brain barrier [72].

A recent study [73] on transgenic rabbit *Apoe* knockouts (*Apoe* -/-) showed that apoE is essential for cholesterol homeostasis under stress conditions. Under normal conditions, the transgenic animals were able to maintain a stable, physiological level of plasma cholesterol. However, when the animals were transitioned to a diet with high cholesterol content, its level increased dramatically (1070 ± 61 mg/dL in apoE KO vs 169 ± 79 mg/dL in the wild type, $p < 0.001$). Another study [74] showed that increased content of fat and cholesterol in the diet increased

apoE production, probably due to transcriptional and posttranscriptional mechanisms. This suggests that functional apoE is essential for cholesterol regulation in mammals and protects against diet-induced atherosclerosis. The various variants of *APOE* have a distinct influence on apoE function and effect. Studies on humans and in apoE transgenic mice suggest that lipidation of apoE depends strongly on the *APOE* genotype, and that apoE ε2 and ε3 are significantly more lipidated than apoE ε4. Interestingly, cholesterol and proper apoE lipidation are essential for apoE function in sustaining synapses [75].

A study [76] analyzing the association of plasma and CSF apoE concentrations showed that the CSF/serum ratios of apoE levels were associated with progression of dementia. Schmidt et al. observed that "the lower the ratio, the faster the deterioration," as measured by the MMSE, instrumental activities of daily living (iADL), or Geriatric Depression Scale (GDS). Subsequently, another study [77] showed that plasma apoE may be a biomarker of dementia, as patients suffering from memory decline had lowered concentrations of plasma apoE.

The first reports indicating that *APOE* may be associated with dementia were published more than 20 years ago. Detailed studies of this gene were carried out mainly in AD; however, the literature data support *APOE* influence on memory in people with no symptoms of dementia. It is believed that *APOE* E3 is the most common allele in the population and does not modify the risk of memory decline. The *APOE* E4 variant was shown to be overrepresented in dementia patients, especially those with AD [78]. So far, *APOE* E4 remains the most significant risk factor of sporadic AD and accounts for 30% of cases [79]. *APOE* E2 was shown to be associated with reduced age-dependent cognitive decline. This observation occurred independently of age-related neuroinflammation and synaptic changes or the Aβ burden [80]. The described effect may be explained by a higher apoE level in *APOE* E2 carriers as well as by more efficient Aβ clearance [81]. Moreover, according to study [75], the effects of *APOE* E2 and E4 counteract, and in transgenic mice the introduction of the E2 allele decreased Aβ deposition, while the E4 allele increased the Aβ burden.

According to [82], the most significant *APOE* effect on the onset of dementia may be observed in patients over the age of 60 ($p = 0.006$). This was visible as an accelerated memory decline in *APOE* E4 carriers. However, other authors [83] reported that characteristic changes in MRI may be observed even in healthy infants carrying the *APOE* E4 allele. According to authors of [84], the *APOE* E4 allele is not only responsible for a decline in episodic memory with age in cognitively normal adults but may also induce impaired olfaction due to deterioration of medial temporal lobe. This is consistent with the neuroimaging data, suggesting that in non-demented *APOE* E4 carriers, mediotemporal atrophy occurs prior to clinical dementia onset.

It is also interesting that multiple studies have confirmed that the *TOMM40*/*APOE* locus is associated not only with the risk of dementia but also with longevity. The GWAS confirmed that the rs4420638 polymorphism on chromosome 19q13.32 was significantly related to living longer than >85 years (OR = 0.72, $p = 3.40 \times 10^{-36}$) [85]. Similarly, according to a recent study [86], with the use of more sophisticated integrative GWAS (iGWAS) method to couple data from 14 meta-analyses, the locus housing *APOE* and *TOMM40* remained significantly associated with longevity, even when the false discovery rate (FDR) was set at 10%, which indicates that

the *APOE*/*TOMM40* locus holds the key for healthy senescence without a pathological memory decline.

6. *APOE* in a healthy Polish population under 60 years of age

Despite the many years of research, AD remains a disease that is difficult to predict and diagnose, with few blood-derived biomarkers possible for use in routine clinical setting. As was stated before, *APOE* remains the most significant genetic risk factor of AD. This creates a need for the development of a novel, quick, and reliable method of analyzing the *APOE* genotype and the apoE plasma concentration. The role of *APOE* in the development of dementia and its influence on longevity in elderly people has been studied by [53]. As per our knowledge, *APOE* studies on a younger population in Poland have been neglected and there are no literature data on association of the *APOE* genotype and the apoE plasma level in non-demented Polish adults.

6.1. Aim of the study

In this study, we tried to assess the influence of the *APOE* genotype and the effect of demographic factors on the apoE level in a subset of Polish non-demented volunteers less than 60 years of age.

6.2. Subjects

A total of 83 healthy adults (70 females, mean age: 51.9 ± 7.2; 13 males, mean age: 44.9 ± 11.7) under 60 years of age with no signs of dementia or other neurological disorders were enrolled in the study. All participants provided signed, written consent. The research project was approved by the Bioethical Committee at the Poznan University of Medical Sciences, decision no. 1031/13, dated May 5, 2013.

6.3. Materials

Each volunteer's blood was collected on an anticoagulant—K_3EDTA (Monovette™ vacuum system, Sarstedt, USA). A total of 3 ml of blood was immediately aliquoted, then frozen and stored at −80°C upon nucleic acid isolation. Subsequently, the remaining blood was centrifuged (1400 relative centrifugal force [RCF], 10 min) and the collected plasma was aliquoted and stored at −80°C.

6.4. Methodology

6.4.1. APOE genotyping

First, a subject's DNA was extracted from frozen blood using gravity flow microcolumns (Genomic Micro AX Blood Gravity, A&A Biotechnology, Poland). The DNA concentration was measured by a microplate spectrophotometer (Take3, Epoch, BioTek, USA) and adjusted to 20

ng/μL with Milli-Q® water. Subsequently, genotyping was performed according to a modified mismatch primer method [87]. Briefly, three quantitative polymerase chain reaction (qPCR) specific to each *APOE* allele were performed with the use of four different primers (as shown in **Table 2**). The qPCR included two steps: primary pre-amplification (15 cycles) with annealing at 64°C followed by 30 cycles of secondary amplification with annealing at 62°C. The reactions were performed on a CFX Connect™ Real-Time PCR Detection System (Bio-rad, USA) in 10 μL volumes, with 250 nM primers and 50 ng of genomic DNA using 1× SsoFast™ EvaGreen® Supermix (Bio-rad, USA). The cycling conditions were: 30 s initial denaturation at 98°C followed by cycles of 98°C for 5 s and 64 and 62°C for 10 s. The reaction was considered positive once the products appeared before the 10th cycle of secondary qPCR. The method was validated by Sanger sequencing in an external laboratory.

Reaction	Starter	Sequence	Annealing temperature	Product melting point
APOE E2	APOE112C	CGGACATGGAGGACGTGT	62–64°C	91.4°C
	APOE158C	CTGGTACACTGCCAGGCA		
APOE E3	APOE112R	CGGACATGGAGGACGTGC		91.6°C
	APOE158C	CTGGTACACTGCCAGGCA		
APOE E4	APOE112R	CGGACATGGAGGACGTGC		91.8°C
	APOE158R	CTGGTACACTGCCAGGCG		

Table 2. Starters used for genotyping of *APOE*.

6.4.2. ApoE quantification

Determination of the plasma apoE concentration was performed by the enzyme-linked immunosorbent assay (ELISA) method. The analysis was performed according to the manufacturer's protocol (Human apoE ELISA Kit, Mabtech, Sweden) using 10,000× diluted plasma samples. Absorbance was measured by an EPOCH microplate reader (BioTek, USA). The concentrations were calculated from a four-parametric standard curve ($R = 0.998$) by Gen5 ver. 2.01 software (provided with the reader).

6.5. Results

Our study on Polish subjects showed that the observed genotype frequencies of *APOE* are in line with the Hardy-Weinberg equilibrium ($p = 0.9365$). The dominating allele was *APOE* E3 (83.7%) and the least common allele was *APOE* E2 (3.0%), as is shown in **Figure 1**. Interestingly, we did not observe any *APOE* E2/E2 homozygotes, as is shown in **Table 3**.

Our results indicate that the apoE plasma concentration depends on the *APOE* genotype (one-way analysis of variance [ANOVA], $p = 0.021$). Generally, in *APOE* E3/E3 carriers we recorded the highest mean concentrations of apoE, while in the *APOE* E4/E4 homozygotes we recorded the lowest mean concentrations. In females with the *APOE* E2/E3 allele, the concentration of apoE was slightly lower than in the E3 homozygotes. Interestingly, in a single case of an

E2/E4 carrier we observed an increased level of plasma apoE. Subsequently, the plasma apoE concentration in *APOE* E3/E3 carriers was higher in males than in females. Conversely, in *APOE* E3/E4 carriers the recorded apoE concentration was higher in females. Hence, the decrease in apoE due to the *APOE* E4 genotype was more pronounced in males than in females (41% vs 16%), as shown in **Table 3**. Overall, the apoE concentration was insignificantly higher in males than in females (2.54 vs 2.24 mg/dL; $p = 0.194$, Student's *t*-test). The observed positive trend of increasing apoE in older individuals did not reach statistical significance ($r = 0.201$, $p = 0.0687$; Pearson correlation coefficient). However, after stratification according to gender, we observed significant correlation of the apoE plasma level and age in females ($r = 0.348$, $p = 0.00128$; Pearson correlation coefficient). The concentrations of apoE stratified according to *APOE* status, gender, and age are shown in **Table 4** and **Figure 2**, respectively.

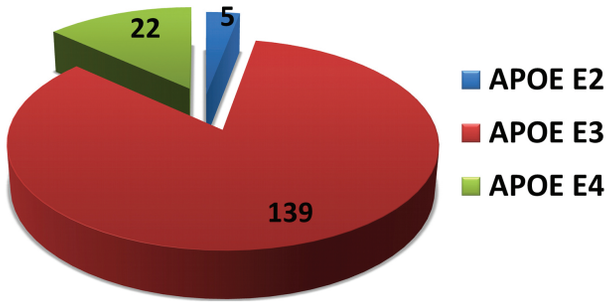

Figure 1. Frequencies of *APOE* alleles in Polish, cognitively normal volunteers under 60 years of age.

Genotypes	*APOE* E2/E2	*APOE* E2/E3	*APOE* E3/E3	*APOE* E3/E4	*APOE* E4/E4	*APOE* E2/E4
Observed frequencies	0	4	58	19	1	1
	0.0%	4.8%	69.9%	22.9%	1.2%	1.2%
Expected frequencies	0.08	4.19	58.20	18.42	1.40	0.66
	0.1%	5.0%	70.1%	22.2%	1.8%	0.8%

Note: Hardy-Weinberg equilibrium calculations, $p = 0.9365$, $n = 83$.

Table 3. Hardy-Weinberg equilibrium calculations of *APOE* variants in Polish, cognitively normal volunteers less than 60 years of age.

Gender	*APOE* E2/E3	*APOE* E2/E4	*APOE* E3/E3	*APOE* E3/E4	*APOE* E4/E4
Female	1.98 ± 0.67	2.91	2.35 ± 0.78	2.02 ± 0.52	0.69
Male	–	–	2.91 ± 0.63	1.72 ± 0.22	–
Combined	1.98 ± 0.67	2.91	2.43 ± 0.79	1.95 ± 0.49	0.69

Note: Mean concentration ± SD (mg/dL) or (single result).

Table 4. Mean plasma apoE concentration (mg/dL) in Polish, cognitively normal volunteers under 60 years of age stratified according to gender and *APOE* genotype.

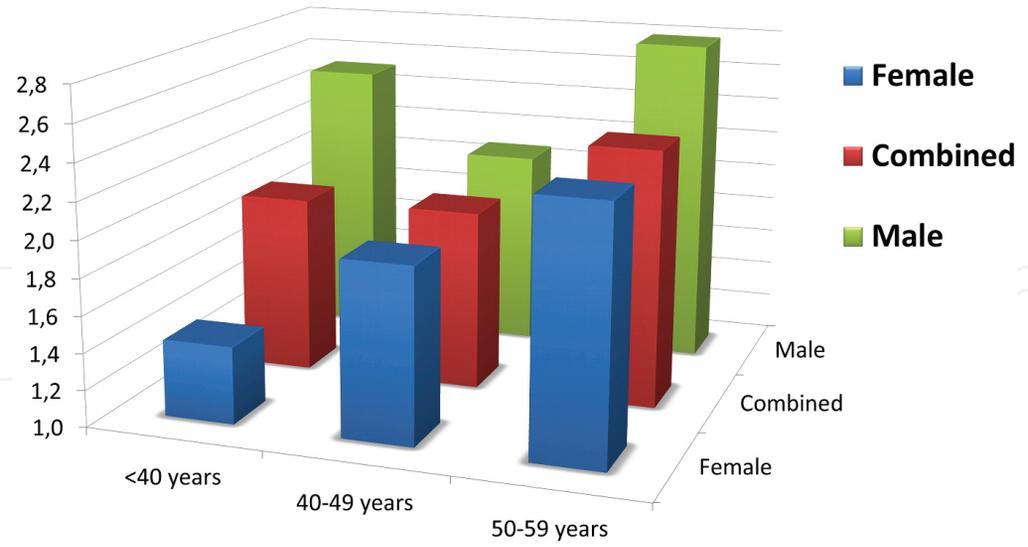

Figure 2. Mean apoE plasma concentration (mg/dL) in Polish, cognitively normal volunteers under 60 years of age stratified according to gender and age.

6.6. Discussion

As was stated before, the *APOE* E4 allele is associated with increased risk of developing dementia.

According to our results, the *APOE* E3 genotype was the most prevalent genotype in the studied group, while the E2 genotype was the least common. Similar results were reported in the Polish population [53].

Our study shows that *APOE* E4 variant is associated with a decreased concentration of plasma apoE in cognitively normal Polish volunteers less than 60 years of age. Our results are supported by the results of other authors [77], who analyzed plasma apoE concentrations and *APOE* status in a cohort of 75,708 participants in the Copenhagen General Population Study and the Copenhagen City Heart Study. The authors also showed that apoE is dependent on the *APOE* genotype, as they found substantial differences in plasma apoE concentrations between carriers of distinct *APOE* genotypes. However, contrary to our results, in their study the highest level of apoE was observed in *APOE* E2 homozygotes and decreased in E4 carriers in a dose-dependent manner: E2/E2 > E2E3 > E2/E4 > E3/E3 > E3/E4 > E4/E4. The plasma concentration of apoE in E4/E4 homozygotes was up to 65% lower as compared to *APOE* E2/E2 carriers. This partial incompatibility with our results may be explained by the utilization of various methods: the authors used the nephelometry and turbidimetry methods, whereas we used the well-established ELISA method. In another study [88], the authors showed that apoE concentrations in plasma apoE increased with age in a healthy population. We observed a similar trend; however, it was significant only in the female group.

The plasma concentration of apoE may be a valuable dementia biomarker because it is easily available and, according to literature data, decreased apoE may be a risk factor for developing dementia. The above-mentioned Australian follow-up cohort study, comprising mostly

Caucasian subjects, showed that the reduced apoE plasma level may be a predictor of a transition from MCI to AD. Moreover, the plasma apoE concentration correlates positively with cognitive function, and patients with a lower apoE level tend to perform worse in neuropsychological tests assessing spatial memory and language abilities [89].

Hence, the assessment of the plasma apoE concentration and the *APOE* status may give valuable information to physicians trying to predict the rate of cognitive decline in the course of dementive disease as well as in normally ageing adults and elderly persons.

7. Summary

The appearance of dementia in old age is influenced by both biochemical and genetic factors leading to structural disorders in the brain of elderly persons. The level of Aβ is mentioned among the other biochemical factors associated with dementia. The deposition of Aβ in the brain is controlled by *APOE* and by genes associated with the amyloid cascade (*APP*, *PSEN1*, and *PSEN2*). Subsequently, Aβ toxicity is modified by the *TOMM40*. In the elderly, also abnormal cholesterol, glucose levels, and the weakening of protective and repair mechanisms leading to the generation of ROS (mediated, e.g. by *PON1*) may cause a reduction in cognitive functions. However, the role of genes associated with longevity (e.g. *FOXO3A, CETP*) and normal aging (e.g. *SIRT1, AKT1, CDKN1A*) is not clearly defined in the occurrence of diseases typical for this age group, as shown in **Figure 3**.

Finding a way to control the genetic factors and their protein products may contribute to the prevention of diseases of old age, including depression and dementia, and to improve the quality of life of elderly people.

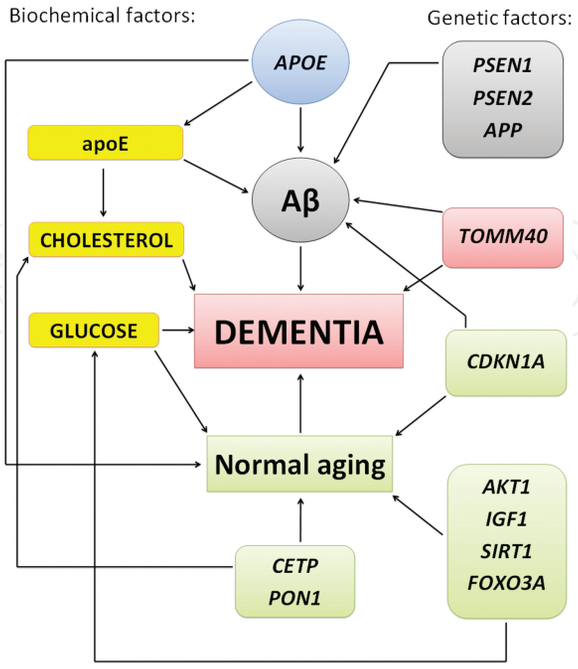

Figure 3. The genetic and biochemical factors associated with normal aging and dementia; β-amyloid—Aβ.

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