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Review article

Effects of DNA methylation and demethylation on LRP1 and RAGE genes as crucial factors in Alzheimer's disease pathophysiology

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Abstract

Presently there is a growing number of scientific papers that indicate a strong connection between epigenetics and the Alzheimer's disease (AD) pathophysiology. This study also concentrates on some events related to this serious, chronic, and lethal neurodegenerative disease. Actually, this disease, with a still incomplete knowledge of its etiology and closely connected to the ageing process and old age, in conditions of intensive ageing of the world population (so called senectual explosion), is increasingly becoming a burden for contemporary society. The most recent scientific papers related to AD pathophysiology indicate the complex interaction of a number of genes and oxidative and glycative stress, as well as epigenetic phenomena. The two mentioned types of stress have been under intensive research for a longer period of time; however, epigenetics is a new challenging field that penetrates into the essence of this disease with promising results. Epigenetics, as opposed to genetics that is concerned with gene mutations, investigates special events that are not directly connected to genes, but events that affect genes not changing their sequential structure. This study does not analyze histone acetylation (adding the acetyl group CH_3CO- to histones) and deacetylation, nor histone methylation (adding the methyl group -CH_3 to histones) and demethylation; it is primarily concerned with DNA methylation as the crucial epigenetic event essential for the process of the transcription of genes and their adequate expression. Special emphasis is directed to the analysis of the low-density lipoprotein receptor-related protein (LRP1) receptor, the extremely important factor in the amyloid beta (Aβ) drainage from the brain, and to the decline of the LRP1 expression on the membrane abluminal side of the blood brain barrier (BBB) endothelial cells. This paper does not analyze oxidative and glycative damage of the LRP1 receptor, and it does not focus on AD therapy; it is primarily concerned with epigenetics.

KEY WORDS: Alzheimer's disease (AD), LRP1 receptor, RAGE receptor, disorder of Aβ drainage from the brain, epigenetics

Introduction

A short review on genes etiologically linked to Alzheimer's disease

Before analyzing the essential features of the DNA methylation process, it is necessary to emphasize that the aim of the presented study is to explain the role of the low-density lipoprotein1 (LRP1, LRP1 gene location 12q13.3) receptor in the Aβ peptide drainage from the brain, and its decline in Alzheimer's disease (AD). This study does not analyze other mechanisms of the mentioned clearance that primarily include effects of hydroxyl radicals (*OH), belong to a great group of reactive oxygen species; ROS, and oxidative and glycative stress; it is exclusively involved in epigenetics, with reference to the DNA methylation. It should also be stressed that the study does not analyze AD etiologically important genes (APP, amyloid precursor gene, chr. 21.q21; PSEN1 gene, chr. 14q24.2; PSEN2 gene, chr.1q42.13; BACE1 gene, chr. 11q23.3; γ-secretase complex-CTFβ chr3p22.1; PSEN1 gene chr, 14q24.2; Pen2 gene chr.19q13.12; ApH1 gene chr. 1q21.2; ADAM10 gene, chr. 15q21.3; APOE gene, chr.19q13.32, ApoEε4 allele, the strongest genetic risk factor for LOAD); however, it is necessary to mention them. The last two mentioned genes are linked to LOAD (late AD onset, 95% of all cases), which develops after the age of 65, and the former mentioned genes to EOAD (early AD onset, 5% of all cases) which develop before the age of 65 [1)].

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Amyloid beta efflux and influx transport receptor proteins critically involved in Aβ drainage from the brain

The authors of three research articles [2-4] emphasize the generally accepted view that amyloid beta (Aβ) accumulation and aggregation is the trigger for the development of progressive neurodegeneration and dementia associated with AD. They also point out that the sporadic late AD form (LOAD, onset over 65 years, 95% of all cases), is primarily caused by the decreased Aβ clearance from the brain, and not by its stronger production. In the mentioned drainage process, the low-density lipoprotein receptor-related protein 1 (LRP1), the member of the large LDL receptor group, has the crucial role. LRP1 is dominantly located on the abluminal side of the BBB capillary endothelial cell membrane, and by the endocytosis mechanism, it ejects Aβ from the brain tissues into the drainage capillary blood. It should be noted that in the other form of AD, onset (EOAD, 5% of all cases) develops before the age of 65. These studies continually point out the imbalance of Aβ homeostasis which results in the pathological accumulation of cerebral Aβ, the main characteristic of AD. Special attention is given to the results after measuring the mRNA efflux genes (LRP1; P-gp, P-glycoprotein, ABCB1 gene) and the influx gene (RAGE, receptor for advanced glycation end products, gene location 6p21.32), where in the efflux group, in the capillary blood and in the endothelial cells, a significant decline of mRNA LRP1 has been found, and in the influx group, an increase of mRNA RAGE has been found. The first group is accompanied by a significant gene transcription drop, and the second group by its increase [2-4].

Essential features of DNA methylation

Yang J *et al.* [5] emphasize that the DNA methylation process occurs in all cells in the body and it is evolutionarily conserved among insects and mammalian species. The main characteristic of this process is the transfer of the methyl group (-CH_3) from the methyl donor, S-adenosyl-methionine (SAM) onto the active site of the DNA cytosine methyltransferase enzyme and C5 everted cytosine from one chain of the corresponding DNA molecule. The mentioned process can be seen during the DNA base cytosine methylation by the effect of the enzyme human DNA (cytosine-5) methyltransferase M.Hhal (Dnmt M.Hhal). Dnmt M.Hhal is a 5-methylcytosine methyltransferase (m5C-MTase) that forms part of a type II restriction-modification system from *Haemophilus haemolyticus*. The enzyme recognizes the specific tetranucleotide sequence, 5-GCGC-3. The methyltransferase binding to DNA begins with three H-bonds breaking between cytosine (target base) and its pair guanine. Immediately after this event occurs the cytosine eversion, so that it projects out of the double helix, then the nucleophilic attack (Nfa) of Cys81 nucleophile (from the Dnmt) on cytosine C6, and C6 attack (Nfa) on C5 with its activation and rise in nucleophilicity. The activated C5 attacks (Nfa) the SAM methyl group and induces its abstraction. At the same time, three bases (Glu119, Arg163, Arg165) on the other Dnmt side, form H-bonds between NH_2, N_3 and O_2. The nucleophilic attack of Cys81 results in the formation of the covalent adduct, Michael adducts (creation of carbon-carbon bond at the acceptor's β-Carbon). Three amino acids on the other Dnmt side are essential for maintaining the target cytosine in the flipped-out position. Before the C5 nucleophilic attack on target -CH_3, C5 deprotonation occurs. The B-elimination of the C5 proton utilizes as base an OH- derived from conserved crystal water that is part of a proton wire water channel. The methylation results in transcriptional blockade (epigenetic phenomenon) (***Fig. 1***).

In the following analysis of the DNA methylation process, the investigation results and views of some eminent researchers are presented.

Liu Y *et al.* [6] emphasize the importance of the AD-associated promoter DNA hypermethylation, which establishes an epigenetic barrier for transcriptional activation. They point out functional activities of selected neurons, microglia, and astrocyte-enriched genes (AGAP2, DUSP6 and GPR37L1), which are DNA hypermethylated at promoters in AD. According to the authors, in mammals, DNA methylation primarily occurs at the 5-carbon of the cytosine base in the form of 5-methylcytosine (5mC).

Cheng X *et al.* [7] describe the approach and binding of Dnmt to DNA. This induces the eversion (base flipping) of the target nucleotide with its projection out of the double helix and entrance into the Dnmt pocket. The catalytic process induces the nucleophilic attack of Cys81 on cytosine C6 with the formation of the covalent Michael adduct. Cytosine C6 induces a nucleophilic attack on C5, which momentarily induces the same attack on the methyl group of S-adenosyl-L-methionine (AdoMet), which is absorbed and transferred to C5.

Lin CC *et al.* [8] emphasize that DNA methyltransferases are primary enzymes for cytosine methylation at CpG sites of epigenetic gene regulation in mammals. DNMT3B methyltransferase uses two flexible loops to enclose DNA and employs its catalytic loop to evert the target nucleotide to project out of the double helix. DNMT3B recognizes DNA with CpGpG sites via residues Asn779 (asparagine, it contains an α-amino acid which is in the protonated state -NH_3^+) and Lys777 in its target recognition domain loop. This facilitates processive methylation of CpG sites repeated in tandem. The authors also emphasize the importance of the proton wire water channel essential for the C5 deprotonation.

According to Xing P *et al.* [9], the promoter region of LRP1 gene is enriched with CpG islands that govern the sensitivity of the LRP1 gene to DNA methylation. When CpG are completely methylated, the transcriptional activity disappears completely and the expression of LRP1 is silenced. They think that the epigenetic mechanism might be involved as hypermethylation of the LRP1 gene promoter, especially as the promoter of this gene is rich in CpG islands.

Tohgi H *et al.* [10] have investigated the methylation status of cytosines in the promoter region of RAGE in the human cortex autopsy using the bisulfite method, polymerase chain reaction (PCR) and the direct sequencing of PCR products. They have found that the total number of methylcytosines in the mentioned region is significantly reduced with age. This reduction of methylcytosines at transcription factor binding sites can increase the expression of RAGE.

Silverberg GD *et al.* [11] emphasize the decreased methylation in the RAGE promoter region. During the

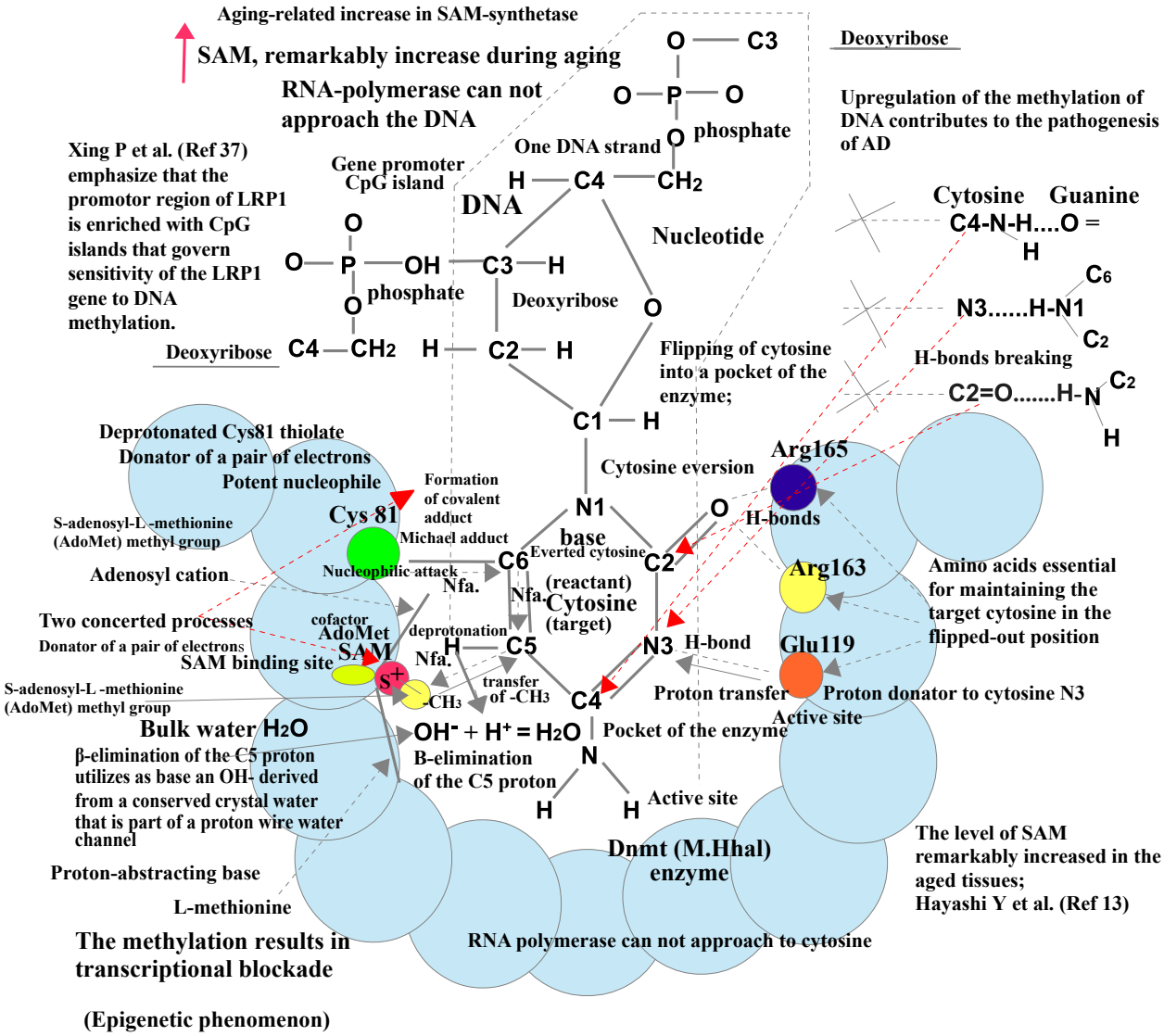

Fig. 1. **Schematic presentation of DNA methylation by human DNA cytosine methyltransferase M.Hhal (epigenetic event).**

The enzyme Dnmt M.Hhal catalyzes the transfer of a methyl group from S-adenosyl- L-methionine (AdoMet) to the C5 position of cytosine. Between cytosine and guanine, there are three H-bonds, according to Yang J *et al.* (Ref 5) and Cheng X *et al.* (Ref 7), and the methyltransferase binding to DNA begins with three H-bonds breaking between cytosine (target base) and its pair guanine, cytosine eversion ("base flipping"), so that it projects out of the double helix, nucleophilic attack (Nfa) of Cys81 nucleophile on cytosine C6, and C6 attack on C5 with its activation and rise in nucleophilicity. C5 attacks the SAM methyl group and induces its abstraction. Concurrently, three bases (Glu119, Arg163, Arg165) on the other Dnmt side, form the H-bonds between NH_2, N_3 and O_2. SAM remarkably increases in the brain tissues (cerebellum, cerebrum, hippocampus) during aging. AD, Alzheimer's disease; Dnmt M.Hhal, DNA methyltransferase M.Hhal; Arg, arginine; Cys, cysteine; Glu, glutamine; SAM, S-adenosyl-L-methionine (AdoMet); Nfa, nucleophilic attack; LRP1, low density lipoprotein receptor-related protein; CpG islands, the CpG sites are regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction and CpG sites occur with high frequency in genomic regions called CpG islands.

ageing process and in AD, there is an increased accumulation of AGEs, as well as increased values of Aβ, cytokines (interleukin 1, tumor necrosis factor), and of nuclear factor ϰB (NFϰB), of which, all can decrease the promoter methylation and elevate the RAGE gene transcription.

It is evident that changes in LRP1 and RAGE genes expression have a crucial role in the Aβ peptide drainage drop from the brain and in the Aβ accumulation and aggregation in the brain tissues, which is the reason for brain neurodegeneration and AD.

SAM cofactor biosynthesis

Following is the explanation of the origin of this important cofactor, the methyl donor required for almost all methyltransferase (MTase) activities. SAM originates by the effect of evolutionally conserved enzyme S-adenosyl-methionine-synthase (SAM-S) that links amino acid methionine and adenosine triphosphate (ATP). Actually, SAM is a complex compound of methionine and adenosyl cation; the latter is the compound of ribose sugar and the adenine purine base.

Experimenting on worms (*Caenorhabditis elegans*), a group of researchers have found that methionine dietary restriction extends their longevity. The same experiments with mammals accelerate their pathology, but accelerating the SAM catabolism by glycine N-methyltransferase (Gnmt), the researchers have suppressed the pathology and extended their longevity. The downstream metabolites of SAM are polyamines and cysteine. They are inductors of autophagy and elevators of oxidative stress resistance (***Fig. 2***)[12].

Here, it is important to point out that the SAM and Sam-S level rises in old-age tissues and in AD. Analyzing age-related changes in SAM biosynthesis in brain tissues (cerebellum, cerebrum, hippocampus) of young and old mice, the authors of a study have found that the elevation of DNA methylation and of histones accelerates the pathogenesis of AD. They conclude that the increase in SAM and Sam-S levels is an age-related phenomenon common across the species and tissues. They emphasize that SAM is the major cellular donor of the methyl group for methylation modifications. However, the factors that induce the aging-related increase in Sam-S levels are not known[13].

The process of DNA methylation in the course of ageing

Xiao FH *et al.*[14] emphasize that DNA methylation represents a link between genetic and environmental signals via the regulation of gene transcription. DNA methylation presents globally the decreasing and site-specific increasing in aging. It is the most common epigenetic modification and it plays the crucial role in many biological processes. It is evident that it is closely associated with aging, age-related and longevity diseases.

In another study, Xiao FH *et al.*[15] present the dynamics of DNA methylation during the human life cycle in detail. The dynamics shows a strong correlation of DNA methylation events with old age and the consequences of ageing. The telomere shortening that occurs during cellular replication has until recently been considered a good predictor for ageing and its consequences. However, exact investigations have shown that the insufficient detection of telomers and their weak correlation with age-related outcomes is a limiting factor in their role in the prediction system. The authors determine the chronological age and predict biological age, especially emphasizing age methylated changes in certain CpG loci. They point out that DNA methylation based on epigenetic clocks serves as a new standard to determine chronological age. The increase in DNA methylation level, associated with age, on selected CpG sites typically located near transcription start sites, has great value in analyzing the character of DNA methylation.

In order to further analyze the DNA methylation process, it is necessary to explain the meaning of the CpG site and CpG island.

A promoter, as related to genomics, is a region of DNA upstream of a gene where relevant proteins (such as RNA polymerase and transcription factors) bind to initiate the transcription of that gene. The resulting transcription produces an RNA molecule (such as mRNA). The transcription start site is the location where transcription starts at the 5'-end of a gene sequence. Transcription factors that include a wide number of proteins, excluding RNA polymerase, initiate and regulate the transcription of genes.

Previously in the text, it has been stressed that the promoter region of LRP1 gene is enriched with CPG islands that govern the sensitivity of the LRP1 gene to DNA methylation. When CpG is completely methylated, the transcriptional activity completely disappears and the expression of LRP1 is silenced[9].

CpG islands (CGIs) are regions of the genome that contain a large number of CpG dinucleotide repeats. In mammalian genomes, CpG islands usually extend by 300–3000 base pairs. They are located within and close to sites of about 40 % of mammalian gene promoters.

The CpG sites or CG sites are regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction. CpG sites occur with high frequency in genomic regions called CpG islands (or CG islands).

Slieker RC *et al.*[16] investigate the tissue-specific character of age-related DNA methylation changes at the level of the CpG functional genomic region and the nearest gene. They have found that the age-related DNA methylation changes are highly tissue-specific. Linear regression analysis between ELOVL2 CpG (cg16867657) with ELOVL2 gene (Fatty acid elongase 2) and age (years) has shown a strong statistically significant linear positive correlation.

Hernandez DG *et al.*[17] have examined DNA methylation at more than 27,000 CpG sites throughout the human genome in the brain structures from 387 human donors between the age of 1 and 102 years. They have identified CpG loci that show a highly significant positive correlation between DNA methylation and chronological age. The major part of these loci is within CpG islands, and there is a positive correlation between age and DNA methylation level. They have also discovered that the CpG sites, where the DNA methylation level is significantly positively associated with age, are located close to genes involved in the DNA binding and in the regulation of transcription.

A growing number of recent studies have shown that DNA methylation plays an important role during mammalian embryogenesis[18-21]. Researchers in these investigations have

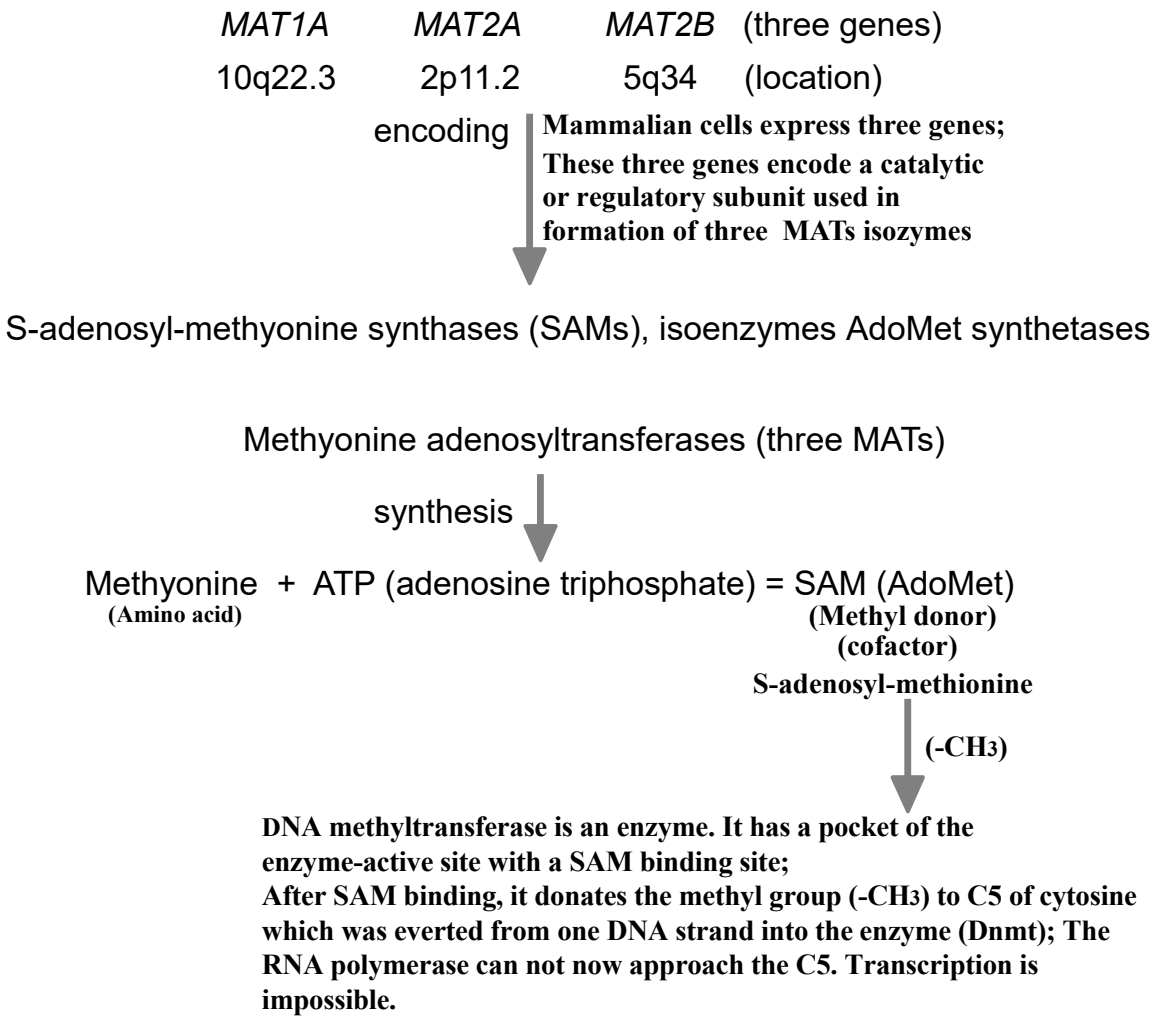

Fig. 2. **Schematic presentation of SAM synthesis**

SAM (AdoMet) acts as a methyl donor necessary for almost all methyltransferase activity. In humans, it is mainly produced from L-methionine and ATP by methionine transadenylase in the liver. ATP, the source of energy for use and storage at the cellular level, is complex, composed from a nitrogenous base adenine, a sugar ribose, and three serially bounded phosphate groups. The three MATs (SAMs, AdoMet synthetases, and methionine adenosyltransferases) convert methionine into SAM. During aging, the systemic SAM levels in the body are increased. SAM acts as a cofactor, a substance whose presence is essential for the activity of an enzyme. SAM consists of adenosyl cation attached to the sulfur (S) of methionine-the linkage is a sulfonium functional group. MAT1A, MAT2A, and MAT2B are three genes which encode a catalytic regulatory subunit used in formation of the MATs isozymes. MAT1A gene is expressed only in the adult liver. MAT2A and MAT2B are expressed in fetal liver and nonhepatic tissues. ATP, adenosine triphosphate; SAM, AdoMet, S-adenosyl-methionine; MAT, S-adenosyl-methionine synthase.

found modifications associated with childhood adversity and epigenetic modifications. They point out DNA methylation modifications in genes that regulate the hypothalamus pituitary adrenal axis as well as the immune system.

The analysis of the mentioned studies, especially of the presented graphs showing the positive linear regression line of DNA methylation and the age of the investigated subjects (Smith ZD *et al.*[18], Hahn MA *et al.*[19], Greenberg MVC *et al.*[20], Hao G *et al.*[21], Hernandez DG *et al.*[17], Slieker RC *et al.*[16], shows the lifelong DNA methylation of a number of genes. These authors point out changes in brain tissues in particular. Considering the increase of the SAM and Sam-S levels related to ageing, it is obvious that there is a program determined by evolution that dictates the scope and speed of the DNA methylation in the genome. The theory of programmed ageing, practically applicable to all living beings, actually determines the duration of certain life cycle phases with the maximally possible lifetime limit of each species. All these phases, as well as the maximally possible duration of life, are crucial conditions for the species to survive in the conditions of severe struggle for survival (***Fig. 3***). It seems that the DNA methylation program represents the crucial link in these events. At many CpG loci, there is a standard average methylation level. However, CpG sites of LRP1 genes have a much more sheer methylation regression line direction, which indicates the accelerated rate of methylation, and increased local ageing with decreased LRP1 gene transcription.

It has been mentioned that SAM occurs through the biosynthesis of methionine and ATP. The biosynthesis occurs by effect of the evolutionally conserved enzyme methionine adenosyltransferase (Sam-S, MAT, SAM-synthetase). It has been found that with ageing there is an aging-related increase in SAM levels.

Hoffert KM *et al.*[22] present human genes which encode methionine adenosyl transferases (MATs), also known as AdoMet synthetases. Three genes, MAT1A (10q22.3), MAT2A (2p11.2), and MAT2B (5q34), each encode a catalytic or regulatory subunit used in formation MATI (homotetramer), MATII (heterotrimer), and MATIII (homodimer) isozymes. MAT1A is expressed only in the adult liver, while MAT2A and MAT2B are expressed in the fetal liver and non-hepatic tissues. The mechanism of their action is not yet well understood. This action is connected with the upregulation or downregulation of some cancers (***Fig. 2***).

Ciccarone F *et al.*[23] have found that the level of DNMT1 (19p13.2) gradually dropped with aging, but this was only observed up to the age of 64 years. The expression of DNMT3B (20q11.21) decreased linearly with increasing age and this association was particularly evident in females. The authors emphasize that age affects the expression of both mentioned DNA methyltransferases as an almost independent variable in respect to all other variables evaluated.

It is necessary to further emphasize that with ageing, and in old age, remarkably elevated levels of SAM and aging-related increases in Sam-S are increasingly manifested. Is it the question of the effect of certain transcription factors that intensify the transcription, or is it the question of the blockade of transcription blockers, or of the positive or negative biofeedback mechanism? In these events, the programmed ageing theory probably has the crucial role. DNA methylation is closely linked to this program. The DNA methylation programmed levels during the individual lifetime cycle are correlated with the tendency for chronic diseases and longevity. The individual terminal old age and the final inevitable death are the constituent parts of the life cycle. DNA methylation is the crucial regulator of this inevitability. The above-mentioned questions require special attention[13, 15, 24-29].

Obviously, the LRP1 gene is subjected to all the mentioned effects. He Y *et al.*[30], analyzing the critical side effects of cisplatin, the cancer chemotherapeutic, have found that in the background of the mentioned events lies the strong LRP1 gene promoter methylation, and the strong decline of this gene expression. If the LRP1 gene expression declines, this also happens to the Sha1/PI3K/AKT signaling pathway important for M2 microglia polarization. M2 microglia phenotype has a strong protective function. Unfortunately, the necessary further investigations of LRP1 gene methylation and transcription suppression are extremely complicated, so that in the available literature there are few facts covering this subject. However, the author of this study believes in the validity of the thesis related to the great importance of the LRP1 gene methylation in AD pathophysiology, and the crucial impact of epigenetics in these events.

Following the profound analysis of the complex DNA methylation process, it is necessary to elaborate on the essential characteristics of the active DNA demethylation process that originates through the interplay of DNA oxidative reactions and DNA repair mechanisms (***Fig. 4***).

In their mini-review, Bayraktar G *et al.*[31] analyze the current knowledge on the regulatory mechanisms controlling the active DNA demethylation. The first step of this process is 5mC oxidation induced by the ten-eleven translocation (TET) family of dioxygenases with the generation of 5-hydroxymethylcytosine (5hmC). The second step involves further hydroxylation of 5hmC by TET enzymes with the generation of 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC). Thymine DNA glycosylase (TDG), after recognizing DNA, induces the 5fC and 5caC excision of the glycosidic bond, resulting in an apyrimidinic (AP) site. AP site can be efficiently repaired by Base Excision Repair (BER) enzymes with the generation of mark-free cytosine. At the end of their report the authors emphasize that the contribution of methylation and active DNA demethylation in AD remains to be determined.

The analysis of the presented graphs in the studies by Slieker RC *et al.*[16] and Hernandez DG *et al.*[17] shows that the methylation level of a series of CpG sites related to old age, practically starts to grow from the moment of birth (on graphs, separately marked for different brain regions). Guo H *et al.*[32] emphasize that during the embryonal period (period from fertilization until the end of 8th week) and the fetal period (from 9th/10th week until birth) of life, there has been an intensive demethylation process of the methylated paternal and maternal genes, and at the same time the major wave of genome-wide demethylation is completed at the 2-cell stage (zygotic stage). Demethylation of the paternal genome is much faster than of the maternal genome. The inverse correlation between promoter methylation and gene expression gradually strengthens during early embryonic development reaching its peak at the post-implantation stage. They conclude that the DNA methylation and demethylation are the crucial elements in the epigenetic regulation of mammalian embryonic development.

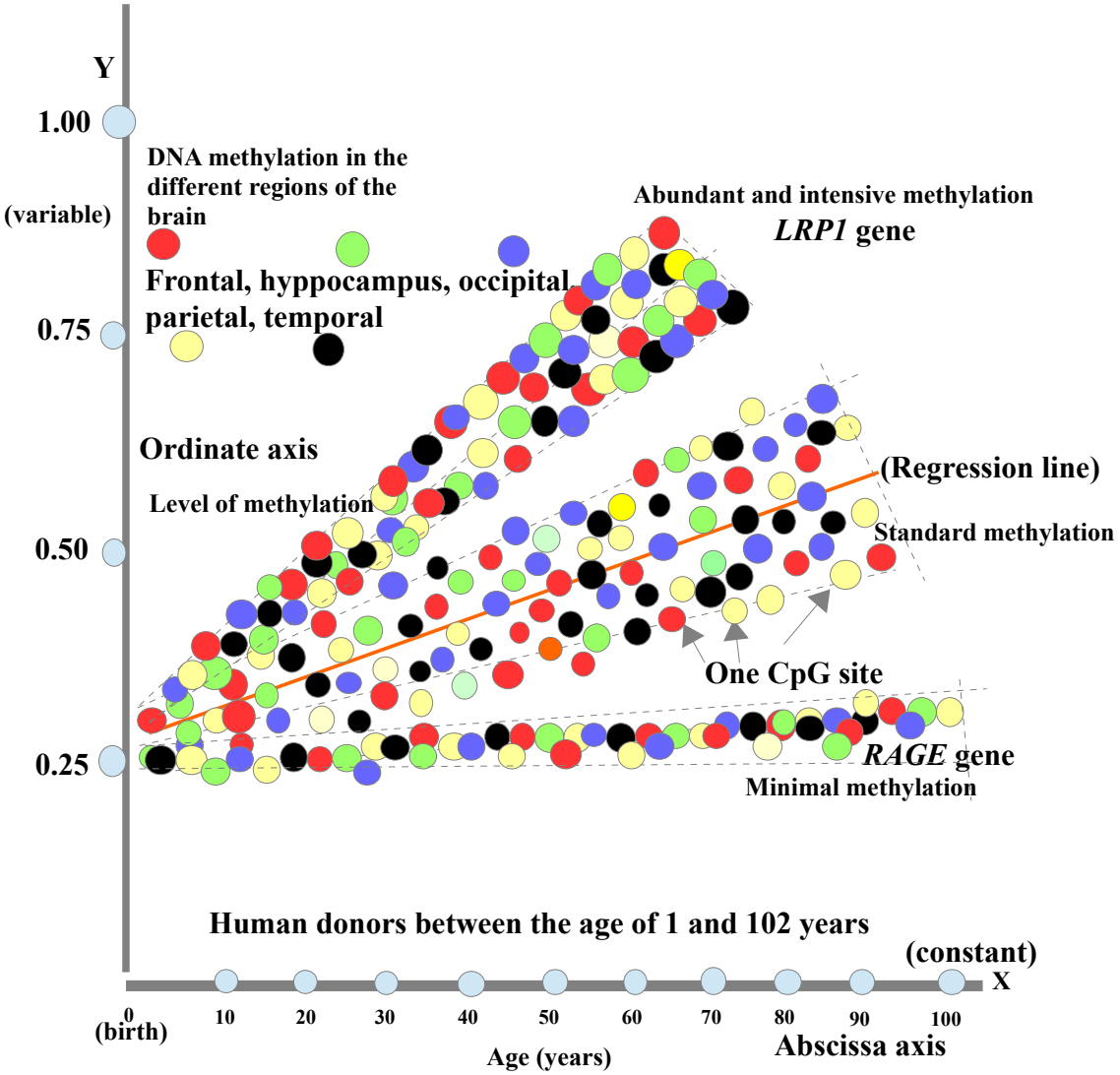

Fig. 3. **Schematic presentation of age-related methylation changes in brain regions.**

The presented graph clearly shows the relation of the level of DNA methylation in some brain regions (y axis) and age (x axis). This hypothetical relation, according to Slieker RC *et al*. (Ref 16) and Hernandez DG *et al*. (Ref 17), presents the methylation of individual CpG loci on the DNA of several genes. Here, the presented graph does not represent the real situation, its purpose is only educational. It is important to emphasize that on Figure 2 in Ref 17, the authors have measured the DNA methylation level at 10 CpG sites. For example, the code for one of these sites is cg06993413. On their graph, the CpG sites in the frontal cortex are green, and on the temporal cortex red. For all 10 sites the methylation levels increase significantly with age in all investigated brain regions (four). On this graph all circles present the same CpG site, only its location is different (different colors). CpG sites, regions of DNA where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction; LRP1, low density lipoprotein receptor-related protein; RAGE, receptor for advanced glycation endproducts.

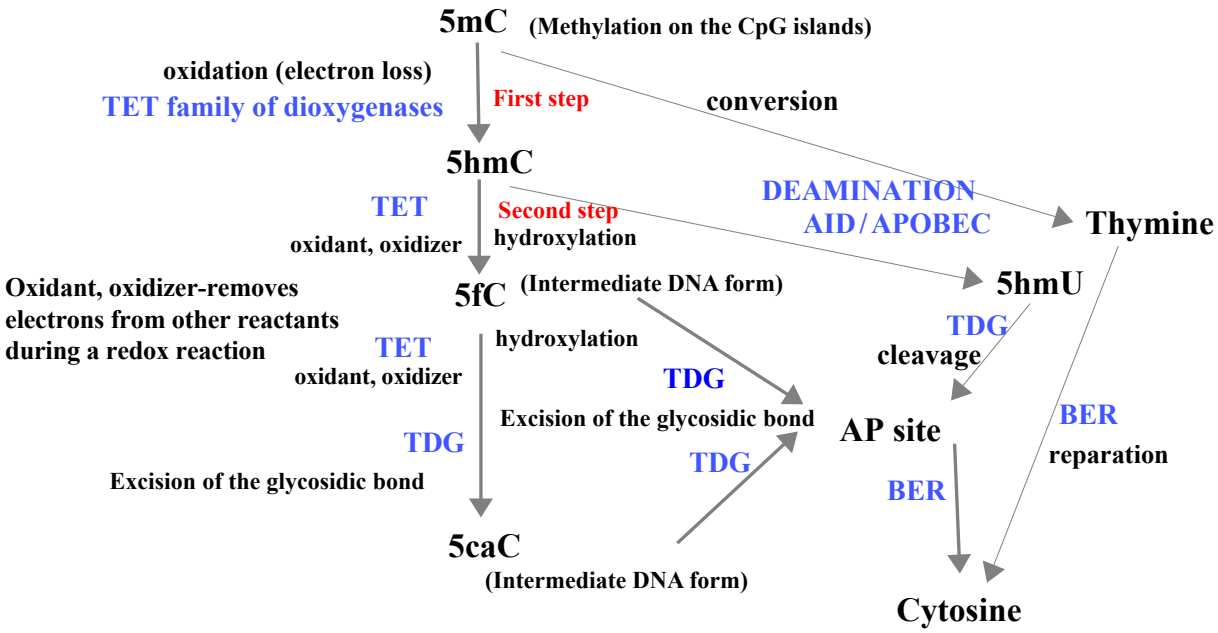

Fig. 4. **Schematic presentation of active DNA demethylation processes.**

The figure from Bayraktar G *et al*. (Ref 31) shows the two active DNA demethylation pathways. Both pathways involve a number of enzymes and compounds. In the first step, 5mC is oxidized by the TET family to produce 5hmC. In the other pathway, TDG induces glycosidic bond cleavage of 5fC and 5caC to generate AP sites, which are repaired by BER to produce cytosine. The active DNA demethylation in AD yet remains to be determined. 5mC, 5-methylcyutosine; 5hmC, 5-hydroxymethylcytosine; 5caC, 5-carboxylcytosine; TET, ten-eleven translocation; TDG, thymine DNA glycosylase; BER, base excision repair enzymes; AP, apyrimidinic; CpG, where a cytosine nucleotide is followed by a guanine nucleotide in the linear sequence of bases along its 5' → 3' direction; AID/APOBEC, activity-induced cytidinedeaminase/apolipoprotein B mRNA editing complex deaminase.

Gkountela S *et al*.[33], using laboratory mice in their investigations, answer the question of the purposefulness of the global DNA demethylation in the preimplantation phase of the mammal embryonal phase, including humans. The mentioned purposefulness lies in the strong prevention of the inherited transmission of abnormal cytosine methylations from parents to children. DNA demethylation in the pre-implant phase of the embryonal development removes the cytosine methylation acquired in the parents' gametes before fertilization. Their findings give the explanation of the regression lines in diagrams by Slieker RC *et al*.[16] and Hernandez DG *et al*.[17].

Bayraktar G *et al*.[31] and Kang J *et al*.[34] intensively investigated the process of active DNA demethylation. They are concerned with this problem in the early phase of mammal embryogenesis, including in humans. Their special interest is directed to the function of the dioxygenases TET family. Three mammalian TET proteins, TET1 (gene,10q21.3), TET2 (gene4q24) i TET3 (gene2p13) successively oxidize 5mC to 5hmC. The embryos without Tet1 i Tet3 show a heavy loss of 5-hydroxymethylcytosine (5hmC), and an increase of 5-methylcytosine (5mC) at eight cell stages. TET proteins maintain the consistency of gene transcription. According to Bernstein C *et al*.[35], the active demethylation reactions depend on the family of 5-methylcytosine oxidases (TET) and on DNA base excision repair enzymes (BER). They have found that in mice, within 6 hours of fertilization, the paternal chromosomes are close to 100% actively demethylated through TET3 and repair activity. Methyl groups on maternal DNA passively become highly diluted over the next four days. Qi Q *et al*.[36] also emphasizes the great importance of dioxygenases as inductors of DNA demethylation during mammalian development (***Fig. 4***).

Conclusion

The analysis of the presented studies undoubtedly shows the crucial role of DNA methylation and demethylation in human embryogenesis. Early zygote demethylation significantly removes possible harmful inherited genomes. Consequently, the transfer of damaging epigenetic features from parents to children is prevented, and there is strong support for the optimal embryonal and fetal development. All this also has a strong effect on the overall further lifetime of the individual. The optimization of epigenetic effects on receptor LRP1 genes and RAGE most probably slows down the AD pathophysiology.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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