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Presymptomatic Diagnosis and Gene Therapy for Alzheimer's Disease: Genomic, Therapeutic, and Ethical Aspects—A Systematic Review

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Abstract

Over the past three decades, genomic and epigenetic sciences have identified more than 70 genes involved in the molecular pathophysiology of Alzheimer's disease (AD). DNA methylation, abnormal histone and chromatin regulation and the action of various miRNAs induce AD. The identification of mutated genes has paved the way for the development of diagnostic kits and the initiation of gene therapy trials. However, despite major advances in neuroscience research, there is yet no suitable treatment for AD. Therefore, the early diagnosis of this neurodegenerative disease raises several ethical questions, including the balance between the principle of non-maleficence and the principle of beneficence. The aims of this research were to present the genomic and ethical aspects of AD, and to highlight the ethical principles involved in its presymptomatic diagnosis and therapy. A systematic review of the literature in PubMed, Google Scholar and Science Direct was carried out to outline the genomic aspects and ethical principles relating not only to the presymptomatic diagnosis of AD, but also to its gene therapy. A total of 16 publications were selected. AD is a multifactorial disease that can be genetically classified into Sporadic Alzheimer's Disease and Familial Alzheimer's Disease based on family history. Gene therapy targeting specific disease-causing genes is a promising therapeutic strategy. Advancements in artificial intelli-

gence applications may enable the prediction of AD onset several years in advance. While early diagnosis of AD may empower patients with full decision competence for early decision-making, it also carries implications for the patient's family members, who are at risk of developing the disease, potentially becoming a source of confusion or anxiety. AD has a significant impact on the life of individuals at risk and their families. Given the absence of disease modifying therapy, genetic screening and early diagnosis for this condition raise ethical issues that must be carefully considered in the context of fundamental bioethical principles, including autonomy, beneficence, non-maleficence, and justice.

Keywords

Neurodegenerative Diseases, Alzheimer's Disease, Molecular Mechanism, Gene Therapy, Presymptomatic Diagnosis Ethics, Gene Therapy Ethics

1. Introduction

Alzheimer's disease (AD) is a neurodegenerative disease (NDD). More than 600 disorders affecting the nervous system are grouped under the term NDD [1]. NDDs are defined as a group of disorders that may have genetic, hereditary, or sometimes sporadic origins. They are characterized by the progressive deterioration of neurons or their myelin sheath, leading to a gradual dysfunction of the nervous system [2]. Neurodegenerative diseases include AD, Parkinson's disease, Huntington's disease among others. Numerous genetic and environmental risk factors are known to increase the likelihood of these diseases occurring. However, as some studies suggest, the primary factor contributing to the development of these pathologies is the natural process of aging, characterized by the gradual and irreversible decline of many essential biological functions [3]. Certainly, thanks to advancements in science, modern technologies, and medicine, the life expectancy of the global population continues to rise steadily. According to Rudnicka *et al.* [4] it was estimated that in 1950, no country in the world had a proportion of people aged over 65 exceeding 11%. Similarly, in 2021, 9.6% of the global population consisted of individuals aged 65 or older. Moreover, based on projections, it is anticipated that by 2050, the population of individuals aged over 65 will exceed 1.5 billion, accounting for more than 16% of the global population, which is a substantial increase from the 8% recorded in 2005 and the mere 5% observed in 1950 [5]. The rise in life expectancy at birth is widely recognized as a significant global public health achievement. However, it also presents new health challenges in the form of chronic diseases, including cardiovascular disease, cancer, and late-onset neurodegenerative diseases [6]. Indeed, on a global scale, the aging population, genetic mutations, and epigenetic molecular mechanisms like DNA methylation, histone modifications, chromatin remodeling, regulation of non-coding RNAs (ncRNAs), and microRNAs may col-

lectively contribute to the molecular pathophysiology of neurodegenerative disorders [7] such as (AD).

While predictive medicine holds promise, presymptomatic screening for AD raises a set of ethical issues. Especially since predictive genetic testing for a neurodegenerative disease in one individual in a family can have enormous implications for all other members of the family, as it can potentially reveal their own genetic status. Considering this, adhering to Mendel's first law, an individual affected by an autosomal dominant neurodegenerative disease, irrevocably transmits the pathology to their offspring with a probability of 50%. Additionally, being a carrier of the disease gene does not provide information about when the disease will manifest, presenting a significant challenge for young individuals in planning their careers and futures. Genetic tools can be used for early diagnosis and gene therapy treatment of AD. Thus, these genetic screenings for late-onset NDD raise ethical issues [8] which must be considered in the light of the fundamental principles of bioethics which are autonomy (pre and posttest counseling, free and informed consent, confidentiality), beneficence (respect for the dignity of the patient), non-maleficence ("*primun*, *non nocere*", protection of personal data) and justice (equity in access to screening, follow-up and care). A synthesis study, based on a specialized scientific bibliography, linked to AD, and including ethical aspects, guided this research. The objectives of the present study were to elucidate both the genomic and ethical aspects of AD and, draw upon recent advancements in molecular genetics and epigenetics, to delineate the ethical principles applicable to presymptomatic genetic screening and therapeutic trials for the condition.

2. Methodology

We conducted a systematic review, using PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.

2.1. Data Sources

A systematic review of the literature was performed to identify relevant articles reporting genomic aspects and ethical principles about the presymptomatic diagnosis of AD, and its gene therapy from 1998 to August 2023. The search was conducted in French and/or English in three databases: PubMed, Google Scholar and Science Direct.

2.2. Study Selection

Studies were included if they reported 1) the diagnosis and therapeutic aspects of AD; 2) the genomic and epigenetics aspects; 3) the clinical and physiological aspects; 4) ethics issue. The key words used were "neurodegenerative diseases", "neuroepidemiology", "Alzheimer's disease", "ethics of presymptomatic diagnostic", "gene therapy for NDD" and "bioethical principles". Two evaluators (TMZ and JS) independently identified articles and sequentially (titles, abstracts, and

then full texts) screened them for inclusion (Figure 1). For articles without abstracts or without enough information in the abstract to make a decision, the full text, and where necessary supplemental materials, were reviewed before a decision was made. They excluded duplicate publications, review articles, studies not reporting genes, miRNA or CRISPR/Cas9 tool involved in gene therapy of the AD, research conducted exclusively in pediatric populations, studies conducted exclusively on migrant populations. Figure 1 shows the study selection process.

2.3. Data Extraction

Two reviewers (TMZ and JS) independently conducted the data extraction from

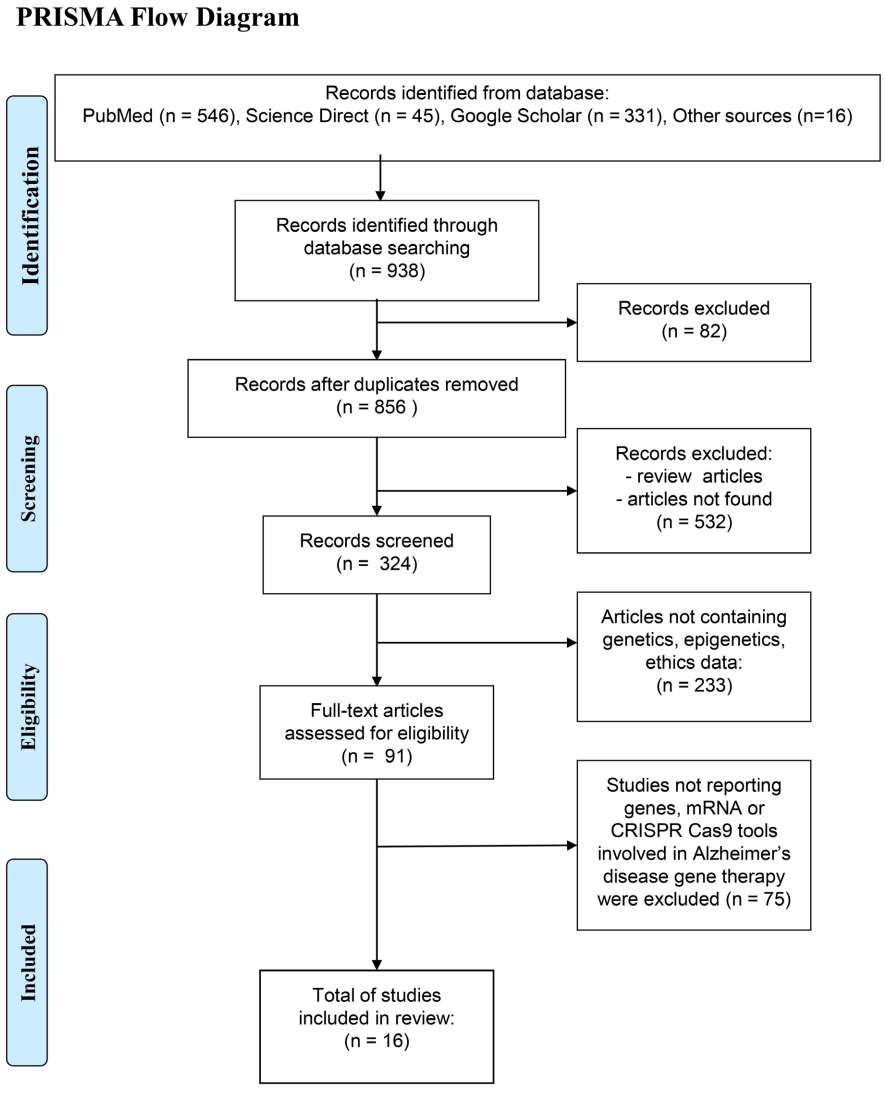

Figure 1. Flow diagram showing the method for the study selection. The database search for the search strategy described in the section was cleaned up to exclude review articles and duplicates. Titles and abstracts were included in the literature review. Review articles, articles with ambiguous data that did not meet the inclusion criteria were then excluded during the full-text review. Sixteen (16) relevant articles were finally included for this review.

included studies. The extracted data on study settings, design, population characteristics, measures of disease occurrence (prevalence), genomic and epigenetics aspects, pathophysiological aspects, diagnostic, therapeutic and ethics issue for the various conditions examined. The data was exported to Endnote X8 software and duplicates were removed. The next stage of the selection process involved a thorough examination of the abstracts for eligible articles. Studies lacking abstracts or presenting irrelevant information were subsequently excluded. The final selection of studies included in this review was accomplished through a comprehensive assessment of the full texts.

2.4. Data Analysis

A detailed inductive thematic analysis and narrative synthesis of the full texts was conducted, and this is then presented under essential sub-themes relevant to this review.

3. Results

3.1. Selection of Articles Examined

Figure 1 shows the flowchart of the process of selecting studies included in the systematic review according to PRISMA (**Figure 1**).

3.2. Alzheimer's Disease

Alzheimer's disease (AD) is a form of dementia, a complex clinical condition that affects both the central and peripheral nervous systems. It is a chronic, progressive pathology that results in the death of nerve cells [9]. Genetic mutations, genetic susceptibilities, and environmental factors such as pesticides, heavy metals, specific medications, addictive drugs, stress, toxins, viruses, and prions are among the factors that, through molecular mechanisms involving apoptosis and oxidative stress, can contribute to neurodegeneration in certain elderly individuals [10]. AD is biologically defined by β-amyloid-containing plaques and tau-containing neurofibrillary tangles (NFT) [11]. It gradually and insidiously impairs the cognitive functions of patients, including memory, language, reasoning, learning, problem-solving, decision-making, perception, and attention. Ultimately, AD leads to a loss of autonomy. These clinical symptoms are primarily associated with neuronal alterations, particularly affecting the hippocampus, known as the seat of memory, and neocortical areas. This is why AD is often referred to as the "memory disease". Neuropathological examination of the brains of AD patients reveals two characteristic types of lesions: neurofibrillary degenerations and senile plaques [12].

Genetically, AD can be classified into Sporadic Alzheimer's Disease (SAD) and Familial Alzheimer's Disease (FAD) based on family history [13]. Genome-wide Analyzes indicate that more than 70 genes or loci contribute to the development of AD [14]. These genes can be divided into pathogenic genes (PSEN1; PSEN2; APP) and risk genes (*APOE ε4*; *TREM2*; *SORL1*; *ABCA7*; CR1; DAPK1;

CLU; PICALM; BIN1; *MS4A6A*; *CD33*; *CD2AP*; *EPHA1*) [15]. Several genetic and molecular mechanisms contribute to the pathogenesis of AD. These include the amyloid cascade, tau-dependent pathology, synaptic dysfunction, neuroinflammation, oxidative stress, and lipid metabolism [11]. With the understanding of various genotypes and phenotypes of AD, numerous possibilities for gene therapy interventions emerge. These interventions aim to address the disease's root cause, primarily defective DNA, genes, or proteins, facilitating cellular repair and problem resolution.

Besides genetic mutations that progressively predispose individuals to AD, various epigenetic processes also play potential roles in its pathophysiology [16]. AD-related genes, such as the *APP* gene and microtubule-associated protein tau (MAPT), undergo DNA methylation regulation. Promoter regions of these genes experience age-dependent alterations in DNA methylation in the human cerebral cortex. These alterations can influence gene transcriptional activity and potentially mediate β-amyloid deposition [17]. Furthermore, abnormalities in histone and chromatin regulation [16] along with the actions of various miRNAs, are also involved in the molecular pathophysiology of AD.

3.3. Gene Therapy for Alzheimer's Disease

Gene therapy consists of introducing a transgene or an engineered endogenous gene into the DNA sequences of cells to treat a disease. The objective of this genotherapy is to correct by replacing or complementing a defective mutant allele with a functional allele or to overexpress a protein whose activity would have a therapeutic impact. The modification aims to trigger several types of gene expression. Depending on the case, this may involve inhibiting a defective gene in the patient, compensating for its absence, or even directly repairing a gene using a genome editing process to make it functional again. Candidate genes targeted for CRISPR Cas9 gene therapy in AD are for example: *PSEN2*, *BACE1*, *MAPT*, *PSEN1M1*, *APPS*, *APP*, *PSEN1*, *APOE* [15]. Certain genes increase the risk of developing AD, while others tend to reduce the risk of this pathology [18]. Numerous clinical trials investigating novel therapeutic drugs has yielded discouraging results in terms of enhancing cognitive performance. Gene therapies rapidly emerged as a robust therapeutic strategy for several neurodegenerative disorders. However, due to the failure of some early clinical trials to achieve satisfactory therapeutic effects, efforts are underway to enhance effectiveness through the identification of new vectors, novel therapeutic targets, and reliable delivery routes for transgenes.

3.3.1. Preclinical Studies on AD Gene Therapy

Gene therapy for AD is a therapeutic strategy which consists of penetrating genes or exogenous or endogenous DNA sequences, using molecular techniques, into the cells or tissues of the patient to treat them. In a preclinical study, peripheral delivery of antisense oligonucleotides (ASOs) targeting Amyloid-β Protein Precursor (AβPP) would decrease its expression and that of low-density li-

poprotein-related protein 1 (LRP-1) in aged SAMP8 mice with AD and thus improve memory [19]. Furthermore, treatment of AD mice with a single dose of ASO increased exon 19 splicing and corrected APOE receptor 2 splicing improving synaptic function, learning, and memory [20]. Similarly, delivery of the peroxisome proliferator-activated receptor (PGC1-α) gene using an engineered virus to mouse brain cells reduced the development of AD. Mice thus treated showed better memory and had very few amyloid plaques after four months of injection [21]. According to Offen *et al.*, 2018 [22], the APOE ε3 and ε4 alleles differ by only one nucleotide; and the APOE ε4 allele could be selectively targeted using CRISPR/Cas9 after lentiviral administration [23]. Because it was found that APOE ε4 protein levels decreased by 56%, without affecting APOE ε3, in mouse astrocytic cells expressing both human alleles. Additionally, in patients with AD, concentrations of 24S-hydroxycholesterol in plasma and cerebrospinal fluid are lower than those in healthy controls. Injection of the AAV-CYP46A1 vector into the hippocampus of THY-Tau22 mice led to normalization of CYP46A1 and 24S-hydroxycholesterol content [24]. Ultimately, patients carrying the APOE4 allele are at increased risk of developing AD. According to Krishnamurthy *et al.*, 2020, It was found that after transient treatment with CN-105 of male APP/PS1/APOETR mice, for 40 days, a reduction in Aβ pathology and a decrease in memory deficits was obtained in male APP/PS1/APOETR mice [25]. Furthermore, miRNAs belonging to the miR-15 family have been shown to be potent regulators of extracellular signal-regulated kinase 1 (ERK1) expression in mouse neuronal cells and are co-expressed with ERK1/2 *in vivo*. Additionally, Hébert *et al.*, 2010, showed that miR-15a is specifically downregulated in the AD brain [25]. Furthermore, a study carried out in vitro by Gupta *et al.*, 2022 [26] showed that inactivation of Glycogen synthase kinase-3 beta (GSK3β) by siRNA reduced the expression of amyloid pathway genes (APP and BACE1) and therefore, offers a reduction in beta-amyloid (Aβ) levels. Ultimately, it is interesting to note that the study by Park *et al.* [27] presents the *in vivo* use of nonviral vectors for CRISPR/Cas9 gene editing using AD models. Intrahippocampal injection, selected as the route of administration in this study, delivers the nanocomplexes directly into the site of action. However, applying this technique to human subjects faces some challenges, as the procedure is invasive and requires deep anesthesia.

3.3.2. Clinical Trials of Gene Therapy against AD

1) ***Gene therapy for Alzheimer's disease targeting the expression of MAPT (Microtubule Associated Protein Tau)***

The pathology of AD is linked to the accumulation of abnormal proteins, amyloid and tau proteins, in different brain regions. In vitro studies in animals have suggested that reducing the amount of tau protein, which plays a key role in the pathophysiology of AD in the brain, could reduce signs of the disease. Thus, one of the gene therapies developed can target the expression of MAPT (Microtubule Associated Protein Tau) to reduce the concentrations of MAPT

messenger RNA. By inhibiting this expression, researchers reduce the total concentration of tau in the cerebrospinal fluid (CSF) and consequently improve the clinical condition of AD patients [28]. There are different approaches to gene therapy for Alzheimer's disease (Table 1).

2) *The Nerve growth factors (NGF) promote neuron survival*

The Nerve growth factor (NGF) is thought to promote neuron survival. Intracerebral administration of NGF using recombinant AAV into the basal forebrain of AD patients showed safety and good tolerability [31]. However, the efficacy endpoints were not met in the subsequent phase2 study [33].

3) *The use of microRNAs in gene therapy for Alzheimer's disease*

MicroRNAs (miRNAs) are small non-coding RNA molecules that regulate gene expression post-transcriptionally via RNA interference. They play an indispensable and irreplaceable role in the regulation of all events such as temporal development, cell proliferation, asymmetric development, dendritic crest development, early embryonic development, tumor development, stem cell differentiation and antiviral effects. Recently, some studies have reported that some miRNAs also play an important role in the development of neurodegenerative diseases by regulating the expression of target genes [34]. MiRNA-485 (miR-485), for example, would be a highly conserved miRNA enriched in the brain. It has been demonstrated that miRNAs involved in AD regulate gene expression by

Table 1. Different genes used in gene therapy for Alzheimer's disease.

Reference	Type of vector	Gene	Therapeutic effects	Ethics and the use of viral vectors in gene therapy
Fol *et al.*, 2016 [29]	AAVvirus APPsα, TREM2,	APP	APPsα, TREM2 and IDE, leading to a decrease in Aβ levels.	Viral vectors used in gene therapy can cause genetic mutations, deletions, substitutions and inversions. So, gene therapy raises some concerns, as its reliance on viral vector delivery of therapeutic transgenes can cause both insertional oncogenesis and immunogenic toxicity [30]. In the application of gene therapy using viral vectors, we must apply not only the principle of ethical precaution but also the fundamental bioethical principles of beneficence and non-maleficence.
Rafii *et al.*, 2014 [31]	AAV2-NGF	NGF	NGF transport in the NBM leads to continuous NGF expression by cholinergic neurons.	
Revilla *et al.*, 2014 [31]	Lentiviral vectors	GDNF	Transduction of astrocytic cells with lenti-GDNF in the hippocampus resulted in substantial overexpression of BDNF, leading to maintenance of spatial learning and memory.	
Burlot *et al.*, 2015 [24]	AAV5-CYP46A1	CYP46A1	In THY-Tau22 mice: Normalization of 24S-hydroxycholesterol and CYP46A1 levels.	
Krishnamurthy *et al.* 2020 [25]	Vectastain Elite ABC/DAB	APOE	Reduced s-Aβ and Aβ plaques; improved memory	
Gupta *et al.*, 2022 [26]	PEG-PEI-siRNA-Carboxylated graphene oxide (GO) nanosheets	GSK3β	Decreased expression of amyloid pathway genes	
Zhou *et al.*, 2020 [32]	Gal-NP@siRNA	BACE1	Restore cognitive functions	

binding to mRNA, either blocking its translation, leading to its degradation, blocking protein synthesis, or leading to mRNA degradation. Thus, deregulation of microRNA expression would cause AD. MiRNm-9 was found in the anterior temporal cortex of AD patients, and its specific target was identified as BACE-1 [35]. Furthermore, in another study, miRNA-9 was reported to be upregulated in the hippocampus of AD patients [36]. Other miRNAs such as miRNA-15a, miRNA-124, and mRNA-181c are downregulated in the brains of people with AD and target mRNAs important for tau phosphorylation, the alternative splicing of AβPP and tau [37]. For this reason, more and more researchers are carrying out *in vitro* or *in vivo* therapeutic trials on AD. Beta-secretase 1 (BACE1) [38] is the enzyme that controls the rate of Aβ-amyloid formation, and miRNAs such as miR-328 and miR-298 that regulate BACE1 expression to affect production of Aβ-amyloid [34]. In monocytes, increased expression of an NF-κB-regulated miRNA-146a downregulates the expression of interleukin-1 receptor-associated kinase-1 (IRAK-1), an essential component of Toll-like receptor/IL-1 signaling. Combinatorial use of NF-κB inhibitors with miRNA-146a or antisense miRNA-146a could provide a two-pronged therapeutic strategy directed against IRAK-2-driven pathogenic signaling [39]. While exosomes microglial cells that increased miR-124-3p (Exo-124) prevented Aβ irregularities in damaged hippocampal neurons by targeting avian retoendotheliosis RELA or v-rel, which is an important APOE suppressing transcription factor (TF) [40]. Thus, miRNAs hold great promise for the treatment of AD through specific inactivation of BACE1. Table 2 shows new techniques that can deliver miRNAs to the brain to perform AD gene therapy.

4) ***The use of CRISPR Cas9 technology in gene therapy for Alzheimer's disease***

In the management of AD, CRISPR/Cas9 genome editing shows great promise. It has been well established that both FAD and SAD involve impaired Aβ metabolism [2]. Therefore, correcting increased Aβ production could be a therapeutic approach regardless of the genetic background. Table 3 provides an overview of studies that have applied CRISPR/Cas9 technology to therapeutic strategies for experimental models of AD (FAD or SAD).

5) ***The Swedish mutation, KM670/671NL APP*** **(APP^{swe})** ***and gene therapy by CRISPR/Cas9***

Treatment of FAD with genome editing can target mutations in three genes, *APP*, *PSEN1*, and *PSEN2*, as well as interfere with Aβ production. In a proof-of-concept study, György *et al.* [44] investigated the therapeutic potential of CRISPR/Cas to decrease the concentration of pathogenic Aβ by selectively disrupting the so-called Swedish mutation, KM670/671NL APP (APP^{swe}), which is a mutation in the *APP* gene located at the β-secretase cleavage site. This mutation causes hyperactivity of the β-secretase enzyme, leading to elevated brain levels of Aβ. Viral vectors containing sgRNA targeting the APP^{swe} allele and Cas9 enzyme were injected into the hippocampus of an AD mouse model expressing the APP^{swe}

Table 2. New techniques show how to deliver miRNA to the brain to prevent or to cure Alzheimer's disease.

Reference	miRNA	Levels	Target genes	Pathological implication	Ethics and the use of miRNA in gene therapy
Ouyang *et al.*, 2022 [37]	miR-124	Down AD patient hippocampus	BACE1, APP	MiR-124 was delivered by DFs, which decreased BACE1 and APP and stopped the production of Aβ in the hippocampi of APP/PS1 mice	Small interfering RNA (siRNA), although designed to be specific, also act on other unwanted targets in unpredictable ways. They can exhibit cytotoxicity by three main mechanisms - MIELE *et al.* (2012): - saturation of the RNA interference system (RISC complex, mainly), - competition with endogenous microRNAs, stimulation of the immune system. - stimulation of the immune system [41]. So, the use of microRNAs in gene therapy can damage the human organism. Consequently, their use in gene therapy requires caution and scrupulous observance of the ethical principles of precaution and the fundamental bioethical principles of beneficence and non-maleficence
Hébert *et al.*, 2008 [35]	miR-15	Down AD patients anterior temporal cortex	BACE-1, ERK1	Increased amyloidosis	
Ge *et al.* 2020 [40]	MiR-124-3p	Microglial exosomes	APOE	Through a novel, RELA-APOE route, Exo-124 reduces the detrimental effects of Aβ and expedites the reversal of neurodegeneration	
Hébert *et al.*, 2008 [35]	miR-15	Down AD patient temporal cortex, frontal lobe	AβPP	Increased amyloidosis Putative; Increased tau phosphorylation	
Cui *et al.*, 2010 [39]	miR-146a	Up AD patients hippocampus, superior temporal cortex	CFH	Increased inflammation	

mutation (Tg 2576 mouse line). One month after DNA sequencing the injection revealed approximately 2% InDels in the APP^{swe} allele [2].

6) ***The application of the CRISPR/Cas9 system using non-viral Cas9 nanocomplexes also constitutes a potential therapeutic approach against AD***

CRISPR/Cas9 was applied to target BACE1 in two mouse models: *5xFAD* mice (expressing human *APP* and *PSEN1* transgenes with a total of five AD-related mutations) and *App* knock -in mice [2]. In this study, the BACE1 gene-specific negatively charged Cas9/sgRNA complex was complexed with the R7L10 peptide to form nanocomplexes. These nanocomplexes were injected into the mouse hippocampus. Genome sequencing and Aβ quantification were performed 8 and 12 weeks later. Sanger sequencing revealed approximately 70% lower BACE1 expression in the hippocampi of treated mice. Additionally, a significant reduction in Aβ levels and cognitive deficits was observed in mice injected with Cas9 nanocomplexes compared to control mice. This study showed that the application of the CRISPR/Cas9 system using non-viral Cas9 nanocomplexes constitutes a potential general therapeutic approach. However, this delivery method does not allow widespread targeting of neural circuits and therefore most likely cannot stop the progression of AD.

7) ***The Icelandic APP A673T gene would prevent Alzheimer's disease***

AD is the result of abnormal processing of amyloid precursor protein (APP) by β-secretase and γ-secretase, which leads to the formation of toxic β-amyloid

Table 3. Overview of studies involving CRISPR/Cas9 technology in Alzheimer's disease treatment.

References	Targeted Gene	Consequences	Advantages	Disadvantages	Ethics and the use of CRISPR/Cas9 in gene therapy
Park *et al.*, 2019 [27]	BACE1	Cas9-Bace1 nanocomplex treatment of these App knock-in mice decreased the number of Bace1+ cells, reduced Aβ42 secretion, and improved cognitive deficits compared to controls. Taken together, the data indicate that targeting Bace1 by the Cas9 nanocomplex ameliorates Aβ-associated pathologies and cognitive deficits in 5XFAD and App knock-in mouse models of Alzheimer's disease.	Both Cas9 protein and sgRNA are carried on the same vector; ensures that both are expressed in the same cell. This system offers improved stability, especially during handling and manufacturing, compared to the other two strategies.	However, despite these positive results, challenges remain in translating such an approach to a clinical setting. Cas9 delivery cannot target widespread dysfunction in neural circuits; thus, is not likely to prevent disease progression in Alzheimer's disease patients. I	Genome editing techniques used in gene therapy can sometimes be imprecise and uncontrollable, which can lead to unexpected and unpredictable effects. These genetic errors, including "on-target" and "off-target" effects, can lead to unanticipated results during genome editing [42].
Offen *et al.*, 2018 [37]	APOE ε4	Application of the apoE4 targeted system led to a significant decrease of ApoE4 protein levels (−56%) without any significant changes in ApoE3 levels.	Specific elimination of the ApoE4 allele using a CRISPR Cas9 variant without harming ApoE3.	There are no clinical trials on humans yet Potential for the random insertion of plasmid fragments into the gene.	
Sun *et al.*, 2019 [23]	APP	Attenuating β-cleavage and Aβ production	CRISPR/Cas9 editing of APP C-terminus attenuates β-cleavage and promotes α-cleavage	Although the strategy of Sun *et al.*, 2019 have reciprocal effects on β/α-cleavage in various cells, they did not test the tools in an AD mouse model. Furthermore, their current off-target analyses cannot detect very small DSBs. No deleterious effects were seen in neurophysiologic parameters, though our experiments were in cultured neurons and in vivo effects are unknown.	CRISPR can also induce double-stranded breaks (DSBs) in the target DNA and by genetic breaks, it can trigger apoptosis rather than the planned genetic modification [42].
Moreno *et al.*, 2018 [43]	PSEN2	Chronic insulin administration reduces the Aβ42/40 ratio in the conditioned media of BFCNs harboring FAD mutations Unbiased exploratory analyses revealed that some subtle memory benefits resulted and that the memory changes were associated with changes in Aβ42	The discovery of insulin's ability to correct calcium flux and lower the Aβ42/40 ratio suggests that insulin acts to oppose the pathophysiology of AD.	Potential for the random insertion of plasmid fragments into the gene. One of the advantages of CRISPR/Cas9 gene editing is that it is somatic rather than germline. Thus, the gene editing results will manifest only in the treated individual and will not be passed to future generations Instability of RNA	In the application of gene therapy using viral vectors, we must apply not only the principle of ethical precaution but also the fundamental bioethical principles of beneficence and non-maleficence.

peptides. These toxic β-amyloid peptides induce neuronal death, cause memory problems and consequently the development of AD. Several APP mutations increase the risk of developing early-onset AD. However, the A673T mutation identified in the Icelandic population prevents the development of AD by re-

ducing the cleavage of APP by β-secretase. This mutation would have no known disadvantages for people who carry it and would reduce the risk of suffering from AD. Using an improved version of the CRISPR genome editing tool, Tremblay *et al.* managed to edit the genome of human cells to insert this mutation [18]. The results of this gene therapy obtained in patients suffering from AD using the A673T mutation from the Icelandic population, show a very significant reduction in amyloid plaques in their brains. Thus, the introduction of the Icelandic APP A673T gene into the genome of people at risk of developing AD could prevent the onset of the pathology or slow its progression.

The relationships between advantages (benefits) of gene therapy for different forms of AD and disadvantages (risks), between safety and imperative necessity, and between innovation and access to care for genetic pathologies, must be taken into consideration for everything that has relating to human genome editing. With this in mind, the ethics of presymptomatic diagnosis and gene therapy of AD range from evaluating the reliability of screening kits and gene therapy technologies, through the process of testing subjects in the laboratory to lead to medical treatment of the patient.

3.4. Bioethics and Ethical Principles Applicable to Presymptomatic Genetic Diagnosis and Medical Management of Alzheimer's Disease

3.4.1. Ethics Issue of Reagents and Presymptomatic Diagnostic Tests

Genetic testing for AD may pose certain risks and limitations. This is because AD encompasses both SAD and FAD, involving over 70 known genes contributing to its pathophysiology. The primary challenge lies in developing a diagnostic kit, possibly in the form of a cassette, capable of identifying most of these multiple genetic mutations associated with AD. Currently, predictive tests based on biomarkers are used for AD diagnosis. In the future, advancements in artificial intelligence (AI) applications may enable the prediction of AD's onset several years in advance [32]. However, it is important to note that genetic testing may not always provide clear-cut answers and, at times, can lead to confusion or anxiety. Test results can be challenging to interpret and may necessitate consultation with a medical genetics expert and counselor for a comprehensive understanding. Furthermore, genetic testing may have implications for the patient's family members, who could also be at risk of developing the disease [45]. In this regard, as noted by the Alzheimer Society of Canada, there is currently no reliable genetic test available for the common sporadic form of AD. Beyond these bioethical concerns regarding the limitations of genetic testing for AD and the application of human genome editing technologies in the context of gene therapy for this neurodegenerative condition, it is essential to underscore the fundamental principles of research ethics. Attempting to rank these principles in order of importance proves challenging, as they are all significant and interconnected. In each situation, a balance among these principles may be necessary to make an ethical decision. Health professionals should collaborate with patients and their

families to explore various options and make informed decisions that respect their rights and values [46].

3.4.2. The Principle of Autonomy

Genetic counseling is a process through which individuals or parents who are at risk of a hereditary disease receive counseling and information about the nature and implications of the disease, the likelihood of its development or transmission to their offspring, and the available options for life and family planning. This process aims to prevent illness or improve their situation [47]. This initial step allows potential patients to exercise their autonomy by providing free and informed consent, which is a fundamental ethical principle in medical practice, including the context of AD. However, obtaining free and informed consent may be more complex in AD, where cognitive abilities may be compromised. It's crucial to consider local laws and guidelines pertaining to informed consent for individuals with cognitive disabilities.

Before proceeding with genetic testing or gene therapy for AD, patients should receive comprehensive information about the risks and benefits of the test and therapy, as well as how the results will be utilized and shared. Patients must provide informed consent before undergoing testing or initiating care. If necessary, they may also meet with a psychologist to help prepare them for the diagnosis and prognosis. Healthcare professionals must respect patients choices and provide clear and complete information to help them make accurate decisions. The ideal would be for each citizen to be able to think and plan in advance for the possibility of this and other illnesses, especially since young people can be affected early by neurodegenerative diseases.

3.4.3. The Principle of Beneficence

This underscores the responsibility of healthcare professionals to maximize benefits for patients while minimizing risks and potential harms. However, it's crucial to acknowledge that the principle of beneficence often needs to be balanced with other ethical principles, such as respect for patient autonomy and fairness in the allocation of available resources, in the context of quality care.

There may arise situations where an ethical dilemma emerges, pitting the potential benefits of a medical intervention against other considerations like respect for autonomy or equitable resource distribution. In such cases, decisions must be made by considering all pertinent ethical principles and taking into account the values and preferences of the patients.

In the context of AD diagnosis and treatment, beneficence entails offering patients an accurate and early diagnostic assessment along with suitable treatment options to slow the progression of the disease and enhance their quality of life. This may involve cognitive testing, brain imaging scans, and clinical assessments to confirm the diagnosis [48].

After a diagnosis is confirmed, various treatment options can be explored, including specific medications aimed at managing the disease symptoms, non-drug

therapies designed to support cognitive functions, and emotional and psychological support for both the patient and their family.

It's crucial to underline that, in the realm of medical ethics, the foremost consideration is the respect for the dignity of patients. This means that diagnostic and treatment decisions should be collaboratively made, closely involving the patient and their family. These decisions should take into account their values, preferences, and interests.

3.4.4. The Principle of Non-Maleficence

The principle of non-maleficence, also known as "*primum non nocere*" in English "above all, do no harm," is a fundamental ethical principle in medicine and patient care. It highlights the importance of avoiding harm, injury or endangering the lives of patients and ensuring that medical treatment is provided with their best interests in mind [48]. This principle serves as the foundation of medical ethics and is vital for upholding the trust patients have in the medical field. Healthcare professionals must prioritize the prevention of harm to patients, which includes refraining from offering inaccurate or deceptive information that may lead to confusion or unnecessary stress [49]. In the context of neurodegenerative diseases such as Alzheimer's, it becomes crucial to carefully assess the potential advantages of early testing against potential drawbacks, including anxiety and stress experienced by family members. Nevertheless, it's important to underscore that early testing can offer valuable insights into the disease's presence, enabling early intervention that can significantly improve the quality of life for both the patient and their family [50].

However, it is also crucial to consider the psychological and emotional implications that such tests may have on family members. The decision to proceed with early testing must be made in an informed manner while respecting the autonomy of patients and their families. Healthcare professionals must be prepared to provide clear information and support family members throughout the decision-making process. Striking a balance between the potential benefits of early testing and the psychological well-being of family members is paramount. In the context of AD, it becomes challenging to delineate the threshold of harm when making an early diagnosis, as it may inadvertently introduce vulnerable individuals to undue stress.

3.4.5. The Principle of Justice

The concept of universal justice in diagnosis, testing, and healthcare embodies the belief that every person should be able to avail themselves of high-quality medical services, irrespective of their social or economic circumstances. This entails ensuring that all patients have equitable access to diagnostic tests, treatment, and healthcare, with no discrimination or bias related to factors like race, age, gender, or socioeconomic status.

Universal justice in the field of medicine also entails ensuring that healthcare resources and services are fairly allocated, with a focus on providing sufficient attention and assistance to individuals with more significant needs. This could

involve giving priority to those facing severe or disabling illnesses, as well as addressing the healthcare requirements of disadvantaged or marginalized population segments.

By upholding the principle of universal justice, healthcare professionals guarantee that each patient is provided with consistent care and attention, regardless of their individual circumstances. This commitment aids in achieving equitable access to healthcare services and mitigating health inequalities [50].

That's why healthcare, including genetic testing for AD, should be within reach for all individuals, irrespective of their financial capacity or societal position. Healthcare professionals should actively strive to diminish disparities in healthcare access and advocate for fairness.

4. Conclusion

Beyond understanding the genetics and pathophysiology of AD, some of the ultimate goals of genomics research include advancing neuroscience, molecular techniques for presymptomatic diagnostics, neurotechnology, and improving the effectiveness of gene therapy. There is no doubt that this biotechnological and therapeutic convergence poses great challenges for *Homo sapiens*. Despite this, making the best use of scientific progress to benefit global public health constitutes a moral obligation and an ethical duty for researchers. For individuals likely to carry an AD gene and who are recommended by a clinician for presymptomatic diagnostic testing, and those participating in clinical trials of gene therapy in the field of AD, their free and informed consent must first be obtained and respected; their human dignity must be unviolated, respected and protected. From this perspective, strong measures must be taken so that the bioethical principles of autonomy, beneficence, non-maleficence, and justice are applied. In the era of convergence of genomic and neurogenetic sciences, biotechnology and neurotechnology research will continue to advance our knowledge of the molecular pathophysiology and clinical trials of gene therapy for AD. It would therefore be imperative that governments, WHO, NGOs and civil societies develop global standards for the governance and oversight of NDD gene therapy and human genome editing.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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