# EFFECT OF GENE EDITING ON AIZHEIMER'S DISEASE

Thesis

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Noha Nageh Mohammed Ali

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# List of Abbreviations

| Abb. | Full term |
|---|---|
| ***21q21.3*** | *Long (q) arm of chromosome 21 at position 21.3* |
| ***A673T*** | *Alanine is replaced by threonine at position 673* |
| ***A673V*** | *Alanine is replaced by valine at position 673* |
| ***AA*** | Alzheimer's Association |
| ***AAVs*** | *Adeno - associated viruses* |
| ***ACH*** | *Amyloid cascade hypothesis* |
| ***ACTB*** | *Beta actin* |
| ***AD*** | *Alzheimer's disease* |
| ***ADRDA*** | *Alzheimer's Disease and Related Disorders Association* |
| ***AMA*** | *APP matrix approach* |
| ***APOE*** | *Apolipoprotein E* |
| ***APP*** | *Amyloid precursor protein* |
| ***Aβ*** | *Amyloid Beta* |
| ***ATP*** | *Adenosine triphosphate* |
| ***BACE1*** | *Beta-secretase 1* |
| ***BM*** | *Bone marrow* |
| ***bp*** | *Base pair* |
| ***CA*** | *Chromosomal abnormalities* |
| ***CAA*** | *Cerebral amyloid angiopathy* |
| ***CARs*** | *Chimeric antigen receptors* |
| ***Cas*** | *CRISPR associated protein* |
| ***Cat no*** | *Catalogue number* |
| ***CD*** | *Cluster of differentiation* |
| ***cDNA*** | *Complementary DNA* |
| ***$CO_2$*** | *Carbon dioxide* |
| ***CPL1*** | *C-terminal domain phosphatase-like 1* |
| ***CRISPR*** | *Cluster of regularly interspaced palindromic repeats* |
| ***CRISPRa*** | *CRISPR activation* |

# List of Abbreviations cont...

| Abb. | Full term |
|---|---|

***CRISPRi*** ..... *CRISPR interference*
***crRNA*** ..... *CRISPR RNA*
***CT*** ..... *Computerized tomography*
***$C_t$*** ..... *Cycle threshold*
***CVD*** ..... *Cerebrovascular disease*
***DAPI*** ..... *4',6-Diamidino-2-phenylindole*
***dCas9*** ..... *Dead cas9*
***DEPC*** ..... *Diethyl pyrocarbonate*
***DJ*** ..... *Diverse and joining gene*
***dL*** ..... *Deciliter*
***DMSO*** ..... *Dimethyl sulfoxide*
***DNA*** ..... *Deoxyribonucleic acid*
***Ds*** ..... *Double strands*
***DSBs*** ..... *Double strands DNA breaks*
***DSM criteria*** ..... *Diagnostic and Statistical Manual of Mental Disorders*
***dT*** ..... *Deoxy thymine*
***DTT*** ..... *Dithiothreitol*
***E. coli*** ..... *Escherichia coli*
***E+03*** ..... $\times 10^3$
***E+05*** ..... $\times 10^5$
***E693K*** ..... *Point, Missense mutation mutation GAA to AAA Within codon 693 of APP(*Italian *type)*
***E693Q*** ..... *Point, Missense mutation GAA to CAA Within codon 693 of APP(Dutch type ).*
***EB buffer*** ..... *Elution buffer*
***EDTA*** ..... *Ethylenediaminetetraacetic acid*
***FACS*** ..... *Fluorescence-activated cell sorting*
***FAD*** ..... *Familial alzheimer's disease*
***FBS*** ..... *Fetal bovine serum*
***FDA*** ..... *Food and Drug Administration*
***FDC*** ..... *Follicular dendritic cells*

# List of Abbreviations cont...

| Abb. | Full term |
|---|---|
| ***FokI*** | *Flavobacterium okeanokoites 1 endonuclease* |
| ***G*** | *Gram* |
| ***GC*** | *Germinal center* |
| ***gRNA*** | *Guide RNA* |
| ***HBG*** | *Hemoglobin gamma gene* |
| ***HC*** | *Heavy chain* |
| ***HCL*** | *Hydrochloric acid* |
| ***HD*** | *Huntington disease* |
| ***HDR*** | *Homology directed repair* |
| ***HLA*** | *Human leukocyte antigen* |
| ***HO*** | *Homothallic switching endonuclease* |
| ***HPA*** | *Hypothalamic, pituitary and adrenal axis* |
| ***HR*** | *Homologous recombination* |
| ***Hr*** | *Hour* |
| ***Hs*** | *Homosapien* |
| ***Ig*** | *Immunoglobulin* |
| ***indels*** | *Insertion or deletion mutations* |
| ***iPS*** | *Induced pluripotent stem* |
| ***ISS*** | *International staging system* |
| ***IWG -2*** | *International Working Group* |
| ***KCl*** | *Potassium chloride* |
| ***KEGG*** | *Kyoto Encyclopedia of Genes and Genomes* |
| ***L*** | *Liter* |
| ***LB*** | *Lysogeny broth* |
| ***LDH*** | *Lactate dehydrogenase* |
| ***MACS*** | *Magnetic cell separation* |
| ***MCI*** | *Mild cognitive impairment* |
| ***Mg*** | *Milligram* |
| ***MGUS*** | *Monoclonal gammopathy of undetermined significance* |
| ***mHTT*** | *Mutant huntingtin* |

# List of Abbreviations cont...

| Abb. | Full term |
|---|---|

***Min*** ..... *Minute*
***mL*** ..... *Milliliter*
***mM*** ..... *Millimolar*
***MM*** ..... *Multiple myeloma*
***mmol*** ..... *Millimole*
***MRI*** ..... *Magnetic resonance imaging*
***mRNA*** ..... *Messenger RNA*
***MTS*** ..... *3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium salt*
***NECS*** ..... *Neuromicrovascular endothelial cells*
***NFTs*** ..... *Neurofibrillary tangles*
***Ng*** ..... *Nanogram*
***NHEJ*** ..... *Non-homologous end joining*
***NIA*** ..... *National Institute on Aging*
***NIH*** ..... *National institute of health*
***Nm*** ..... *Nanometer*
***nM*** ..... *Nanomolar*
***Nt*** ..... *Nucleotide*
***OFP*** ..... *Orange fluorescence protein*
***Oligo*** ..... *Oligonucleotide*
***OR*** ..... *Odds ratio*
***PAM*** ..... *Protospacer adjacent motif*
***PB*** ..... *Phosphate buffer*
***PBS*** ..... *Phosphate buffer solution*
***PCR*** ..... *Polymerase chain reaction*
***PE*** ..... *Phycoerythrin*
***PEG*** ..... *Polyethylene glycol*
***PES*** ..... *Phenazine ethosulfate*
***PET*** ..... *A positron emission tomography*
***PET-CT*** ..... *Positron emission tomography – computed tomography*

# List of Abbreviations cont...

| Abb. | Full term |
|---|---|
| ***pGCS vector*** | *Plasmid of Genomic Cleavage Selection kit* |
| ***PMNL*** | *Polymorphonuclear leukocytes* |
| ***pmol/µL*** | *Picomole per microliter* |
| ***PSEN1*** | *Presenilin proteins 1* |
| ***PSEN2*** | *Presenilin proteins 2* |
| ***PSH*** | *Presenilin hypothesis* |
| ***qPCR*** | *Quantitative polymerase chain reaction* |
| ***RNA*** | *Ribonucleic acid* |
| ***RPMI*** | *Roswell Park Memorial Institute* |
| ***RQ*** | *Relative quantification* |
| ***RR*** | *Relapsed / refractory* |
| ***RT*** | *Reverse transcription* |
| ***S.*** | *Seconds* |
| ***S.O.C*** | *Super Optimal broth with Catabolite repression* |
| ***SaCas9*** | *Staphylococcus aureus Cas9* |
| ***SAD*** | *Sporadic alzheimer's disease* |
| ***sgRNA*** | *Single guide RNA / synthetic guide RNA* |
| ***SpCas9*** | *Streptococcus pyogenes Cas9* |
| ***SPSS*** | *Statistical Package for the Social Science* |
| ***SpyCas9*** | *S. pyogenes Cas9* |
| ***ssDNA*** | *Single-stranded DNA* |
| ***TALENs*** | *Transcription-activator-like effector nucleases* |
| ***TALENs*** | *Transcription-activator-like effector nucleases* |
| ***Tau protein*** | *A protein that is an important component of nerve cells* |
| ***TBI*** | *Traumatic brain injury* |
| ***TCR*** | *T-cell receptor* |
| ***TE buffer*** | *Tris and EDTA buffer* |
| ***tracrRNA*** | *Trans-activating CRISPR RNA* |

# List of Abbreviations cont...

| Abb. | Full term |
|---|---|

***U/mL*** ..... *Units per milliliter*
***V(D)J*** ..... *Variable, diverse and joining gene segments*
***V/V*** ..... *Volume per volume*
$V_HD_HJ_H$ ..... *Heavy chain variable, diverse and joining regions of gene segments*
***W/V*** ..... *Weight per volume*
***WT*** ..... *Wild type*
***ZFNs*** ..... *Zinc finger nucleases*
***Δ absorbance*** ..... *Delta absorbance*
$\Delta C_t$ ..... *Delta* $c_t$
$\Delta\Delta C_t$ ..... *Delta delta* $c_t$
***λ5*** ..... *Lambda5*
***M*** ..... *Mu*
***Mg*** ..... *Microgram*
***µmol*** ..... *Micrmole*

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# INTRODUCTION

Alzheimer's disease (AD) is referred as one of the most common causes of dementia and frailty. Typically, the symptoms of the disease begin with mild memory difficulties and evolve towards cognitive impairment, dysfunctions in complex daily activities, and several other aspects of cognition ***(Mantzavinos & Alexiou, 2017).***

Alzheimer's disease is currently ranked as the sixth leading cause of death in the United States, but recent estimates indicate that the disorder may rank third, just behind heart disease and cancer, as a cause of death for older people ***(National Institute on Aging, 2020).***

Current estimates suggest that 44 million people live with dementia worldwide at present. This is predicted to more than triple by 2050 as the population ages, when the annual cost of dementia in the USA alone may exceed US$600 billion. In coming years, the largest increase in dementia prevalence is expected in low and middle income countries, which show patterns of increasing cardiovascular disease, hypertension and diabetes. AD is the single biggest cause of dementia, accounting for 50%–75%, and is primarily a condition of later life, roughly doubling in prevalence every 5 years after age 65 ***(Lane et al., 2017).***