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A proposal of new biomarkers for Alzheimer's Disease non-invasive diagnosis through gene expression and image processing

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurological disorder that causes brain atrophy. The current diagnosis is based on cognitive tests. Nevertheless, these techniques are not conclusive, and the disease can be diagnosed indubitably postmortem. For this reason, we proposed a set of new biomarkers to improve Alzheimer's non-invasive diagnosis based on three major factors: First, the analysis of specific expression genes in blood. Second, patient data of their medical history. And third, asking for an MRI image to be analyzed. That is why we performed a gene expression analysis and a genome-wide association study from free datasets studies on AD in blood samples in R to find biomarkers that were later used in a Multilayer Perceptron to diagnose patients. Subsequently, we tested different physiological parameters such as sex, age, or level of education to prove its prediction significance through a logistic regression model with data from the National Alzheimer's Coordinating Center. Finally, we processed magnetic resonance images made by the Austrian Science Fund and German Research Foundation. As a result, a set of 55 genes directly related to AD were identified. The logistic regression model showed that the significant variables correspond to age, the presence of other cognitive diseases and the existence of mutations in the APOE gene. And a decrease in intracranial volume of white matter in hippocampus was detected in patients with the disease.

Keywords: Alzheimer, GWAS, Gene Expression, Image Processing, MRI, Software development

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurological disorder that causes the brain to shrink, causing damage to neurons (Autoridad Nacional del Servicio Civil, 2021). This constitutes the most frequent pathology, accounting for around 60% and 70% of all dementia cases in

the world (OMS, 2015). Symptoms of AD can vary from other types of dementia and vary depending on the personality, general health, and social status of the individual. However, there are broad similarities such as memory loss, poor or impaired judgment, disorientation in time and place, changes in mood or behavior, problems with language, concentration, planning or organization, and with social activities (Pérez , 2018).

Diagnosing AD begins with obtaining information about the development of symptoms. It is based on performing cognitive tests such as tests of memory, attention, visual or spatial skills, verbal, and language fluency; in addition, a family member is consulted about the patient's health status. Other types of tests are also considered, such as brain scans through tomography, MRI, among others. However, these techniques are not conclusive, and, on many occasions, it is definitively diagnosed after death, by performing an examination of brain tissue in an autopsy (Ustárroz, 2007). The uncertainty for the diagnosis is very wide, in medicine it becomes a challenge, especially in the initial stages when the symptoms are not clear. For this reason, efforts are added, and biological diagnosis has occasionally begun (Custodio *et al*., 2019).

The biological diagnosis is based on the analysis of biomarkers that participate in the molecular mechanism of the disease; for this reason, these allow the identification of the disease state and understand the physiological process in the progression of AD. These biomarkers make use of current diagnostic tools in the brain, such as magnetic resonance imaging (MRI), positron emission tomography (PET) and the detection of cerebrospinal fluid (CSF) biomarkers (Aβ and tau), which they are invasive and expensive (Lee *et al*., 2019). The main disadvantages of this diagnosis are the high training of clinicians, positioning in late stages of the disease and the risk of causing harm, anxiety, and fear to the patient.

MATERIAL AND METHODS

Gene expression as peripheral biomarkers for Alzheimer's disease

Data obtained from the Gene Expression Omnibus (GEO) via the accession identifier GSE63060 was analyzed to characterize the expression profile in patients with AD. The samples correspond to the peripheral blood of Alzheimer's cases and controls originate from the AddNeuroMed cohort, the cases are patients with Alzheimer's disease, subjects with mild cognitive impairment or controls matched by age and sex (Sood *et al.*,2018; Liang *et al.*,2006).

Expression profiling was performed using R (version 3.5.0) software. The differentially expressed genes (DEGs) were determined using day 0 or BL as a baseline. Up and down-regulated genes were those with a log2 fold-change greater and less than 0.5 and a p-value<0.05, respectively. For DEGs calculation and visualization limma package was used. Regarding the gene-set enrichment analysis (GSEA) a p-value<0.05 and q-value<0.2 as the threshold was used and the analysis was displayed using the DOSE and ClusterProfiler package (Yu *et al.,* 2015; 2012). It was decided to analyze a study of enrichment of diseases through the Disease Ontology Semantic enrichment (DOSE) analysis with the up-regulated genes found in the expression profile and select those ones related to Alzheimer.

Deep post-GWAS analysis

With the aim of biomarkers associated with genetic architecture of additional AD-related phenotypic traits to elucidate deeper clinical insights into the genetic basis of AD, a Genome-wide meta-analysis (GWAS) was performed. Additionally, to determine specific tissues where samples for the diagnosis of AD identified in the GWAS analysis could be carried out, a tissue expression enrichment analysis was carried out. The GWAS regions associated with AD were obtained from the GWAS Catalog under the accession number GCST007511 (Kunkle *et al.* 2015). The researchers conducted a GWAS meta-analysis of non-Hispanic Whites using a larger Stage 1 discovery sample (17 new, 46 total datasets; n = 21982 cases and 41944 cognitively normal controls). GWAS analysis and Manhattan plot was using the Functional mapping and annotation of genetic associations with FUMA (Watanabe, and Taskesen, 2017), and subsequently a gene-based analysis was carried out on all SNP output using the MAGMA software (de Leeuw, 2015).

Clinical Variables signatures of patients with Alzheimer's Disease

For the third study aim, participants with Alzheimer's Disease (n =84) and Mild Cognitive Impairment or MCI

due to presumed Alzheimer's disease (n =123) were analyzed against a control population sample (n=133) with a logistic regression model where we compared demographic, psychological, and functional variables. Data was obtained from a large cohort study of older adults of the National Alzheimer's Coordinating Center (NACC) uniform data set (n =44359) (Li *et al.*, 2018). Variables compared corresponded to the sex, age, the presence of seizures, if the patients had another cognitive disease, the years of education, if the subject was left or right and if they presented a mutation in the APOE gen, which previously was demonstrated and reported to be associated to AD.

Image Processing of MRI

The mass reduction of the brain is one of the main characteristics of Alzheimer disease and thanks to the Magnetic Resonance Imaging (MRI) can be visually demonstrated. Thus, the brain MRI images were obtained from the study made by Alzheimer's Disease Neuroimaging Initiative (ADNI) and there were chosen in total 5 cases of Alzheimer's Disease and 5 cases of Controls (Petersen *et al.* 2010; Mueller *et al.* 2010). Then each file was compressed from NII format to NII.GZ format using MATLAB. With this compress file it was processed in the software BrainSuite to obtain the 3D brain form, so it was found the Cortical Thickness and the different volumes of the brain. After the 3D brain reconstruction, BrainSuite provides a table with the volume values of each part of the brain. With them, it was processed in R to analyze the difference between the Alzheimer and Control patients (Shattuck and Leahy, 2002).

RESULTS

DEGs related to Alzheimer's Disease

As a result of the Disease Ontology Semantic enrichment, it was found that the significant diseases corresponding to Alzheimer's disease, dementia, bacterial infection disease, hepatitis, rheumatoid arthritis, blood coagulation, osteoarthritis, spondylitis, brollosis, etc. (**Fig. 1**). The network displayed shows a strong association between diseases with exception of urticaria, dementia and Alzheimer's disease due to the non-existence of overlapping genes with the main network. Subsequently, those genes related mainly to Alzheimer and dementia were plotted in a cneplot (**Fig. 2**), In total it was found 53 significant up-regulated genes and some respiratory, kidney and reproductive diseases with a weak relationship to those genes. Finally, the protein-protein interaction shows one that all the genes differentially expressed in AD have a relationship, in the protein-protein interaction analysis it was found that the AKT1, GAPDH, and IL1B genes were those that had a greater number of degrees of freedom associating with more than 25 genes each of it (**Fig. 3**). Those 53 genes used in the SMVs showed levels of accuracy of 87% for the training data and 65% for the testing data. On other hand, the same genes used in the perceptron multilayer showed an accuracy of 99% for the training and 87% for the testing data.

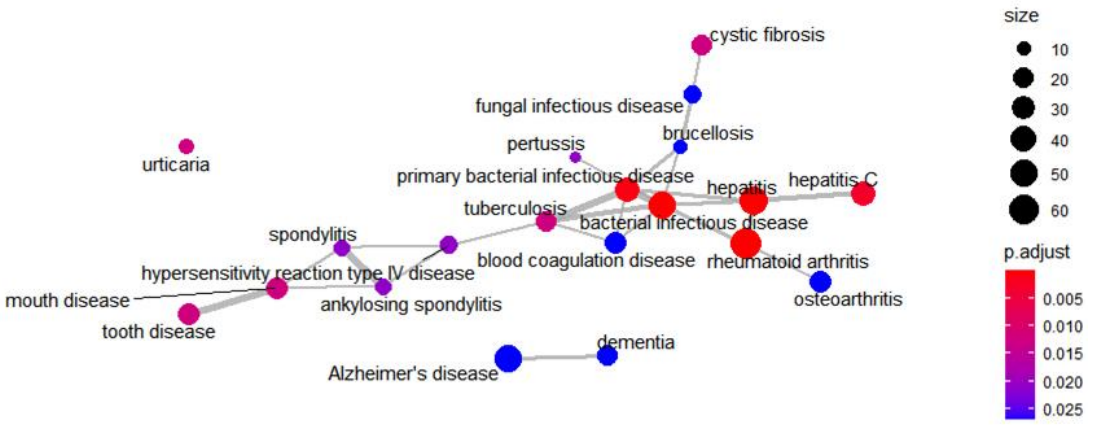

Fig. 1 Emapplot of Alzheimer's Disease Ontology Semantic enrichment with up-regulated genes in GSE63060. Among 20 significant pathways of up-regulated meta-analysis genes for Alzheimer's disease pathway and enriched gene ontology with other top significant pathways in this study have been visualized with DOSE and ClusterProfiler packages in R

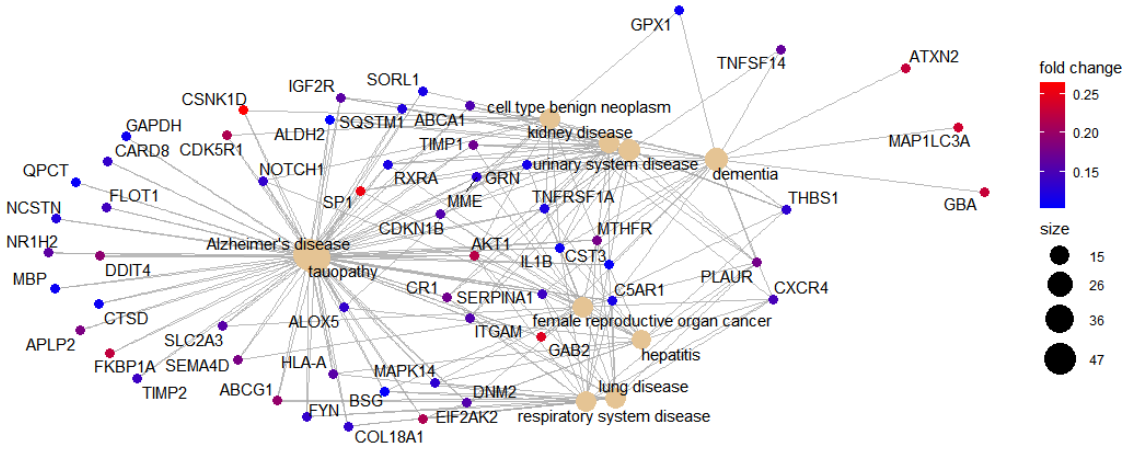

Fig. 2 Cneplot of Alzheimer's disease Disease Ontology Semantic Enrichment with up-regulated genes regarding AD and dementia. Among 53 significant up-regulated genes for Alzheimer's disease and dementia pathways in this study have been visualized with DOSE and ClusterProfiler packages in R.

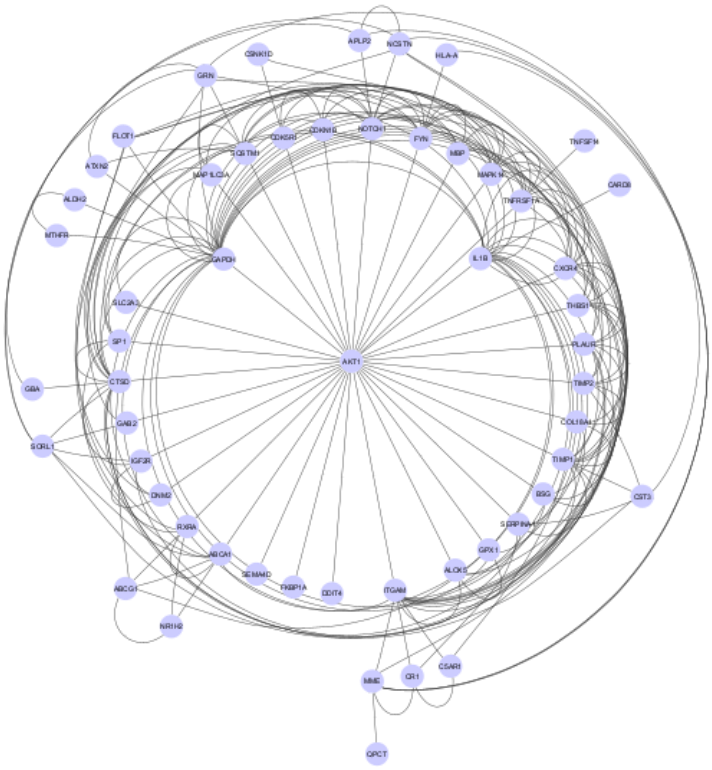

Fig. 3 Protein-protein interaction network of Alzheimer's disease Disease Ontology Semantic enrichment with up-regulated genes regarding AD and dementia. Among 53 significant up-regulated genes for Alzheimer's disease and dementia pathways in this study have been visualized with DOSE and ClusterProfiler packages in R.

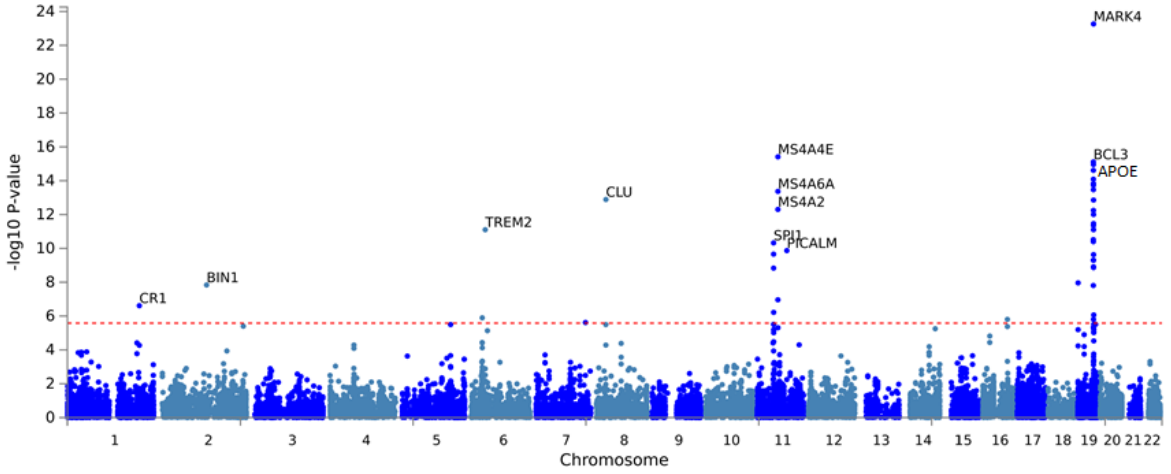

Fig. 4 Manhattan plots of A SNP level and B gene-level genome-wide association results in AD patients. All plots include gene assignments made with FUMA. Dotted red lines represent the threshold for genome-wide significance, i.e., α = 5.0E-08 for SNP-based for gene-based analyses.

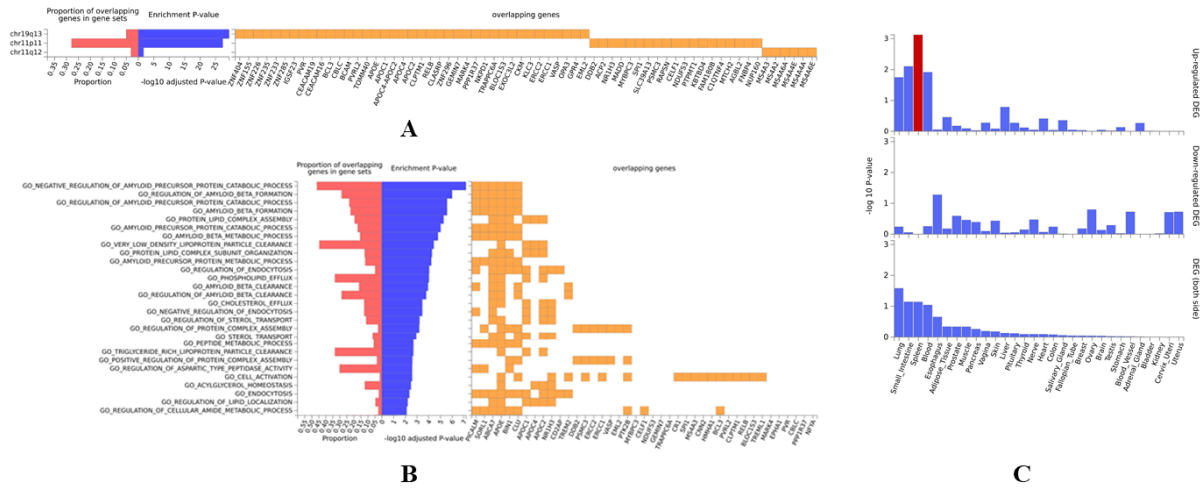

Fig. 5 Enrichment GWAS analysis (A) Positional gene sets (MsigDB c1). The gene sets in the Molecular Signatures Database (MSigDB) are divided into 3 major collections for chr19q13, chr11p11, and chr11q12. (B) GO biological processes (MsigDB c5) Well-annotated gene sets grouped according to gene ontology (GO) categories (C5). (C) Tissue-Specific Enrichment Analysis (TSEA) of GWAS from GTEx v8 30 general tissue types in significantly enriched DEG sets (Pbon < 0.05). Red bar indicates the tissue with greater DEG sets.

Potential risk genes and risk variants for Alzheimer's disease provided by the deep post-GWAS analysis

The meta-analysis of the AD GWAS data identified 78 lead SNPs and 224 independent significant SNPs with P < 5.0E-08 from 14 genomic risk loci (**Fig. 4**). The majority (n = 45) of the lead SNPs were in the gene-dense 44377739-46125148 locus on chromosome 19 (**Fig. 5a**). An additional enrichment analysis in R suggests that a total of 37 genes were affected and that most SNPs affected the protein NECTIN2, MS4A4A, MARK4, CLPTM1, APOE, and TOMM40. The enrichment analysis shows that there exists a negative regulation of the catabolic process, metabolic process, and formation of the amyloid precursor protein (APP) during the disease. Furthermore,

the pathways of cholesterol efflux and endocytosis were negatively affected (**Fig. 5b**). Regarding the tissue expression enrichment analysis, it was found that most of the genes expressed belong to the spleen, the small intestine, and the blood (**Fig. 5c**).

Clinical factors associated with Alzheimer's Disease

The logistic regression model for patients with MCI and AD showed that the significant variables correspond to age, the presence of other cognitive diseases and the existence of mutations in the APOE gene due to having a p-value less than 0.05 (**Fig. 6**). The model was created through the system of GAAIN. The age variable had an odds ratio (OR) of 1.13 and 1.08 for AD and MCI respectively, which would describe a positive relationship with the onset of the disease. The presence of other cognitive diseases also had a positive relationship with ODs of 2.7 and 2.13 for AD and MCI respectively. Finally, mutations in the alleles of the APOE gene contribute to the development of AD and MCI with ODs of 1.77 and 1.30.

Volume of white matter at hippocampus as neuroimaging biomarker

The 3D reconstruction of the brain given by the BrainSuite Software gives different views such as the Volume Segmentation where each color represents a specific brain part and the Cortical Thickness which is a kind of heat map that varies depending on the depth of the zone (**Fig. 7**). The plots in R showed that Alzheimer's cases have a lower volume both in Gray and White matter as in the Hippocampus (**Fig. 8**).

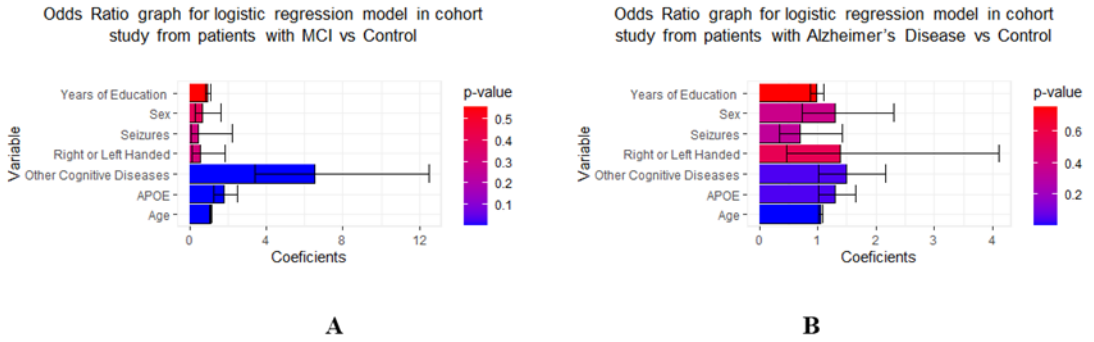

Fig. 6 Significant variable identification in logistic regression for patients with Mid Cognitive Impairment vs Alzheimer's Disease (A) and for patients with Alzheimer's Disease vs healthy patients.

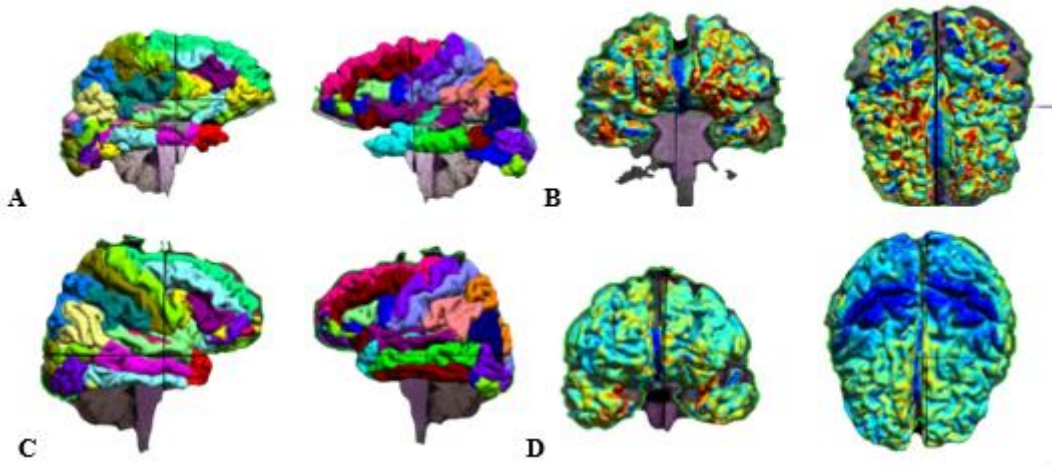

Fig. 7 Alzheimer's Disease MRI Analysis (A) volume segmentation and (B) cortical thickness. (C) Healthy Patient: volume segmentation and (D) cortical thickness

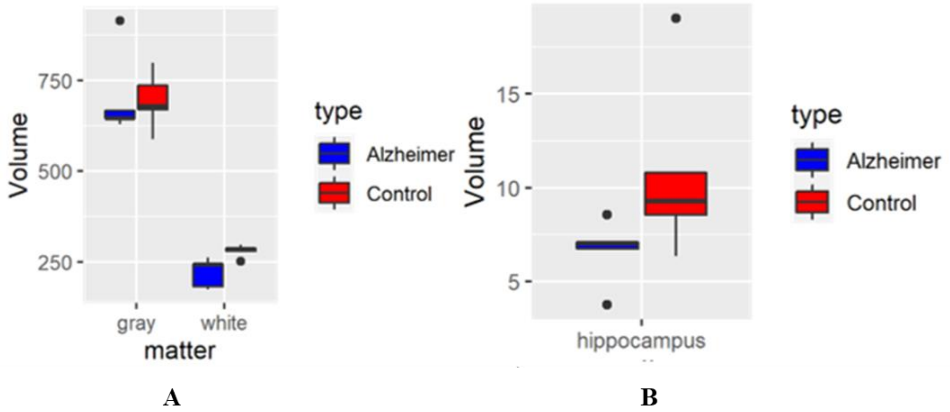

Fig. 8 Comparation of volume of gray and white matter in patients with Alzheimer's Disease vs control. In the whole brain (A) and in the Hippocampus (B).

DISCUSSION

Regarding the enrichment analysis of the overexpressed genes in peripheral blood in Alzheimer's disease direct genes were found related to the disease and dementia. Nevertheless, some genes were found directly related to diseases such as bacterial infections, this could be explained because it has been proven that there exists a strongly positive association between AD and bacterial infections by Spirochetal Bacteria or Chlamydophila Pneumoniae (Maheshwari and Eslick, 2015; Sochocka, 2017). In regard to the hepatitis as a detected disease, it is believed that this suggested relationship is due the amyloid-β (Aβ) deposits, this accumulate as plaques in the brain of an AD patient and the liver is involved being the origin of the Aβ by presenting an impairment in Aβ clearance via the peripheral system (Bassendine *et al*., 2020; Chiu *et al*., 2014). Usually, deposits of Aβ bind to fibrinogen and fibrin, leading to blood coagulation, also detected in enrichment, that are structurally abnormal and harder to degrade (Strickland, 2018).

Some other diseases found such as rheumatoid arthritis, osteoarthritis, and osteoporosis have been proven to significantly increase the risk of and putatively accelerate cognitive decline in AD-related neurodegeneration (Culibrk and Hahn, 2020). Considering the gene sets corresponding to each of the diseases, it results inconvenient due the vast number of them and these diseases are found strongly related to others that are not necessarily present in an AD patient. That's the reason the second enrichment analysis was realized only with the genes related to AD and dementia.

The analysis of the set of genes filtered showed that the genes also are related to tauopathy. It has been reported that in AD brain tau is three to four-fold more hyperphosphorylated than the normal adult brain tau and in this hyperphosphorylated state it is polymerized into paired helical filaments (PHF) admixed with straight filaments (SF) forming neurofibrillary tangles (Iqbal *et al*., 2010). It has been found in the second analysis after the exclusion of the gene sets of other diseases that filtering the genes allowed the identification of a disease indicative of AD.

The GWAS meta-analysis confirmed 37 previous late-onset AD risk loci and demonstrates that these can be detected from samples in tissues such as the spleen, lung, blood, and small intestine. Unlike current studies where it is shown that the apolipoprotein E (APOE) gene is the strongest gen risk for AD (Moreno-Grau *et al*., 2018). In our study, the gene with the highest number of SNPs corresponded to receptor-related poliovirus 2 or nectin-2 (NECTIN2). However, this can be explained given that it was located in close vicinity of the APOE locus on chromosome 19 as well as the first set of genes identified in the GWAS analysis (Porcellini *et al*., 2010), and shown in the Manhattan plot (**Fig. 2.1**). Other risk genes identified were SPI1 and CR1, that have been associated with lower expression in monocytes and macrophages

(Patel *et al.*, 2021), and ABCA7 for having the same regulation directions across tissues (Li *et al.*, 2018).

The results on the logistic regression model corresponding to age, presence in other cognitive diseases, and mutations in the APOE gene coincide with the current literature. For instance, AD is the most common type of dementia, accounting for at least two-thirds of cases of dementia in people aged 65 and older (Kumar and Tsao, 2018). The results indicate a significant importance of age this is not an isolate case, for example in previous research, age at onset has been described as highly heritable in AD families in which they have mutations such as the PSEN1 mutation (Guerreiro and Bras, 2015). Mutations in the APOE gene were found to be strongly related to the development of AD, this is expected as it is still considered as the strongest genetic risk factor associated with sporadic AD. For example, carriers of APOE ε4 allele, have an earlier age on onset of cognitive impairment. Besides the APOE ε2 allele is the most genetic protective factor against AD delaying the age on onset (Serrano-Pozo *et al*., 2021).

On the other hand, while sex and education in the results are not positively related, despite the fact that in other studies it is indicated that these factors are correlated with the symptoms corresponding to dementia (Laurin *et al*., 2021). There could be other factors not included in this research explaining that relationship, such as excluding other causes for dementia or that there isn't an association between education and AD. Women may have lower resilience to tau, being manifested by a higher degree of metabolic dysfunction in the entorhinal cortex in response to tau pathology (Ramanan *et al.,* 2019).

Finally, regarding to the brain imaging it was confirmed the reduction of the brain mass caused by AD. There exist investigations that confirm that this condition can be detected in early Alzheimer (Burns *et al*., 2010). Besides, since the MRI images are cross-sectional, the parts of the brain that present the greatest size change are mainly the anterior and posterior horn of the lateral ventricle. The lateral ventricle represents a part of the cerebral ventricular system, and their function is to house the cerebrospinal fluid and allow its circulation (Fame *et al*., 2020). In fact, there are some cohort studies that affirm the relation between Alzheimer's disease and this ventricular enlargement because this condition is strongly related with the loss of the cognitive functions (Apostolova *et al.*, 2012). Also, it was investigated that this biomarker can give an early and possibility of a presymptomatic disease diagnosis; even so, it needs more research to corroborate because this enlargement is commonly presented in old people (Apostolova *et al.*, 2012). However, it would be a perfect biomarker for the young population.

CONCLUSION

DOSE analysis indicates a strong relationship between Alzheimer's disease and dementia. Protein-protein interaction indicate that AKT1, GAPDH and IL1B genes has more than 25 genes associated with them. GWAS analysis identified a majority of the lead SNPs located on chromosome 19. The genes that have most of the SNPs affect the NECTIN2, MS4A4A, MARK4, CLPTM1, and TOMM40 proteins.

Demographic variables indicate distinct odds ratio between Alzheimer's disease and sex, presence of other cognitive diseases and allele mutations in the APOE gene. Lower brain volume both in Gray and White matter in the hippocampus were found confirming brain reduction caused by Alzheimer's disease. Further research is advised in these areas.

Author contributions:
Luis Quesada Velarde: Software, Validation, Writing- Original draft preparation **Amalia Villena Romani:** Data curation, Writing- Original draft preparation **Nathaly Dongo Mendoza:** Conceptualization, Methodology, Software, Visualization, Investigation

Conflicts of Interest: The authors stated that no conflicts of interest.

REFERENCES

Apostolova LG *et al.* (2012) Hippocampal atrophy and ventricular enlargement in normal aging," Alzheimer Dis. Assoc. Disord., vol. 26, no. 1.

Autoridad Nacional del Servicio Civil, "Hoja informativa sobre la Enfermedad de Alzheimer," Angewandte Chemie International Edition, 6(11), 2021, 951–952.,. https://www.nia.nih.gov/espanol/hoja-informativa-sobre-enfermedad-alzheimer.

Bassendine MF, Taylor-Robinson SD, Fertleman M, M. Khan, and D. Neely, (2020) Is Alzheimer's Disease a Liver Disease of the Brain?," Journal of Alzheimer's Disease, vol. 75, no. 1. 2. doi: 10.3233/JAD-jad190848.

Burns JM, Johnson DK, Watts A, Swerdlow RH and Brooks WM, (2010) Lean mass is reduced in early Alzheimer's diseasea and associated with brain atrophy," Arch. Neurol., vol. 67, no. 4, 2010.

Chiu WC, Tsan YT, Tsai SL, Chang CJ, Wang JD, and Chen PC (2014) Hepatitis C viral infection and the risk of dementia," Eur. J. Neurol., vol. 21, no. 8, 2014, doi: 10.1111/ene.12317.

Culibrk RA and Hahn MS (2020) The Role of Chronic Inflammatory Bone and Joint Disorders in the Pathogenesis and Progression of Alzheimer's Disease," Frontiers in Aging Neuroscience, vol. 12. 2020, doi: 10.3389/fnagi.2020.583884.

Custodio N, Montesinos R and Alarcón JO (019) Evolución histórica del concepto y criterios actuales para el diagnóstico de demencia.," Rev. Neuropsiquiatr., vol. 81, no. 4, 2019, doi: 10.20453/rnp.v81i4.3438.

de Leeuw CA, Mooij JM, Heskes T and Posthuma D (2015) "MAGMA: Generalized Gene-Set Analysis of GWAS Data," PLoS Comput. Biol., vol. 11, no. 4. doi: 10.1371/journal.pcbi.1004219.

Fame RM, Cortés-Campos C and Sive HL (2020) Brain Ventricular System and Cerebrospinal Fluid Development and Function: Light at the End of the Tube: A Primer with Latest Insights," BioEssays, vol. 42, no. 3. doi: 10.1002/bies.201900186.

Guerreiro R and Bras J (2015) The age factor in Alzheimer's disease," Genome Med., vol. 7, no. 1, 2015, doi: 10.1186/s13073-015-0232-5.

Kumar A and Tsao JW (2018) Alzheimer Disease: REVUE," StatPearls, 2018.

Kunkle BW *et al*. (2019) "Genetic meta-analysis of diagnosed Alzheimer's disease identifies new risk loci and implicates Aβ, tau, immunity and lipid processing," Nat. Genet., vol. 51, no. 3. doi: 10.1038/s41588-019-0358-2.

Laurin D, Verreault R, Lindsay J, MacPherson K and Rockwood K (2001) "Physical activity and risk of cognitive impairment and dementia in elderly persons," Arch. Neurol., vol. 58, no. 3. doi: 10.1001/archneur.58.3.498.

Lee JC, Kim SJ, Hong S and Kim YS (2019) Diagnosis of Alzheimer's disease utilizing amyloid and tau as fluid biomarkers," Experimental and Molecular Medicine, vol. 51, no. 5. 2019, doi: 10.1038/s12276-019-0250-2.\

Li X *et al*., (2018) Systematic Analysis and Biomarker Study for Alzheimer's Disease," Sci. Rep., vol. 8, no. 1, 2018, doi: 10.1038/s41598-018-35789-3.

Liang WS *et al*., (2007) Gene expression profiles in anatomically and functionally distinct regions of the normal aged human brain," Physiol. Genomics, vol. 28, no. 3, 2007, doi: 10.1152/physiolgenomics.00208.2006.

Maheshwari P and Eslick GD (2015) Bacterial infection and Alzheimer's disease: A meta-analysis," J. Alzheimer's Dis., vol. 43, no. 3. doi: 10.3233/JAD-140621.

Moreno-Grau S *et al*. (2018) Genome-wide significant risk factors on chromosome 19 and the APOE locus," Oncotarget, vol. 9, no. 37, 2018, doi: 10.18632/oncotarget.25083.

Mueller SG *et al*., (2005) "The Alzheimer's disease neuroimaging initiative," Neuroimaging Clinics of North America, vol. 15, no. 4. doi: 10.1016/j.nic.2005.09.008.

OMS, "OMS | Demencia," Nota descriptiva N°362. 2015.

Patel D *et al*. (2021) Cell-type-specific expression quantitative trait loci associated with Alzheimer disease in blood and brain tissue," Transl. Psychiatry, vol. 11, no. 1, 2021, doi: 10.1038/s41398-021-01373-z.

Pérez Á (2018) Deterioro cognitivo leve y depresión en el adulto mayor," Investig. y Pensam. Crítico, vol. 6, no. 2, doi: 10.37387/ipc.v6i2.84.

Petersen RC *et al*. (2010) Alzheimer's Disease Neuroimaging Initiative (ADNI): Clinical characterization," Neurology, vol. 74, no. 3, 2010, doi: 10.1212/WNL.0b013e3181cb3e25.

Porcellini E, Carbone I, Ianni M and Licastro F (2010) Alzheimer's disease gene signature says: Beware of brain viral infections," Immun. Ageing, vol. 7, 2010, doi: 10.1186/1742-4933-7-16.

Ramanan VK *et al*. (2019) Association of Apolipoprotein e ϵ4, Educational Level, and Sex with Tau Deposition and Tau-Mediated Metabolic Dysfunction in Older Adults," JAMA Netw. Open, vol. 2, no. 10, 2019, doi: 10.1001/jamanetworkopen.2019.13909.

Serrano-Pozo A. Das S and Hyman BT (2021) APOE and Alzheimer's disease: advances in genetics, pathophysiology, and therapeutic approaches," The Lancet Neurology, vol. 20, no. 1. 2021, doi: 10.1016/S1474-4422(20)30412-9.

Shattuck DW and Leahy RM (2002) Brainsuite: An automated cortical surface identification tool," Med. Image Anal., vol. 6, no. 2. doi: 10.1016/S1361-8415(02)00054-3.

Sochocka M, Zwolińska K and Leszek J (2017) The Infectious Etiology of Alzheimer's Disease," Curr. Neuropharmacol., vol. 15, no. 7. doi:10.2174/1570159x15666170313122937.

Sood S *et al*., (2015) A novel multi-tissue RNA diagnostic of healthy ageing relates to cognitive health status," Genome Biol., vol. 16, no. 1, 2015, doi: 10.1186/s13059-015-0750-x.

Strickland S (2018) Blood will out: Vascular contributions to Alzheimer's disease," Journal of Clinical Investigation, vol. 128, no. 2. 2018, doi: 10.1172/JCI97509.

Tauopathies," Curr. Alzheimer Res., vol. 7, no. 8, 2010, doi: 10.2174/156720510793611592.

Tirapu Ustárroz J (2007) "La evaluación neuropsicológica," Interv. Psicosoc., vol. 16, no. 2, 2007, doi: 10.4321/s1132-05592007000200005.

Watanabe K, Taskesen E, A. Van Bochoven, and D. Posthuma, (2017) "Functional mapping and annotation of genetic associations with FUMA," Nat. Commun., vol. 8, no. 1, , doi: 10.1038/s41467-017-01261-5.

Yu G, Wang LG, Han Y and He QY (012) ClusterProfiler: An R package for comparing biological themes among gene clusters," Omi. A J. Integr. Biol., vol. 16, no. 5. doi: 10.1089/omi.2011.0118.

Yu G, Wang LG, Yan GR and He QY (2015) DOSE: An R/Bioconductor package for disease ontology semantic and enrichment analysis," Bioinformatics, vol. 31, no. 4, doi: 10.1093/bioinformatics/btu684.