IRON RELATED GENE EXPRESSION AND BIOCHEMICAL PHENOTYPE SUPPORT IRON HOMEOSTASIS DYSREGULATION IN ALZHEIMER'S DISEASE

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Introduction

The distinction between normal aging and Alzheimer's disease (AD) is a first and relevant step to combat this disease efficiently. Because of the clinical interest in predicting patient evolution and prognosis, the identification of biomarkers and the unraveling of genetic factors underlying AD are of crucial importance.

Several lines of evidence implicate an imbalance of the redox-active biometals, as iron in AD. Metal-catalyzed hydroxyl radicals are potent mediators of cellular injury and are central to the oxidative injury hypothesis of AD pathogenesis [1,2].

In this study, we seek to further investigate this hypothesis through the identification of serum biomarkers/endophenotypes related to Fe metabolism and candidate genes involved in Fe homeostasis.

This integrative approach is planned to deal with heterogeneity in these complex disorder and further elucidate the contribution of Fe metabolism disruption to the etiopathogenesis of AD.

Methods

Subjects and sample collection

A total of 116 AD patients and 98 healthy volunteers were recruited at Hospital de Santa Maria, Hospital Fernando Fonseca and Hospital Magalhães Lemos. Blood samples were collected by venipuncture in serum gel and EDTA tubes. The study was submitted and approved by the local ethics committee and each donor or legal representative signed an informed consent before blood collection.

Genetic Analysis

A total of 74 SNPs were evaluated by highthroughput genotyping in *APOE* (apolipoprotein E) and 9 Fe metabolism-related genes: *CYBRD1* (cytochrome b reductase 1), *HAMP* (hepcidin), *HFE* (hemochromatosis gene), *IREB1/2* (iron responsive element binding protein 1/2), *SLC11A2* (divalent metal transporter 1), *SLC40A1* (ferroportin), *TF* (transferrin) and *TFR2* (transferrin receptor 2). SNPassoc® package for R 2.8.1® (1999-2006 R Development Core Team) was used for logistic regression analysis of the genotyping data for association with AD (adjusted for sex and gender). *P*-values <0.05 were considered statistically significant.

Biochemical measurements

Serum iron (Fe), transferrin (Tf), ferritin (Ft) concentration and transferrin saturation (Tf Sat.) were measured by standard methods. PASW Statistics 18.0® (SPSS Inc.) software was used for MANCOVA and logistic regression analysis of all biochemical data.

Gene expression evaluation

Total RNA was extracted from PBMCs (peripheral blood mononuclear cells) of 59 control and 53 AD individuals and the expression of *HPRT1, TFRC, TFR2, SLC40A1, HAMP and SLC11A2* genes was analysed by real-time PCR.

PASW Statistics 18.0® (SPSS Inc.) software was used to perform the analysis of variance, adjusted for age and gender (ANCOVA), of gene expression data.

Results

Genetic analysis

Table 1. Results of significant SNP allelic association in *iron-related* genes and *APOE* with AD.

Gene	Chromosome	SNP reference	Position (Mb)	Alleles[a]	Gene region	Associated Allele numbers (frequency) AD (2n = 232)	Associated Allele numbers (frequency) CTRL (2n = 178)	Associated Allele	P-value	OR(95% CI)	*P*-value$_{corr}$
SLC40A1	2q32	rs1439816	190152875	C/G	Intron 1*	44 (0.190)	19 (0.107)	C	**2.10E-02**	1.96 (3.49-1.10)	NS
TF	3q22.1	rs4428180	134949064	G/A	Intron 1	32 (0.138)	37 (0.210)	G	**5.37E-02**	0.60 (1.01-0.36)	NS
TF	3q22.1	rs1358024	134966878	T/C	Intron 11	24 (0.104)	30 (0.174)	T	**4.15E-02**	0.55 (0.98-0.31)	NS
TF	3q22.1	rs8177277	134967520	C/T	Intron 11	7 (0.030)	15 (0.085)	C	**1.47E-02**	0.33 (0.84-0.13)	**NS**
TFR2	7q22	rs7385804	100073906	A/C	Intron 3	90 (0.395)	83 (0.539)	C	**5.50E-03**	0.56 (0.37-0.84)	**1.65E-02**
IREB1	9p21.1	rs10970973	32414900	C/G	Intron 10	76 (0.349)	42 (0.244)	C	**2.58E-02**	1.66 (2.59-1.06)	NS
APOE	19q13.2	rs429358	50103781	C/T	Exon 4	58 (0.271)	18 (0.123)	C	**7.00E-04**	2.64 (4.71-1.48)	**7.00E-04**

Mb: Megabases; OR: odds ratio; 95% CI: 95% confidence interval; NS: not significant; [a]: minor/major allele; *: rs1439816 in *SLC40A1* gene is located in intron 1 in a boundary region to exon 2 (at 24 bp) . Significant P-values (<0.05) and significant Bonferroni corrected P-values (P-valuecorr; <0.05) are highlighted in bold.

- ✓ **Six SNPs in iron-related genes have been shown associated with AD.**
- ✓**The associated alleles in *TFR2* and *TF* SNPs confer protection to AD while the ones in *SLC40A1* and *IREB1* are associated to AD risk.**
- ✓ Only the *TFR2* association survives Bonferroni correction at the gene level.
- ✓ *APOE* ε4 association with AD risk was confirmed in our study.

Biochemical markers

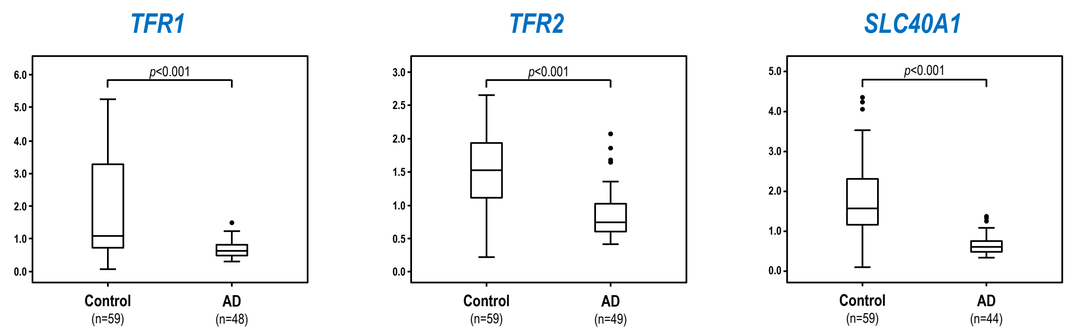
Table 2. Biochemical parameters measured in serum from AD cases and controls and respective multivariate analysis of variance.

Parameters	AD Cases n	AD Cases mean ± SD	Controls n	Controls mean ± SD	Normal range	Significance (F)	Overall MANCOVA (F)
[Iron] (µg/dL)	116	76.63 ± 26.36	84	86.67 ± 25.18	37.0 – 158.0	**0.003** (4.19)	
[Transferrin] (mg/dL)	114	250.96 ± 43.23	84	267.86 ± 44.28	200.0 – 400.0	**0.016** (3.16)	**0.003** (6.27)
Transferrin Saturation (%)	113	23.43 ± 8.17	83	25.70 ± 7.91	25.0 – 50.0	0.066 (2.25)	
[Ferritin] (ng/µL)	101	126.91 ± 70.00	81	138.40 ± 83.91	6.0 – 397.0	**0.033** (2.69)	

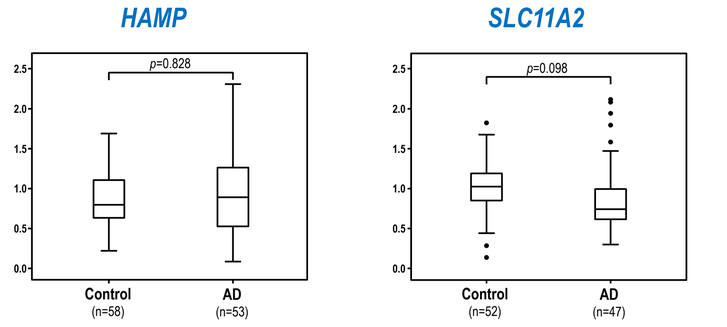
Significant values (<0.05) are highlighted in bold.

- ✓ **Both serum iron, ferritin and transferrin levels were found to be significantly decreased in AD patients.**
- ✓ **No significant differences have been found with the Transferrin Saturation levels.**

RT-qPCR



- ✓ **The expression of *TFR1*, *TFR2* and *SLC40A1* genes in PBMCs from AD patients is significantly decreased when compared to controls.**



- ✓ The expression of *HAMP* and *SLC11A2* genes were not significantly different when comparing AD patients to controls.

The quantification was performed by normalizing the sample to a pool of individuals and using *HPRT1* as a housekeeping gene. Dots represent the mild outliers. The number of individuals analyzed is indicated in brackets.

Conclusions

- ✓ **The overall results obtained in this work provide additional evidence that strengthen the hypothesis of an altered Fe homeostasis in Alzheimer's disease.**
- ✓**Fe metabolism biomarkers measured in serum (Fe, Tf and Ft concentration) were found significantly decreased in AD patients when compared to controls. Also, associations with AD were found for three SNPs in *TF*, one SNP in *TFR2* and for the first time in *IREB1* and *SLC40A1* genes. In addition, evidence for the down-regulation of *TFR1*, *TFR2* and *SLC40A1* gene expression in AD patients was found.**
- ✓ **Importantly, this study is in agreement with previous reports of an association of *APOE ε4* with AD, thus reproducing well-accepted results and reinforcing the validity of our work.**

Discussion

✓ **We hypothesize that the lower serum Fe concentration observed in AD patients can be due to impaired Fe excretion from cells, since ferroportin (Fpn) codified by *SLC40A1* is the only known Fe exporter in mammalian cells. Also, the *TFR2* polymorphism found to be associated with AD is located at the putative promoter region of the TfR2_beta isoform which is involved in *SLC40A1* transcriptional regulation. On the other hand, *IREB1* codifies a cytosolic protein which binds to iron-responsive elements (IREs) found in RNA from several iron metabolism-related proteins as ferritin, TfR, Fpn itself and importantly amyloid precursor protein (APP). The intracellular accumulation of Fe, particularly in the brain where Fpn is also expressed, would lead to a rise in oxidative damage, contributing to the AD physiopathology.**

References

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Acknowledgements

We thank the Unidade Laboratorial Integrada from Departamento de Promoção da Saúde e Doenças Crónicas, Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA) for the measurement of biochemical parameters. We also thank João Costa from the Genotyping Unit of Instituto Gulbenkian de Ciência.

This research was supported by the *Fundação para a Ciência e a Tecnologia* (FCT) [SFRH/BPD/29354/2006 to MM, SFRH/BD/60718/2009 to BS, and SFRH/BD/48671/2008 to LM]; Fundação Astrazeneca (Research Grant awarded through the "Programme of Support to Research"); Instituto Nacional de Saúde Dr. Ricardo Jorge; Portuguese Ministry of Health (Research Grant 53/2007 of the "Comissão de Fomento da Investigação em Cuidados de Saúde"); Lundbeck Portugal, Lda (Research Grant).