Iron/copper metabolism in Alzheimer's disease: genetics and biomarkers in a Portuguese population

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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 unravelling of genetic factors underlying AD are of crucial importance.

Several lines of evidence implicate an imbalance of the redox-active biometals, copper and 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/Cu metabolism and candidate genes involved in Fe/Cu homeostasis.

In fact, an important genetic component has been recognized for AD [3]. Several candidate genes involved in Fe/Cu metabolism were evaluated for their contribution to AD susceptibility and to the assessed biological marker distributions.

This integrative approach is planned to deal with heterogeneity in these complex disorder, and will power phenotypic data expressed in AD to identify susceptibility loci, and further elucidate the contribution of Fe/Cu metabolism disruption to the etiopathogenesis of AD.

Materials and Methods

Subjects and sample collection

A total of 117 AD patients (24 men, 92 women) and 91 healthy volunteers (38 men, 51 women) were recruited at Hospital de Santa Maria (HSM), Hospital Fernando Fonseca (HFF) and Hospital Magalhães Lemos (HML). Blood samples were collected by venipuncture under no fasting conditions 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.

Biochemical measurements

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

Genetic Analysis

A total of 108 SNPs were evaluated through highthroughput genotyping in *APOE* (apolipoprotein E) and 12 Fe/Cu metabolism-related genes: *CALR* (calreticulin) , *CP* (ceruloplasmin), *CYBRD1* (cytochrome b reductase 1) , *FB19* (protein phosphatase 1), *HAMP* (hepcidin), *HFE* (hemochromatosis gene), *IREB1/2* (iron responsive element binding protein 1/2), *SLC11A2* (divalent metal transporter 1) , *SLC40A1* (ferroportin), *TF* (transferrin), *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, and for mean difference calculation (adjusted for gender) of biochemical/immunological parameters' values between carriers of different genotypes for each associated SNP. p-values <0.05 were considered statistically significant.

Results

Iron metabolism biomarkers are impaired in Alzheimer's disease

Results of iron metabolism markers measured in peripheral blood from AD patients and controls are presented in Table 1.

Table 1 Means, standard deviation and normal range of each biochemical parameter measured in serum from AD cases and controls, after removal of outliers.

Parameters	AD Cases		Controls		
	n	mean ± SD	n	mean ± SD	Normal range
[Iron] (µg/dL)	116	76.63 ± 26.36	84	86.67 ± 25.18	37.0 – 158.0
[Transferrin] (mg/dL)	114	250.96 ± 43.23	84	267.86 ± 44.28	200.0 – 400.0
Transferrin Saturation (%)	113	23.43 ± 8.17	83	25.70 ± 7.91	25.0 – 50.0
[Ferritin] (ng/µl)	101	126.91 ± 70.00	81	138.40 ± 83.91	6.0 – 397.0

Legend: Fe - Iron concentration; Tf - transferrin concentration; Tf Sat. - transferrin saturation; Ft - ferritin concentration

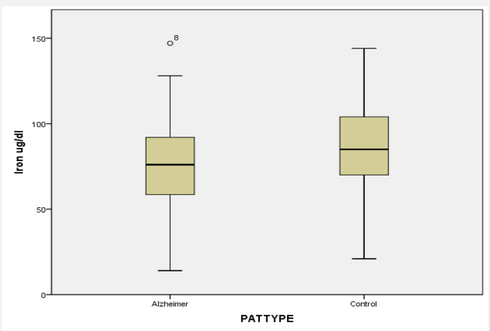

Figure 1 – Boxplots showing the median and dispersion of the iron concentration (µg/dL) measured in serum of AD patients and controls after removal of outliers. * - significant differences in the means (p-value<0.05, see Table 1).

Table 2. Multivariate analysis of variance of the biochemical parameters between AD patients and controls. Sex and age are used as co-variables in the analysis

Parameters	F	Significance
Overall MANCOVA	6.27	0.003 (S)
[Iron] (µg/dL)	4.19	0.003 (S)
[Transferrin] (mg/dL)	3.16	0.016 (S)
Transferrin Saturation (%)	2.25	0.066 (NS)
ln [Ferritin] (ng/µl)	2.69	0.033(S)

Legend:. ln[Ferritin] - Ferritin concentration values were logaritmized before the test. S – significant; NS – non-significant.

Higher risk of AD is associated with specific iron metabolism genes SNPs

Three SNPs were found to be significantly associated with AD: rs429358 (p-value=0.0004), which distinguishes APOE ε4 from the other two alleles (APOE ε2/ε3) in the *APOE* gene, rs1049296 (p-value = 0.0082), which differentiates TFC1 allele from TFC2 allele of *TF* gene, and rs1439816 (p-value = 0.0354), which lies in intron 1 of *SLC40A1* gene. The model with higher significance for these 3 SNPs was the dominant model, meaning that the presence of the allele itself, even in heterozygosis, causes a significant association with AD.

Table 4. SNPs significantly associated with higher risk of AD

Gene	Chr	SNP reference	Position (Mb)	Assoc Allele	Chi Square	p value	OR[95% CI]
APOE	19q13.2	rs429358	50103781	C	11.375	7.00E-04	2.64 (1.48-4.71)
IREB1	9p21.1	rs10970973	32414900	C	4.97	0.0258	1.66 (1.06-2.59)
TFR2	7q22	rs7385804	100073906	A	7.716	0.0055	1.79 (1.19-2.71)
TF	3q22.1	rs1358024	134966878	C	4.155	0.0415	0.55 (0.31-0.98)
TF	3q22.1	rs8177277	134967520	T	5.946	0.0147	0.33 (0.13-0.84)
SLC40A1	2q32	rs1439816	190152875	C	5.324	0.0210	1.96 (1.10-3.49)

Legend: SLC40A1 – ferroportin gene; TF – transferrin gene; APOE –apolipoprotein E gene; Genot. – genotype; Freq. – frequency; Abs. – absolute; Rel. – relative; OR – odds ratio.

Concluding Remarks

The overall results obtained in this work provide additional evidence to strengthen the hypothesis of an altered Fe homeostasis in Alzheimer's disease.
In fact, significant differences in serum Fe metabolism biomarkers (Fe, Tf and Ft concentration) were found between AD patients and controls.
Also, significant associations with AD were found for two SNPs in *TF*, one SNP in *TFR2* (*P*=0.0055) and for the first time in *IREB1* (*P*=0.0258) and *SLC40A1* (*P*=0.0210) genes.
Importantly, this study is in agreement with previous reports of an association of *APOE ε4* with this disease, thus reproducing well-accepted results and reinforcing the validity of our work.

We hypothesize that the lower serum Fe concentration observed in AD patients can be due to impaired Fe excretion from cells, since 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 seems to be 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 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.

Noteworthy, an integrative approach was followed to deal with heterogeneity in this complex disorder, and new directions were raised related to the study of the involvement of Fe metabolism in AD.

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