

CROSSTALK BETWEEN GENETICS, GENE EXPRESSION AND BIOCHEMICAL MARKERS SUPPORTS SYSTEMIC IRON HOMEOSTASIS DYSREGULATION IN ALZHEIMER 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 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.

AIM

- To screen a set of SNPs in Fe metabolism-related genes and APOE in a sample of 116 AD patients and 98 healthy controls;
- To compare serum biomarkers of Fe metabolism in the same samples;
- To analyze the expression level of several Fe metabolism genes in the peripheral blood mononuclear cells (PBMCs).

RESULTS

To determine whether Fe metabolism-related gene variants were associated with susceptibility to AD in a Portuguese group, allelic, and genotypic frequencies were compared between patients with AD and healthy control subjects.

The results show that specific iron metabolism related gene variants are associated with AD susceptibility.

IRON METABOLISM BIOMARKERS IN ALZHEIMER'S DISEASE

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
Transferin 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

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)
Transferin Saturation (%)	2.25	0.066 (NS)
In [Ferritin] (ng/µl)	2.69	0.033 (S)

SPECIFIC IRON METABOLISM GENE VARIANTS IN AD

Table 3: SNPs significantly associated with higher risk of AD

Gene	Chr	SNP reference	Position (Mb)	Assoc Allele	Chi Square	p value	OR [95%]
APOE	19q13.2	rs429358	50103781	C	11.375	7.00E-04	2.64 (1.48-4.71)
IRBE1	9p21.1	rs10970973	32414900	C	4.97	0.0258	1.66 (1.06-2.59)
TRF2	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)

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METHODS

SUBJECTS AND SAMPLE COLLECTION

A total of 116 AD patients (76.6 6.9, 24 men and 92 women) and 89 healthy volunteers (68.2 7.7, 38 men and 51 women) were recruited at Hospital de Santa Maria, Hospital Fernando Fonseca and Hospital Magalhães Lemos. 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 MANCOVA and logistic regression analysis of all biochemical data.

GENETIC ANALYSIS

SNPs were evaluated by highthroughput genotyping in APOE (apolipoprotein E) and Fe/Cu 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), 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 age and gender). P-values <0.05 were considered statistically significant.

CONCLUSIONS

We hypothesize that the low systemic Fe status profile observed in AD patients could be due to impaired regulation of cellular Fe efflux.

The intracellular accumulation of Fe, particularly in the brain would lead to a rise in oxidative damage, contributing to the AD pathophysiology.

References

- Zecca L, Youdim MBH, Riederer P, Connor JR, Crichton RR (2004) Iron, Brain Ageing and Neurodegenerative Disorders. Nature Reviews 5:863-873
- Strausak D, Mercer JFB, Dieter HH, Stremmel W, Multhaup G (2001) Copper in disorders with neurological symptoms: Alzheimer's, Menkes, and Wilson diseases. Brain Research Bulletin 55(2):175-185
- Cacabelos R (2002) Pharmacogenomics in Alzheimer's Disease. Min Reviews in Medicinal Chemistry 2:59-84