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Support for the Committee for Aid and Education in Neurochemistry (CAEN) Report
CATEGORY 1B: Research supplies for use in the applicant's home laboratory
April 2015 round

APPLICANT INFORMATION

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RESEARCH REPORT

Project Title: *Are rare variants in TREM2 associated to Late-Onset Alzheimer's Disease in Argentine population?*

Recently, a rare variant in the *TREM2* gene (rs75932628) was identified as a novel risk factor for Late-Onset Alzheimer's Disease (LOAD) in two independent studies. Although the *TREM2* R47H variant is rare, the risk of AD conferred by this SNP has been compared to the effect size of *APOE* e4. This has been replicated by other studies in different Caucasian populations, and also in African American population while lack of association between *TREM2* R47H and LOAD was observed in Asiatics. Indicating that the associated LOAD-risk of this variant depends on the genetic background of the population. In addition, other variants within the *TREM2* gene, like R62H (rs143332484) and T96K (rs2234253) substitutions have been recently reported and their associated risk of LOAD is still controversial. The main aim of the proposed investigation was to determine the frequency of R47H, R62H, and T96K *TREM2* variants in Argentine population in order to test if these rare variants are genetic risk factors for LOAD in this population.

Patients with cognitive impairments (cases), and individuals without obvious clinical dysfunction (controls) were recruited from the outpatient Neurology Department of Instituto de Investigaciones Médicas A. Lanari (Buenos Aires, Argentina). The study was approved by the institutional ethical committee, and has been carried out with the family members' informed consent. The median of age (range) in cases and controls were 76 (62-92) and 78 (60-94) years old, respectively; and the age at onset in cases was 72 (50-92) years old. The mini mental state examination (MMSE) results presented a mean of 20 in cases, and 29 in controls. The incidence of APOE alleles between cases and controls were similar to the reported for Hispanic populations (http://www.alzgene.org/meta.asp?geneID=83). Frequencies observed in cases were *APOE2* = 0.040, *APOE3* = 0.673, and *APOE4* = 0.287; whereas in controls were *APOE2* = 0.056, *APOE3* = 0.846, and *APOE4* = 0.098.

This Argentine population sample was genotyped for 97 biallelic SNPs associated to ancestry, using VeraCode, GoldenGate platform (Illumina). Ancestry of the studied group was determined using Admixture and Structure softwares, observing that it is an admixture of European and Native populations, with a higher Caucasic proportion (Figure 1). Cases and controls are similarly distributed in a principal components analysis (PCA); hence, there is not obvious association between ancestry and disease (Figure 1), however this relationship needs to be further studied. These results were presented in the Argentine Society of Biochemical and Molecular Biology Investigations (SAIB) meeting, published in BIOCELL 39 (Suppl. 2) 2015.

On the other hand, R47H, R62H, and T96K *TREM2* polymorphisms were genotyped using a custom-designed TaqMan assays (Life Technologies). Association between case-control status and variant carrier/noncarrier status was calculated using Fisher exact test for 2 x 2 contingency tables. The statistical analyses were performed using the implementations in R (www.rproject.org). The genotyping data were inspected to detect deviations from Hardy-Weinberg equilibrium. The minor allele frequencies (MAF) obtained showed that these variants are rare, being R47H the most rare one, which was absent in all control samples examined (Table 1). Although we could observe a tendency of these variants to be associated to LOAD (odds ratios, OR > 1), results were statistically non-significant ($p > 0.05$). To overcome this problem, it is necessary to increase sample size to get enough statistical power, and be able to reach a conclusion. Recently, we incorporated more

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recruitment centers to the project (Hospital Milstein, Hospital Eva Perón, and Hospital de Clínicas); consequently, we expect to progress in this regard shortly.

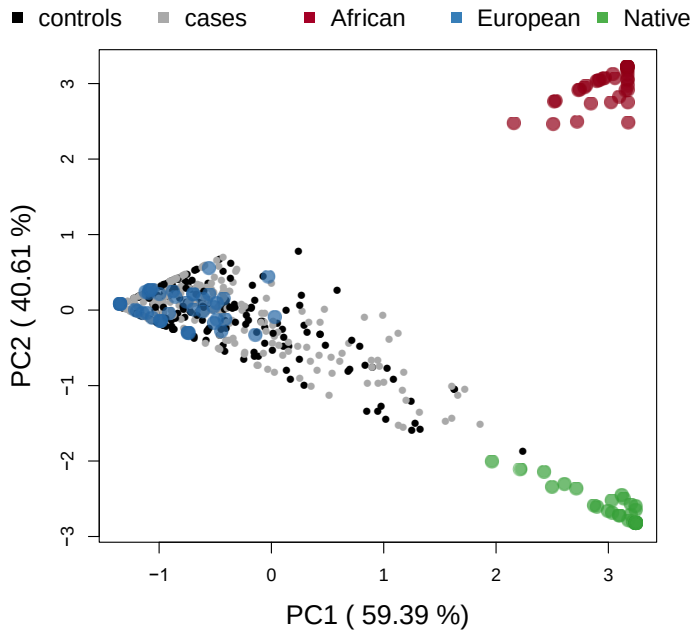

Figure 1. Ancestry of the studied population. PCA representation of results obtained with Admixture software. Red, blue and green points are African, European, and Native ancestors, respectively. Black and grey points are the studied controls and cases, respectively. Percentages in parenthesis represent the proportion of data explained by the corresponding principal component (PC).

Table 1. TREM2 variants in our case-control study

		Cases		Controls			
Mutation	**MAF**	**# alleles A**	**# alleles B**	**# alleles A**	**# alleles B**	**OR**	**95% CI**
R47H	0.001	1	433	0	326	NA	NA
R62H	0.014	7	427	4	322	1.32	0.38 - 4.55
T96K	0.005	3	431	1	325	2.28	0.24 - 21,98

MAF, minor allele frequency; **OR**, odds ratio; **95% CI**, 95% confidence interval.

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FINANCIAL REPORT

Expenditures	US dollars (US$)
Reagents for patient's DNA purification: DNA purification kit (QIAamp DNA Mini Kit x 250 det, Quiagen).	940
TaqMan SNP Genotyping Assay for *TREM2* SNPs: rs75932628, rs143332484, and rs2234253 (Life Technology) + Sequencing service	3,860
Total amount	**4,800**

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