In this study, we investigated the associations between single-nucleotide polymorphisms in GAB2 (rs2373115), GSK3B (rs6438552) and SORL1 (rs641120) and Alzheimer’s disease (AD), both alone and in combination with the APOE*4 allele.

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INTRODUCTION

Alzheimer’s disease (AD) is a multifactorial neurodegenerative disorder that is caused by the interaction of multiple genetic and environmental factors (1). In the early stages, Alzheimer’s disease is clinically characterized by short-term memory impairment, which evolves to widespread cognitive decline and dementia. There is unequivocal evidence that genetic factors contribute to the pathogenesis of Alzheimer’s disease, including the sporadic form (2). Currently, apolipoprotein E is the only well-established genetic risk factor for sporadic Alzheimer’s disease, and the APOE*4 allele has been consistently shown to be associated with an increased risk of Alzheimer’s disease (3,4). There is little doubt that other – most likely multiple – polymorphisms play an important role in the pathophysiology of Alzheimer’s disease, given that the presence of one or even two copies of APOE*4 is neither a necessary nor sufficient condition for developing the disease.

Several new single-nucleotide polymorphisms (SNPs) associated with Alzheimer’s disease have recently been identified in genome-wide association studies, namely PICALM, CLU, CRI and SORL1 (5-7). None of these SNPs can be regarded as etiological factors; rather, they serve as susceptibility modifiers, i.e., factors with independent or additive effects in the interactions among several genetic variants (mostly SNPs) at multiple genomic loci. These variants may not be deleterious per se, but they may modify disease outcomes as a result of direct and indirect interactions with other genetic and environmental factors (8,9).

Polymorphisms in the SORL1, GAB2 and GSK3B genes have been shown to be associated with Alzheimer’s disease in recent studies. Association studies have yielded conflicting data regarding the role of SORL1 rs641120 in Alzheimer’s disease (7,10,11-13). A recent study showed that there were age-dependent differences in SORL1 expression and promoter methylation in an AD cohort, with possible implications for the disease (14). Likewise, two studies suggested that there is an association between GAB2 polymorphisms and AD in Caucasians (15,16), but other studies failed to confirm this association in European (17) and Asiatic populations (18,19). Only one study to date has addressed the association between GSK3B polymorphisms and AD; the results of that study suggest that rs6438552 has a significant effect on disease risk (20). Therefore, the objective of the present study was to determine the effects of GAB2 (rs2373115), GSK3B (rs6438552) and SORL1 (rs641120) polymorphisms on the risk for AD and to investigate the interactions of these SNPs with APOE*4 in a sample of 201 older Brazilian adults.

MATERIALS AND METHODS

Subjects were recruited from two university-based memory clinics in Sao Paulo, Brazil. All participants underwent comprehensive clinical and neuropsychological evaluations. The diagnosis of probable AD (n = 130, mean age 77 ± 8.3, 66% females) was established according to the NINCDS-ADRDA criteria (21). The comparison group included healthy volunteers (n = 71, mean age 71.8 ± 6.7, 79% females) with no signs of cognitive or functional impairment. No
The OR for APOE was calculated by comparing APOE*4 carriers with non-carriers. The ORs for other genes compared the homozygous risk allele genotype with the remaining cohort (e.g., GG vs. GT + TT).

### RESULTS AND DISCUSSION

Our results are consistent with the well-established role of the APOE*4 allele as a risk factor for sporadic AD (p<0.0001) (3, 5, 6, 23-25)(7). Data regarding the genetics of AD in the Brazilian population remain scarce (26, 27), underscoring the importance of our findings. We call attention to the positive association of all the studied SNPs, namely GAB2 rs2373115, GSK3B rs6438552 and SORL1 rs641120, with AD (Table 1). The association of the GG genotype of SORL1 with AD (p = 0.047, OR = 2.07, CI95% [1.17 - 3.68]) was independent of APOE, and the binomial logistic regression analysis showed no interaction effect between APOE*4 and any of the SORL1 genotypes (Table 2). We conclude that SORL1 has an independent role in AD, irrespective of the presence of the APOE*4 allele.

We found a positive association between the GG genotype of GAB2 (rs2373115) and the diagnosis of AD (p = 0.021, OR = 1.8, CI95% [1.01-3.18]). This genotype was associated with a greater odds ratio (OR) for AD in the APOE*4 carriers (p = 0.006, OR = 5.08, CI95% [1.45-18.98]). We further used logistic regression to investigate the interaction between the APOE*4 and GAB2 polymorphisms (GG vs. non-GG genotypes, given the small proportion of individuals with the TT genotype in our sample), and we observed a robust increase in the effect as a result of the interaction between GAB2 GG and APOE*4 (p = 0.014, ORinteraction = 7.95, ORmain = 1.44) (Table 2).

With respect to the association between the GSK3B polymorphism (rs6438552) and AD diagnosis, we found that the GG genotype was approximately twice as common in the AD group (28.8%) than in the controls (13.8%) and that this genotype had a significant effect on the OR (p = 0.018, OR = 2.48, CI95% [1.19-5.20]). Interestingly, this effect was even more pronounced in the absence of APOE*4.

### Table 1 - Polymorphisms associated with Alzheimer’s disease and sample stratification based on the presence or absence of the APOE*4 allele.

| Gene    | DbSNP rs ID | Risk Allele | Freq. Cases | Freq. Controls | OR (95% CI)     | p-value |
|---------|-------------|-------------|-------------|----------------|-----------------|---------|
| APOE    | 429358 7412 | E4          | 0.29        | 0.11           | 3.33 (1.73-6.63) | 0.0001  |
| GAB2    | 2373115     | G           | 0.83        | 0.78           | 1.79 (1.01-3.18) | 0.021   |
| GSK3B   | 6438552     | G           | 0.46        | 0.44           | 2.48 (1.19-5.20) | 0.018   |
| SORL1   | 641120      | G           | 0.72        | 0.60           | 2.07 (1.17-3.68) | 0.047   |
|         |             | APOE*4      |             |                | 2.02 (0.58-7.31) | 0.260   |
|         |             | APOE*4      |             |                | 2.01 (0.94-4.34) | 0.054   |

The OR for APOE was calculated by comparing APOE*4 carriers with non-carriers. The ORs for other genes compared the homozygous risk allele genotype with the remaining cohort (e.g., GG vs. GT + TT).

### Table 2 - Logistic regression analysis of the risk genotype for LOAD in APOE*4 individuals.

| Gene    | DbSNP rs ID | Interaction | OR interaction | OR main effects | p-value |
|---------|-------------|-------------|----------------|-----------------|---------|
| GAB2    | 2373115     | APOE*4:GG   | 7.95           | 1.44            | 0.014*  |
|         |             | APOE*4:TT   |                | -               | -       |
| GSK3B   | 64384552    | APOE*4:GG   | 1.61           | 0.65            | 0.211   |
|         |             | APOE*4:AA   | 1.10           | 0.19            | 0.024*  |
| SORL1   | 641120      | APOE*4:GG   | 1.64           | 0.49            | 0.140   |
|         |             | APOE*4:AA   | 5.39           | 31.03           | 0.989   |

* p<0.05. The OR interaction values were obtained by logistic regression evaluating the interaction between APOE*4 and the given genotype. ‡ Because there were very few individuals who were homozygous for the T allele, this interaction was discarded.
APOE*4 is involved in the abnormal cleavage of the amyloid-precursor protein (APP), leading to the accumulation of the amyloid-beta peptide, which in turn favors the hyperphosphorylation of Tau. These pathological changes ultimately disrupt axonal transport and neuronal viability (29, 30). GAB2 and GSK3B (rs6438552, AA genotype) have been shown to increase Tau phosphorylation (15, 28). The studied GSK3B and GAB2 polymorphisms are located in intrinsic regions of these genes and may thus have subtle effects on transcription, with biological consequences that are yet to be defined. It is also possible that these SNPs are in linkage disequilibrium with other polymorphisms that may contribute to the observed effects. GAB2 is a scaffolding protein with important roles in several growth and differentiation signaling pathways, including the phosphorylation of kinases that participate in core neurobiological pathways related to AD (15,16,31,32). GAB2 and presenilin 1 both activate PKB, leading to the activation of PKB and the further inactivation of GSK3B (33). Because the inactivation of GSK3B prevents Tau hyperphosphorylation in neurons (34), it is reasonable to assume that any decrease in GAB2 expression and/or function would increase Tau phosphorylation (15). Supporting this hypothesis, in vitro studies have shown that the inhibition of GAB2 expression using siRNA increases Tau phosphorylation (15).

We conclude that interactions between the GAB2 and GSK3B polymorphisms and the well-established genetic factor APOE may modify the overall risk of AD. These effects are by no means linear or cumulative, given that the protective effect of a one studied polymorphism (e.g., the AA genotype of GSK3B) may increase the odds ratio for AD in the presence of APOE*4. Our results support the hypothesis that there is no single genetic cause for late-onset AD; instead, the development of AD depends on the interaction of several genes, environmental factors and age. Further evaluation of the interactions between distinct genes and of the respective implications on neuronal homeostasis may provide insight into the complex neurobiology of AD.

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author contributions
All authors contributed to the present work and consent to the publication of the findings. Gattaz WF and Ojopi EB were responsible for the initial concept. The patients were recruited by Bertolucci HFF, Forlenza OV and Gattaz WF. The experimental analyses were performed by Izzo G and Kerr DS. The statistical analyses were performed by Santos B and Kerr DS. Izzo G wrote the first draft of the manuscript. The literature review was performed by Izzo G and Kerr DS. The manuscript was prepared and formatted and the tables were prepared by Kerr DS and Forlenza OV. All authors have reviewed and approved the final manuscript.

tables

[Table 1: Gene interaction in Alzheimer's disease]

| Gene Interaction | Odds Ratio (95% CI) |
|------------------|---------------------|
| APOE*4 | 4.45 (1.47-16.39) |
| GSK3B | 0.19 (0.024-0.84) |

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