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RAPID COMMUNICATION

Single-nucleotide polymorphisms of *GSK3B, GAB2* and *SORL1* in late-onset Alzheimer's disease: interactions with the *APOE* genotype

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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 on Alzheimer's disease have recently been identified in genome-wide association studies, namely *PICALM*, *CLU*, *CR1* 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

No potential conflict of interest was reported.

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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
		*APOE*4+			5.08 (1.45-18.98)	0.006
		*APOE*4-			1.10 (0.51-2.35)	0.859
GSK3B	6438552	G	0.46	0.44	2.48 (1.19-5.20)	0.018
		*APOE*4+			0.76 (0.22-2.88)	0.768
		*APOE*4-			4.45 (1.47-16.39)	0.003
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).

relatives of AD patients were included in the control group. No statistically significant differences were observed with respect to the age distribution or self-reported ethnic background between the patients and controls, but there was a greater percentage of females in the control group. However, we believe that this gender difference should not negatively affect the findings, as similar results were obtained in a preliminary analysis of gender-matched samples.

The *GSK3B*, *GAB2* and *SORL1* SNPs were analyzed using a Real-Time PCR SNP genotyping system (*TaqMan*® Assays – Applied Biosystems, CA, USA) *TaqMan* PCR Master Mix 1x, *TaqMan* SNP genotyping assay 1x, genomic DNA 10 ng/μL and ultrapure water to a volume of 5 μL were mixed in each well of an optical plate. Allelic discrimination was performed using a 7500 Real-Time PCR system (Applied Biosystems, CA, USA) by comparing the fluorescence levels before and after amplification (45 cycles of 15 seconds at 95 ºC and 1 min at 60 ºC). Two SNPs (rs7412 and rs429358) were evaluated to determine the *APOE* genotype, as previously described (22). The real-time PCR reactions were run using the protocol presented above.

Pearson's Chi-squared test with simulated *p*-values was used to compare the genotype distributions between cases and controls. The interactions between the *GSK3B*, *GAB2* and *SORL1* SNPs and *APOE*4* were tested in two ways: first, each group was stratified into *APOE*4*-positive and *APOE*4*-negative subgroups, and the association between each SNP and the diagnosis of AD was assessed separately in each group. In the second step, a binomial logistic regression model was used to compare the interactions between *APOE*4* and each of the three SNPs in the entire sample. The statistical analysis was conducted using R software version 2.12.2.

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, $CI_{95\%}$ [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, $CI_{95\%}$ [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* ($p=0.014$, $OR_{interaction}=7.95$, $OR_{main}=1.44$) (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, $CI_{95\%}$ [1.19-5.20]). Interestingly, this effect was even more pronounced in the absence of *APOE*4*

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.

($p = 0.003$, OR = 4.45, $CI_{95\%}$ [1.47-16.39]). In contrast, the A allele was associated with a protective effect, irrespective of the *APOE* status ($p = 0.018$, OR = 0.40, $CI_{95\%}$ [0.19-0.84]); however, the logistic regression analysis showed that *APOE*4*-positive carriers of the AA genotype displayed an increased OR for AD ($p = 0.024$, $OR_{interaction} = 1.10$, $OR_{main} = 0.19$) (Table 2). This finding is noteworthy because it indicates that the A allele of the *GSK3B* gene may represent either a protective factor or a risk factor for AD, depending on the *APOE* genotype. We speculate that this dual role may occur because the rs6438552 polymorphism is intronic and may affect the transcription and splicing of *GSK3B*. In fact, splice variants of *GSK3B* arising from the AA genotype have been shown to favor Tau protein hyperphosphorylation, which is one of the pathological hallmarks of AD (28).

*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 intronic 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 PI3K, 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 PHF, 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 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.

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