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ORIGINAL PAPER

rs3851179 Polymorphism at 5′ to the *PICALM* Gene is Associated with Alzheimer and Parkinson Diseases in Brazilian Population

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Abstract Alzheimer's (AD) and Parkinson's diseases (PD) share clinical and pathological features, suggesting that they could have common pathogenic mechanisms, as well as overlapping genetic modifiers. Here, we performed a case–control study in a Brazilian population to clarify whether the risk of AD and PD might be influenced by shared polymorphisms at *PICALM* (rs3851179), *CR1* (rs6656401) and *CLU* (rs11136000) genes, which were previously identified as AD risk factors by genome-wide association studies. For this purpose, 174 late-onset AD patients, 166 PD patients and 176 matched controls were genotyped using TaqMan® assays. The results revealed that there were significant differences in genotype and allele frequencies for the SNP *PICALM* rs3851179 between AD/PD cases and controls, but none for *CR1* rs6656401 and *CLU* rs11136000 intronic polymorphisms. After stratification by *APOE* ε4 status, the protective effect of the *PICALM* rs3851179 A allele in AD cases remains evident only in *APOE* ε4 (−) carriers, suggesting that the *APOE* ε4 risky allele weakens its protective effect in the *APOE* ε4 (+) subgroup. More genetic studies using large-sized and well-defined matched samples of AD and PD patients from mixed populations as well as functional correlation analysis are urgently needed to clarify the role of rs3851179 in the AD/PD risk. An understanding of the contribution of rs3851179 to the development of AD and PD could provide new targets for the development of novel therapies.

Keywords Alzheimer's disease · Parkinson's disease · *PICALM* · *CLU* · *CR1* · Single nucleotide polymorphisms

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Introduction

The ongoing increase in life expectancy has led to an aging population and a subsequent increase in age-related conditions. Alzheimer's disease (AD) is the leading cause of dementia in the elderly, characterized by global cognitive decline, accumulation of amyloid beta protein (Aβ) deposits and neurofibrillary tangles of phosphorylated tau protein in the brain. Even so, cumulative evidence supports that increased Aβ production and/or decreased Aβ clearance is an upstream event of neurodegeneration, which is directly affected by AD risk gene products (for review, see Scheltens et al. 2016). So far, rare and highly penetrant mutations in three Aβ-related genes were associated with familial AD: Aβ precursor protein (*APP*), presenilin 1

(*PSEN1*) and presenilin 2 (*PSEN2*), whereas for the more common late-onset AD (LOAD) form, the primary genetic variant that influences disease risk in different geographical populations is the ε4 allele of the apolipoprotein E gene (*APOE*). Nevertheless, genome-wide association studies (GWAS) and next-generation sequencing (NGS) technologies have been gradually unraveling that the genetic architecture involving the LOAD form is much more complex (Cuyvers and Sleegers 2016). In 2009, two GWAS conducted in large cohorts of AD patients identified risk variants in three novel candidate genes, beyond the well-established *APOE* association: phosphatidylinositol-binding clathrin assembly (*PICALM*; rs3851179), complement component (3b/4b) receptor 1 (*CR1*; rs6656401) and clusterin (*CLU*; rs11136000) (Harold et al. 2009; Lambert et al. 2009). *PICALM* gene is involved in the endocytic internalization of cell surface APP for β- and γ-secretase cleavages to generate Aβ, while *CLU*, *CR1* and also *PICALM* participate in Aβ clearance through multiple mechanisms (Rosenberg et al. 2016). During the last few years, even though independent replicative studies concerning polymorphisms in such genes are in progress, great discrepancy exist among them and their potential use as prodromal AD biomarkers remains elusive. Furthermore, the frequency of these AD-based GWAS findings in ethnically mixed populations and the relationship between the risk variants and other neurodegenerative disorders, such as Parkinson's disease (PD), are poorly studied.

PD is the second most common neurodegenerative disorder after AD, causing inexorably progressive parkinsonian motor and nonmotor dysfunctions, being characterized by intracellular protein inclusions (Lewy bodies) and neuronal loss in the substantia nigra and other brain regions (for review, see Kalia and Lang 2016). In the last decade, highly penetrant mutations producing rare, monogenic forms of the disease have been discovered in singular genes such as *SNCA*, *Parkin*, *DJ*-1, *PINK1*, *LRRK2* and *VPS35* (Hernandez et al. 2016), but susceptibility *loci* that modify the risk of developing sporadic PD still persist to be identified. PD and AD share many clinical and pathological features, suggesting that these neurodegenerative disorders could have, at least in part, overlapping risk factors and common pathogenic mechanisms. Both conditions are progressive with abnormal protein deposition (Ross and Poirier 2004), and more than half of patients with PD develop dementia (Caballol et al. 2007). Besides, parkinsonism signs are commonly found in patients with AD (Chung et al. 2013; Macleod et al. 2013), and some studies suggest that individuals with familial PD exhibit a higher risk of developing AD (Rosen et al. 2007; Costello et al. 2010).

Herein, we performed a case–control study in a Brazilian population to clarify whether the risk of late-onset AD and PD might be influenced by shared polymorphisms at *PICALM*, *CLU* and *CR1 loci*.

Methods

Subjects

Our cohort consisted of 174 late-onset (onset age over 65 years) AD patients (77.15 ± 6.36, females = 68.93%), 166 late-onset (onset age over 50 years) PD patients (69.0 ± 8.4 years, males = 62.21%) and 176 controls (70.7 ± 6.04 years, females = 73.44%). Unrelated AD and PD patients were recruited by specialized clinicians from major public care centers for elderly, whereas healthy elderly controls were recruited from either spouses/husbands of patients or from the University of Third Age at the State University of Rio de Janeiro. Controls had a negative family history of neurological diseases in first-degree relatives and were all unrelated. Probable AD cases were ascertained on the basis of the criteria of the National Institute of Neurological and Communicative Disorders, and Stroke-Alzheimer's Disease and Related Disorders (NINCDS/ADRDA) (McKhann et al. 1984), while PD patients fulfilled the diagnosis criteria established by Hughes et al. (2001). Patients were classified into Caucasian (European origin) and non-Caucasian (Africans and mulattos) subgroups by self-declaration. The research protocol was approved by the institutional ethics committee in accordance with the Declaration of Helsinki, and a written informed consent was obtained from all subjects.

SNPs Genotyping

Genomic DNA was isolated from peripheral blood using standard procedures, and rs3851179 (*PICALM*), rs3818361 (*CR1*) and rs11136000 (*CLU*) polymorphisms were genotyped using TaqMan® genotyping assays (Thermo Fisher Inc., San Diego, CA, USA) as recommended by the manufacturer. The assays were run in a 7500 Fast Real-Time PCR system (Thermo Fisher Inc.) and analyzed with SDS software version 2.0.6 (Thermo Fisher Inc.). *APOE* genotyping was performed by the restriction fragment length polymorphism (RFLP) or Sanger sequencing approaches, according to a modified method (Hixson and Vernier 1990).

Statistical Analysis

Hardy–Weinberg equilibrium was assessed by the Chi-square test. Frequencies were calculated for each genotype/allele, and differences between case and control individuals were determined using the Chi-square test. Odds ratios

were calculated by using Fisher's exact test for each genotype/allele separately and in the AD patients for potential interaction with *APOE*-ε4 allele using the genotype/allele with major frequency as reference. Statistical comparisons were made using the Instat Graph Pad Software (version 3.0, San Diego, USA), and p values <0.05 were adjusted through Bonferroni correction.

Results

The distribution of *PICALM*, *CR1* and *CLU* genotypes in both AD/PD case and control groups was found to be in Hardy–Weinberg equilibrium ($p > 0.05$). There were no significant differences between groups in terms of ethnicity and socioeconomic status. In our AD patient's subgroup, 65% were Caucasians and 35% non-Caucasians, whereas in the PD patients, 70% were Caucasians and 30% non-Caucasians. Table 1 indicates genotype and allele frequencies comparisons. Genotype and allele frequencies for *CR1* rs3818361 and *CLU* rs11136000 polymorphisms were similar among AD, PD and control groups, even in the combination of heterozygous and homozygous variant genotypes. Conversely, for *PICALM* rs3851179, there were significant differences in both genotype (AA + GA vs. GG, $p = 0.04$, OR = 0.64) and allele (A vs. G, $p = 0.01$, OR = 0.66) frequencies between AD cases and controls, as well as from PD cases and controls (AA vs. GG, $p = 0.01$, OR = 0.34; A vs. G, $p = 0.02$, OR = 0.67) (Table 1). Furthermore, after stratification of our AD dataset according to the *APOE* ε4 status, significant association for *PICALM* rs3851179 A allele was found only in the *APOE* ε4 (−), but not in the *APOE* ε4 (+) subgroup (Table 2). *APOE* ε2 allele did not show a more significant protective effect in the interaction with *PICALM* rs3851179 GA + AA genotypes (Supplementary Table 1).

Discussion

In the last few years, contradictory findings from different geographical populations have been published on the evaluation of *PICALM*, *CLU* and *CR1* polymorphisms as modulating factors for developing AD (Harold et al. 2009; Lambert et al. 2009; Yu et al. 2011; Wijsman et al. 2011; Ferrari et al. 2012; Miyashita et al. 2013). Nevertheless, the conclusions provided might not be so far generalized to ethnically mixed populations (particularly composed by Africans descendants) due to the lack of studies in such cohorts. Brazil is a continental-sized country that forms one of the most heterogeneous populations in the world. This miscegenation profile results from five centuries of interethnic crosses of peoples from three continents: the European colonizers, mainly represented by the Portuguese, the African slaves and the autochthonous Amerindians (Parra et al. 2003). Therefore, data stratification by ethnicity was not performed cautiously, since the ethnic origin was self-reported and it could lead to misinterpretation due to the high level of miscegenation, mainly between Europeans and Africans (Brazilian mulattos). Besides, many individuals ignore/omit interethnic crosses in previous generations, considering only their skin color as a wrong prerogative of their ancestral origin. It may have led to an underestimation of non-Caucasians rate.

In our Brazilian cohort, intronic SNPs at *CR1* (rs3818361) and *CLU* (rs11136000) *loci* were not associated neither with AD nor with PD risk. Conversely, carriers of the *PICALM* rs3851179 A allele had approximately 34% lower odds of having AD compared to carriers of rs3851179 G allele (OR = 0.66; $p = 0.01$), but the presence of the risk factor *APOE* ε4 ($p = 0.24$) neutralized the protective effect of the rs3851179 A allele. For PD, the rs3851179 A allele also represented a protective factor with odds similar to AD (OR = 0.67; $p = 0.02$) in our population. The minor allele frequency (MAF) of rs3851179 A allele in our control group (32.7%) is in agreement with the global MAF reported in 1000 Genomes Project Phase 3 (31%), which is lower in Africans (11%) and higher in Americans (39%) and Europeans (37%) (1000 Genomes Project Phase 3).

The association between *PICALM* rs3851179 and AD has been suggested in Caucasian and Asian populations, although some studies reported inconsistent results in both populations (Supplementary Table 2). Until now, only one study focused on the role of *PICALM* rs3851179 on AD risk in Brazil (Belcavello et al. 2015). The authors found a preponderance of the variant rs3851179 A in both case (66%) and control (73%) groups, and considering the A allele as reference, they suggested that rs3851179 G allele acts as a risk factor for AD, which is the opposite of the effect observed in the original publication of Harold and colleagues (2009) and other subsequent replication studies (Supplementary Table 2). The study, however, was conducted in a different geographical area from ours (Espirito Santo), and the samples were genotyped by PCR–restriction fragment length polymorphism (PCR–RFLP).

PICALM gene (11q14.2) encodes the ubiquitously expressed clathrin adaptor protein involved in clathrin-mediated endocytosis (CME), which is an essential step in the intracellular trafficking of proteins, lipids, growth factors and neurotransmitters. PICALM protein is indispensable for neurotransmitter release at the presynaptic membrane, being important for memory formation and neuronal function (Harel et al. 2008). Reduced expression of *PICALM* in AD and murine brain endothelium correlates with Aβ pathology and cognitive impairment. Moreover,

Table 1 Genotype and allele frequencies, odds ratios and p value of *PICALM* (rs3851179 G > A), *CR1* (rs6656401 G > A) and *CLU* (rs11136000 T > C) polymorphisms in patients with AD or PD and control individuals

Polymorphism	Genotypes	AD[a] ($n = 174$) n [%]	PD[a] ($n = 166$) n [%]	Controls[a] ($n = 176$) n [%]	AD X controls		PD X controls	
					Odds ratio (CI 95%)	p value	Odds ratio (CI 95%)	p value
PICALM (rs3851179)	GG	99 [58.2]	88 [55.7]	83 [47.2]	1.0	Reference	1.0	Reference
	GA	59 [34.7]	62 [39.2]	71 [40.3]	0.69 (0.44–1.09)	0.13	0.82 (0.52–1.29)	0.42
	AA	12 [7.1]	8 [5.1]	22 [12.5]	0.45 (0.21–0.97)	0.06	0.34 (0.14–0.81)	0.01[$]
	GA + AA	71 [41.8]	70 [44.3]	93 [65.3]	0.64 (0.41–0.97)	0.04	0.71 (0.46–1.09)	0.12
	Frequency of G	257 [75.6]	238 [75.3]	237 [67.3]	1.0	Reference	1.0	Reference
	Frequency of A	83 [24.4]	78 [24.7]	115 [32.7]	0.66 (0.47–0.92)	0.01[$]	0.67 (0.48–0.94)	0.02[$]
CR1 (rs6656401)	GG	124 [71.7]	122 [76.3]	124 [71.3]	1.0	Reference	1.0	Reference
	GA	42 [24.3]	32 [20.0]	46 [23.4]	0.91 (0.56–1.48)	0.80	0.7 (0.42–1.18)	0.19
	AA	7 [4.0]	6 [3.7]	4 [2.3]	1.75 (0.49–6.13)	0.54	1.52 (0.41–5.53)	0.74
	GA + AA	49 [28.3]	38 [23.7]	50 [25.7]	0.98 (0.61–1.56)	1.0	0.77 (0.47–1.26)	0.32
	Frequency of G	290 [83.8]	276 [86.25]	294 [84.5]	1.0	Reference	1.0	Reference
	Frequency of A	56 [16.2]	44 [13.75]	54 [15.5]	1.05 (0.69–1.58)	0.83	0.86 (0.56–1.33)	0.58
CLU (rs11136000)	CC	53 [30.5]	52 [31.3]	50 [29.6]	1.0	Reference	1.0	Reference
	CT	94 [54.0]	82 [49.4]	95 [54.3]	0.93 (0.57–1.51)	0.81	0.83 (0.51–1.35)	0.45
	TT	27 [15.5]	32 [19.3]	30 [17.1]	0.84 (0.44–1.62)	0.74	1.02 (0.54–1.92)	1.0
	CT + TT	121 [69.5]	114 [68.7]	125 [71.4]	0.91 (0.57–1.44)	0.72	0.87 (0.55–1.39)	0.63
	Frequency of C	200 [57.5]	186 [56.02]	195 [55.7]	1.0	Reference	1.0	Reference
	Frequency of T	148 [42.5]	146 [43.98]	155 [44.3]	0.93 (0.69–1.25)	0.64	0.98 (0.72–1.33)	0.93

[a] The number of genotyped individuals in case and control samples slightly differs among the selected polymorphisms due to some amplification fails

[$] Significance maintained after Bonferroni correction

deficiency in PICALM diminished Aβ clearance and accelerated Aβ pathology, which has implications for Aβ brain homeostasis and clearance therapy (Zhao et al. 2015). Recently, PICALM levels were also correlated with levels of phosphotau and autophagy-related proteins, being associated with tau inclusions in AD (Ando et al. 2016). SNP rs3851179 is located approximately 90 kb upstream from *PICALM*, and the protective variant A was associated with modestly increased *PICALM* expression in the microvasculature, which was likely to facilitate the clearance of Aβ and may reduce AD risk (Parikh et al. 2014). Also, *PICALM* rs3851179 A allele was associated with a better cognitive performance in individuals with 92–93 years at intake, called "oldest old" (Mengel-From et al. 2011), and rs3851179 GA or AA genotype displayed a significant effect protecting against AD rapid progression during pharmacogenetic assays compared to GG carriers (Ruiz et al. 2013).

After adjustment of our AD dataset according to the *APOE* ε4 status, significant association for *PICALM* rs3851179 A allele remained only in the *APOE* ε4 (−) subgroup. These results were also found in Chinese AD patients by Chen et al. (2012) and could suggest that the powerful *APOE* ε4 risky allele weakens the protective effect of polymorphism rs3851179 in the *APOE* ε4 (+) subgroup. Like *PICALM*, *APOE* is known to be involved in the regulation of Aβ clearance from the brain in an isoform-dependent manner (Kim et al. 2014). In addition, a neural mechanism for *APOE–PICALM* interactions in patients with AD was recently proposed, indicating that the *PICALM*

Table 2 Analysis of multiplicative interaction between *PICALM* (rs3851179 G > A) and *APOE* ε4 status in patients with AD and control individuals, odd ratios and p values

APOE status	*PICALM* rs3851179 G > A	AD[a]	Controls[a]	Odds ratio (CI 95%)	*P* value
APOE ε4 −	GG	39	41	1.0	Reference
	GA + AA	30	56	0.56 (0.30–1.05)	0.08
	G	102	125	1.0	Reference
	A	32	69	0.57 (0.35–0.93)	0.02[$]
APOE ε4 +	GG	60	16	1.0	Reference
	GA + AA	37	16	0.62 (0.27–1.38)	0.30
	G	151	45	1.0	Reference
	A	43	19	0.67 (0.36–1.27)	0.24
All sample	GG	99	57	1.0	Reference
	GA + AA	67	72	0.54 (0.34–0.85)	0.009[$]
	G	253	170	1.0	Reference
	A	75	88	0.57 (0.40–0.82)	0.003[$]

[a] The number of genotyped individuals in case and control samples slightly differs among the studied polymorphisms and from Table 1 due to some *APOE* amplification fails

[$] Significance maintained after Bonferroni correction

genotype modulates both brain atrophy and cognitive performance in *APOE* ε4 carriers (Morgen et al. 2014).

Concerning PD, to our knowledge, this is the first attempt to analyze the role of *CLU*, *CR1* and *PICALM* SNPs on disease risk in an ethnically mixed population. So far, *CLU*, *CR1* and *PICALM* GWAS AD-based findings were only explored in five studies involving patients with PD from USA (Gao et al. 2011; Barrett et al. 2016), Greece (Kalinderi et al. 2012), Korea (Chung et al. 2013) and China (Wang et al. 2016) (Supplementary Table 3). In the study of Gao et al. (2011), *CLU* rs11136000 polymorphism was the unique associated with PD risk under the recessive model (TT vs. CC + CT; OR = 0.71, 95% CI 0.55–0.92), whereas in the populations of Greece, Korea and China, none of the selected AD-susceptibility *loci* showed statistically significant association with PD susceptibility (Kalinderi et al. 2012; Chung et al. 2013; Wang et al. 2016). Finally, Barrett et al. (2016) recently associated *PICALM* rs3851179 with cognitive impairment in PD subjects >70 years old, but not in PD subjects ≤70 years old, suggesting that *PICALM* rs3851179 could contribute to cognitive impairment in older patients with PD. However, these findings were not corroborated by Wang et al. (2016) that also investigated the AD GWAS top findings in cognitive function among PD patients, showing no evidence of an obvious linkage.

The potential role of *PICALM* in PD pathogenesis was poorly addressed. However, some functional data could link *PICALM* to PD. A clathrin-dependent endocytic mechanism is essential for the maintenance of synaptic transmission, since synaptic vesicles (SV) need to be recycled after releasing a neurotransmitter, including dopamine, glutamate, acetylcholine and g-aminobutyric (Kalinderi et al. 2012). PD predominantly affects the dopamine-producing neurons residing at the substantia nigra, and α-synuclein was found to play a role in regulating dopamine homeostasis through its involvement in clathrin-mediated endocytosis (Kisos et al. 2014). PICALM is likewise involved in directing the trafficking of VAMP2, an SV protein, which has a prominent role in the fusion of SVs to the presynaptic membrane in neurotransmitter release, a process that is crucial to neuronal function in general and could also explain the involvement of *PICALM* rs3851179 in neurodegenerative disorders other than AD (Harel et al. 2008; Harold et al. 2009). Besides, Cheng et al. (2011) demonstrated that α-synuclein promotes clathrin-mediated N-methyl-D-aspartate (NMDA) receptor endocytosis and attenuates NMDA-induced dopaminergic cell death. Finally, Oh et al. (2016) recently found that mesenchymal stem cells inhibited α-synuclein transmission by blocking the clathrin-mediated endocytosis of extracellular α-synuclein via modulation of the interaction with NMDA receptors, which led to a pro-survival effect on cortical and dopaminergic neurons with functional improvement of motor deficits in α-synuclein-enriched models. An additional explanation for the association of *PICALM* rs3851179 with PD could be mediated by the effect of this polymorphism over cognitive impairment or dementia. Nonetheless, data concerning cognitive function/dementia were not part of all PD clinical records, making impossible to address this issue in our study. For the same reason, it was not possible to interrogate the influence of *PICALM* rs3851179 into comorbidities and other conditions among AD/PD patients.

In conclusion, there are still many divergences about the relationship between *PICALM* rs3851179 and AD/PD in different populations. Taken together, the discordant data from distinct geographical areas (in the same or different

countries) may reflect genetic heterogeneity and also disparities related to screening methodologies and selection criteria (including age at onset, familial or sporadic profiles, diagnostic criteria, sample size and differences between case and control samples). By this way, more genetic studies using large-sized and well-defined matched samples of AD and PD patients as well as functional correlation analysis are urgently needed to clarify the role of *PICALM* rs3851179 in the AD/PD risk. Moreover, further studies with large-scale longitudinal follow-up of PD patients are needed to address the relationship between *PICALM* rs3851179 and cognitive function/dementia in PD. An understanding of the contribution of rs3851179 to the development of both neurodegenerative disorders could provide new targets for novel therapies.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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