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Aspectos nutrigenéticos da memória: influência do micronutriente selênio e de polimorfismos em selenoproteínas

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"*Dizem que antes de um rio entrar no mar, ele treme de medo. Olha para trás, para toda a jornada que percorreu, para os cumes, as montanhas, para o longo caminho sinuoso que trilhou através de florestas e povoados, e vê à sua frente um oceano tão vasto, que entrar nele nada mais é do que desaparecer para sempre. Mas não há outra maneira. O rio não pode voltar. Ninguém pode voltar. Voltar é impossível na existência.*

O rio precisa se arriscar e entrar no oceano. E somente quando ele entrar no oceano é que o medo desaparece, porque apenas então o rio saberá que não se trata de desaparecer no oceano, mas de tornar-se oceano."

Osho

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LISTA DE ABREVIATURAS E SIGLAS

CCL - Comprometimento cognitivo leve

DA- Doença de Alzheimer

SNPs- Polimorfismos de nucleotídeo único

GPX- Glutationa peroxidase

QI - Quoeficiente de Inteligência

H_2O_2- Peróxidos de hidrogênio

ERO – Espécies Reativas de Oxigênio

PRO- Prolina

LEU- Leucina

RESUMO

A memória episódica parece ser a mais sensível ao processo do envelhecimento e pode ser classificada de acordo com dois estilos distintos: visual e verbal. O Comprometimento cognitivo leve (CCL) representa o estado entre as alterações cognitivas normais decorrentes da idade e o estágio inicial de demência, e está associado com aumento de risco para doença de Alzheimer (DA). A DA é multifatorial, mas o envelhecimento é apontado como principal fator de risco devido ao aumento do estresse oxidativo. O selênio é um micronutriente antioxidante com potencial interesse para a função cerebral e é essencial para a síntese de selenoproteínas, como a glutationa peroxidase (GPX) 1 e 4. Polimorfismos de nucleotídeo único (SNPs) em genes que codificam selenoproteínas podem alterar as necessidades individuais para selênio. Os objetivos desse estudo consistiram em investigar a influência genética e a influência da concentração sérica de selênio sobre a memória episódica, comprometimento cognitivo leve e doença de Alzheimer através da avaliação de polimorfismos em *GPX1* (rs1050450) e *GPX4* (rs713041). As análises moleculares foram feitas através da reação em cadeia da polimerase em tempo real utilizando sondas de hidrólise e as concentrações séricas de selênio foram comparadas entre os genótipos.

Para a associação dos polimorfismos genéticos com memória ou déficits de memória, os fenótipos foram analisados da seguinte forma: 1) cada memória como um traço quantitativo; 2) presença de déficit em uma memória específica; 3) presença de CCL; 4) presença de DA. Assim, o estudo foi composto por um delineamento transversal (278 sujeitos) e um estudo caso-controle (108 controles saudáveis e 103 com DA). Os homozigotos TT (rs1050450 - *GPX1*) apresentaram menores escores de memória visual de longo prazo do que o grupo CC / CT (-0,28 ± 1,03 vs. 0,13 ± 1,03, respectivamente, p=0,017). No entanto, em uma regressão logística multivariada, os homozigotos CC para o mesmo polimorfismo apresentaram 2,85 maiores chances de desenvolver DA (OR=2,85, CI95%=1,04-7,78, p=0,041) em comparação com o genótipo de referência. Para o rs713041 (*GPX4*), a frequência do genótipo TT foi maior no grupo com escores normais do que no grupo com déficits para memória visual de longo prazo (p=0,025). Não foram observadas diferenças significativas quanto ao grupo CCL entre variantes genéticas.

No delineamento transversal os níveis séricos de selênio foram avaliados em 156 idosos saudáveis. Foram encontradas correlações significativas entre o nível sérico

de selênio e as memórias verbais e um usando um modelo de regressão linear múltipla verificou-se que os níveis de selênio são um preditor para memória de aprendizagem verbal (coeficiente de regressão=0,541, erro padrão=0,244, p=0,028), representando 3,28% de variabilidade de memória de aprendizagem verbal. Além disso, observamos uma correlação significativa entre escolaridade e concentração de selênio (r=0,211, p=0,009). Não foram observadas associações significativas entre os genótipos *GPX1* e *GPX4* e a concentração sérica de selênio. Esses resultados indicam que o selênio está associado ao desempenho da memória, especialmente as verbais e que os polimorfismos exercem efeitos singulares sobre a memória episódica, DA, mas não há associação com CCL.

Palavras chave: Memória, comprometimento cognitivo leve, doença de Alzheimer, selênio, gene *GPX1*, gene *GPX4*.

ABSTRACT

Episodic memory seems to be the most sensitive to the aging process and can be classified according to two distinct styles: visual and verbal. Mild Cognitive Impairment (MCI) represents the state between normal cognitive changes due to age and the early stage of dementia, and is associated with an increased risk for Alzheimer's disease (AD). AD is multifactorial, but aging is the main risk factor due to increased oxidative stress. Selenium is an antioxidant micronutrient with potential interest for brain function and is essential for the synthesis of selenoproteins, such as glutathione peroxidase (GPX) 1 and 4. Single nucleotide polymorphisms (SNPs) in genes encoding selenoproteins may alter individual needs for selenium. The objectives of this study were to investigate the genetic influence and the influence of serum selenium concentration on episodic memory, mild cognitive impairment and Alzheimer's disease through the evaluation of polymorphisms in *GPX1* (rs1050450) and *GPX4* (713041). Molecular analyzes were done through real-time polymerase chain reaction using hydrolysis probes and serum selenium concentrations were compared between the genotypes. For the association of the genetic polymorphisms with memory or cognitive loss, the phenotypes were analyzed as follows: 1) each memory as a quantitative trait; 2) presence of deficit on a specific memory; 3) presence of MCI; 4) presence of AD. Thus, the study consisted of a cross-sectional design (278 subjects) and a case-control study (108 healthy controls and 103 with AD). TT homozygotes (rs1050450 - *GPX1*) had lower long-term visual memory scores than CC/CT group (-0.28±1.03 vs. 0.13±1.03, respectively, p=0.017). However, in a multivariate logistic regression, *GPX1* CC homozygotes had a 2.85 higher chance of developing AD (OR=2.85, CI95%=1.04–7.78, p=0.041) in comparison to the reference genotype. For the rs713041 (*GPX4*), the frequency of the TT genotype was higher in the group with normal scores than in the group with long-term visual memory deficits (p = 0.025). No significant differences were observed regarding the MCI group. In the cross-sectional design serum selenium levels were evaluated in 156 healthy elderly subjects. Significant correlations were found between serum selenium level and verbal memories, and a multiple linear regression model indicated that selenium levels are a predictor of verbal learning memory (regression coefficient=0.541, standard error=0.244, p=0.028), representing 3.28% of verbal learning memory variability. In addition, we observed a significant correlation between schooling and selenium concentration (r=0.211, p=0.009). No

significant associations between GPX1 and GPX4 genotypes and serum selenium concentration were observed.

These results indicate that selenium is associated with memory performance, especially verbal ones, and that polymorphisms exert unique effects on episodic memory, AD, but there is no association with MCI.

Keywords: Memory, mild cognitive impairment, Alzheimer's disease, selenium, *GPX1* gene, *GPX4* gene.

1 INTRODUÇÃO

A memória humana pode ser definida de maneira geral e concisa como a capacidade neurocognitiva para codificar, armazenar e recuperar informações. O declínio de memória figura como uma das queixas mais frequentes na população idosa, e o reconhecimento dos mecanismos envolvidos no curso do déficit de memória ao longo do tempo ainda é um desafio para a ciência. Entretanto, por se tratar de uma característica multifatorial, fatores genéticos, Quoeficiente de Inteligência (QI), *status* socioeconômico, nível educacional e fatores nutricionais estão envolvidos nos processos metabólicos relacionados com o comprometimento cognitivo e surgimento de doença demencial (RÖNNLUND et al. 2005; DEL PARIGI et al. 2006; ZAMROZIEWICZ, et al 2017, ARPAWONG et al. 2017).

O declínio de memória no idoso é uma condição preocupante, pois pode afetar negativamente as atividades diárias e a independência desse indivíduo. No entanto, nem todo declínio de memória é caracterizado como doença demencial. Diante disso, foi introduzido na década de 1980 por Reisberg et al. (1988) e definido por Petersen et al. (1999), o conceito de "comprometimento cognitivo leve" (CCL) que é um termo amplamente utilizado para indicar comprometimento cognitivo que não pode ser explicado por nenhuma condição médica ou psiquiátrica reconhecida (PETERSEN, 1999). Assim, este conceito refere-se ao declínio cognitivo em idoso mais acentuado que no envelhecimento normal, porém não configura uma demência detectável, representando então um estado intermediário entre o envelhecimento normal e demência. As estimativas da prevalência de CCL variam de 3% para indivíduos a partir dos 60 anos chegando a 15% para indivíduos a partir dos 75 anos (FRISONI et al. 2000, RITCHIE; ARTERO; TOUCHON, 2000; ESHKOOR et al. 2017).

Em relação à Doença de Alzheimer (DA), estima-se que o número de pessoas acometidas por essa patologia atingirá 65,7 milhões em 2030 e quase duplicará em 2050 (FERRI et al. 2005; RIZZI; ROSSET; RORIZ-CRUZ, 2014). Em contraste com o CCL, um diagnóstico clínico para a DA pode ser formulado a partir de características como demência lentamente progressiva com lesões neuro-histológicas, incluindo placas senis e emaranhados neurofibrilares. Esta doença neurodegenerativa geralmente leva à

completa dependência psicológica e física e a morte dentro de uma a duas décadas (AMERICAN PSYCHIATRIC ASSOCIATION, 1994).

Vários estudos apontam que a prevalência geral de DA varia muito entre os países, sendo influenciada por fatores culturais, socioeconômicos e de estilo de vida. A ingestão de alimentos antioxidantes tem sido apontada como um fator protetivo (SUH; SHAH, 2001; PARLETTA; MILTE; MEYER, 2013).

O selênio é um elemento essencial para as atividades do sistema nervoso central e a deficiência tem sido associada com declínio cognitivo (BERR et. al., 2000; GAO et al., 2007; CARDOSO et al., 2010). Este mineral exerce seu papel antioxidante por meio de selenoproteínas, com destaque para a enzima glutationa peroxidase 1 (GPX1) e 4 (GPX4) que são expressas abundantemente no cérebro. Visto que o estresse oxidativo está intimamente relacionado com a progressão a demência, o selênio apresenta-se como um possível alvo terapêutico a fim de evitar o desenvolvimento de déficits de memória e doenças demenciais (ZHANG et al., 2010).

Entretanto, poucos estudos são encontrados na literatura a respeito dos benefícios do selênio sobre a cognição de adultos maduros e idosos saudáveis, e na maior parte dos trabalhos, a suplementação com esse mineral está associada a outros componentes (SCHELTENS et al., 2010; KESSE-GUYOT et al., 2011; DA ROCHA et al., 2014). Outra questão importante a ser avaliada diz respeito aos efeitos que os polimorfismos genéticos em genes que codificam as selenoproteínas podem ter sobre a função cognitiva, uma vez que podem alterar o metabolismo de selênio e as necessidades desse nutriente (MEPLAN 2011; HESKETH 2008; FERGUSON; KARUNASINGHE, 2011). Nesse sentido, estudos apontam que o polimorfismo rs1050450 no gene *GPX1* estaria associado a um risco aumentado de desenvolvimento de DA (PAZ-Y-MIÑO et al., 2010; CARDOSO et al., 2012). Além disso, pesquisas desenvolvidas em modelo animal apontam para o relevante papel neurobiológico da enzima GPX4 (CHEN et al., 2008; YOO et al., 2010; ZHANG et al., 2016), e embora o gene que a codifica seja altamente polimórfico, até o momento não há pesquisas que avaliem o impacto do polimorfismo sobre a memória humana e distribuição sérica de selênio.

Desse modo, este trabalho visou avaliar os efeitos dos polimorfismos rs1050450 (*GPX1*) e rs713041 (*GPX4*) sobre a memória episódica, sobre o

comprometimento cognitivo leve e doença de Alzheimer. Além disso, investigamos a influência desses polimorfismos sobre a distribuição sérica de selênio.

Estruturalmente, esta tese é apresentada na forma de capítulos, sendo os primeiros referentes à revisão da literatura, a justificativa e objetivos. Na sequência, os dados obtidos da associação entre os polimorfismos em *GPX1* e *GPX4*, doença de Alzheimer, comprometimento cognitivo leve e memória são apresentados, e o segundo artigo discorre sobre as correlações entre escores de memória em adultos maduros e idosos e concentração sérica de selênio.

2 REVISÃO DA LITERATURA

2.1 Envelhecimento populacional

Nas últimas décadas o envelhecimento populacional transformou-se em uma característica mundial. Em 2000, aproximadamente 10% da população global era idosa, com previsão de crescimento acima de 20% para 2050. No Brasil, a projeção é que para o ano de 2050 cerca 65 milhões de indivíduos sejam considerados idosos (IBGE 2016). Ainda, segundo as projeções do Instituto Brasileiro de Geografia e Estatística (IBGE, 2016) a expectativa de vida dos brasileiros alcançará o patamar de 81,29 anos em 2050. Este índice de vida pode ser comparado ao de países desenvolvidos e de alta densidade demográfica, como a Islândia (80,80 anos), a China (82,20 anos) e o Japão (82,60 anos). Dentre as capitais brasileiras, a cidade de Porto Alegre é a segunda com maior número de indivíduos idosos, com 211.896, correspondendo a uma taxa de 13,5% da população total (IBGE 2016).

Dentre as alterações fisiológicas decorrentes do processo de envelhecimento, as funções do sistema nervoso central, principalmente as envolvidas no processo cognitivo, como o aprendizado e memória, constituem os principais alvos de pesquisas realizadas sobre senescência, em virtude das constantes queixas por essa população (NYBERG et al. 2012).

Entretanto, estudos prospectivos como, por exemplo, o *Maastricht Aging Study - Mass* (JOLLES et al, 1998), realizado na Holanda, demonstram que somente um quinto de todos os idosos com idade superior a oitenta anos apresenta demência, e, portanto, a grande maioria dos idosos não chega a desenvolver demência. Infelizmente, dados similares não estão disponíveis no Brasil. Desta maneira os problemas mais frequentes relacionados com memória não são os quadros demênciais, mas sim outras características com gravidades variáveis e que podem ou não se tornar patológicas.

2.2 Memória

Memória pode ser definida como um processo de aquisição, formação, conservação e evocação de informações. A aquisição é também chamada de aprendizado ou aprendizagem, visto que só “gravamos” o que foi aprendido. A evocação é também chamada de recordação, lembrança, recuperação, uma vez que só é possível lembrar aquilo que foi aprendido (IZQUIERDO 2011).

Dentre todas as funções cognitivas, a memória é considerada uma das mais importantes, pois é fundamental para o desenvolvimento da linguagem, da consciência de quem somos e do reconhecimento de pessoas e objetos (YASSUDA et al. 2006).

A primeira etapa para a formação da memória é a aquisição, também denominada aprendizagem, que começa por volta dos 2 a 3 anos de idade. Os seres humanos utilizam a linguagem para adquirir, codificar, guardar ou evocar memórias; as demais espécies não. Apesar da quantidade e complexidade dos processos que envolvem sua formação, usamos praticamente as mesmas regiões do cérebro e mecanismos moleculares semelhantes para a construção de memórias distintas, exceto as áreas de linguagem (IZQUIERDO 2011).

O armazenamento é a consolidação da memória. No entanto, as memórias não são adquiridas imediatamente na sua forma final, pois nossa memória individual necessita descartar o trivial e ao longo dos anos perdemos fatos, lembranças ou conhecimentos que não nos interessam. A descoberta da consolidação surgiu de duas fontes, uma de cunho popular, já no século XIX, de que após um traumatismo craniano os indivíduos esquecem seletivamente aquilo que havia acontecido nos minutos anteriores. A outra fonte de observação sobre o armazenamento foi inicialmente feito por dois pesquisadores alemães, Müller e Pilzecker, no ano de 1900, em que observaram que muitas memórias interferem em outras adquiridas imediatamente antes (McGAUGH 1966; IZQUIERDO 1989; McGAUGH 2000).

Ambas as observações indicam que aquilo que se aprende inicia processos nervosos que duram tempo além do ato de aprendizado em si, sem os quais não haverá memória. Este armazenamento de informações ocorre de maneira sistemática pelo cérebro, que através de mecanismos diversos é capaz de reconhecer, processar e separar as informações de extrema importância daquelas que não serão mais utilizadas. Considerando toda a informação sensorial captada, normalmente cerca de 99% dela é descartada pelo cérebro como sendo irrelevante (IZQUIERDO 2006).

A última etapa é a evocação de informações retidas, também chamada de recordação ou lembrança. No momento da evocação, o cérebro deve recriar, em instantes, memórias que levam horas para ser formadas, e nesse momento, ocorre uma reativação das redes sinápticas de cada memória específica. È bem reconhecido o fato de que quanto mais informações ou “dicas” forem dadas a respeito da memória a ser evocada, mais rápido e fácil ela será lembrada, por uma maior ativação destas redes

neuronais (IZQUIERDO 1998; IZQUIERDO 2006). Em alguns casos podem acontecer os chamados “brancos” da memória, que são déficits associados à evocação, e não afetam a memória consolidada, que permanece intacta, somente não podendo ser expressa. Estes eventos podem ocorrer devido a situações de estresse ou ansiedade excessiva, ou também serem causados pela atuação de glicocorticóides em excesso no hipocampo ou na amígdala basolateral (IZQUIERDO 2006).

A observação do indivíduo é fundamental para a suspeita de alterações em sua memória. Pessoas que repetem ideias, colocações e histórias, ou se perdem em seu raciocínio, bem como quando apresentam dificuldade na organização de uma ideia ou pensamento, ou em associar e adicionar informações novas podem estar apresentando alterações na construção da memória (IZQUIERDO 2011).

Diversas mudanças no funcionamento da memória já foram bem documentadas, e são esperadas durante o envelhecimento saudável (YASSUDA et al. 2002; GRADY 2013). Embora tais mudanças possam ter impactos negativos, isto não ocorre de maneira uniforme e devastadora, geralmente os indivíduos se mantêm intactos neste período da vida, de maneira suficiente para que permaneçam independentes (ARGIMON; STEIN 2005). Além disso, há grande variabilidade entre os indivíduos no que se refere à intensidade dos efeitos do envelhecimento na memória, especialmente por se tratar de uma característica multifatorial (YASSUDA et al. 2006).

Por exemplo, sabemos hoje que a velocidade do processamento das informações, importante à formação de novos traços de memória, é menor nos adultos mais velhos do que nos jovens. Isto é, em média, um senhor de 65 anos necessitará de mais tempo para processar (ler, compreender, memorizar) as informações da primeira página do jornal do que seu neto de 20 anos. Sabemos também que o declínio que ocorre com a idade na memória para fatos, também chamada de memória episódica, é mais acentuado do que o declínio que ocorre na memória semântica, que usamos para materiais linguísticos (CANÇADO; HORTA 2006).

Outro dado documentado e bem aceito pelos pesquisadores é que a memorização consciente, também chamada de memória explícita, é mais sensível aos efeitos do envelhecimento do que a memorização realizada inconscientemente (memória implícita) (YASSUDA et al. 2005).

2.2.1 Classificações dos tipos de memória

Há muitas classificações das memórias: de acordo com sua função, tempo de duração e conteúdo.

Em relação à definição da memória de acordo com a função, esta é denominada de memória de trabalho, também chamada de memória operacional. A memória de trabalho pode ser referida como aquela capaz de gerenciar a realidade e determinar o contexto em que os diversos fatos, acontecimentos ou outros tipos de informações ocorrem. A memória de trabalho serve para manter durante alguns segundos, no máximo poucos minutos, a informação que está sendo processada no momento, e também avalia se é pertinente ou não fazer uma nova memória disso. A memória de trabalho também nos auxilia no contexto da percepção do instante, trazendo informações de onde estamos ou o que estamos fazendo a cada momento, ou onde estávamos no momento anterior. Ou seja, a memória de trabalho dá continuidade aos nossos atos e permite ainda o ajuste fino do comportamento enquanto este está acontecendo (IZQUIERDO 1988; SQUIRE 2004).

No entanto, a memória de trabalho não é capaz de formar arquivos, e é nesse quesito que ela se diferencia das outras memórias (SQUIRE 2004). Muitas vezes essa memória só dura o tempo suficiente para que se faça uso dela na solução de problemas, na tomada de decisões ou em questões mais simples de escolha como, por exemplo, a procura de um número telefônico e sua posterior discagem (IZQUIERDO 2002; BERTOLUCCI 2005; DE FREITAS et al., 2006).

A memória de trabalho é processada fundamentalmente pelo córtex pré-frontal, que depende basicamente, da atividade elétrica dos neurônios dessas regiões, sendo que este processo não provoca alterações bioquímicas importantes. Comparada com os demais tipos de memória, é considerada menos complexa, já que não produz “arquivos” e dura poucos segundos. É considerada a memória mais rápida em questão de duração, no entanto, é importante para a formação dos diversos tipos de memória existentes (IZQUIERDO 1998).

Em relação a classificação das memórias de acordo com a duração, a memória de curta duração ainda não está totalmente compreendida, e pode ser caracterizada como a memória recente, capaz de reter informações por alguns minutos, somente o tempo necessário para que a memória de longa duração se consolide. Estende-se do início do aprendizado até 3 a 6 horas após o mesmo, e é regulada pelo hipocampo e por

algumas regiões do córtex, principalmente por receptores serotoninérgicos, dopaminérgicos, noradrenérgicos e colinérgicos (IZQUIERDO; IZQUIERDO 2004; CANÇADO, HORTA 2006). Possui uma importante função, que é a de manter o indivíduo capaz de responder a estímulos ou condições variadas, através de uma cópia da memória a ser consolidada, enquanto esta ainda não está formada (IZQUIERDO 2002).

Este tipo de memória depende da análise prévia das informações realizadas pela memória de trabalho. Para que ocorra a compreensão da linguagem oral e escrita a memória de curta duração é considerada essencial, mostrando que há uma influência mútua entre esta memória e a de longa duração. Ou seja, a informação adquirida, considerada importante, terá o mesmo conteúdo na memória de curta duração, como na de longa duração (IZQUIERDO 2002). Esta memória difere em dois aspectos principais da memória de longa duração, em relação à capacidade e à duração das informações (COWAN 2008). A memória de curta duração, de acordo com a própria denominação, dura pouco tempo, de alguns minutos a poucas horas, já a memória de longa duração pode durar a vida toda (IZQUIERDO 2002). De acordo com a capacidade, a memória de curta duração também é mais limitada que a memória de longa duração. Pode processar somente alguns itens ou informações ao mesmo tempo, por um tempo limitado. Após este período estes dados são descartados ou atravessam um processo em direção a consolidação da memória (YASSUDA et al. 2006).

A memória de longa duração ou tardia está principalmente relacionada à nossa capacidade de manter as informações já adquiridas, armazenadas por longos períodos de tempo, de dias a anos. Geralmente é mais estável e menos atingida pelo envelhecimento (IZQUIERDO 2002; YASSUDA 2006).

Em relação à definição da memória de acordo com o seu conteúdo a classificação pode ainda ser feita em memórias implícitas (procedimento) ou explícitas (declarativa). As memórias explícitas ainda se subdividem em memórias semânticas e episódicas.

As memórias implícitas são adquiridas de maneira automática, sem que o sujeito perceba de forma clara que está aprendendo e são principalmente relacionadas a conhecimentos contraídos na infância. Durante a vida adulta e em idosos a sua formação é mais prejudicada, visto que é durante a infância que o maior número de informações

novas e comportamentais é adquirido. É dependente de atenção ou de um contato mínimo com o estímulo, manifestando-se através do desempenho, geralmente atividades motoras e sensoriais, não havendo acesso consciente ao conteúdo da informação (IZQUIERDO 2002; YASSUDA et al 2006; CANÇADO , HORTA 2006; PARENTE et al., 2006). Exemplo de memória implícita inclui saber andar de bicicleta, lavar pratos, executar tarefas que dependem do treinamento repetitivo, diário e cuja aquisição é gradual (YASSUDA et al. 2006).

As memórias explícitas são relacionadas aos fatos e eventos que vivenciamos, e ao conhecimento adquirido de forma consciente. Na idade adulta e na velhice este tipo de memória é mais afetado, havendo maior declínio de informações do que a memória implícita. Uma fração das memórias explícitas é denominada de memória semântica, e está correlacionada ao registro de informações linguísticas, verbais, de conhecimentos gerais, de vocabulário, idiomas, significados, sem contexto temporal específico (IZQUIERDO 2002; YASSUDA et al. 2006; CANÇADO, HORTA, 2006).

A memória episódica, outro subtipo da memória explícita, está relacionada com a capacidade de consolidar informações sobre eventos e fatos ocorridos e situá-los no tempo. Ligada às informações e experiências pessoais, chamada também de memória autobiográfica, parece ser mais sensível ao envelhecimento do que a memória semântica (YASSUDA 2006 et al.; CANÇADO, HORTA 2006). Comparando os dois subtipos de memória explícita, sabe-se que durante o envelhecimento a memória episódica tende a sofrer mais alterações e perdas que a memória semântica (PARENTE et al., 2006).

A memória episódica pode ser classificada ainda de acordo com dois estilos distintos: a visual e a verbal. A memória episódica visual está envolvida diretamente na percepção do ambiente, estando relacionada à capacidade de recordar imagens, como símbolos, desenhos, fotos ou outros recursos gráficos. Já a memória episódica verbal consiste na capacidade de armazenar fatos ou eventos (WESCHLER 2004). Entre as principais estruturas nervosas envolvidas na formação da memória semântica e episódica encontram-se o hipocampo e algumas regiões do córtex cerebral. Na modulação destas memórias estão envolvidas outras regiões do cérebro humano, como a amígdala, substância negra, o núcleo de rafe e o núcleo basal de Meynert (IZQUIERDO 2002; CANÇADO; HORTA, 2006).

No presente estudo, a avaliação dos diferentes tipos de memória foi realizada a fim de se avaliar a memória episódica visual e verbal, tanto imediata quanto tardia, descritas acima. Além disso, foi avaliado outro tipo de memória que está envolvida na capacidade de aprendizado verbal, onde é examinada a capacidade do indivíduo de armazenar novas informações.

2.3 Comprometimento cognitivo leve (CCL) e Doença de Alzheimer (DA)

O termo comprometimento cognitivo leve foi introduzido como uma entidade clínica há mais de vinte anos. Inicialmente, Reiserg e colaboradores usaram esse termo para descrever pacientes que estavam no estágio intermediário entre envelhecimento normal e estado de demência. Mais tarde, um conjunto de critérios diagnósticos foram revistos e definidos (REISERG et al.,1988; PETERSEN et al., 1999), conforme descrição no Quadro 1.

a) Queixa cognitiva referida pelo paciente ou por informante, ou observado pelo médico; b) Distúrbio cognitivo evidenciado pela avaliação clínica; c) Alteração de um ou mais domínios cognitivos quando comparado ao esperado para o indivíduo; d) Preservação da independência para a realização das atividades da vida diária; e) Ausência de critérios para diagnóstico de demência.

Quadro 1: Critérios para diagnóstico de Comprometimento Cognitivo Leve (AMERICAN PSYCHIATRIC ASSOCIATION, 2013; PETERSEN et al., 2014).

Mais recentemente, as investigações com perspectivas clínicas, genéticas, e epidemiológicas sugerem que o CCL deve ser expandido para incluir outros domínios cognitivos como os de função executiva ou habilidades viso-espaciais (PETERSEN 2014).

De acordo com essa nova abordagem, o CCL foi definido como um declínio no funcionamento cognitivo maior do que seria esperado para a idade do paciente e formação educacional e que vai além das mudanças normais observadas no envelhecimento (PETERSEN 2004). Esse declínio pode incluir uma variedade de domínios cognitivos, incluindo aprendizado e memória, atenção, funções executivas, linguagem, domínio perceptivo-motor e comportamento social, embora seja comum que o declínio se manifeste em apenas um único domínio (KNOPMAN; PETERSEN, 2014).

Embora essas mudanças cognitivas não sejam severas o suficiente para interferir com as atividades do dia a dia, tarefas funcionais complexas, como por

exemplo, a interpretação de instruções, planejamento de atividades subsequentes ou tomada de decisões, pode exigir o envolvimento de estratégias compensatórias pelo indivíduo.

O CCL é uma entidade clínica heterogênea e, conforme a etiologia e o prognóstico pode ser classificados em subtipos, considerando-se a base cognitiva. O CCL do tipo amnésico se caracteriza pela perda evidente de memória, além de apresentar biomarcadores mais condizentes com a DA, enquanto que o CCL não amnésico, em que não se identificam prejuízos na memória, se correlaciona com patologias cerebrovasculares (PETERSEN et al., 2009). O CCL também pode ser distinguido em CCL de múltiplos domínios ou de único domínio, conforme o número de domínios cognitivos prejudicados. Assim, o indivíduo pode ser classificado em um dos quatro subtipos clínicos possíveis: a) CCL amnésico de único domínio; b) CCL amnésico de múltiplos domínios; c) CCL não-amnésico de único domínio; d) CCL não-amnésico de múltiplos domínios (PETERSEN 2014).

A prevalência relatada para o CCL aumenta com o envelhecimento, e assim percebe-se um aumento na prevalência de 19% entre indivíduos com idade abaixo de 75 anos para 29% entre aqueles com idade superior a 85 anos (LOPEZ et al., 2013). Embora o CCL não esteja relacionado com o comprometimento da independência das atividades de vida diária, está associado com aumento do risco para demência, em particular Doença de Alzheimer. Em termos do conceito de CCL como fase intermediária de DA demonstrou-se que 15-41% dos casos de CCL por ano evoluem para DA ou outras demências (ALBERTS et al., 2011). Por exemplo, em meta-análise que inclui 13 estudos clínicos envolvendo um total de 4301 indivíduos, a taxa de conversão anual de CCL para demência foi de 9,6% e, durante todo o período de acompanhamento, 39,2% converteram para a demência (MITCHELL et al., 2014). No entanto, esta entidade clínica pode ser vista como instável (RITCHIE 2004; GANGULI, 2004). Por exemplo, em um estudo longitudinal de oito anos, Anstey et al. (2013) descobriram que 45% dos diagnósticos eram instáveis para o grupo de participantes jovens de 62 a 64 anos. Assim, o CCL pode ser visto como uma fase transitória e de alto risco, e as intervenções para promover uma melhor saúde ou funcionamento cognitivo podem ser particularmente pertinentes para este grupo. No entanto, esses dados também indicam que o diagnóstico ou rastreio de CCL pode ainda ser impreciso.

A DA é o tipo mais comum de demência, caracterizada por perda de memória e de outros domínios cognitivos. O termo "demência" refere-se a um grupo de distúrbios que causam declínio cognitivo como resultado da morte ou por danos às células cerebrais. Por definição, a demência causa um declínio em pelo menos duas das quatro funções cognitivas essenciais: (1) memória; (2) capacidade de falar ou entender a linguagem; (3) capacidade de planejar, fazer julgamentos sonoros e realizar tarefas complexas; e (4) capacidade de processar e interpretar a informação visual. O declínio deve ser suficientemente grave para interferir na vida cotidiana (MOLLER 1998; AMERICAN PSYCHIATRIC ASSOCIATION, 2000).

O padrão típico de sintoma de Alzheimer começa com perda de memória para eventos recentes (memória de curto prazo). Patologicamente, muitas lesões moleculares foram detectadas na doença de Alzheimer, como por exemplo, a deposição de proteínas no cérebro, resultando em danos oxidativos e inflamatórios, que, por sua vez, levam à falha de energia e disfunção sináptica. Além disso, outro mecanismo fisiopatológico da DA é o acúmulo de emaranhados neurofibrilares, que são inclusões filamentosas em neurônios (QUERFURTH 2010).

O envelhecimento populacional é, hoje, um proeminente fenômeno mundial, que resulta em uma transformação no perfil de saúde da população idosa. Dessa maneira, observa-se um aumento de novos casos de doenças demenciais e, de acordo com Ferri et al. (2005), 4,5 milhões de novos casos de demência serão diagnosticados a cada ano e, caso estratégias de prevenção não sejam desenvolvidas, em 2040 o número de indivíduos afetados ultrapassará os 80 milhões de pessoas.

A DA constitui aproximadamente 70% de todos os casos de demência sendo que a incidência aumenta com a idade, dobrando a cada cinco/dez anos. A prevalência também aumenta exponencialmente com a idade, passando de 3% entre os 65-74 anos, para quase 50% entre os mais de 85 anos (HEBERT et al., 1995; FERRI et al., 2005). No Brasil, projeções indicam que a prevalência média se apresenta mais alta que a mundial. Entre a população com 65 anos ou mais, projeta-se um aumento de 7,6% para 7,9% entre 2010 e 2020, ou seja, 55.000 novos casos por ano (HERRERA et al., 2002; BURLÁ et al., 2013).

Com relação a genética da DA, os fatores genéticos podem explicar cerca de 58 a 79% do risco, e o padrão de herança autossômica dominante corresponde apenas 1% da totalidade dos casos da doença (TANDON; FRASER, 2002). Nesse caso, a proteína

precursora da beta-amiloide tem grande impacto na predisposição à DA familiar de início precoce (DE STROOPER et al.; 1998; KWOK et al., 2000), enquanto polimorfismos de nucleotídeo único (SNP) em genes que codificam apolipoproteína E e α_2-macroglobulina estão associados com a DA de início tardio (SCHELLENBER; MONTINE, 2012). A maioria dos casos de DA são esporádicos, apresentam uma genética complexa, que é resultado de múltiplas interações entre genes e ambiente (SCHELLENBERG; MONTINE, 2014).

Partindo da premissa que o estresse oxidativo tem um papel central na DA (RAO; BALACHANDRAN, 2002; CHEN; ZHONG, 2014), é importante entender o papel dos polimorfismos envolvidos na codificação de enzimas antioxidantes, como por exemplo, as glutationas peroxidases 1 e 4 (MEPLÁN, 2011).

2.4 Selênio, selenoproteínas e sistema nervoso central

O selênio foi descoberto em 1817 pelo químico sueco Jöns Jacob Berzelius, como um elemento químico relacionado com o enxofre e o telúrio, apresentando propriedades químicas semelhantes a este último. Como o telúrio foi denominado "tellus", que no latim significa terra, Berzelius chamou o novo elemento de "selene", que no grego significa lua. Atualmente, depois de 200 anos do descobrimento do selênio, o conhecimento sobre as propriedades biológicas do selênio é imenso (BROWN; ARTHUR, 2001).

No entanto, até a década de 1930, o conceito que predominava a respeito do selênio era de um elemento tóxico, em virtude de um episódio ocorrido em uma fazenda do estado americano de Dakota do sul. Nessa fazenda, os ovos de galinha apresentavam baixa eclodibilidade e, quando nasciam pintos, muitos eram deformados e morriam. Após investigação do Departamento de Agricultura dos Estados Unidos da América, identificou-se que uma parte da fazenda apresentava solo selenífero (solo com elevada concentração de selênio). Assim, os primeiros estudos sobre a relevância do selênio para os organismos vivos apontavam para um elemento de ocorrência natural que seria tóxico (PRAUCHNER 2014).

Após, Schwarz e Foltz (1957) identificaram a função essencial do selênio para a saúde animal ao verificar que esse mineral atuava na prevenção de necrose em ratos (OLDFIELS 2002). No caso de seres humanos, sua essencialidade foi comprovada em 1979, quando um paciente com distrofia muscular em razão de longa permanência sob

nutrição parenteral total apresentou melhora do quadro clínico após suplementação com o mineral (PRAUCHNER 2014). Anteriormente, em 1973, Flohé et al. (1973) identificaram o que atualmente é considerado de importância primordial: o selênio faz parte do sítio ativo da enzima glutationa peroxidase (GPX).

Atualmente, o selênio atua como um mineral versátil, sendo reconhecido por seu envolvimento em diversas funções fisiológicas em mamíferos, tais como: defesa antioxidante, fertilidade, metabolismo do hormônio da tireoide e resposta imune (SCHOMBURG 2012; HADASZADEH; BEGGS, 2006, YOUN et al., 2008; TONDO et al., 2010).

Como outros elementos traços, o selênio é um constituinte natural da crosta terrestre, estando presente também na água. O teor de selênio nos alimentos varia consideravelmente, uma vez o conteúdo de selênio nos alimentos depende do teor regional de selênio. Isso pode variar de níveis tóxicos em regiões de solos seleníferos a regiões formadas por solos pobres, onde os seres humanos sofrem de problemas de saúde por deficiência de selênio (RAYMAN 2008). No Brasil, os estudos demonstraram que o país apresenta divergências no conteúdo mineral nos solos, o que tem reflexos diretos na ingestão alimentar de selênio. Estados como São Paulo e Mato Grosso apresentaram os menores níveis do mineral em refeições analisadas em laboratório (FAVARO et al., 1997; BOAVENTURA 1991). Por outro lado, no Amazonas e em Santa Catarina, foram encontradas as maiores concentrações (YUYAMA; AGUIAR; MACEDO; GIOLA 1997). Para manter um balanço de selênio de zero (onde o selênio consumo é igual à excreção de selênio na urina e fezes), a ingestão necessária para homens é de 80 μg/dia e de mulheres de 57 μg/dia (RAYMAN 2008). De maneira geral a ingestão média de selênio no Brasil é adequada (102,34 μg/dia), no entanto quanto se avalia o estágio de vida e estado nutricional, os maiores percentuais de ingestão insuficiente foram encontrados nos indivíduos com baixo peso, especialmente nos idosos (TURECK et al., 2007). Na tabela 1, encontram-se todos os valores detalhados para cada estágio de vida. O nível de admissão tolerável (UL) é de 400 μg/ dia, ocorrendo reações adversas em níveis mais altos (NAVARRO-ALARCON; CABRERA-VIQUE, 2008). Globalmente, estima-se que a ingestão de selênio varia muito, apresentando intervalo 3-7000 μg por dia (RAYMAN 2008; FAIRWEATHER-TAIT et al., 2011).

Assim, o equilíbrio do selênio no corpo é crítico porque os déficits podem levar a problemas neurológicos, problemas cardiovasculares, câncer e deficiências imunológicas, enquanto níveis mais altos resultam em toxicidade (RAYMAN 2012).

Tabela 1. Recomendações de ingestão de selênio em diferentes estágios de vida

Estágio da vida	AI*/EAR µg/dia	RDA µg/dia	UL µg/dia
Recém-nascidos e crianças			
0-6 meses	*15	-	45
7-12 meses	*20	-	60
1-3 anos	17	20	90
4-8 anos	23	30	150
9-13 anos	35	40	280
Adolescentes			
14-18 anos (M)	45	55	400
14-18 anos (F)	45	55	400
Adultos			
19-70 anos (M)	45	55	400
19-70 anos (F)	45	55	400
Gestantes			
14-50 anos	49	60	400
Lactantes			
14-50 anos	59	70	400

Fonte: IOM, 2000 (INSTITUTE OF MEDICINE, 2000). AI=ingestão adequada; EAR=necessidade média estimada; RDA=ingestão dietética recomendada, UL= limite máximo tolerado de ingestão diária.

Há duas formas de compostos de selênio na natureza: a orgânica e a inorgânica. Vegetais absorvem o selênio em sua forma inorgânica a partir do solo, a qual é convertida para a forma orgânica, gerando compostos metilados de baixo peso molecular, além de selenometionina e selenocisteína. A selenometionina é a principal fonte de compostos de selênio presente em produtos vegetais como grãos, legumes e leguminosas. A biodisponibilidade da selenometionina parece ser maior em ratos e

humanos e é provável que seja a principal fonte de selênio para organismos vivos (RAYMAN; INFANTE; SARGENT, 2008; SCHOMBURG; SCHWEIZER; KOHRLE 2004). Ainda, a selenometionina é o principal precursor para a síntese de selenocisteína, a forma mais abundante em produtos de origem animal (RAYMAN, 2012; PAPP; LU; HOLMGREN; KHANNA 2007).

A função biológica do selênio ocorre através da sua incorporação em três formas de proteínas contendo selênio (BEHNE; KYRIAKOPOULOS 2001). Primeiramente o selênio pode ser incorporado não especificamente em proteínas ricas em metionina, como as encontradas na castanha do Brasil (PAPP; LU; HOLMGREN; KHANNA 2007). Em segundo lugar, o selênio pode ser especificamente incorporado em selenoproteínas. Isto requer uma decodificação do códon UGA em resíduos de selenocisteína (Sec) e a necessidade de uma estrutura secundária de "stem-loop" na região 3' não traduzida (3'UTR) do mRNA de selenoproteína, também conhecida como sequência de inserção Sec (SECIS). Há um tRNA específico ($Cys^{[Sec]}$-tRNA) para Sec e um complexo de proteínas de ligação de RNA que reconhecem a estrutura secundária de "stem-loop" e facilita a decodificação do códon UGA, que classicamente atua como um códon de terminação (BOCK et al., 1991).

Em terceiro lugar, existem proteínas específicas contendo selênio que possuem a habilidade de sequestrar o selênio através de um mecanismo desconhecido, exemplos incluem a proteína do fígado não caracterizada 56K (SLP-56) e a proteína de ligação aos ácidos gordurosos do fígado (SLP-14) (BEHNE et al., 1994).

O selênio incorporado na forma de selenocisteína é reconhecido como vigésimo primeiro aminoácido, essencial por se diferenciar da cisteína pela presença de um átomo de selênio no lugar de enxofre. Ao analisar todo o genoma para presença do SECIS, verificou-se que existe um total de 25 selenoproteínas em seres humanos (KRYUKOV et al., 2003), que são organizadas em grupos distintos, de acordo com a localização e as propriedades funcionais da selenocisteína, sendo que aproximadamente metade das selenoproteínas caracterizadas apresenta função antioxidante. São elas: cinco glutationa peroxidases (GPX), três iodotironina deiodinases (DI), três tioredoxina redutases (TRR), uma selenoproteína de 15-kDa (SEP15), selenoproteínas H, I, K, M, N, O, P, R, S, T, U, V e seleofosfato sintetase 2 (HATFIELD et al., 2014).

As GPX constituem uma família com cinco enzimas dependentes de selênio e essas enzimas são encontradas em todos os tecidos de mamíferos onde ocorrem

processos oxidativos. Essas enzimas podem prevenir a produção de espécies reativas de oxigênio, contribuindo para a proteção das macromoléculas e biomembranas do organismo contra a oxidação (BRIGELIUS-FLOHÉ; MAIORINO 2013). A glutationa peroxidase clássica (GPX1) é a mais abundante selenoproteína em mamíferos e apresenta expressão mais elevada em tecidos com alta produção de peróxidos, como rins, fígado e eritrócitos. A GPX1 foi a primeira a ser identificada e está presente no citosol das células, onde funciona como antioxidante, reduzindo peróxidos de hidrogênio (H_2O_2) e hidroperóxidos orgânicos livres e transformando-os, respectivamente, em água e álcool. Devido à sua capacidade de redução de hidroperóxido, GPX1 foi classificada como a enzima que diminui o estresse oxidativo (FLOHÉ; GÜNZLER; SCHOCK 1973; ROTRUCK et al., 1973).

A GPX2, ou gastrointestinal, possui a mesma estrutura e o mesmo substrato da GPX1, sendo encontrada principalmente em órgãos do aparelho digestivo, como estômago e intestino, enquanto a GPX3 tem sua maior expressão nos rins. A GPX4 é encontrada principalmente nos testículos, nos espermatozoides, no coração, no fígado, nos rins e no cérebro, nas isoformas mitocondrial, citosólica e nuclear. Ao contrário do restante das selenoproteínas, a GPX4 é capaz de reduzir hidroperóxidos de fosfolipídeos e colesterol diretamente, sendo sua isoforma mitocondrial quem medeia a resposta apoptótica frente ao estresse oxidativo. A GPX6 foi recentemente identificada no epitélio olfatório e em tecidos embrionários (FLORIAN et al., 2001; SAREWKO et al., 2002; TAPIERO et al., 2003; LEI et al., 2007; HERBETTE et al., 2007; COMINETTI et al., 2011).

O selênio é essencial para o sistema nervoso central, como demonstrado em trabalhos experimentais que identificaram que os animais alimentados com dietas deficientes em selênio mantiveram os níveis relativamente elevados de selênio em seus cérebros, enquanto a concentração de selênio em outros órgãos, incluindo fígado e rim, foi severamente esgotado (CHEN; BERRY, 2003). Além disso, quando selênio foi administrado para animais deficientes em selênio, uma grande parte do selênio foi rapidamente confiscado pelo cérebro (TRAPP; MILLAM, 1975; NAKAYAMA et al., 2007, BURK; HILL 2009).

Estes e outros estudos sugerem que o selênio desempenha um papel importante na fisiologia e funções do cérebro e desse modo ocorre uma hierarquia quanto aos órgãos em relação ao *status* de selênio. Nesse sentido, o cérebro é o último órgão a ser

depletado com a deficiência de selênio e, na repleção, este é o primeiro a estabelecer seus níveis adequados do mineral, mostrando a importância do selênio no funcionamento cerebral (BENTON 2002).

A síntese de selenoproteínas é totalmente dependente da disponibilidade de selênio, e desse modo, também há uma hierarquia na sensibilidade ao consumo do mineral. Nesse sentido, observa-se que algumas selenoproteínas respondem rapidamente à deficiência de selênio com diminuição da sua atividade frente à prolongada e notável depleção (BRIGÉLIUS-FLOHÉ 1999). Assim, a deiodinase 1 é a selenoproteína menos vulnerável e encontra-se no topo da hierarquia; e, dentro da família das GPX, verifica-se uma prioridade para GPX2 e 4 em relação às GPX1 e 3, enquanto que a selenoproteína P se encontra em posição intermediária. Uma provável interpretação para essa hierarquia entre as GPX sugere que estas são classificadas conforme a importância do seu desempenho para o organismo (SHWEIZER 2004; SCHOMBURG; SCHWEIZER 2009).

Considerando a atividade antioxidante espacialmente no cérebro, algumas selenoproteínas foram caracterizadas como protetoras contra a neurodegeneração através da regulação redox, dentre elas as GPX1 e GPX4. Para isso, estas enzimas estão envolvidas numa sequência de reações que visam à redução de peróxidos de hidrogênio e hidroperóxidos orgânicos (GARCIA 2009; ZHANG 2010).

A GPX1 é a mais abundante entre as GPX e é expressa em vários tipos de células neuronais, incluindo neurônios, astrócitos e células gliais (TAKIZAWA 1994, LINDENAU; NOACK; ASAYAMA; WOLF 1998). Crack et al. (2006) ao utilizarem isolados de cultura de neurônios de ratos Gpx $^{-/-}$ demonstraram que a GPX1 protege contra o estresse oxidativo induzido por H_2O_2 e por toxicidade dos peptídeos amilóides (Aβ), o principal componente das placas senis e a causa da doença de Alzheimer.

A proteína GPX4 é uma selenoproteína antioxidante, intracelular que catalisa uma diminuição de H_2O_2, hidroperóxido orgânico e peróxidos lipídicos dentro de membranas e lipoproteínas, reduzindo os níveis de glutationa e protege as células contra danos oxidativos. A GPX4 apresenta três isoformas, todas codificadas pelo mesmo gene, que se localizam no citoplasma, mitocôndria e núcleo (URSINI; MAIORINO; GREGOLIN, 1985). Nos cérebros de ratos adultos, as isoformas de GPX4 mitocondriais e citosólicas são expressas principalmente em células neuronais, mas não

são expressas em células gliais, como evidenciado por análises de imuno-histoquímica e mRNA (SAVASKAN; BORCHERT; BRAUER; KUHN, 2007).

Além disso, observou-se que a ablação neurônio-específica da biossíntese de selenoproteínas em camundongos produziu um fenótipo neurológico grave, caracterizado por convulsões, ataxia e prejudicou o desenvolvimento de interneurônios corticais que foi acompanhado por uma perda da expressão de GPX4 e de várias outras selenoproteínas no cérebro (WIRTH et al., 2010). Recentes trabalhos forneceram uma visão sobre um papel para GPX4 como um sensor de estresse oxidativo e um transdutor de sinais de morte celular. Isto é consistente com a noção de que a depleção de GPX4 no hipocampo demonstrou resultar em neurodegeneração, sendo GPX4 diretamente envolvida na sinalização pró-apoptótica. Além disso, demonstrou-se que a baixa ingestão de selênio ou níveis insuficientes de GPX4 no cérebro contribuiram para patogênese da doença de Parkinson e da doença de Alzheimer (REEVES; HOFFMANN, 2009).

As informações sobre a função das selenoproteínas no cérebro estão resumidas na tabela 2.

Tabela 2: Função de diferentes selenoproteínas no cérebro.

Selenoproteína	Função do cérebro normal	Doenças neurológicas
Família Glutationa peroxidase (GPx)		
GPX1	Redução ERO/Peróxidos (ZHANG; ROCOURT; CHENG; 2010)	D. de Alzheimer (ANSARI et al., 2006; CHEN et al., 2008); D. de Parkinson (PANEE et al., 2007)
GPX4	Redução dos hidroperóxidos de fosfolipídeos (URSINI; MAIORINO; GREGOLIN, 1985)	D. de Alzheimer (CHEN et al., 2008); D. de Parkinson (BELLINGER et al., 2011)
GPX6		D. Huntington (SOROLLA et al., 2008)
Família Tireodoxina redutase (TRXR)		
TRXR1	Redução do peróxido de hidrogênio e do estresse oxidativo, regulam os fatores de transcrição sensíveis ao redox que controlam mecanismos de transcrição celular (SELENIUS et al., 2010)	D. de Alzheimer (LOVELL; XIE; GABBITA; MARKESBERY 2000); epilepsia (YUZBASIOGLU et al., 2009).
TRXR2	Redução do peróxido de hidrogênio e do estresse oxidativo (20), regulam os fatores de transcrição sensíveis ao redox que controlam mecanismos de transcrição celular (SELENIUS et al., 2010)	
Deiodoxina DIO1	Deodinação de T4 e T3 (BATES; GALTON, 1999).	
Selenoproteina H (SelH)	Detecção de Redox, localização nuclear e ligação de DNA (PANEE; STOYTCHEVA; LIU; BERRY, 2007; NOVOSELOV et al., 2007)	

Continuação Tabela 2

Selenoproteína	Função do cérebro normal	Doenças neurológicas
Selenoproteína K (SelK)	Resposta ao estresse ER, regulação do cálcio (REEVES; BELLINGER; BERRY, 2010)	
Selenoproteína M (SelM)	Proteína retículo endoplasmático (RE), regulação de cálcio (REEVES; BELLINGER; BERRY, 2010)	D. de Alzheimer (YIM et al., 2009; DU et al., 2013)
Selenoproteína P (Sepp1, SelP)	Retenção de selênio dentro do cérebro (NAKAYAMA et al., 2007)	D. de Alzheimer (BELLINGER et al., 2008) D. de Parkinson (PERRY; YONG, 1985)
Selenoproteína S (SelS)	Retrotranslocação de proteínas dobradas de RE, resposta ao estresse RE (FRADEJAS et al., 2008)	
Selenoproteína T (SelT)	RE proteína, regulação do cálcio (FRADEJAS et al., 2008)	Epilepsia (STEFANIUK; LUKASIUK, 2010)
Selenoproteína W (SelW)	Localização sináptica (RAMAN et al., 2013)	Epilepsia (YUZBASIOGLU et al., 2009)
Selenoproteína R (SelR)	Redução de resíduos de metionina oxidados, polimerização de actina (LEE et al., 2013)	D. de Alzheimer (MOSKOVITZ et al., 2011)

Tabela adaptada de PILLA; UYEHARA-LOCK; BELLINGER, 2014.

2.5 Selênio e neurodegeneração

Os estudos sobre a relação do selênio com patologias neurodegenerativas fornecem evidências sobre a função do selênio para o SNC. A neuroproteção pode ser definida como a manutenção da maior integridade possível de células e interações no cérebro que resultam em função neural não perturbada (CHEN; BERRY, 2003; PILLAI; UYEHARA-LOCK; BELLINGER, 2014).

Uma vez que pelo menos metade das selenoproteínas estão envolvidas na redução do estresse oxidativo, o principal mecanismo presumido de neuroproteção inclui ERO e remoção de espécies de nitrogênio reativo. O cérebro é suscetível ao estresse oxidativo devido ao baixo nível de antioxidantes, alto teor de ácidos graxos poliinsaturados e aumento da demanda de oxigênio (RAYMAN 2012). Assim, as propriedades neuroprotetoras do selênio podem incluir a estimulação de biossíntese de selenoproteínas antioxidantes. Outro mecanismo de neuroproteção descrito para o selênio é atribuído à sua capacidade de modular o influxo de Ca^{2+} através de canais iônicos, gerando efeito anti-inflamatório (NAZIROGLU 2009; MCKENZIE; ARTHUR; BECKETT, 2002).

Os estudos que avaliam a relação entre os níveis de selênio e o declínio cognitivo sugerem que a deficiência de selênio pode ser um risco para as demências (BERR 2000; GAO 2007, BERR 2012), entretanto os resultados ainda são contraditórios. Cardoso et al. (2010) e Vural et al., (2010) verificaram que os pacientes com DA apresentavam grande deficiência de selênio quando comparados a idosos saudáveis, ao passo que Ceballos-Picot et al. (1996) encontraram níveis aumentados de selênio plasmático em pacientes com DA quando comparados ao grupo controle. Em um estudo de associação entre elementos-traços e desempenho cognitivo em diferentes grupos com doenças neurodegenerativas, Smorgon et al. (2014), encontraram correlação direta entre concentração de selênio plasmático e nível de função cognitiva e, dessa maneira, os pacientes com DA apresentaram níveis reduzidos desse mineral quando comparados ao grupo controle.

Mesmo que grande parte dos estudos transversais não detectaram uma diminuição significativa dos níveis de selênio no plasma/soro em pacientes com Alzheimer (LOEF; SCHRAUZER; WALACH, 2011; CARDOSO et al., 2010; MESEQUEIR et al., 1999) importantes estudos epidemiológicos indicam o contrário. As evidências desses estudos indicam que o baixo *status* nutricional de selênio em

idosos pode ser associado com o declínio mais rápido das funções cognitivas e mau desempenho nos testes de coordenação motora e de velocidade (SHAHAR et al., 2010; GAO et al., 2007; CHEN; BERRY, 2004; BERR et al., 2004). Um estudo longitudinal francês com 1.166 participantes com idades entre 60-70 anos, encontrou uma associação de baixos níveis de selênio no início do estudo, com o aumento no risco de declínio cognitivo durante os primeiros quatro anos de acompanhamento. Depois de 9 anos, a probabilidade de declínio cognitivo foi maior nos sujeitos que apresentaram maior redução dos níveis de selênio no plasma (CHEN; BERRY, 2004).

É importante salientar que os exemplos encontrados na literatura abordam fenótipos cognitivos extremos como os encontrados na Doença de Alzheimer. Com relação à memória e os hábitos alimentares, mais especificamente a importância do selênio na prevenção dos déficitis de memória, esta abordagem ainda é pouca abordada na literatura (DA ROCHA et al., 2014). Além disso, os estudos citados não investigaram o perfil genético dos idosos, em relação à presença de polimorfismos em genes que codificam as selenoproteínas que podem acarretar em modificações nas taxas de distribuição plasmática do selênio (MATHERS; MEPLAN; HESKET, 2010).

2.6 Polimorfismos nos genes *GPX1* e *GPX4*

As principais questões quando se considera o papel do selênio dietético e os fatores genéticos na determinação da susceptibilidade à doença multifatorial, são se existem variações genéticas comuns em selenoproteínas que possuem consequências funcionais e se estas têm efeitos fenotípicos isolados ou apenas em combinação com fatores alimentares (HESKETH 2008). Nesse sentindo, uma vez que o metabolismo do selênio ocorre de maneira hierárquica, em que a depleção de selênio gera manutenção da síntese de algumas selenoproteínas em detrimento de outras, um polimorfismo em gene que codifica determinada selenoproteína pode alterar sua própria função ou até mesmo de outras selenoproteínas (MUTCH 2005; MATHERS; MÉPLAN; HESKETH, 2010).

Dessa forma, a combinação de polimorfismos em genes que codificam selenoproteínas com o consumo inadequado de selênio poderia influenciar, de forma negativa, os mecanismos antioxidantes, aumentando a suscetibilidade para algumas doenças. Por outro lado, variações em genes de selenoproteínas poderiam também

interferir significativamente na resposta frente ao consumo de selênio (FERGUSON; KARUNASINGHE, 2011).

No ano de 1999, Forsberg e colaboradores, desenvolveram um estudo cujo objetivo era encontrar novos polimorfismos em genes que codificam para enzimas antioxidantes. Detectaram um ponto de mutação na posição 593 C/T que causa a substituição de uma prolina (Pro/C) por uma leucina (Leu/T) no códon 198 no gene *GPX1* (rs1050450). Em relação à frequência genotípica, os estudos indicam que os homozigotos T ocorrem em uma frequência de apenas 7-11% em europeus saudáveis, mas em maior frequência (~15%) em afro-caribenhos saudáveis (FORSBERG 2000; HU, DIAMOND 2003; RATNASINGHE et al., 2000).

A mudança de aminoácido causada por este SNP produz mudanças funcionais, com a variante Leu da proteína com menor atividade em lisados celulares transfectados. Não há dados disponíveis sobre se este SNP afeta a função de GPX1 *in vivo* ou qual seria a ingestão de selênio necessária para manter a atividade GPX1, no entanto os estudos sugerem uma associação da variante leucina deste SNP com a suscetibilidade para o desenvolvimento de doenças como câncer de pulmão, mama e bexiga (ICHIMURA et al., 2004; HU, DIAMOND, 2003; RATNASINGHE et al., 2000).

A influência do rs1050450 foi investigada em pacientes brasileiros com DA. Cardoso et al. 2012 buscou determinar a frequência do polimorfismo Pro198Leu no gene *GPX1* em pacientes com Doença de Alzheimer e verificar sua relação com a atividade enzimática da GPX e alterações na concentração de selênio. Neste estudo caso controle realizado com somente 28 pacientes portadores da Doença de Alzheimer, não houve diferença entre os grupos na distribuição dos genótipos, assim como na atividade da enzima GPX. No entanto, em relação à concentração de selênio no sangue, níveis menores foram encontrados em pacientes com o genótipo Pro/Pro em relação ao grupo controle, sendo que essa característica não foi encontrada entre os grupos portadores do genótipo Pro/Leu. Além disso, a associação entre a concentração de selênio no sangue e atividade da GPX foi afetada pelo genótipo Pro198Leu (CARDOSO et al., 2010). Outro estudo, realizado por Paz-Y- Miño et. al 2010, apontou uma associação entre o alelo T e o aumento do risco de DA.

O gene que codifica a GPX4 apresenta sete éxons e está localizado no braço curto do cromossomo 19 (19p13.3) (MEPLAN et al., 2008). Em 2002, Villete et al. identificaram um polimorfismo de nucleotídeo único na região 3’UTR na posição 718

(rs713041) que influenciava o metabolismo da lipoxigenase. Este polimorfismo também se mostrou associado com câncer colorretal (BERMANO et al., 2007) e a sua funcionalidade foi demostrada por meio de estudos *in vitro* com plasmídeos contendo ambos os possíveis alelos (GAUTREY et al., 2011).

A importância desse polimorfismo deve-se ao fato de ele estar localizado próximo ao sítio de incorporação do selênio durante o processo de tradução. Bermano et al. (2007) observaram que a variante C na região 3'UTR não traduzida do gene *GPX4* é mais responsivo que a variante T na biossíntese do gene repórter na selenoproteína. Meplan et al., observaram que os portadores do genótipo CC conseguem manter as concentrações séricas de selênio por mais tempo do que os portadores do genótipo TT após a retirada da suplementação com selênio. Até o momento este é o primeiro estudo que investigou a participação do polimorfismo rs713041 em déficits cognitivos.

A tabela 3 resume os principais resultados para os polimorfismos rs1050450 (*GPX1*) e rs713041 (*GPX4*).

Tabela 3. Descrição dos principais resultados funcionais para os polimorfismos rs1050450 (*GPX1*) e rs713041 (*GPX4*).

Gene - SNP	Posição	Efeitos descritos em cultura celular	Efeito funcional *in vivo*	Referências
GPX1 rs1050450	c.599C>T Pro198Leu	Menor atividade do alelo Leucina	Estudos de associação com várias doenças, mas nem todos, sugerem que T (Leu) aumenta a suscetibilidade.	FORSBERG et al, 2000; FOSTER et al., 2006; RAVN-HAREN et al., 2006.
GPX4 rs713041	c.660T>A 3'UTR	Estudos do gene repórter e com RNA e proteína demonstram que alelo C apresenta maior atividade	Afeta a atividade de GPX1 e GPX4 dos linfócitos, e nos níveis de lipoxigenase. Dois estudos sugerem que o alelo C aumenta a susceptibilidade ao câncer.	BERMANO et al., 2007; FOSTER et al., 2006; UDLER et al., 2007.

3'UTR: região 3' não traduzida.

3 JUSTIFICATIVA

Frente à tendência atual de envelhecimento populacional, é necessário o desenvolvimento de pesquisas para uma das queixas mais frequentes durante o envelhecimento: o declínio da memória. Para melhorar a qualidade de vida da população de idosos e prevenir futuras desordens fisiopatológicas, estudos voltados para a prevenção e para a investigação das variáveis que interferem no bem-estar da população fazem-se necessários. Uma vez que dentre os vários fatores que influenciam a memória humana se encontram os hábitos alimentares, e levando em conta que a memória é uma característica multifatorial, é perfeitamente possível que a dieta possa interagir com o perfil genético individual para determinar a suscetibilidade de um indivíduo desenvolver déficit de memória.

A literatura demonstra que o selênio é um elemento traço nutricional multifacetado, ora capaz de ser tóxico, ora revela-se como um importante agente biológico para as mais diversas funções orgânicas, incluindo a remoção de espécies reativas de oxigênio no cérebro, por exemplo. Ademais, estudos sugerem que o declínio cognitivo está associado com a concentração sérica de selênio. Diante disso, surge a hipótese de que o selênio poderia ser utilizado para reduzir ou até mesmo retardar o déficit de memória, no entanto, SNPs em genes que codificam selenoproteínas podem alterar as necessidades individuais para selênio. Assim, esta pesquisa justifica-se por abranger em sua investigação fatores ambientais e genéticos que podem conduzir aos déficits de memória na população de adultos maduros e idosos. Conhece-los, poderá conduzir a estratégias para retardar ou até mesmo reduzir os déficits de memória. Para estudar a influência genética, propusemos em nossa investigação, a avaliação dos polimorfismos em *GPX1* (rs1050450) e *GPX4* (rs713041). Tais polimorfismos foram associados a doenças neurodegenerativas, no entanto, não havia registro na literatura sobre a influência desses polimorfismos sobre fenótipos como o CCL e memória episódica.

Além disso, propusemos em nossa investigação, avaliar o efeito dos polimorfismos em *GPX1* (rs1050450) e *GPX4* (rs713041) em pacientes com doença de Alzheimer. Para o gene *GPX1*, os estudos evidenciam a associação do polimorfismo rs1050450 e a doença de Alzheimer, no entanto, há resultados contraditórios acerca das associações alélicas, o que reforça a necessidade de mais investigações. Também constatamos a inexistência de estudos de associação e desfecho cognitivo para o

polimorfismo rs713041 do gene *GPX4*, sendo este estudo pioneiro nesse sentido. Assim, os dados provenientes deste estudo auxiliarão na construção de conhecimentos essenciais para a compreensão dos mecanismos moleculares subjacentes do micronutriente selênio sobre à memória humana.

4 OBJETIVOS

4.1 Objetivo geral

Investigar a influência genética na memória episódica através da avaliação de polimorfismos em genes de selenoproteínas em adultos maduros e idosos.

4.2 Objetivos específicos

- Determinar a influência dos polimorfismos rs1050450 do gene *GPX1* e rs713041 do gene *GPX4* sobre a variabilidade em escores de memória;
- Determinar a influência dos polimorfismos rs1050450 do gene *GPX1* e rs713041 do gene *GPX4* em indivíduos com e sem déficits para cada memória episódica avaliada;
- Determinar a influência dos polimorfismos rs1050450 do gene *GPX1* e rs713041 do gene *GPX4* sobre o comprometimento cognitivo leve e Doença de Alzheimer;
- Determinar a influência dos polimorfismos rs1050450 do gene *GPX1* e rs713041 do gene *GPX4* sobre as concentrações séricas de selênio;
- Determinar a influência das concentrações séricas de selênio sobre a memória episódica.

5 ARTIGO CIENTÍFICO 1

Association of GPX1 and GPX4 polymorphisms with episodic memory and Alzheimer's disease.

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Research article

Association of *GPX1* and *GPX4* polymorphisms with episodic memory and Alzheimer's disease

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ABSTRACT

It is well established that healthy aging, mild cognitive impairment (MCI), and Alzheimer's disease (AD) are associated with substantial declines in episodic memory. However, there is still debate about the roles of *GPX1* and *GPX4* polymorphisms. The aim of this study was to investigate the association of rs1050450 and rs713041 polymorphisms with memory. This research was composed of a cross-sectional study (334 subjects) and a case-control study (108 healthy controls and 103 with AD-NINCDS/ARDA, DSM-IV-TR criteria). For the association of the genetic polymorphisms with memory or cognitive loss, the phenotypes were analyzed as follows: 1) each memory as a quantitative trait; 2) presence of deficit on a specific memory; 3) presence of MCI; 4) presence of AD. To assess verbal learning and the ability to store new information, we used the Rey Verbal Learning Test. Scores were recorded as a function of age as in the WMS-R testing battery. DNA was obtained from whole blood, and genotypes for *GPX1* (rs1050450) and *GPX4* (rs713041) were detected by allelic discrimination assay using TaqMan® MGB probes on a real-time PCR system. *GPX1* TT homozygotes had lower long-term visual memory scores than CC/CT group (-0.28 ± 1.03 vs. 0.13 ± 1.03, respectively, $p = 0.017$). For the *GPX4* rs713041, the frequency of the TT genotype was higher in the group with normal scores than in the group with long-term visual memory deficits ($p = 0.025$). In a multivariate logistic regression, *GPX1* CC homozygotes had a 2.85 higher chance of developing AD (OR = 2.85, CI95% = 1.04–7.78, $p = 0.041$) in comparison to the reference genotype. No significant differences were observed regarding the MCI group between genetic variants. This study is one of the first to show that polymorphisms in *GPX1* and *GPX4* are significantly associated with episodic memory and AD in a South Brazilian population.

1. Introduction

Aging is associated with changes in a variety of cognitive functions, including executive function, episodic memory, and working memory. Studies of the cognitive neuroscience of aging have demonstrated that episodic memory is considered the form of long-term memory that displays the largest degree of age-related decline [1,2]. Episodic memory is considered a major neurocognitive system that enables conscious recollection of past experiences and, from a genetic standpoint, can be defined as a complex behavioral trait with substantial heritability estimates [3]. Specific life-style and genetic factors are related to the risk of memory deficits and neurodegenerative diseases, such as Alzheimer's disease (AD). AD, the most common form of dementia, is characterized by the loss of episodic memory and other cognitive domains [4]. However, some people do not have explicit clinical symptoms of dementia but rather exhibit an intermediate state between normal aging and dementia. Mild cognitive impairment (MCI) refers to cognitive decline in the elderly that is more pronounced than in normal aging but does not constitute detectable dementia, although it may be referred to as the preclinical stage of AD [5]. In a *meta*-analysis that included 13 clinical studies involving 4301 subjects, the annual conversion rate of MCI to dementia was 9.6%, and throughout the

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follow-up period, 39.2% converted to dementia [6]. Although there is a high degree of heritability for AD, with estimates ranging from 58 to 79% [7], a number of genetic risk factors are shared with MCI [5]. Mutations that predispose one to the occurrence of early-onset familial Alzheimer's disease are already well characterized [8]. Genome-wide association studies (GWAS) have identified several additional single nucleotide polymorphisms (SNPs) that appear to predict the susceptibility of memory performance [9,10] but do not explain all of the heritability.

Oxidative stress has a central role in memory deficits, and understanding the function of SNPs involved with antioxidant enzymes is fundamental. The glutathione peroxidase (GPx) family comprises eight sequentially numbered isoenzymes that catalyze the reduction of H_2O_2 and organic hydroperoxides by glutathione (GSH) or other biological reductants [11]. In the brain, GPx enzymes are expressed in neurons and glial cells, where their free-radical-scavenging role protects against oxidative stress. The human *GPX1* gene is located at chromosome 3p21, and a SNP at nucleotide 593 (rs1050450), which is a C to T substitution in exon 2, results in an amino acid change from proline (Pro) to leucine (Leu) at codon 198 [12]. In a study of an Ecuadorian population [13], the Leu allele of *GPX1* increased risk for AD. However, Cardoso [14] did not observe differences in genotype frequencies between patients with AD and a control group in a Brazilian population. The human GPx4 protein, unlike GPx1, is capable of metabolizing fatty acid hydroperoxides esterified to phospholipids, which are likely to occur in cell membranes undergoing oxidative stress [15]. Furthermore, GPx4 is the most widely expressed isoform in brain tissue and is found in neurons of the cerebellum, hippocampus and hypothalamus, thus supporting the hypothesis that GPx4 plays a role in preventing neurodegeneration [11]. A study with mouse model suggest that mice with a neuron-specific deletion of GPx4 had mild neurological dysfunction [16]. The *GPX4* gene is located at chromosome 19p13.3 and has a SNP at nucleotide 718, associated with a C to T substitution (rs713041) located in the 3′ untranslated region (3′UTR), that modulates the synthesis of GPx4 by changing the affinity of the SeCys insertion machinery [15]. Moreover, recent studies associated this polymorphism with antioxidant function [17] and cancer [18]. However, to our knowledge, no previous study has examined the effect of *GPX4* polymorphisms on episodic memory, AD and MCI. Therefore, the purpose of this study was to evaluate the implications of *GPX1* rs1050450 and *GPX4* rs713041 on memory by investigating a) individuals with and without deficits for each episodic memory evaluated, b) individuals with and without MCI, and c) AD patients and a control group. All participants were from a South Brazilian population.

2. Material and methods

2.1. Study design and participants

This research was composed of a cross-sectional study and a case-control study.

The cross-sectional study was composed of men and women (456 individuals in total). The selection of the participants was performed according to the following criteria: minimum age of 50 years, fluency in Portuguese, and the absence of any neurological or psychiatric illness such as psychosis or depression.

The volunteers were asked about the occurrence of neurological disease or psychiatric disorders and the use of anxiolytic drugs. The Beck Depression and Anxiety Inventory and the Lipp Inventory of Stress [19] were used to exclude volunteers who presented the indicated symptoms. Volunteers were excluded if they presented a lower estimate of intellectual function (IQ) ≤ 70. Using these exclusion criteria, 122 individuals were removed from the study. Evaluations of verbal and visual memory were performed using the Wechsler Memory Scale-Revised (WMS-R) [20]. This test allows the evaluation of 4 types of episodic memory: short-term visual memory, long-term visual memory, short-term verbal memory and long-term verbal memory. To assess verbal learning and the ability to store new information, we used the Rey Verbal Learning Test [21]. Scores were recorded as a function of age as in the WMS-R testing battery. For each test, the final data were transformed into standard deviations of the mean as a function of age, resulting in a range from -4 to $+4$. In order to evaluate the association of the genetic polymorphisms with memory or cognitive loss, we analyzed the phenotypes as follows: 1) each memory as a quantitative trait; 2) presence of deficit on a specific memory (cognitive loss in relation to each evaluated memory was determined when the score was ≤ -1 standard deviation below the mean values normalized for age according WMS-R testing battery); 3) presence of MCI (subjects with two or more scores ≤ -1 in the evaluated memories). The diagnosis of MCI was also based on neuropsychological evaluations that included cognitive alteration but not dementia, and evidence of cognitive loss was verified by subjective interviews and by objective evaluations, such as evaluations of the preservation of activities of daily living.

For the case-control study, we compared a group of Alzheimer's disease patients to a group of healthy control subjects. A total of 103 elderly adults were diagnosed with probable AD according to the NINCDS-ADRDA and DSM-IV-TR. This diagnosis, as well as the inclusion and exclusion criteria for the AD group, was described in detail in Pezzi et al. [22]. AD patients were recruited for convenience from two academic outpatient neuropsychiatric services located in a southern Brazilian city.

A control group of 108 healthy elderly volunteers with normal cognitive function and gender matched to those individuals in the AD group were enrolled in the study and recruited from the catchment areas of the same academic services. The inclusion criteria were as follows: age greater than 65 years, clinical dementia rating (CDR) of 0–27, Mini-Mental State Examination (MMSE) score higher than 26 and independence in completing activities of daily living (ADL) [23]. Control volunteers were excluded if they presented chronic renal disease, a history of significant head injury or stroke, a history of cancer, a family history of dementia, other psychiatric conditions such as major affective disorder or evidence of current depression, uncorrectable vision or hearing loss or other conditions such as substance abuse or use of medications that could impair cognitive function. All participants of both groups were of European ancestry from southern Brazil.

Both studies were approved by the Ethics Committees of the participating institutions and were performed in compliance with the Declaration of Helsinki. All participants or their proxies in AD cases provided written informed consent.

2.2. Genotyping

Genomic DNA was extracted from 500 µL of EDTA-treated whole blood using the salting out method [24], and the concentration was assessed using a Biospec® Nano spectrophotometer (Shimadzu do Brasil). The final concentration of DNA used was 10 ng/µL.

Polymorphisms of *GPX1* (rs1050450) and *GPX4* (rs713041) genes were genotyped with the use of TaqMan Genotyping Master Mix and TaqMan SNP Genotyping assays (Applied Biosystems®). The assays were predesigned for rs713041 from Applied Biosystems (ID Assays C_2561693_20) and custom-made for rs1050450 through the Custom TaqMan® Genomic Assays service.

For each reaction plate, genomic DNA control samples and non-template controls (water) were included. A control of the TaqMan SNP genotyping assay was also performed (25% of randomly chosen samples from both groups) to check for genotyping accuracy, and identical genotypes were identified in all repeated samples. The researchers who performed the genotyping were blinded to the patients' diagnostic status.

2.3. Statistical analysis

The results were entered into a database, and the statistical package SPSS® version 19.0 was used to perform the analyses. Continuous variables were expressed as the mean ± standard deviation. Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy–Weinberg equilibrium expectations was tested using chi-square tests. Genotype distributions between groups (memory deficit x normal memory and MCI group x no-MCI group) were compared by chi-square or Fisher's exact test. After adjustment for sex and level of education through linear regression, specific memory scores (short-term and long-term verbal memories, short-term and long-term visual memories and verbal learning) were compared among genotypes by Student's *t*-test or ANOVA. In case of significant associations, the strength of the association was evaluated using Cramer's V (chi-square test) and partial Eta squared value (ANOVA). Univariate analyses to verify the associations between the polymorphisms in the genes *GPX1* and *GPX4* and Alzheimer's disease were carried out by chi-square association tests with a dominant model. Multivariate logistic regression analysis was performed to estimate the AD outcome, with polymorphisms as independent variables. The confounding variables entered in the model were age and education based on the literature review [25]. A two-tailed $p < 0.05$ was considered significant for all analyses.

3. Results

3.1. Cross-sectional study

After application of the exclusion criteria, 122 subjects were excluded from the study. A total of 334 subjects remained in the study, and the average of each episodic memory score, socio-demographic and lifestyle characteristics of the study population are described in Table 1. Overall, deficits in short-term verbal, long-term verbal, short-term visual and long-term visual memories were observed in 24.4%, 38.4%, 20.2%, and 19.3% of the sample population, respectively. Verbal learning deficits were observed in 26.8% of the sample population (data not shown). Mild cognitive impairment was observed in 45.9% of the sample.

The genotypic frequencies observed did not show statistically significant differences compared to those expected under Hardy–Weinberg equilibrium. The allelic frequencies for the polymorphisms were 33% for rs1050450T (*GPX1*) and 44.5% for rs713041T (*GPX4*). The observed allelic frequencies in this study were similar to those reported in SNP databases (Entrez SNP, International HapMap Project and 1000 Genomes) and in previous studies analyzing European or European-derived populations.

Table 1
Demographic characteristics of the cross-sectional study group.

Characteristic	
n	334
Age (years)	63.4 ± 7.9
Sex (% men)	69 (20.6)
Years of education	10.5 ± 4.8
Hormonal replacement therapy, women only (%)	65 (19.4)
Age at completion of education (years)	25.9 ± 13.5
Years without studying	35.1 ± 17.7
Cigarette smoking	
Never	231 (69%)
Past	90 (26.9%)
Current	14 (4.1%)
Mild cognitive impairment	146 (45.9%)
Memory scores	
Short-term verbal memory	0.03 ± 1.30
Long-term verbal memory	−0.08 ± 1.28
Short-term visual memory	0.05 ± 1.02
Long-term visual memory	0.06 ± 1.03

The values shown are the mean ± standard deviation or numbers and percentages in parentheses.

After adjusting each specific type of episodic memory for level of education and sex, the comparison of the three genotypes of *GPX1* rs1050450 showed a borderline association with scores for long-term visual memory ($p = 0.051$, partial Eta squared = 0.1364, Table 2). Using a recessive model, TT homozygotes had lower long-term visual memory scores than CC/CT genotypes (−0.28 ± 1.03 vs. 0.13 ± 1.03, respectively, $p = 0.017$). For the *GPX4* SNP rs713041, the chi-square test demonstrated a significant association with deficit of long-term visual memory. The frequency of the TT genotype is higher in the group with normal scores than in subjects with deficits in long-term visual memory ($p = 0.025$, Cramer's V = 0.155) (data not show). No significant differences were observed regarding the MCI group among genetic variants.

3.2. Case-control study

The subjects of this study (n = 211) consisted of 103 individuals with AD (61.5% females; aged 76.67 ± 7.34 years) and 108 healthy elderly individuals (71% females; aged 74.96 ± 7.73 years). Demographic characteristics, as well as the genotype and distribution of *GPX1* and *GPX4* for each group, are shown in Table 3. Individuals with AD showed significantly lower scores on the MMSE (p 0.0001) and lower educational level ($p < 0.0001$) than healthy control subjects. *GPX1* TT homozygotes were more frequent among individuals in the control group (15.7%) than in patients with AD (7.8%, $p = 0.034$) (Table 3). In a multivariate logistic regression, *GPX1* CC homozygotes had a 2.85 higher chance of developing AD (OR = 2.85, CI95% = 1.04–7.78, $p = 0.041$) compared to the reference genotype.

4. Discussion

In this study, we investigated the association of polymorphisms in the *GPX1* gene (rs1050450) and *GPX4* gene (rs713041) with memory and AD. The results of the cross-sectional study, conducted on 334 individuals, revealed that episodic memory scores may be associated with *GPX1* and *GPX4* genotypes. We showed that for the *GPX1* gene, TT homozygotes presented lower scores of long-term visual memory. Moreover, for *GPX4* gene, qui-square test demonstrated that the TT genotype is present at a higher frequency in the group with normal scores for long-term visual memory. For the case-control study, the *GPX1* CC homozygotes had a higher chance of developing AD. No association was found with MCI.

The rs1050450T (leucine) allele on *GPX1* has been associated with a higher risk of some disease conditions, such a lung and bladder cancers [12,26,27], and the authors suggest that an important change in the conformation of GPx1 may be responsible because Pro is the only amino acid without a free unsubstituted amino group on the alpha carbon atom [28]. However, the studies are not conclusive as the association of Pro198Leu and the enzymatic activity of GPX1. Three studies measuring the GPX1 activity (with respect to the rs1050450) in human erythrocyte extracts have been reported the activity of CT/TT extracts was found to be 9% lower than the activity in CC extracts [29] and the activity was 13% lower in TT males as compared to CT/CC males [30], while others did not verify differences between the genotypes [31–33]. Forsberg et al. suggested that GPX1 presents high stability and that its activity can be stimulated in the presence of reactive oxygen species, which would compensate for the reduction caused by the presence of the variant allele [31]. Soerensen et al. also used this hypothesis in their cohort study to explain the reduction in mortality in carriers of the T allele, in addition, the author suggests that there may be an antagonistic pleiotropic effect of the SNP in Danes since mortality reduction was observed only in the elderly to very old age [34]. Based on this

Table 2
Polymorphisms in *GPX1* and *GPX4* and the five types of episodic memories.

Genotype	n*	Short-term Verbal Memory	Long term Verbal Memory	Short-term Visual Memory	Long-term Visual Memory	Verbal Learning Memory
GPx1 (rs1050450)						
CC	138	0.11 ± 1.30	−0.00 ± 1.33	0.01 ± 0.94	0.13 ± 0.98	0.01 ± 1.22
CT	132	0.00 ± 1.23	−0.12 ± 1.21	0.10 ± 1.10	0.14 ± 1.08	−0.08 ± 1.13
TT	41	−0.04 ± 1.30	−0.19 ± 1.34	−0.20 ± 0.98	−0.28 ± 1.03	−0.41 ± 1.21
P		0.615	0.586	0.203	0.051**	0.104
GPx4 (rs711031)						
CC	89	0.07 ± 1.28	−0.06 ± 1.27	−0.01 ± 1.00	−0.05 ± 0.98	0.06 ± 1.27
CT	144	0.01 ± 1.35	−0.01 ± 1.32	0.07 ± 1.08	0.08 ± 1.14	−0.14 ± 1.23
TT	73	0.08 ± 1.25	0.08 ± 1.20	0.13 ± 0.95	0.24 ± 0.88	−0.12 ± 1.12
P		0.932	0.393	0.636	0.185	0.636

Each specific memory score was adjusted for sex and level of education through linear regression. * Sample composed by all subjects from the cross-sectional study. ** TT versus CT/CC, p = 0.017.

Table 3
Demographic characteristics and genotype frequencies of *GPX1* and *GPX4* polymorphisms of the healthy control and Alzheimer's disease groups.

Variable	Control Group (n = 108)	AD Group (n = 103)	P
Sex (female)	77 (71)	64 (61.5)	0.146**
Age (years) means/ SD)	74.96 (7.73)	76.67 (7.34)	0.099*
Education (years)	7.95 (4.17)	5.03 (3.06)	< 0.0001*
MMSE	27.55(2.02)	12.72 (5.55)	< 0.0001**
GPX1 (rs1050450)			
CC (%)	44 (40.7)	59 (57.3)***	
CT (%)	47 (43.5)	36 (35)	
TT (%)	17(15.7)	8 (7.8)	0.034
GPX4 (rs713041)			
CC (%)	34 (32.4)	28 (27.5)	
CT (%)	42 (40)	49 (48)	
TT (%)	29 (27.6)	25 (24.5)	0.504

AD, Alzheimer's disease; MMSE, Mini Mental State Examination score
*Mann-Whitney test, ** Chi-square test, *** OR = 2.84, CI95% = 1.13–7.20, p = 0.027; Adjusted OR = 2.85, CI95% = 1.04–7.78, p = 0.041. Adjusted OR calculated considering age and educational level as confounders.

observation, we may presume that memory scores will change in a different way in individuals with different *GPX1* genotypes and that this change may be a reflection of change enzymatic activity.

Recently, there has been considerable interest in the biological and clinical consequences of *GPX1* polymorphisms on AD risk [14], although the precise mechanism by which the SNP influences AD and cognitive decline are unclear. Paz-y-Miño [13] determined the prevalence of the rs1050450 polymorphism in an Ecuadorian population and observed that the frequency the T allele is higher in the AD group and that the TT genotype provides a relative risk of 7.2 for AD. In contrast, we observed that the frequency of T homozygotes was significantly higher in our control group and that the CC genotype was associated with a 2.85-increase in the risk of AD (CI95% = 1.04–7.78, p = 0.041). One of the reasons for these results might be the difference in ethnic backgrounds and lifestyles of the study populations. In addition, if we assume that the T allele is responsible for the decrease in memory scores, we will observe an antagonistic effect of rs1050450 in the AD group, since, that the *GPX1* TT shows some deficits in particular memory domains with ageing, but then appears to be associated with reduced risk of dementia. Here, we determined the genotype frequency in a South Brazilian population, and the results regarding the homozygous T frequency were quite similar to those of other studies [28–30], including an additional study of a Brazilian population. Cardoso [14] did not observe differences in genotype frequencies between patients with AD and the control group. An important point to consider in these previous studies [13,14] is the limited number of affected and control individuals who were analyzed. Our case-control study consisted of 103 affected and 108 control individuals, a sample size almost twice bigger the size of the studies mentioned above. Moreover, in our case-control study, the frequency of C homozygotes was higher in patients with AD, in contrast to the hypothesis raised by Paz-Y-Miño [13] that this allele may have a protective effect against the disease.

Numerous studies characterizing the function of GPx4 have demonstrated that this selenoenzyme is protective against oxidative stress. In neurodegenerative diseases, accumulating data suggest that increased lipid peroxidation is an early symptom of AD. For example, Williams [35] reported that subjects with MCI and early AD had significantly elevated levels of biomarkers of lipid peroxidation in brain regions. Ferroptosis, a newly identified oxidative cell death mechanism triggered by massive lipid peroxidation, is implicated in the degeneration of neuron populations such as spinal motor neurons and midbrain neurons. Recently, Hambright et al. [16] reported the participation of this mechanism in neurodegeneration. Adult mice (3–4 months of age), with neuronal GPx4 conditionally ablated in neurons via tamoxifen treatment, presented a striking paralysis phenotype associated with motor neuron degeneration with ferroptosis-like features, whereas neurons in the cerebral cortex were not affected. It is important to highlight that these cited studies also serve to illustrate the absence of neurobiological studies for *GPX4* in humans.

Animal study model support the hypothesis of the antioxidant and neuroprotective role of GPX4; however, there is a very important gap to be filled regarding the effects of *GPX4* polymorphisms on episodic memory and neurodegenerative diseases. This is the first study that evaluated the association of the rs713041 polymorphism in *GPX4* with human episodic memory in healthy subjects and patients with AD. Our findings suggest that T homozygotes are more frequent in the subjects without long-term memory deficits, which may reveal a neuroprotective role for this genotype. However, the genotype frequencies were not significantly different between the AD and control groups.

Episodic memory is a major neurocognitive system that enables conscious recollection of experiences. As described in this paper the episodic memory can be classified according to their content to two forms: visual and verbal. Visual episodic memory is directly involved in the perception of the environment, being related to the ability to remember images, such as symbols, drawings, photos or other graphic resources. On the other hand, verbal episodic memory consists of the ability to store facts or events. Healthy aging is associated with impairment of episodic memory, although not all forms of episodic memory are equally affected by advancing age. In our study, we found the participation of *GPX1* and *GPX4* polymorphisms only on visual memory. This suggests that both polymorphisms selectively influence only one component of memory. In this regard, it may be possible that *GPX1* and *GPX4* genes variations may combine with other gene polymorphisms, as with other genes [36,37] to influence memory performance.

In our cross-sectional study, we evaluated five kinds of episodic memory in mature and elderly adults, and the results indicate that long-term visual memory showed lower scores and that this was genotype

dependent. We did not identify genotypic associations for the MCI group, and our hypothesis for this conflicting result is that a longitudinal study is necessary to detect an association since the rate of conversion from MCI to dementia is estimated to be between 5 and 10% per year [38]. Moreover, there is controversy if MCI is a clinical entity or prodromal dementia [39,40]. Many subjects identified with MCI do not worsen over the time and MCI presents high heterogeneity, with different clinical and etiological subtypes.

For the case-control design, individuals with AD also showed significantly lower scores on the MMSE ($P < 0.0001$) and had a lower educational level ($P < 0.0001$) than healthy control subjects. The possibility of a connection between life experience and the prevalence of dementia has long been discussed [41]. Epidemiologic studies suggest that lifetime exposure, including educational and occupational attainment and leisure activities late in life, could slow cognitive aging or reduce the risk of dementia [42,43].

Despite a well-controlled protocol, this study had some limitations. We did not measure oxidative stress markers such as malondialdehyde and lipid peroxidation or determine the enzymatic activity of GPx1 and GPx4, which is important since some studies suggest that cognitive decline is associated with reduced enzyme activity. This is an exploratory study, which a small sample size that suggests association between polymorphisms of *GPX1* and *GPX4* and memory and AD. Therefore, these results need to be replicated in other samples. Moreover, the analyzed observed *p*-values are marginal significant and the effect size of each analyzed polymorphism is small.

In addition, as with any complex genetic trait, episodic memory is a result of an assembly of phenotypes with gene–gene and gene-environment interactions in addition to epigenetic mechanisms. Despite this obvious complexity, the literature supports the notion that behavioral genetic studies of episodic memory successfully identify genes and pathways associated with traits. In conclusion, our findings provide the foundation for additional research regarding *GPX1* and *GPX4* and the identification of molecular pathways related to human episodic memory and Alzheimer's disease.

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6 ARTIGO CIENTÍFICO 2

Effects of selenium serum in memory performance in population of mature and elderly adults.

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Abstract:

Selenium (Se) is an antioxidant micronutrient with potential interest for brain function. However, the effects of serum Se in memory performance in healthy population are largely unknown. Various functional single-nucleotide polymorphisms (SNPs) may affect the efficacy of Se utilization. These include the glutathione peroxidases *GPX1* rs1050450 and *GPX4* rs713041. This cross-sectional study measured serum Se levels of 156 Brazilian healthy elderly. Serum Se was measured using atomic absorption spectrophotometry. The SNPs *GPX1* rs1050450 and *GPX4* rs713041 were determined by PCR real-time. The evaluations of verbal and visual memory were performed using the revised Weschler Memory Scale and the Rey's verbal learning test. Significant correlations between serum Se level and verbal memories were found and a multiple linear regression model indicated that Se levels are a predictor for Verbal Learning memory (multivariate regression coefficient = 0.541, standard error = 0.244, p=0.028), accounting for 3.28% of variability of verbal learning memory. In addition, we observed a significant correlation between schooling and concentration of Se (r=0.211, p=0.009).No significant effects of *GPX1* and *GPX4* genotypes on Se concentration were observed. These results indicate that Se is associated with memory performance, especially verbal ones.

Abbreviations:

Se, Selenium; GPX, Glutathione peroxidases; SNP, Single-nucleotide polymorphisms ; AD, Alzheimer disease; WMS-R, Weschler Memory Scale-revised ; RDA, Recommended daily allowance.

1.Introdution

Selenium (Se) has been widely recognized as a vital trace element of our diet with multiple and complex effects on human health through its antioxidant action. In the central nervous system, Se is an essential element involved in diverse functions [1,2] its deficiency has been associated with cognitive decline [3,4]. Se is incorporated as selenocysteine amino acid residue in a small but vital family of proteins, namely the selenoproteins. In human, twenty five selenoprotein genes have been characterized [5], and many selenoproteins are of paramount interest in relation to neurobiology due to their antioxidant function, such as glutathione peroxidase (GPX) 1 and 4 [6,7]. GPX1 and GPX4 are major forms of GPX in brain [8]. Se concentration variability may be associated to single nucleotide polymorphisms (SNPs) in genes encoding selenoproteins and negatively influence the normally protective roles against oxidative stress [9]. Pay-Y-Miño et al. reported an association between ther rs1050450SNP in *GPX1* and increased risk for Alzheimer disease (AD) and Jablonska et al. suggested that SNPs in selenoprotein encoding genes might affect the plasma selenium concentration [10,11].

GPX4 functions in various locations of the neuron including the cytosol, mitochondria and nucleus [6] and seems to play a particularly important role in the glutathione-dependant antioxidant system [12]. Although the level of GPX4 expression is correlated with antioxidant function of central nervous system, no link has been found between a series of genetic variations and cognitive decline. The *GPX4* rs713041 C to T polymorphism can modulate GPx4 activity by altering selenocysteine insertion and protein binding to the 3'UTR, [13,14] and although research suggest the beneficial neurobiology properties of GPX4,

to date there is no investigation of the role of polymorphism on memory in healthy individuals. The term memory covers different systems that can be differentiated by the type of information processed. Declarative memory is a major neurocognitive system that involves the conscious recollection about facts (semantic memory) and events (episodic memory) [15]. Decline in memory performance, particularly episodic memory, is a major characteristic of dementia, particularly in early-middle stages. However, it is also well established that there is normal aging-associated decline of episodic memory in the absence of dementia [16,17].

From a genetic standpoint, memory can be defined as a genetically complex behavioral trait with substantial influence on the life-style including education level and diet [18]. Although findings are not unanimous, observational studies generally pointed out a positive correlation between cognitive functions and antioxidant dietary intakes [19,20]. About Se levels, studies in humans have reported negative correlations between cognitive decline and Se levels [21]. Such insight assists in informing the development of strategies to increase resistance to memory decline in older adults. Since Se was shown to be involved in diverse functions of the central nervous system, we suppose is that Se concentration is correlated to memory performance, and that *GPX1* and *GPX4* polymorphisms may influence Se levels.

2. Materials and methods

2.1. Subjects

The sample was composed of men and women (334 individuals in total) who were selected according to the inclusion criteria: minimum age of 50 years, absence of dementia, owning intellect enough to continue production, as

determined by the National Institute of Mental Health [22]. The volunteers were asked about the occurrence of neurological disease or psychiatric disorders and the use of anxiolytic drugs. The Beck Depression [23], Anxiety Inventory [24] and the Lipp Inventory of Stress [25] were used to exclude volunteers who presented the indicated symptoms. Volunteers were excluded when presenting with lower estimate intellectual function (QI) ≤70, and individuals who use vitamin supplements containing Se were also excluded. Using these exclusion criteria, 122 individuals were removed from the study. This study followed the principles outlined in the Declaration of Helsinki of 1975 as revised in 1983, and registered at Brazil platform number 1.596.199.

2.2 Neuropsychological measures

Evaluations of verbal and visual memory were performed using the Weschler Memory Scale-revised (WMS-R) [26]. For each test, the final data were transformed into standard deviations of the mean as a function of age, resulting in a range from -4 to + 4. To assess verbal learning and the ability to store new information, we used Rey Auditory Verbal Learning Test (RAVLT) [27]. Scores were recorded as a function of age, just as in the WMS-R testing battery.

2.2 Selenium concentration

For the analysis of Se, blood was stored in metal-free tubes (BD Vacutainer, Trace Elements Serum). Blood was allowed to clot at room temperature for 30 min according to the manufacturer's instructions. Once the blood had clotted completely, it was centrifuged for 10 min at 2,500 rpm. After centrifugation, the serum was fractionated into specific transport tubes and frozen at -80ºC until the completion of concentration measurements. The

samples were sent to a third-party laboratory that analyzed the samples using standardized methods, as previously described [28]. Se concentration was measured for atomic absorption spectrometry in a graphite furnace, with a linearity of 40 µg/dL, standard curve from 4 to 40 µg/dL, and a detection limit of 0.1 µg/dL. Serum reference values were 4.6–14.3 µg/dL for selenium [29]. A Se level below the cutoff concentrations was considered low, levels between the reference values were considered normal. These cutoff points were established in order to determine differences in serum concentrations between genotype groups on memory scores.

2.3 *DNA extraction and genotyping*

Genomic DNA was isolated from peripheral blood leukocytes using a standard salting-out procedure [30]. DNA quality and purity were analyzed by spectrophotometry using the Nanodrop ND-1000. Polymorphisms of *GPX1* (rs1050450) and *GPX4* (rs713041) genes were genotyped with the use of TaqMan Genotyping Master Mix and TaqMan SNP Genotyping assays (Applied Biosystems®).The assays were obtained as pre-designed from Applied Biosystems for rs713041 (ID Assays C__2561693_20) and custom-made through Custom TaqMan ® Genomic Assays service for rs1050450.

2.4 Statistical analysis

The results were entered into a database, and statistical package SPSS® version 19.0 was used to perform the analyses. Continuous variables were expressed as the mean ± standard deviation. Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy–Weinberg equilibrium expectations was tested using χ^2 tests. Univariate analyses to verify the associations between the polymorphisms in the genes

GPX1 and *GPX4* and selenium concentration were carried out by ANOVA. Pearson correlation and Student t-test were used to verify the association between each evaluated memory and Se concentration. A multivariate regression model was constructed to evaluate the contribution of sex, schooling (years) and Se concentration (< and ≥ 4.7µg/dL) on verbal memories. A two-tailed $p < 0.05$ was considered significant for all analyses.

3. Results

A total of 156 healthy men and women aged 50 to 85 (mean 64.3 ± 8.4 years) participated in the study. The mean serum Se concentration was 5.32 ± 1.43 µg/dL and 22.2% of the sample present Se concentration below the reference value. There was no difference between the Se concentration and the sexes (data not shown). We observed a significant correlation between schooling and concentration of Se. Pearson correlation indicated that the higher the schooling the higher the concentration of serum Se ($r=0.211$, $p=0.009$). Overall, deficits in Short-term verbal, Long-term verbal, Short-term visual, and Long-term visual memories were observed in 22.1%, 27.5%, 18.1% and 17.4% subjects, respectively. Verbal learning deficits were observed in 26.8% of the sample. Baseline characteristics the population study are shown in Table 1.

3.2 Allele, genotype frequency and selenium concentration

The genotypic frequencies observed did not show statistically significant differences compared to those expected under Hardy–Weinberg equilibrium. The allelic frequencies for the polymorphisms were 27% for rs1050450T (*GPX1*) and 50% for rs713041T (*GPX4*). The observed allelic frequencies in this study were similar to those reported in SNP databases (1000genomes) and

in previous studies analyzing European or European-derived populations [31] . No significant effect of *GPX1* and *GPX4* genotypes on Se concentration were observed (supplementary Table 1).

3.3 Correlation between selenium concentration and memories

In our study, we tested the effect of Se concentration on memory using two approaches. In the first approach, Se concentration was positively correlated with all analyzed verbal memories (Table 2). Using Se cut-off values (≤4.6 µg/dL and ≥ 4.7µg/dL), we observed that subjects with decreased Se concentration (≤ 4.6 µg/dL) showed lower scores of verbal memories (Table 3).

Considering that Se concentration, schooling and sex individually affect memory, a multiple linear regression model was created to verify the effect of these factors on each of verbal memory analyzed (Table 4). In this analysis, we observed that, after correction for sex and schooling, Se levels remained a statistical significant predictor for Verbal Learning memory (regression coefficient = 0.541, standard error = 0.244, p=0.028), accounting for 3.28% of variability of verbal learning memory.

4. Discussion.

In this study, we examined the possible association of Se serum concentration with performance memory in elderly subjects. In addition, we evaluated the association of *GPX1* and *GPX4* polymorphisms with Se concentration. Our major findings were the positive correlation of Se and verbal memories that suggest a beneficial effect of Se on memory.

Se could be implicated in the protection of cognitive function, however, the influence of Se on cognitive performance has been investigated in cross-sectional and prospective studies, and regardless of the design, there are

contradictory results. Cardoso et al [21] and Vural et al [32] verified that AD patients had lower Se levels than healthy elderly people whereas Ceballos-Picot et al [33] found increased levels of plasma Se in patients with AD when compared to the control group. On the other hand, Smorgon et al [34], when evaluating the association between trace element and cognitive performance in different groups with neurodegenerative diseases, found a direct correlation between plasma Se concentration and cognitive function level, and, therefore, patients with AD presented reduced levels of this mineral when compared to the control group. In our population, Se had a consistent, dose-response relation with verbal memories performance, such that higher selenium levels were associated with better cognition. After correction for sex and schooling, Se levels remained a statistical significant predictor for verbal learning memory accounting for 3.28% of variability of verbal learning memory. Our data corroborate with the cross-sectional study in Chinese population. The neuropsychological evaluation of this group measured in general, verbal memory, and identified that lower selenium levels were significantly associated with lower cognitive scores [36]. The concentration of Se in plasma or serum is often used as an index of Se status in epidemiologic and clinical studies [4, 37,38]. Our investigation is also in agreement with the longitudinal follow-up of the Etude du Vieillissement Arteriel cohort [4] which cohort reported a significant protective plasma Se level on cognitive decline. Our data also show that volunteers with a better cognitive function have higher serum Se concentration.

The US recommended daily allowance (RDA) for Se is 55 µg/day being the tolerable intake level the 400µg/day [39]. Thus, the level of Se in the body is critical because low or too high concentration leads to serious health

consequences. Furthermore, different geographic and ethnic population groups showing varied conditions once optimum Se level may vary according to life stage, general state of health and genotype [39,40]. The current study evaluated memory performance in Brazilian healthy elderly that most of them did not present Se deficiency. Although the serum is the favored biomarkers for comparison of Se status among countries [41] few studies about Se status in Brazilian elderly have been developed so far [42- 44], and some studies have investigated Se participation in extreme cognitive phenotypes. Thus, this is the first work that provides clues about Se and cognition in Brazilian healthy elderly population.

The association between low education and dementia syndromes is supported the majority studies [44,45], but little information exists concerning the effects of lifestyle on Se requirements, other than that smoking [9]. In our study, for example, schooling was directly correlated with the concentration of Se. The hypothesis for this finding is that the level of schooling is a variable capable of interfering with the way the population chooses their food, which can be decisive for the quality of self-care and the ability to interpret information health protection [46,47]. In this study, we also evaluated the association of *GPX1* rs1050450 and *GPX4* rs713041 and Se concentration. Various functional SNP may affect the efficacy of Se utilization and *GPX1* and *GPX4* genes were studied and associations described in several pathologies [48-50]. These researches are focused on identifying the possible phenotypic consequences among which may lead to inter-individual variations in response to the micronutrient Se. Thus, the combination of polymorphisms in selenoprotein-encoding genes with inadequate Se consumption could negatively influence

antioxidant mechanisms, increasing susceptibility to some diseases. On the other hand, variations in selenoprotein genes could also significantly interfere in the response to Se consumption [38,39]. Although there is evidence of gene-nutrient association for polymorphism *GPX1* rs1050450, a limited number of studies have investigated genetic variations within the *GPX4* gene that could potentially alter its function. To our knowledge, this is the first study examining Se levels together with *GPX1* and *GPX4.*

The limitations of our study include the small sample size for some of the comparisons, the low number of SNP, the absence of a 24-hour diet recall to estimate the intake of Se. We did not use the 24-hour diet recall to estimate the intake of Se because dietary intake accuracy based on 24-hour recalls is known to be influenced by memory errors. This could result in overreporting or underreporting of food intake, especially among the elderly.Finally, another limitation, up to now there is no transparency whether the concentration of Se in the brain can be adequately determined by measuring its content in hairs, toenails, plasma, serum, erythrocytes and even cerebrospinal fluid and how the different measurements relate to each other [51,52].

As the life stage is an important parameter to establish the ideal amount of intake, and given the importance of Selenium to the central nervous system, further studies in healthy elderly population are necessary to determine the appropriate level for neuroprotection. Despite these limitations, this study is the first to demonstrate the influence of Se in verbal memory, in healthy elderly. Thus, are results of our study suggest that more research must be performed to elucidate the role of Se in performance memory and polymorphisms in the serum concentrations of Se.

5. Duality of interest

All authors have declared no conflict of interest relevant to this article

Appendix A. Supplementary data

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Table 1. Demographic characteristics of the study group (n=156).

Characteristic	%
Age (years), mean (SD)	64.3 ± 8.4
Sex (male)	13.1
Smoking status	
Never smoker	68.0
Former smoker	28.7
Current smoker	3.3
European ancestry	71.2
Selenium (µg/dL)[a], mean (SD)	5.32 ±1.43
≤ 4.6	22.2
4.7 – 14.3	77.8
Years of study, mean (SD)	11.5 ± 4.6
Age at completion of education (years) , mean (SD)	27.3 ± 13.1
Years without studying, mean (SD)	33.4 ± 17.4
Deficit in memory	
Short-term Verbal Memory	22.1
Long-term Verbal Memory	27.5
Short-term Visual Memory	18.1
Long-term Visual Memory	17.4
Verbal Learning Memory	26.8

The values shown are percentages or mean ± standard deviation (SD).

[a]The cutoff reference values were set according to [29]

Table 2. Pearson correlation between selenium concentration (µg/dL) and memory scores.

	Verbal memories			Visual memories	
	Short-term	Long-term	Verbal Learning	Short-term	Long-term
r	0.299	0.270	0.199	0.078	0.038
p	**<0.001**	**0.001**	**0.015**	0.346	0.648

Table 3. Association between selenium concentration and memories scores.

Selenium (µg/dL)	n	Verbal memories			Visual memories	
		Short-term	Long-term	Verbal Learning	Short-term	Long-term
≤ 4.6	38	-0.46±1.53	-0.49±1.41	-0.81±1.16	-0.17±1.03	-0.21±0.80
4.7-14.3	118	0.13±1.16	0.06±1.20	-0.11±1.28	0.01±0.97	-0.00±0.93
p		**0.017**	**0.025**	**0.005**	0.357	0.242

Table 4. Multivariate analysis for verbal memories scores.

Covariates	Regression coefficient ± standard error	Partial R^2 x 100	P
Verbal Learning Memory			
Constant	-1.629 ± 0.293		
Female Sex	0.763 ± 0.295	4.45	0.011
Years of education	0.072 ± 0.022	6.71	0.002
Selenium ≥ 4.7 µg/dL	0.541 ± 0.244	3.28	**0.028**
Short-term verbal Memory			
Constant	-1.351 ± 0.297		
Female Sex	0.185 ± 0.298	0.27	0.535
Years of education	0.093 ± 0.023	10.49	<0.001
Selenium ≥ 4.7 µg/dL	0.325 ± 0.247	1.18	0.190
Long-term verbal memory			
Constant	-1.536 ± 0.286		
Female Sex	0.144 ± 0.287	0.18	0.616
Years of education	0.111 ± 0.022	15.05	<0.001
Selenium ≥ 4.7 µg/dL	0.248 ± 0.238	0.75	0.189

Supplementary table 1. Levels of serum selenium according to genotype *GPX1* and *GPX4.*

Genotype	Selenium µg/dL (mean ± SD)
GPX1 rs1050450	
CC	5.35 ± 1.55
CT	5.08 ± 5.08
TT	5.57 ± 1.38
P	0.423
GPX4 rs713041	
CC	5.21 ± 1.10
CT	5.10 ± 1.35
TT	5.58 ± 1.66
P	0.212

7 CONCLUSÕES

O trabalho apresentado investigou a influência genética sobre a memória episódica através da avaliação de polimorfismos em genes de selenoproteínas em adultos maduros e idosos bem como em portadores de doença de Alzheimer.

A quantidade de estudos que avalia desordens neuropsicológicas e principalmente a memória tem aumentado e ganhado importância, em especial aqueles que focam em indivíduos com doença de Alzheimer e doenças cerebrovasculares.

Em relação à memória, têm sido investigados fatores que possam estar associados a desordens ou alterações cognitivas, sendo que alguns fatores têm sido frequentemente associados a essas alterações, como por exemplo, a genética individual, a idade, o fator educacional e ingestão de alimentos antioxidantes como o selênio. Nosso estudo realizou a abordagem desses fatores através da investigação dos polimorfismos em *GPX1* e *GPX4* e para avaliação da influência do selênio, realizamos a dosagem sérica desse elemento.

Assim como indica a literatura científica, a concentração sérica de selênio exerceu efeito positivo sobre a memória, no entanto, em nosso estudo, esse efeito foi observado apenas sobre as memórias verbais. Além disso, observamos uma correlação significativa entre escolaridade e concentração de selênio.

Diferente do esperado, os polimorfismos rs1050450 e rs713041 não refletiram mudanças na concentração sérica de selênio, mas observamos que existe diferença em relação aos genótipos e escores de memória. Por exemplo, homozigotos TT para o polimorfismo investigado em *GPX1* apresentaram menores escores de memória visual de longo prazo do que o grupo CC/CT. Para o rs713041 (*GPX4*), a frequência do genótipo TT foi maior no grupo com escores normais do que no grupo com déficits para memória visual de longo prazo.

Este estudo apresenta algumas limitações, em especial por não ter avaliado a atividade enzimática de GPX1 e GPX4. Também é importante mencionar que múltiplos polimorfismos em genes de selenoproteínas e de outras enzimas antioxidantes podem cooperar para gerar diferentes respostas fenotípicas, e assim os efeitos dos SNPs podem ser alterados pela presença de outros SNPs. Nesse contexto, estudos que associam polimorfismos em genes de selenoproteínas e outros SNPs, relacionados a diferentes condições ambientais e fatores dietéticos, são de grande valor.

Torna-se evidente a necessidade de futuras investigações para determinar o valor dos efeitos fenotípicos dos polimorfismos em GPX1 e GPX4 aqui investigados, mas deve-se reconhecer que os resultados aqui gerados fornecem uma melhor compreensão sobre o papel dos polimorfismos na neurobiologia da memória, apontando para novas oportunidades de prevenção de déficits de memória. Ao nosso conhecimento, este estudo forneceu a primeira evidência neurobiológica de associação do rs713041 em humanos.

Algumas considerações são importantes acerca do instrumento utilizado para a avaliação da memória. A escala de Memória-Revisada WMS-R (do inglês *Weschler Memory Scale-Revised)* é bastante utilizada internacionalmente para as avaliações de memória, principalmente se tratando da memória visual e verbal, e foi o instrumento escolhido para a avaliação da memória no nosso estudo. Este teste é realizado em duas partes, denominadas I e II. No teste de memória visual, se pretende verificar a capacidade de retenção de material visual, fazendo uso de desenhos geométricos impressos em cartões individuais. Cada um dos cartões é apresentado individualmente, por 10 segundos, para cada indivíduo. Solicita-se que ele reproduza a imagem em seguida (forma I, correspondendo à memória de curto prazo) e novamente, depois de 30 minutos (forma II, correspondendo à memória de longo prazo). O teste de memória verbal (também denominado memória lógica) foi realizado para verificar a capacidade que cada indivíduo possui em reter o conteúdo de duas histórias. Cada uma destas histórias é lida oralmente pela pessoa responsável por realizar o teste, o examinador, de uma maneira pausada, e em seguida o indivíduo deve reproduzir o texto o mais fielmente possível (forma I, correspondendo à memória imediata). É solicitada nova evocação das mesmas histórias após 30 minutos (forma II, correspondendo à memória tardia).

Neste momento, a fim de concluir, torna-se útil ressaltar que as considerações desta Tese, sugerem que um novo enfoque na questão de avaliação da memória deve ser realizado, observando o *status* para o selênio também em outras populações, principalmente no papel individual e em conjunto que variadas atividades possuem sobre a memória. Além disso, a crescente investigação de fatores genéticos pode contribuir para a avaliação da influência conjunta entre os polimorfismos rs1050450 e rs713041 e hábitos de vida, vislumbrando no futuro formas personalizadas e individuais de intervenções e seus efeitos sobre memória humana.

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ANEXOS

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THE JOURNAL OF NUTRITIONAL BIOCHEMISTRY

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objetivo da pesquisa é Investigar a influência de interações nutrigenéticas selecionadas sobre escores de memória em indivíduos com mais de 50 anos de idade.

Avaliação dos Riscos e Benefícios:

O risco dessa pesquisa está relacionado à coleta de sangue que será realizada em uma das veias do braço, com material descartável, podendo causar desconforto semelhante a uma injeção na veia, e em alguns casos pode aparecer uma mancha que desaparece em algumas horas ou dias após a coleta.
Os dados dessa pesquisa auxiliarão, no futuro, para a detecção de indivíduos que estejam sob um risco aumentado de desenvolver perda de memória.

Comentários e Considerações sobre a Pesquisa:

Trata-se de um estudo em colaboração e com um tema bastante relevante.

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Os participantes serão recrutados a partir de anúncios em veículos de comunicação e redes sociais (jornal, facebook) ou através de divulgação em clubes e associações de terceira idade através da fixação de folder na recepção. Não será feita abordagem direta dos participantes, motivo pelo qual não há autorização escrita dos locais.O contato dos sujeitos com os pesquisadores será através de email.

Considerações sobre os Termos de apresentação obrigatória:

O TCLE está bastante objetivo e compreensível. Também apresenta as autorizações dos laboratórios onde a pesquisa será realizada.

Recomendações:

O projeto apresenta um orçamento de 28 mil reais, contudo não diz qual a fonte de financiamento. Sugiro acrescentar no projeto.

O contato dos pacientes através de email pode causar um viés amostral, onde apenas os participantes com acesso a internet serão incluídos na pesquisa. Sugiro acrescentar no cartaz telefone e endereço para contato.

Conclusões ou Pendências e Lista de Inadequações:

Nenhuma pendência foi verificada.

Considerações Finais a critério do CEP:

De acordo com o parecer do relator.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_604907.pdf	04/05/2016 19:14:13		Aceito
Outros	Folder.pdf	04/05/2016 19:02:11	Marilu Fiegenbaum	Aceito
Projeto Detalhado / Brochura Investigador	projeto_CEP.pdf	04/05/2016 19:01:41	Marilu Fiegenbaum	Aceito
Outros	termo.pdf	01/12/2015 14:09:34	Marilu Fiegenbaum	Aceito
Declaração de Instituição e Infraestrutura	AUTO2.pdf	27/11/2015 13:41:19	Marilu Fiegenbaum	Aceito
Declaração de Instituição e Infraestrutura	AUTO1.pdf	26/11/2015 15:48:02	Marilu Fiegenbaum	Aceito

Orçamento	DESPESASDECUSTEIO.doc	16/11/2015 15:42:18	Marilu Fiegenbaum	Aceito
Cronograma	cronogramaatividades.doc	03/11/2015 18:26:25	Tatiane Jacobsen da Rocha	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE2015.doc	02/11/2015 19:42:12	Tatiane Jacobsen da Rocha	Aceito
Folha de Rosto	FOLHAROSTO.pdf	02/11/2015 11:52:53	Tatiane Jacobsen da Rocha	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

PORTO ALEGRE, 17 de Junho de 2016

Assinado por:
Julia Fernanda Semmelmann Pereira Lima
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Research article

Association between DNA methyltransferase gene polymorphism and Parkinson's disease

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HIGHLIGHTS

- This study first investigated the association among *DNMT1* and *DNMT3B* polymorphisms with Parkinson Disease (PD) and showed an original result.
- The *DNMT3B* polymorphism rs2424913 was associated with Parkinson's Disease (PD). The presence of the T allele increased the odds ratio to PD.
- No significant difference was observed for others SNPs investigated in *DNMT3B* and *DNMT1*.
- This association suggests a role for a DNA methylation enzyme in PD, highlighting the hypothesis of epigenetic mechanisms to PD pathogenesis.

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ABSTRACT

Parkinson's disease (PD) is a common and complex neurodegenerative disorder, the second most prevalent, only behind Alzheimer's disease. Recent studies suggest that environmental factors may contribute for neurodegeneration through induction of epigenetic modifications, such as DNA methylation, that is carried out by enzymes, such as DNMT1 and DNMT3B.

This present study targeted to investigate the association among *DNMT1* and *DNMT3B* polymorphisms with PD.

Five hundred and twenty-two participants (214 PD patients following UK Brain Bank criteria and 308 healthy individuals) were evaluated. DNA was obtained from whole blood and genotypes were detected by an allelic discrimination assay using TaqMan® MGB probes on a real-time PCR system. The polymorphisms studied were rs2162560 and rs759920 (*DNMT1*) and rs2424913, rs998382 and rs2424932 (*DNMT3B*).

Was found association between *DNMT3B* rs2424913 in T allele carriers with PD. The presence of the T allele was associated with PD (OR = 1.80, 95% CI 1.16–2.81, p = 0.009). No significant difference was observed for others *DNMT3B* SNPs. Also, no association between PD and the control group were observed for *DNMT1* polymorphisms.

This is the first study addressing an association between *DNMT3B* polymorphism and PD. The polymorphism may play a role in the pathogenesis of PD.

1. Introduction

In the last years, it was arisen a bunch of studies regarding epigenetic in neuropsychiatric disorders, such as depression with suicide risk [25], schizophrenia [30], and Alzheimer's disease (AD) [27]. Parkinson's disease (PD) is a progressive age-related neurodegenerative disorder, being the second most prevalent after AD [34]. Most of the cases of PD are sporadic [19] and, like AD, PD is understood as a multi-factorial disorder, where environmental and genetic factors are complexly linked [23]. So, epigenetic mechanisms may have a role mediating gene-environment interaction mechanisms in PD.

DNA methylation is one of the most important epigenetic modifications and modulates gene expression [30]. An increased global

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methylation was suggested as an inducing factor to parkinsonism in animal model [3], as well as, changes in the DNA methylation of human α-synuclein (*SNCA*) in brain [15,24] and in leukocytes [33] of Parkinson's disease patients have been reported. A tendency for hypomethylation of the tumor necrosis factor alpha gene (*TNF-α*), a key inflammatory cytokine associated to dopaminergic cell death in PD, was also described [28]. Recently, a meta-analysis of Parkinson's disease genome-wide association studies identified six *loci* associated with both methylation and expression changes in brain tissues [26]. Additionally, in other study, it was demonstrated hypermethylation of enhancers in dopaminergic neurons from PD patients that were related to gene or protein downregulation of relevant PD transcription factors [10].

This process of DNA methylation is driven by DNA methyltransferases enzymes (DNMTs) which add a methyl group to position 5 of the cytosine pyrimidine ring in the CpG dinucleotide [4]. DNA (cytosine-5)-methyltransferase 1 (DNMT1) is a maintenance enzyme and DNA (cytosine-5-)-methyltransferase 3 beta (DNMT3B) is responsible to establish de novo methylation patterns during embryonic development. Mutations in *DNMT1* gene were associated with a myriad of neurodegenerative disorders, probably mediated through its role in protein homeostasis and autophagy [1]. Polymorphisms in *DNMT3B* gene were associated with an increased DNA methylation in *post-mortem* brain tissue in patients with psychiatric diseases and suicide attempt [25]. Recently, our group found an association between polymorphisms in gene encoding the enzyme *DNMT3B* and AD. Individuals carrying the *DNMT3B* TGG haplotype (rs2424932, rs998382 and rs2424913) presented an increased risk of Alzheimer's disease (OR = 3.03, 95% CI 1.63–5.63, $p < 0.001$) [27]. A possible association between *DNMTs* gene polymorphisms and PD has not yet been studied. Taking into account that epigenetic process may be enrolled either in AD and PD, this study aims to evaluate if our previous finding was specific for AD or if it is also present in another neurodegenerative disorders, like PD.

2. Methods and materials

2.1. Participants

A case-control study was conducted. Two hundred and fourteen PD patients were recruited from an outpatient Movement Disorder clinic and underwent a structured interview for collecting clinical and demographic data. The diagnosis of PD was determined based on UK Brain Bank criteria [14]. Evaluation of PD clinical features was accessed by modified Hoehn-Yahr Scale [13,31] and Schawb-England Scale [21]. A control group of 308 age-matched healthy individuals were recruited from the same catchment area. The inclusion criteria were absence of any Parkinson symptom and independence for activities of daily living (ADL) [16,20]. Controls were excluded if they presented cognitive deficit, chronic renal disease, history of significant head injury or stroke, history of cancer, family history of dementia, psychiatric conditions such as major mood disorder, evidence of current depression or substance abuse, and finally uncorrectable vision or hearing loss.

All participants were of European ancestry from southern Brazil. The study was performed in compliance with the Declaration of Helsinki. All participants provided written informed consent.

2.2. Genotyping

Genomic DNA was extracted from 500 μL of EDTA-treated whole blood using the salting out method [17]. After extraction, the DNA was quantified on a UV visible spectrophotometer (Biospec® Nano). The final concentration of DNA used was from 10 ng/mL. The selection criteria and genotyping methods for DNMT1 rs2162560 (C___2774680_30, Applied Biosystems), rs759920 (C___2355506_20, Applied Biosystems) and DNMT3 B rs2424932 (C___2488770_10, Applied Biosystems), rs998382 (C___7500184_10, Applied Biosystems) and rs2424913 (C__25620192_20, Applied Biosystems) polymorphisms were described in previous report [27]. Briefly, the selection was performed using the HapMap (HapMap Genome Browser release #24) (Phases 1 and 2—full dataset) using the following settings for the tool "annotate TagSNP Picker": European population (CEU), minimum frequency of the rarer allele of 20% and a coefficient of determination (R2) of 80%. The five polymorphisms were genotyped with the use of TaqMan® Genotyping Master Mix and TaqMan® SNP Genotyping assays (Applied Biosystems). A control on the genotyping assay was also performed (25% of randomly chosen samples from both groups) to check for genotyping accuracy, and identical genotypes were identified in all repeated samples. The researchers who performed the genotyping were blinded to the patients' diagnostic status.

Table 1
Demographic variables: descriptive and comparative analyses.

Variable	Controls	PD	*P*
N	308	214	
Age (years), mean (SD)	66.7 (9.6)	67.8 (9.9)	0.190[a]
Sex (male), %	20.1	51.9	<0.001[b]
Education (years), median (p25-p75)	11 (5–13)	5 (4–9)	<0.001[c]

Note: PD: Parkinson disease.
[a] Student *t*-test.
[b] Chi-square test.
[c] Mann-Whitney test.

2.3. Statistical analysis

Descriptive analyses were firstly carried out. Normality distribution was tested by the Kolmogorov–Smirnov test. Age and education difference between cases and controls were calculated by Mann–Whitney test, and sex were compared by chi-squared association test.

Genotype frequency was used to estimate allelic frequencies, and agreement with Hardy-Weinberger equilibrium was ascertained by chi-square test. Pairwise linkage disequilibrium (LD) statistics (D' and r^2) and haplotype frequencies were estimated with Haploview 4.2 [2] and Gabriel definitions of linkage disequilibrium were used to determine haplotype blocks [11].

Univariate analyses to verify the associations between the polymorphisms in the genes encoding the enzymes *DNMT1* and *DNMT3B* and PD were carried out by chi-square association test with a dominant model. The Bonferroni correction was performed for multiple comparisons.

Carriers of the T allele were pooled together due to previous studies showing that CT had similar characteristics of TT subjects [12,25,27]. In all of these studies, T carriers confer a disease risk.

Multivariate logistic regression analysis was performed for PD outcome, with polymorphisms as independent variable. The confounders entered in the model were education and sex.

Statistical analyses were performed with SPSS (version 18.0) (SPSS, Chicago, IL, USA), considering $p < 0.05$ as significant.

3. Results

Demographic sample's characteristics are summarized in Table 1. The mean age of PD patients and healthy controls was similar. Educational attainment was significantly lower in the PD group. PD was more prevalent in male population (51.5%). Among the PD patients, 84% were physically independent and scored stage 3 or bellow on modified Hoehn-Yahr Scale. The mean score on Schawb-

Table 2
Genotype and Allelic Frequencies of *DNMT3B* gene polymorphisms rs2424913, rs998382, rs2424932 and *DNMT1* rs2162560, rs759920 in PD and healthy control groups: descriptive and univariate analyses.

		Genotype Frequency			Allelic Frequency	
		%	%	%	%	%
DNMT3B	**rs2424913**	CC	CT	TT	C	T
	Control	37.0	44.5	18.5	59.3	40.7
	Parkinson	24.2	50.2	25.6	49.3	50.7
	P	0.0179[a] (0.006[b])			0.006[a] (0.002[b]) (OR = 1.5; 95% CI = 1.16–1.94)	
	rs998382	AA	AG	GG	A	G
	Control	40.9	41.2	17.9	61.5	38.5
	Parkinson	31.9	48.1	20.0	56.0	44.0
	P	0.113			0.082 (OR = 1.26; 95%CI = 0.97–1.63)	
	rs2424932	AA	AG	GG	A	G
	Control	15.3	46.1	38.6	38.3	61.7
	Parkinson	11.2	44.2	44.7	33.3	66.7
	P	0.258			0.112 (OR = 1.25; 95%CI = 0.95–1.63)	
DNMT1	**rs2162560**	AA	AG	GG	A	G
	Control	16.6	48.7	34.7	40.9	59.1
	Parkinson	12.4	46.2	41.4	35.5	64.5
	P	0.236			0.093 (OR = 1.26; 95%CI = 0.96–1.64)	
	rs759920	AA	AG	GG	A	G
	Control	24.0	51.3	24.7	49.7	50.3
	Parkinson	24.4	51.9	23.6	50.5	49.5
	P	0.958			0.849 (OR = 0.97; 95%CI = 0.76–1.24	

Note: Control: Control Group. Parkinson: Parkinsonís disease Group.
[a] Bonferroni correction.
[b] Uncorrected p values.

Table 3
Multiple logistic regression analysis for outcome PD.

Variables in the Model	B	OR	95% CI	*P*
DNMT3B rs2424913*	0.590	1.80	1.16–2.81	0.009
Education (years)	−0.184	0.83	0.79–0.87	<0.001
Sex (male)	1.367	3.92	2.58–5.98	<0.001

Note: **DNMT3B* rs2424913 categories: T carriers (TT+CT) × CC (reference); OR: Odds Ratio; B: estimated coefficient; 95% CI: Confidence Interval 95%.

England Scale was 75.5% (±23.8%) and the first symptoms were observed at 59.1 ± 10.8 years.

The genotypic frequencies of *DNMT1* and *DNMT3B* polymorphisms were consistent with Hardy-Weinberg equilibrium ($P > 0.05$). The haplotype structure and pairwise LD values (r^2) for *DNMT3B* gene demonstrated strong linkage disequilibrium between rs998382-rs2424932 (D' = 0.95), but a low determination coefficient ($r^2 = 0.36$). Three haplotypes were observed in the LD block (haplotype GG, frequency = 0.402; AA, frequency = 0.357; AG, frequency = 0.235). For rs2424913-rs998382 and rs2424913-rs2424932 pairs the 95% confidence interval of D' was lower than 90%, indicating evidence of historical recombination.

Table 2 describes genotypic and allelic frequencies related to each gene polymorphism and their association with PD. Univariate analyses showed significant association between the *DNMT3B* polymorphism rs2424913 genotype and PD ($P = 0.0179$) and that the T allele of *DNMT3B* polymorphism rs2424913 was associated with PD ($P = 0.006$). There was no significant association of polymorphisms with age of disease onset or with PD disability, measured by Hoehn-Yahr and Schawb-England scales (data not shown).

In order to verify whether the effect of the *DNMT3B* rs2424913 was independent of age, sex and education, a multivariate logistic regression analysis was performed. For this analysis we categorized the *DNMT3B* rs2424913 in T carriers (individuals with genotypes TT and CT) and individuals with CC genotype. The presence of the T allele (TT+CT) was associated with PD (OR = 1.80, 95% CI 1.16–2.81, $P = 0.009$). These results are shown in Table 3.

4. Discussion

The present study evaluated the relation of polymorphisms in DNA methyltransferase enzymes, which are responsible for important epigenetic mechanism, and Parkinson disease. The main result described herein was the association between T allele of the rs2424913 polymorphism in the *DNMT3B* gene and PD. No association was found for polymorphisms in *DNMT1*.

To the best of our knowledge, this is the first study showing an association between polymorphisms of DNA methyltransferase and PD. Previous studies have shown a relationship between polymorphisms of DNA methyltransferases with aging [6], cancer [18], psychiatric [25] and neurodegenerative syndromes [5,7,27]. Our previous study shows a positive association between *DNMT3B* TGG haplotype and Alzheimer's disease [27]. PD shares similar mechanisms with AD: both disorders are age-related and resulted from an abnormal handling of proteins. Considering these results, one might hypothesize that a common dysfunctional methylation pattern, influenced by *DNMT3B* polymorphisms, could contribute to abnormal protein accumulation and neurodegenerative process in a broader way.

A variety of studies suggested epigenetic mechanisms in PD. In the context of gene-specific methylation process, DNA methylation of CpG islands of human α-synuclein (*SNCA*) intron 1 and demethylation of the *SNCA* CpG in brains of PD patients can regulate *SNCA* gene expression [15,33]. Besides, studies regarding methylation status of *SNCA* in leukocytes have demonstrated controversial results. While one investigation in one hundred patients with PD, paired with one hundred healthy control subjects, indicated that CpG-2 island was hypomethylated [33], other study reported no evidence for differential methylation of alpha-synuclein in leukocyte DNA of Parkinson's disease patients [29]. On the other hand, an increased global methylation was observed in aging animal model with a consequent dopamine, norepinephrine, and serotonin depletion and acetylcholine increase, causing hypokinesia and tremor. These findings suggested increased methylation as an inducing factor in parkinsonism [3].

The rs2424913 is located in noncoding region of *DNMT3B* and its functionality is still unclear, however in vitro essays showed an increase of 30% in *DMNT3B* promoter activity [32]. Therefore we may hypothesize that this genetic variability could upregulate the *DNMT3B* expression with consequent increase of methylation and expression of several PD candidates genes.

Another potential epigenetic deregulation in PD was related to the brain-derived neurotrophic factor (*BDNF*) gene. Reduction of BDNF levels has been verified in patients with PD and other neurodegenerative disorders [35], also is known that BDNF expression is regulated by histone acetylation as well as DNA methylation [22].

Interestingly, the presence of T allele of rs2424913 was previously associated with decreased risk of cancer, the same allele that in our sample was associated with AD and PD [9]. Recent epidemiological studies are depicting an inverse correlation between cancer and neurodegenerative disorders [8]. Epigenetic mechanisms are a possible explanation for the fact that individuals with neurodegenerative disorders, like PD, are protected against some types of cancer. The same methylation patterns that could predispose to cell death in PD might protect the individual against abnormal cell proliferation in cancer.

Some limitations need to be pointed out. There is a sex difference between the groups. However, we controlled this possible confounding factor in the multivariate analysis. This is an exploratory study, which suggests association between the polymorphism of *DNMT3B* and PD. This results need to be replicated in other samples.

The findings described in this paper suggest a role for an important DNA methylation enzyme in Parkinson disease. Understanding the complexity of PD neurodegeneration, the influence of environmental factors on phenotypic constitution as well as the attractive hypothesis of epigenetic mechanisms contributing to PD pathogenesis, justifies further researches on this field.

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