UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM PSIQUIATRIA E CIÊNCIAS DO COMPORTAMENTO

Letícia Sanguinetti Czepielewski

Trajetórias de Transtornos Mentais Graves: Contribuições da Pesquisa em Esquizofrenia

Porto Alegre, 2016

Letícia Sanguinetti Czepielewski

Trajetórias de Transtornos Mentais Graves: Contribuições da Pesquisa em Esquizofrenia

Tese apresentada como requisito parcial para a obtenção do título de Doutor em Psiquiatria e Ciências do Comportamento, à Universidade Federal do Rio Grande do Sul, Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento

Orientador: Profª. Drª. Clarissa Severino Gama

Porto Alegre, 2016

CIP - Catalogação na Publicação

Czepielewski, Letícia Sanguinetti
Trajetórias de Transtornos Mentais Graves: Contribuições da Pesquisa em Esquizofrenia / Letícia Sanguinetti Czepielewski. -- 2016.
120 f.

Orientadora: Clarissa Severino Gama.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Médicas: Psiquiatria, Porto Alegre, BR-RS, 2016.

1. Esquizofrenia. 2. Cognição. 3. Funcionamento Intelectual. 4. Telômero. 5. Substância Cinzenta. I. Gama, Clarissa Severino, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

À minha orientadora, Profª. Clarissa Severino Gama, pelo acolhimento, confiança e inspirações constantes.

Aos professores e colegas do Laboratório de Psiquiatria Molecular, pelo companheirismo e pelos grandes ensinamentos ao longo dos últimos anos.

À Profª Deanna Marie Barch e aos colegas do Cognitive Control and Psychopathology Laboratory e do Department of Psychology and Brain Sciences da Washington University in St. Louis, por terem me mostrado como se pode produzir ciência em psicologia.

Aos CAPES, CNPq e FIPE/HCPA, pelas bolsas de estudo e pelo apoio financeiro ao longo do doutorado.

Ao PPG Psiquiatria e Ciências do Comportamento, à Universidade Federal do Rio Grande do Sul e ao Hospital de Clínicas de Porto Alegre, pela excelência em ensino e educação.

À minha família, em especial aos meus pais Ivanice e Mauro, ao meu irmão Rafael, e ao meu amor Luiz Felipe, pelos incentivo, suporte, compreensão, parceria e amor incondicionais.

Por fim, à banca avaliadora por sua generosa disponibilidade em avaliar esta tese.

RESUMO

Transtornos mentais graves são doenças crônicas altamente incapacitantes que geram um alto custo para a sociedade. Indivíduos acometidos por essas doenças apresentam maior morbidade e mortalidade. Dentre elas, a esquizofrenia parece possuir os piores desfechos. Portanto, estudar a esquizofrenia pode trazer contribuições importantes para o entendimento e manejo de transtornos mentais graves como a depressão maior e o transtorno bipolar. Esse trabalho buscou compreender mecanismos fisiopatológicos da esquizofrenia ao longo de quatro artigos que exploram aspectos de funcionamento cognitivo, funcionamento intelectual, de biomarcadores e de estrutura cerebral. O primeiro artigo investigou as alterações de performance de memória em indivíduos com esquizofrenia em estágios iniciais e tardios da doença comparadas ao transtorno bipolar e a sujeitos saudáveis. Os resultados mostraram que indivíduos com esquizofrenia apresentaram precoces prejuízos cognitivos de memória, diferentemente de indivíduos com transtorno bipolar quando comparados a controles. O segundo artigo investigou as influências das performances cognitiva e intelectual em estruturas cerebrais de indivíduos com esquizofrenia comparados a controles saudáveis. Os resultados indicaram que o funcionamento intelectual pré-morbido estava relacionado ao volume de estruturas globais, enquanto o funcionamento cognitivo estava relacionado ao volume e espessura de massa cinzenta cortical, sugerindo influências diferentes e complementares relacionadas a neurodesenvolvimento e neurodegeneração. O terceiro artigo investigou um biomarcador de envelhecimento precoce, um possível mecanismo para a neuroprogressão na esquizofrenia. Os resultados monstraram que indivíduos com esquizofrenia apresentaram encurtamento de telômero quando comparados a controles, mas não houveram diferenças entre o tamanho de telômero de pacientes e seus irmãos não afetados pela doença. Por fim, o quarto artigo buscou investigar a teoria do envelhecimentoa patológico acelerado na esquizofrenia, integrando os achados dos artigos anteriores. Os resultados demonstraram correlações entre comprimento de telômero, níveis de CCL11, performance de memória, volume de massa cinzenta e tempo de doença em indivíduos com esquizofrenia. Esses achados sugerem que a esquizofrenia seria uma doença do neurodesenvolvimento associada a uma carga adicional ao longo do curso da doença que levaria a um envelhecimento patológico precoce. A partir dos

achados em esquizofrenia, pode-se ampliar a compreensão de alterações percebidas nas trajetórias de outras psicopatologias. Com adequado entendimento desses mecanismos, será possível o desenvolvimento de novos tratamentos e intervenções mais efetivas e eficazes.

Palavras-chave: esquizofrenia, cognição, funcionamento intelectual, comprimento de telômeros, massa cinzenta.

ABSTRACT

Severe mental disorders are debilitating chronic diseases that have a high cost to society. Individuals affected by these diseases have increased morbidity and mortality. Among them, schizophrenia seems to have the worst outcomes. Therefore, studying schizophrenia may provide important contributions to the understanding and management of severe mental disorders such as major depression and bipolar disorder. The present study aimed to understand the pathophysiological mechanisms of schizophrenia over four articles that explore aspects of cognitive functioning, intellectual functioning, biomarkers and brain structure. The first article investigated changes in memory performance in individuals with schizophrenia in early and late stages of disease compared to bipolar disorder and healthy subjects. The results showed that subjects with schizophrenia had early cognitive deficits of memory, unlike individuals with bipolar disorder compared to controls. The second article investigated influences of cognitive and intellectual performances on brain structures of individuals with schizophrenia compared to healthy controls. The results indicated that premorbid intellectual functioning was related to volume of global structures, while cognitive functioning was related to volume and thickness of cortical gray matter, suggesting different and complementary influences related to neurodevelopment and neurodegeneration. The third article investigated an early aging biomarker, a possible mechanism of neuroprogression in schizophrenia. The results showed that individuals with schizophrenia had shortened telomeres when compared to controls, but there were no differences between the telometer length of patients and their siblings not affected by the disease. Finally, the fourth article sought to investigate the theory of pathological accelerated aging in schizophrenia, integrating the findings of the previous articles. The results demonstrated correlations between telometer length, CCL11 levels, memory performance, gray matter volume and illness duration in individuals with schizophrenia. These findings suggest that schizophrenia is a neurodevelopmental disorder associated with an additional burden over the course of the disease that leads to a pathological accelerated agig.

Key words: schizophrenia, cognition, intellectual functioning, telomere length, gray matter.

SUMÁRIO

1 APRESENTAÇÃO

Este trabalho consiste na tese de doutorado intitulada “Trajetórias de Transtornos Mentais Graves: Contribuições da Pesquisa em Esquizofrenia”, apresentada ao Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento da Faculdade de Medicina da Universidade Federal do Rio Grande do Sul em 04 de novembro de 2016. Está organizada na ordem que segue: Introdução, Objetivos, Artigo #1 (publicado na revista *European Neuropsychopharmacology*), Artigo #2 (publicado na revista *Schizophrenia Bulletin*), Artigo #3 (publicado na revista *Schizophrenia Research*), Artigo #4 (submetido para publicação na revista *Schizophrenia Bulletin*), e Considerações Finais.

Os estudos apresentados nesta tese foram desenvolvidos como parte de um projeto guarda-chuva de avaliação de aspectos clínicos, biológicos, cognitivos, funcionais e de neuroimagem de indivíduos com esquizofrenia e transtorno bipolar em estágios iniciais e tardios das doenças. Na presente tese, buscou-se investigar possíveis mecanismos cognitivos, biológicos e de estrutura cerebral associados ao curso da esquizofrenia. No artigo #1, intitulado “*Verbal Episodic Memory Along the Course of Schizophrenia and Bipolar Disorder: a New Perspective*”, comparou-se a performance de memória de indivíduos com esquizofrenia e transtorno bipolar em estágios iniciais e tardios da doença, e controles saudáveis. No artigo #2, intitulado “*The Relationship of Intellectual Functioning and Cognitive Performance to Brain Structure in Schizophrenia*”, investigou-se as influências das performances cognitiva e intelectual em estruturas cerebrais de indivíduos com esquizofrenia e controles. No artigo #3, intitulado “*Telomere length in subjects with schizophrenia, their unaffected siblings and healthy controls: evidence of accelerated aging*”, avaliou-se as diferenças na medida de um possível biomarcador de envelhecimento patológico precoce entre indivíduos com esquizofrenia, seus irmãos não afetados pela doença e controles. No artigo #4, intitulado “*Telomere Length and CCL11 Levels Are Associated with Gray Matter Volume and Episodic Memory Performance in Schizophrenia: Evidence of Pathological Accelerated Aging*”, buscou-se integrar os achados dos outros estudos trazendo evidências para uma hipótese de envelhecimento patológico precoce na esquizofrenia. Nesses estudos, procurou-se investigar possíveis mecanismos que podem trazer contribuições para um melhor entendimento dos transtornos mentais graves.

2 INTRODUÇÃO

Transtornos mentais graves são doenças crônicas altamente incapacitantes que afetam entre 0.4% a 7.7% da população mundial (Demyttenaere et al, 2004; Kessler et al., 2009) e geram um alto custo para a sociedade (Levinson et al., 2010). Indivíduos com essas doenças tendem a apresentar maior prevalência de comorbidades clínicas quando comparados à população geral, como obesidade, hipertensão, dislipidemia, diabetes, asma, artrite e infecções virais transmitidas pelo sangue (Birkenaes et al., 2006; Razzano et al., 2015; Hughes et al., 2016). Além disso, existem evidências de que esses indivíduos apresentam maiores taxas de mortalidade (Lumme et al., 2016) que levam a uma expectativa de vida reduzida de 8 a 18 anos quando comparados à população geral (Chang et al, 2011). Esses fatores sugerem uma possível vulnerabilidade biológica, o que aponta para a necessidade de se entender esses transtornos como doenças orgânicas que afetam integralmente o indivíduo, e não apenas como alterações de funções mentais (Bloomfield, Buck e Howes, 2016).

Diversos estudos na literatura que apresentam intervenções focadas nas principais condições médicas e em comportamentos de riscos de indivíduos com transtornos mentais graves. Contudo, os níveis de evidência destes tratamentos complementares são baixos (McGinty et al., 2016), o que indica a urgência de se entender melhor os mecanismos fisiopatológicos destas doenças para então poder desenvolver novas e mais adequadas intervenções. Ainda que a associação entre transtornos mentais e problemas na saúde física seja reconhecida há muito tempo, a reintegração entre psiquiatria e medicina clínica representa um dos maiores desafios atuais na busca de promoção de melhores serviços para o atendimento destas tão vulneráveis populações (Fleischhacker et al., 2008).

Além disto, outra discussão da atualidade está no questionamento dos limites entre os transtornos mentais, uma vez que diferentes diagnósticos psiquiátricos compartilham sintomas, heterogeneidade de apresentações e diversos mecanismos patogenéticos (Figura 1) (Craddock e Owen, 2010; Pearlson, 2015). Com isto, surge a ideia de que abordagens dimensionais podem substituir ou melhorar os métodos categóricos vigentes (Owen, Sawa e Mortensen, 2016). Contudo, ainda se demonstram válidos e úteis os estudos focados em psicopatologias específicas, uma

vez que existem características e respostas a estratégias terapêuticas particulares que as diferenciam (Sommer e Carpenter, 2016).

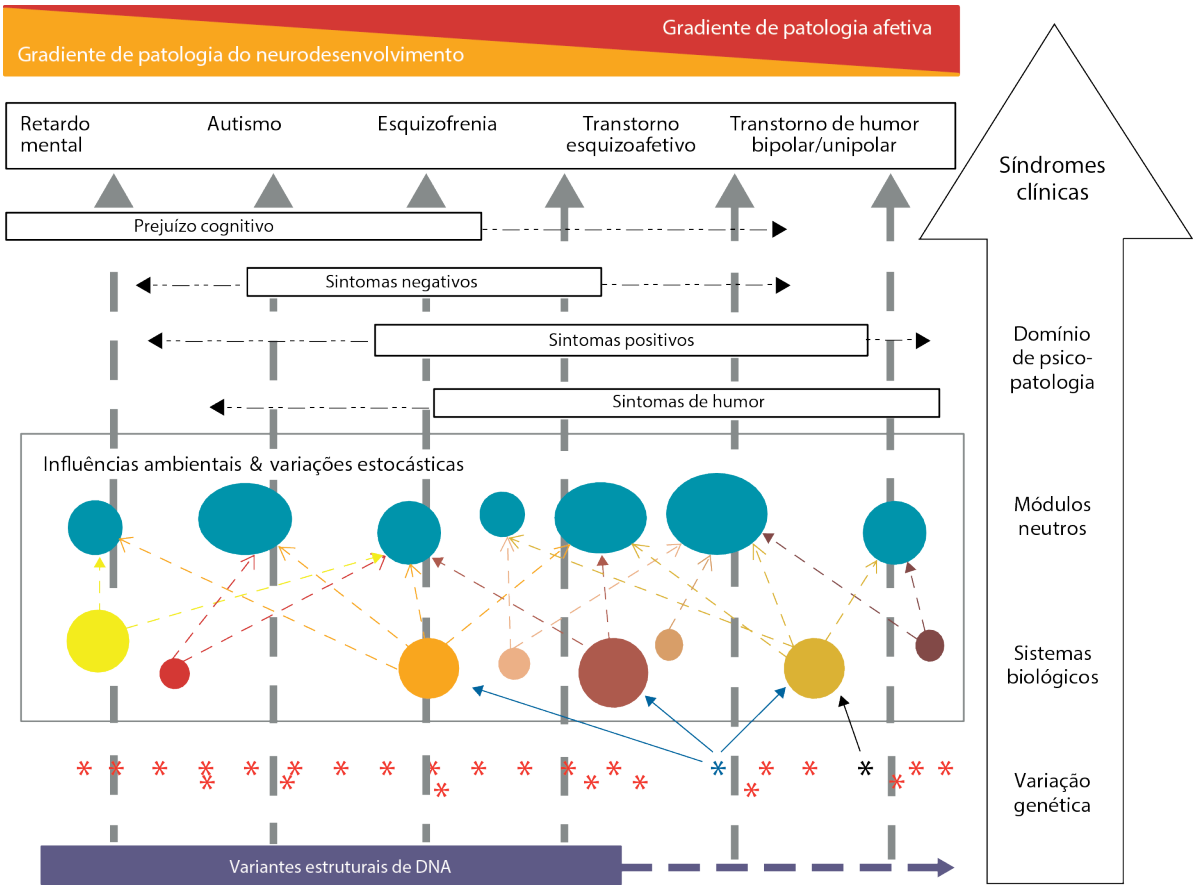

Figura 1. Traduzida e adaptada de Craddock e Owen, 2010: Hipótese do modelo da complexa relação entre variáveis biológicas e psicopatologias

Os transtornos identificados como graves na literatura incluem esquizofrenia, transtorno bipolar e transtorno esquizoafetivo, mas também transtorno depressivo maior crônico e recorrente, e abuso de substâncias. Destes, a esquizofrenia parece apresentar desfechos mais graves considerando diversos aspectos, principalmente relacionados ao prejuízo funcional (Harvey et al., 2012; Zipursky, 2014). Por isto, estudar a esquizofrenia pode trazer contribuições importantes para o entendimento dos mecanismos fisiopatológicos de transtornos mentais graves. A seguir, será apresentada uma revisão da literatura acerca de aspectos da esquizofrenia relevantes à tese.

2.1 Sintomatologia e tratamento

A esquizofrenia é caracterizada pela presença de sintomas que podem ser divididos em três grupos centrais: *sintomas positivos* (psicóticos, ou seja, delírios e alucinações), *sintomas negativos* (embotamento afetivo, prejuízo motivacional, isolamento social, entre outros) e *sintomas cognitivos* (pior performance em diversos domínios cognitivos quando comparados a controles). Os sintomas positivos tendem a ter um curso marcado por relapsos e remissão, ainda que alguns pacientes apresentem sintomas psicóticos residuais de longo-prazo. Contudo, os sintomas negativos e cognitivos tendem a ser crônicos e associados aos importantes déficits de funcionamento social e ocupacional observados na esquizofrenia (Owen, Sawa e Mortensen, 2016).

Para os sintomas positivos, o tratamento com diversos antipsicóticos é efetivo na maior parte dos pacientes (Kreyenbuhl et al., 2010), entretanto, ainda assim alguns pacientes permanecem não-responsivos. Para os pacientes refratários ao tratamento, a clozapina é superior a outros antipsicóticos de primeira e segunda geração para ambos sintomas positivos e negativos (Siskind et al., 2016). Contudo, independente da resposta terapêutica, os sintomas negativos e cognitivos seguem não sendo cobertos pelos tratamentos usuais (Millan et al., 2016). Mais recentemente, buscando preencher esta lacuna, novos alvos terapêuticos vêm sendo explorados, como por exemplo focando nos receptores N-metil D-Aspartato (NMDA) e no receptor α-7 nicotínico da acetilcolina, entre outros (Citrome, 2014). Entretanto, ainda não existem tratamentos aprovados que tenham como alvo os sintomas negativos e cognitivos. Espera-se, portanto, que o progresso na compreensão da neurobiologia desses importantes domínios da esquizofrenia possa ajudar no desenvolvimento de estratégias de tratamento mais adequadas e efetivas (Carbon e Correll, 2014).

Desta forma, os tratamentos hoje disponíveis não conseguem abranger integralmente a complexidade e gravidade da esquizofrenia, e a recuperação do indivíduo com esse transtorno em todos os domínios sintomatológicos é pouco comum, o que leva ao prejuízo psicossocial e laboral importante e característico desta doença (Zink e Englisch, 2016).

2.2 Morbidade somática e mortalidade

Indivíduos com esquizofrenia são amplamente descritos na literatura como tendo uma variedade de doenças somáticas crônicas, como doenças cardiovasculares, doenças respiratórias, diabetes, hepatite, osteoporose, doenças renais, entre outras (Leucht et al., 2007; Laursen, Munk-Olsen e Gasse, 2011), presentes inclusive antes do diagnóstico (Sørensen et al., 2015). Ainda que os estudos analisando as comorbidades tenham limitações, como o uso de psicofármacos e o estilo de vida dos pacientes, existem evidências de que a esquizofrenia compartilha possíveis mecanismos associados a essas comorbidades, como fatores genéticos, do desenvolvimento e de envelhecimento acelerado (Dieset et al., 2016).

A presença do conjunto dessas doenças em indivíduos com esquizofrenia parece estar relacionada com maior mortalidade. O risco relativo de todas as causas de morte na esquizofrenia é de 2.54, o que é significativamente maior que nos transtornos de humor e de ansiedade (Walker et al., 2015). Comparado à população geral, indivíduos com esquizofrenia têm sua expectativa de vida reduzida em 15 a 20 anos (Chang et al., 2011; Nordentoft et al., 2013; Lume et al., 2016). Os possíveis motivos pelos quais esses sujeitos têm maior mortalidade parecem ser principalmente porque ocorre diagnóstico tardio e tratamento insuficiente das doenças físicas, existem muitos efeitos colaterais negativos do uso de antipsicóticos, eles têm um estilo de vida não-saudável (incluindo fumar, sedentarismo, dieta pobre, consumo de álcool em excesso), além do risco de suicídio e de acidentes (Laursen, Nordentoft e Mortensen 2014; Dickerson et al., 2016). Interessantemente, além das doenças físicas, a mortalidade parece estar associada também aos sintomas negativos, mas não aos sintomas positivos (Hayes et al., 2012a). Nos transtornos mentais graves, o risco aumentado de mortalidade geral (por qualquer causa) está associado a um prejuízo na funcionalidade de atividades diárias (Hayes et al., 2012b).

2.3 Funcionalidade

O desfecho funcional de indivíduos com esquizofrenia é comumente prejudicado, afetando amplamente os diversos domínios, como empregabilidade, vida autônoma e relacionamentos interpessoais (Harvey et al., 2004). Pessoas com esquizofrenia usualmente não conseguem se manter em empregos competitivos e

atingir níveis educacionais esperados para o seu contexto. Elas também demonstram dificuldades em usar transporte público, cozinhar, gerenciar dinheiro e aderir de forma correta aos medicamentos prescritos. Assim, usualmente não conseguem se adequar aos papéis sociais básicos, e desta forma podem ficar isoladas socialmente por não conseguirem manter conversações, expressar necessidades e sentimentos ou desenvolver relacionamentos próximos e significativos (Harvey, Velligan e Bellack, 2007).

Além disto, sintomas negativos parecem estar mais relacionados com o desfecho funcional que os sintomas positivos (Ventura et al., 2009). Igualmente, as condições de saúde física descritas anteriormente também parecem influenciar o funcionamento no dia-a-dia (Harvey e Strassnig, 2012). Mas os principais preditores parecem ser as performances nos processos cognitivos, que já há algum tempo na literatura são demonstrados estarem relacionados ao desfecho funcional de indivíduos com esquizofrenia (Green, 1996), com tamanhos de efeito médio a grande em domínios como a memória verbal e funções executivas (Green et al., 2000).

2.4 Alterações cognitivas

Indivíduos com esquizofrenia podem apresentar prejuízos globais e heterogêneos em diversos domínios cognitivos, incluindo processos atencionais, memória episódica, memória de trabalho e funções executivas (Fioravanti, Bianchi e Cinti, 2012). Essas alterações parecem ser semelhantes às de outras psicopatologias, como o transtorno bipolar, apenas diferindo em extensão e severidade. Prejuízos cognitivos também parecem se correlacionar com variáveis sócio-demográficas (como baixa escolaridade), clínicas (como maior número de hospitalizações e maior tempo de doença), e de tratamento (como uso de antipsicóticos), e com pior funcionamento psicossocial (Kuswanto et al., 2016).

Interessantemente, os achados se mantêm mesmo em pacientes em estágio inicial da doença que nunca fizeram uso de psicofármacos, o que demonstra o caráter central destas alterações na esquizofrenia (Fatouros-Bergman et al., 2014). Desta forma, a cognição parece já estar moderadamente prejudicada muito antes do diagnóstico, e existem poucas indicações de que durante o curso da doença exista piora adicional das performances cognitivas (Woodward, 2016). Contudo, o curso

das alterações cognitivas ainda é controverso na literatura, principalmente pela heterogeneidade das suas apresentações (Shmukler et al., 2015).

Um aspecto consistente na literatura é que a maior parte dos prejuízos cognitivos parecem ter origem no desenvolvimento, ou seja, na aquisição e não na perda de habilidades. Portanto, a heterogeneidade poderia ser o resultado dos diferentes insultos ocorridos durante o desenvolvimento do indivíduo com esquizofrenia (Bora, 2015). Um possível marcador dessa alteração do desenvolvimento seria o funcionamento intelectual, que também pode estar reduzido nestes pacientes (Woodberry, Giuliano e Seidman, 2008; Khandaker et al., 2011).

Outra evidência dessa hipótese vem de estudo recente que mostrou que o risco para desenvolver esquizofrenia estava fortemente relacionado ao desvio no desempenho escolar baseado na aptidão cognitiva familiar, e não só à performance cognitiva em si. Ou seja, possivelmente os desfechos cognitivos são influenciados por uma soma de fatores genéticos e ambientais também relacionados às famílias das pessoas com esquizofrenia (Kendler et al., 2016).

2.5 Alterações cerebrais

Há algumas décadas as alterações de estrutura e funcionamento cerebral são consistentemente descritas na literatura. Em meta-análise recente sobre o assunto, com mais de 18.000 pacientes com esquizofrenia, demonstrou-se redução nos volumes cerebrais comparado a controles, tanto globalmente quando em diversas estruturas corticais e subcorticais específicas. Curiosamente, os achados foram semelhantes em pacientes que nunca fizeram uso de antipsicóticos. Uma das alterações com maior tamanho de efeito foi o volume total de massa cinzenta e volume cortical de massa cinzenta (Haijma et al., 2013). Contudo, algumas estruturas subcorticais, como caudato, putamen, palidum e ventrículos laterais apresentam indícios de aumento no volume quando comparado a controles (Okada et al., 2016). Além disso, existem também evidências de alterações do funcionamento de redes neurais de pacientes com esquizofrenia (Kambeitz et al., 2016).

Em suma, o que se pode compreender da literatura é que as evidências das alterações de estrutura cerebral apontam para uma combinação de processos do neurodesenvolvimento – refletido na redução do volume intracranial – e de progressão da doença – refletido na perda de massa cinzenta (Haijma et al., 2013).

2.6 Alterações biológicas

Biomarcadores são medidas objetivas que indicam processos biológicos com potencial para utilizados para avaliação diagnóstica, prognóstica e de resposta terapêutica. Como um dos principais objetivos da pesquisa em esquizofrenia é entender os mecanismos biológicos da doença, o estudo de biomarcadores pode ter papel importante nesta busca. Contudo, a diversidade e a complexidade das alterações observadas em indivíduos diagnosticados dificultam este processo (Goff et al., 2016).

Um dos modelos que tentam explicar as alterações biológicas em doenças psiquiátricas é o modelo vulnerabilidade-estresse-inflamação, que descreve que fatores genéticos criariam uma vulnerabilidade na qual o estresse aumentaria citocinas pró-inflamatórias e contribuiria para um estado pró-inflamatório contínuo (Müller et al., 2015). Na esquizofrenia, existem evidências consistentes de disfunções no sistema imune (Miller et al., 2011), além de alterações de estresse oxidativo (Flatow, Buckley e Miller, 2013). Todavia, ainda não há compreensão de como essas alterações interagem para produzir os desfechos observados ao longo do desenvolvimento da doença, como por exemplo as comorbidades clínicas.

Um recente biomarcador que vem sendo estudado na esquizofrenia e em outros transtornos psiquiátricos é o comprimento de telômeros (Lindqvist et al., 2015). Telômeros são estruturas protetoras das extremidades dos cromossomos. Sua função é de proteger o genoma da degradação, e, portanto, preservar as suas informações. Uma parte pequena do DNA do telômero é perdida a cada divisão celular, o que leva a senescência ou apoptose quando o tamanho atinge uma dimensão muito reduzida. Portanto, o comprimento de telômero pode ser entendido como um “relógio biológico” relacionado ao tempo de vida da célula e do organismo. Ou seja, o comprimento de telômero pode indicar a velocidade do envelhecimento biológico (Reynolds, 2016), ou mesmo de “redundância somática”, que seria a capacidade do corpo de absorver danos ao longo do tempo (Boonekamp et al., 2013). Na esquizofrenia, recente meta-análise mostrou que o comprimento de telômero está levemente diminuído na esquizofrenia se comparado a controles, contudo os dados são ainda inconsistentes (Polho et al., 2015). Além disso, apesar do comprimento de telômeros ser um traço herdado, ele pode ser influenciado pela epigenética, meio ambiente (Lindqvist et al., 2015), inflamação e estresse oxidativo (Baylis et al., 2014; von Zglinicki, 2002).

2.7 *Modelos das trajetórias da esquizofrenia*

Integrando os aspectos descritos até agora, pode-se afirmar que o curso da esquizofrenia é influenciado por diversos fatores que vêm muito antes do diagnóstico da doença (Figura 2). A melhor compreensão dos substratos neurobiológicos está fazendo surgir uma noção de que o curso da esquizofrenia pode ser modificado por novas intervenções. Por isso, torna-se imprescindível compreender os mecanismos envolvidos no curso da doença (Millan et al., 2016).

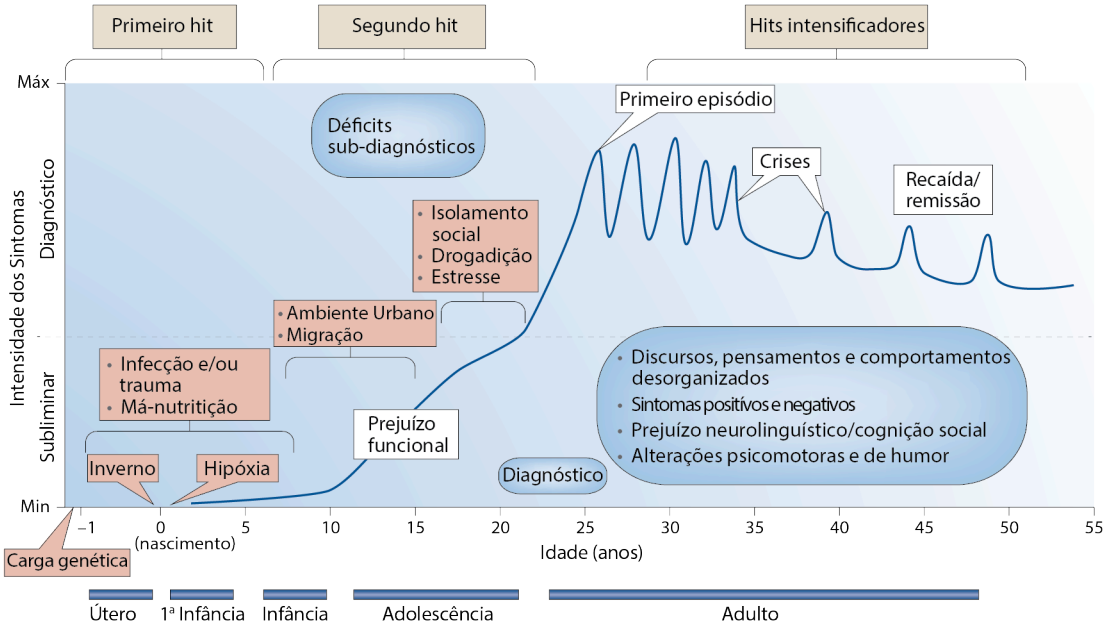

Figura 2. Traduzida e adaptada de Millan et al., 2016

Um dos modelos mais concebidos afirma que o desenvolvimento da esquizofrenia ocorre a partir de dois *hits*. O primeiro seria as vulnerabilidades genéticas, somado ao segundo *hit* de insultos sofridos ao longo do desenvolvimento infantil, que suscitaria o primeiro episódio de sintomas positivos ao fim da adolescência/início da vida adulta. Este modelo também pode ser descrito como o modelo do neurodesenvolvimento, o qual afirma que a esquizofrenia é o fim de processos anormais do neurodesevolvimento que começaram muitos anos antes do que se identifica como início da doença. Existem diversas e consistentes evidências na literatura de fatores de risco associados ao desenvolvimento da esquizofrenia corroborando com este modelo, como fatores pré e perinatais, trauma na infância, imigração, entre outros (Lewis e Levitt, 2002; Fatemi e Folsom, 2009; Rapoport,

Giedd e Gogtay, 2012). Evidências atuais demonstram que o desenvolvimento da esquizofrenia poderia se dar a partir da vulnerabilidade genética interagindo com múltiplos outros fatores ambientais de vulnerabilidade, como infecções virais, quociente de inteligência, uso de *cannabis*, cognição social, entre outros. Esses fatores interagiriam entre si ao longo do desenvolvimento, culminando na expressão da doença (Davis et al., 2016).

Uma hipótese mais recente da esquizofrenia é a do envelhecimento patológico precoce. Essa teoria diz que as observações de morbidade e mortalidade aumentadas, somando-se a prejuízos cognitivos similares aos vistos no envelhecimento normal, poderiam sugerir que um envelhecimento acelerado estivesse ocorrendo em indivíduos com esquizofrenia (Kirkpatrick et al., 2008). Um estudo longitudinal que avaliou mapas de densidade de massa cinzenta demonstrou que a redução do volume total do cérebro era devido ao envelhecimento mais rápido em indivíduos com esquizofrenia comparados a controles, especialmente nos primeiros anos de doença (Figura 3). No *baseline*, a idade do cérebro dos pacientes era 3.36 anos maior que a idade cronológica, e ainda ao longo dos anos demonstrou-se um envelhecimento adicional de 4 meses em cada ano. Sendo assim, os autores então sugeriram a ocorrência de dois processos: envelhecimento acelerado do cérebro e variações individuais, como o uso de medicamentos (Schnack et al., 2016). Contudo, estudos adicionais são necessários para trazer evidências relacionadas a esta teoria.

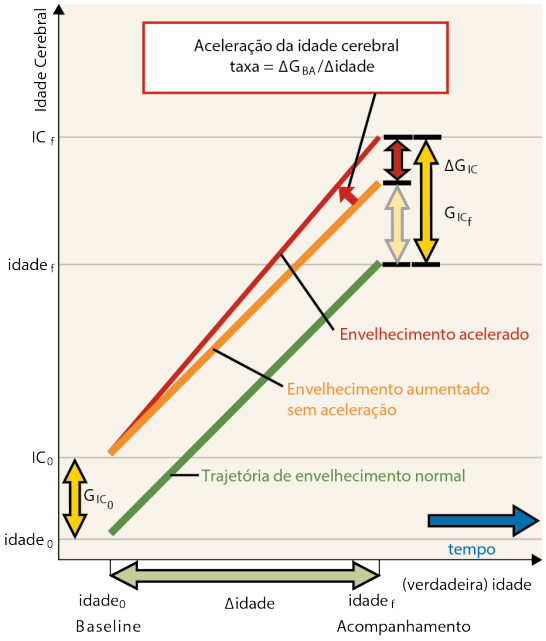

Figura 3. Traduzida e adaptada de Schnack et al., 2016.

2.8 Justificativa

A partir do exposto acima, esse trabalho busca compreender como a trajetória da esquizofrenia influencia seus desfechos tão graves, procurando entender quais os possíveis mecanismos cognitivos, intelectuais, biológicos e de estrutura cerebral envolvidos, com o objetivo maior de construção do conhecimento para posterior investigação de possíveis intervenções focadas nesses mecanismos fisiopatológicos da esquizofrenia e dos transtornos mentais graves.

3 OBJETIVOS

3.1 Objetivo Geral

Estudar mecanismos biológicos, de estrutura cerebral e cognitivos associados ao curso da esquizofrenia.

3.2 Objetivos Específicos

a. Investigar alterações na performance cognitiva, especificamente de memória, em estágios iniciais e tardios de indivíduos com esquizofrenia comparados a indivíduos com transtorno bipolar e controles (artigo #1);
b. Investigar diferenças na performance intelectual e cognitiva de indivíduos com esquizofrenia comparados a controles saudáveis, e sua influência em estruturas cerebrais (artigo #2);
c. Determinar se indivíduos com esquizofrenia apresentam alterações no comprimento de telomero, um marcador biológico de envelhecimento patológico acelerado (artigo #3);
d. Estudar possíveis mecanismos relacionados à teoria de envelhecimento patológico acelerado a partir da análise das interações entre marcadores biológicos, de estrutura cerebral e cognitivos (artigo #4).

4 ARTIGO #1

Publicado no ***European Neuropsychopharmacology***

Fator de Impacto (2015): 4.409

Versão do manuscrito aceita:

Verbal Episodic Memory Along the Course of Schizophrenia and Bipolar Disorder: a New Perspective

Letícia S. Czepielewski[1]; Raffael Massuda[1]; Pedro Goi[1]; Miréia Sulzbach-Viana [1]; Ramiro Reckziegel[1]; Monise Costanzi[1]; Flavio Kapczinski[1]; Adriane R. Rosa[1] [2]; Clarissa S. Gama[1]

[1] *Laboratory of Molecular Psychiatry, INCT for Translational Medicine—CNPq, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Post Graduate Program in Medicine, Department of Psychiatry, Universidade Federal do Rio Grande do Sul*
Porto Alegre, Brazil.

[2] *Department of Pharmacology, Universidade Federal do Rio Grande do Sul Porto Alegre, Brazil*
Centro de Investigación Biomédica en Red de Salud Mental, (CIBERSAM), Madrid

Address for correspondence: Clarissa S Gama, MD, PhD
Hospital de Clínicas de Porto Alegre
Laboratório de Psiquiatria Molecular
Ramiro Barcelos, 2350 - CEP 90035-903
+55- 0xx51-33598845

Abstract

Impairment on episodic memory (EM) has been strongly correlated to psychiatric disorders, including schizophrenia (SZ) and bipolar disorder (BD). Morevover, the effects of course and progression of the illness on cognitive functioning has not been well established. The aim of the present study is to assess performance of episodic memory in BD and SZ according to their clinical stages.

Subjects who met DSM-IV criteria for bipolar disorder (n=43) and schizophrenia (31), on euthymia or clinical remission, were recruited from the outpatients facilities at Hospital de Clínicas de Porto Alegre (Brazil). They were classified into two clinical stages (early or late for BD, and recent onset or chronic for SZ) and compared to 54 healthy controls. Episodic memory performance was assessed by means the Hopkins Verbal Learning Test – Revised (HVLT-R), that measures verbal learning and episodic memory in both disorders. Our results showed that patients in early stage of BD (EBD) performed better performance on the total immediate free recall ($p<0.0001$, $F=12.060$) as well as in delayed free recall ($p<0.0001$, $F=13.914$) compared to late stage (LBD) and SZ groups. In the ability to retain words learned, LBD and chronic (CSZ) were more impaired than other groups. Furthermore, the variation of learning (i.e, learning effects) along the 3 trials of immediate free recall was similar between groups.

In conclusion, we found a cognitive decline alongside with the progression of BD whereas such impairment was evident in the early of SZ. Despite of this, both groups (BD and SZ) seem to maintain the ability to learn. It emphasizes the relevance of studying new therapeutic strategies, in particular, cognitive rehabilitation/remediation techniques' as promissory treatment for psychiatric patients, even in those with moderate disabilities.

Key words: Episodic Memory; Cognitive dysfunction; Bipolar Disorder; Schizophrenia;

1. Introduction

Cognitive impairment has been consistently associated with severe psychiatric disorders like schizophrenia (SZ) and bipolar disorder (BD) (Vöhringer et al., 2013). SZ and BD present a similar cognitive deficits profile, although their degrees of dysfunction may be different (Vöhringer et al., 2013; Schretlen et al., 2013). Multiple factors such as psychotic symptoms, recurrences, chronicity, drug abuse and medication may contribute to cognitive deficits in both disorders (Au et al., 2013; Meyer et al., In Press). Memory impairment is one of the core features of cognitive and functional decline in psychiatric population (Kuswanto et al., 2013; Schaefer et al., 2013; Yatham et al., 2010).

Episodic memory (EM), an independent declarative memory system, is responsible for storage and conscious recall of past personal experiences that contains details about spatial and temporal context of these occurrences - what/when/where happened (Tulving, 2002). There is an interrelation with memory and other important cognitive domains, such as executive components and language. EM is highly sensitive to aging and to neurodegenerative diseases, and its performance is thought to be predictive of long-term outcome (Pause et al., 2013). Furthermore, in either BD or SZ, EM has been correlated to functioning in everyday life domains (Danion et al., 2007), especially in work performance (Tse et al., 2013; Gilbert and Marwaha, 2013).

A novel approach to understand severe mental disorders is to adopt a clinical staging model (Wood et al., 2011). This model is useful as it differentiates early, milder clinical phenomena from those that accompany illness progression and chronicity (McGorry et al., 2010). Staging models for SZ (Agius et al., 2010; Wood et al., 2011) and BD (Vieta et al., 2011; Kapczinski et al., 2009a,b; Berk et al., 2007) have been proposed in order to personalize and optimize treatments (Berk et al., 2009).

Although cognitive functioning is widely studied in SZ and BD, the performance of EM along the course and progression of both diseases has not yet been studied. Therefore, our goal is to ascertain whether SZ and BD show different patterns of episodic memory performance, according to their clinical stages.

2. Experimental Procedures

Subjects

We included 128 subjects that were recruited throughout outpatient facilities at the Hospital de Clínicas de Porto Alegre, Brazil. The study was approved by the Institutional Review Board, and all individuals signed informed consent after the procedures were fully explained. The participants had ages between 18-65 years, and were allocated in six groups: 23 patients with recent-onset schizophrenia (RSZ), 20 patients with chronic schizophrenia (CSZ), 17 patients with early-stage bipolar disorder (EBD), 14 patients with late-stage bipolar disorder (LBD), 28 healthy controls matched with the recent-onset/early-stage patients (EC) and 26 healthy controls matched with chronic/late-stage patients (LC). Patients with BD were in euthymia for at least a month and patients with SZ were in symptomatic remission for at least 6 months. All patients were receiving pharmacological treatment according to previously determined protocols.

The groups of controls (EC and LC) consisted of healthy subjects selected from the pool volunteers at the hospital. They had no current or previous history and no first-degree family history of major psychiatric disorders, including dementia or mental retardation, assessed by the non-patient version of the Structured Clinical Interview for DSM-IV (SCID). They were matched for level of education with the groups of patients, following the early or late distribution.

Patients were diagnosis with BD or SZ according to DSM-IV. Euthymia in BD was defined by the total score on the Young Mania Rating Scale (Young et al., 1978) and Hamilton Depression Rating Scale (Hamilton, 1960) less than 8. Symptomatic remission in SZ was confirmed by Brief Psychiatric Rating Scale (BPRS < 15) (Romano and Elkis, 1996). Early and late classification of BD patients was established in accordance with the staging model described by Kapczinski et al. (2009b). To that end, a semi-structured interview was administered to each patient by two psychiatrists with PhD degrees previously trained in the model. The clinicians collected data on course of illness, presence or absence of psychiatric comorbidities, subjective assessment of work activity and social interactions, and self-care. Patients were stratified into early and late clinical stages by the clinicians considering the self-perception of the patient, regardless of functional status results, as follows: (1) Early stage (stage I), individuals who referred the same functioning in the inter-episodic period as they did before the onset of BD; and (2) Late stage (stage IV), individuals

who referred being unable to maintain personal self-care and to live autonomously. Medical charts were carefully checked and the clinician responsible for each patient consulted in cases of inter-rater disagreement, so that a final decision on clinical staging could be reached. Both clinicians were blind to the results of the clinical evaluation, as well as of cognitive assessment. The same method has been successfully used previously by Rosa et al. (2014).

For SZ patients, recent-onset patients were those within first 5 years of SZ diagnosis while chronic patients had minimum of 20 years after the diagnosis of SZ. This allocation criteria is supported by previous studies (Pedrini et al., 2014).

Assessment

Subjects underwent a psychiatric evaluation to collect sociodemographic, clinical, and pharmacological data by a structured interview and examining the patients' clinical records. Experienced raters administered the scales of symptoms to assess psychiatric status.

Subsequently, the participants were assessed by trained psychologists with the Hopkins Verbal Learning Test – Revised (HVLT-R), which is a word-list task widely used in psychiatric diseases that measures verbal learning and episodic memory (Benedict et al., 1998). HVLT-R was included in MATRICS Consensus Battery for Schizophrenia (Nuechterlein et al., 2008) and in the proposed battery by ISBD for Bipolar Disorder (Yatham et al., 2010), therefore is an appropriated instrument for both SZ and BD. It is comprised by 3 immediate recall trials of 12 words within 3 categories and a delayed recall followed by a recognition task. For the immediate recall, on each trial the subject is asked to say as much words enunciated by the psychologist as possible. For the delayed recall, after 20 minutes of the immediate recall trials, the subject is requested to evoke all words he/she can remember without any cues.

Statistical analysis

Analysis was performed using SPSS Version 20.0 software. Demographic and clinical characteristics were analyzed using Chi-Square and analysis of variance (ANOVA). Descriptive analyses are presented as mean and standard deviation. P-values < 0.05 were considered significant. A general linear model for multivariate was used to control the effect of age and sex in HVLT-R measures. A general linear

model for repeated measures was used to check the variation of learning between groups along the 3 immediate recall trials.

3. Results

Sample characteristics are summarized in Table 1.

Significant differences were found in all HVLT-R measures along the groups (Table 2). For the total immediate free recall, EC and LC performed similarly to EBD, which was significant better than LBD, RSZ and CSZ ($p<0.0001$, $F=12.060$). This same performance was found for delayed free recall ($p<0.0001$, $F=13.914$). In the ability to retain words learned, LBD and CSZ presented worst retention ratios than other groups ($p=0.03$, $F=3.814$). Nevertheless, the variation of learning (learning curve) along the 3 trials of immediate free recall was similar between groups ($p=0.497$, $F=0.879$; Figure 1.).

4. Discussion

As far as we concern, this is the first study to show EM performance of BD and SZ according to their clinical stages. Our findings showed that in SZ, EM impairment occurs in the early stage of illness as patients with a recent-onset performed similar impairment to chronic patients. Contrasting to SZ, early-stage individuals (EBD) and healthy controls experienced similar cognitive performance and better than late-stage BD group (LBD). The same degree of EM dysfunctions was performed by late-stage BD group and both groups of individuals with SZ. Although cognitive performance was different between groups, the variation of learning effects along the trials of immediate free recall was similar.

Cognitive impairment is an important clinical feature of psychiatric patients. In our sample, in line with other studies (Rodríguez-Sánchez et al., 2013; Zhou et al., 2012), patients with SZ at early-onset had a similar performance on EM tests compared to chronic patients. EM performance has been proposed as a potential predictive factor for developing psychosis in individuals at high-risk (Valli et al., 2012). Persistent verbal episodic memory deficits have been associated with poorer clinical progression (Rodríguez-Sánchez et al., 2013) and poor psychosocial function (Sánchez-Morla et al., 2009). Cognitive remediation is a promising approach to improve real world functioning in SZ, it may boost functional and vocational outcomes (Bell et al., 2014) in patients at chronic disability. Nevertheless speculative, it also

should be considered a key strategy for early intervention in the psychoses (Barlati et al., 2013).

Our results corroborate the findings of BD as a progressive disease that would impair cognition along its course (Gama et al., 2013, Grande et al., In Press; Rosa et al., 2014). Impairment of EM in euthymic patients is in line with literature (Radanovic et al., 2013); however, comparisons showing similar performance of early-stage patients compared to controls and worst performance of late-stage group comparable with both groups of individuals with SZ has never been done before.

EM impairment could be mediated by speed or quality of information processing (Maekawa et al., 2013; Tsai et al., 2012). Nevertheless, the primary deficit seems to be due to poorer integration of context information (Talamini et al., 2010) and organization strategies during encoding of episodic representations both in BD (Deckersbach et al., 2004) and SZ (Grillon et al, 2010). However, it does not appear to reflect deficits in retention of information (Deckersbach et al., 2004); neither is attributable to general intellectual deficiency (Kopald et al., 2012). EM dysfunctions may be a consequence of impairment in proactive control that is the ability to guide behavior by actively representing goal information (Barch and Ceaser, 2012). This is supported by the deficit in the frontal activity found in fMRI study during EM task when comparing BD and controls. The lower activation in frontal areas during memory encoding and retrieval in BD euthymic patients was associated with dysfunctional cognitive control in organizing verbal information appropriately during learning (Oertel-Knöchel et al., 2013).

Although both SZ groups and LBD patients showed impairment in total learned words compared to controls, they presented an increasing learning over the trials (more words remembered in the last trial than the first). These results suggest that LBD and SZ patients were able to apprehend, even if significantly lesser than other groups. The most impaired group had, on the third trial, a similar performance than controls on first trial (Figure 1). Thus, it highlights the scope for interventions; suggesting that with training, even the most affected subject could improve EM performance.

Therefore, our study supports the importance of therapeutic interventions, such as cognitive remediation or rehabilitation. In this regard, there is a growing body of evidence showing that memory is a primary improvement outcome following cognitive remediation (Demant et al., 2013), and this type of treatment improves

adaptive competence and real-world skills (Bowie et al., 2014). Taken together, these findings emphasize the importance to implement cognitive remediation to the psychiatric population in order to improve functional recovery.

Our results must be interpreted in light of some limitations. First, we didn't assess general intellectual functioning. However, it doesn't seem to be related with EM performance (Kopald et al., 2012). Second, we didn't control the data analysis for the effect of psychopharmacological treatment and nearly all patients were on polypharmacy. Third, we didn't used the same scales for both disorders to assess clinical stability. Finally, our sample was recruited at a single tertiary hospital in Porto Alegre, southern Brazil, which may limit the generalization of findings to other cultures and countries. Notwithstanding in a large follow-up study, generalized cognitive impairment in SZ has remained robust over the time despite changes in assessment instruments and alterations in diagnostic criteria; besides, it manifests similarly in different regions of the world despite linguistic and cultural differences (Schaefer et al., 2013).

In conclusion, our results showed a progressive cognitive decline along the progression of BD and an early cognitive impairment in SZ. Nevertheless, independent of groups, all individuals seem to maintain the ability to learn. These findings give support to previous studies (Torrent et al., 2013) showing the efficacy of cognitive and functional rehabilitation/remediation techniques' and open a new venue for therapeutic in these highly neglected groups. Moreover, it would help to alleviate the burden of these severe psychiatric illnesses and should become the subject of intensive research.

Author Disclosures

Dr. Gama has been a paid speaker for Lundbeck and a consultant/speaker for Roche, Pfizer and Actelion Pharmaceuticals Ltda. Dr. Kapczinksi has received grant/support from Astra-Zeneca, Eli Lilly, Janssen-Cilag Servier, CNPq, Capes, NARSAD and the Stanely Medical Research Institute; has been a member of the speaker´s boards of Astra-Zeneca, Eli Lilly, Janssen-Cilag Servier and has been served as a consultant for Servier. Dr Rosa has served as speaker for Eli Lilly. The others authors have no potential or other conflicts of interest to report.

Funding support

This study was supported by grants from CNPq (Universal 470326/2011-5 and PQ 302195/2011-4), CNPq (Ciência sem Fronteiras, 40.00032/2012-0), FAPERGS (PqG 1009340-06/2010) and FAPERGS/CNPq (PRONEM 11/2057-2), Brazil. These agencies had no role in the study design, in the acquisition or interpretation of the data, or in writing the report.

Acknowledgements

Dr. Rosa is grateful for the support provided by L'Oréal Brasil, Academia Brasileira de Ciências, and Comissão Nacional da UNESCO, "For Women in Science."

References

Agius, M., Goh, C., Ulhaq, S., McGorry, P., 2010. The staging model in schizophrenia, and its clinical implications. Psychiatria Danubina, 22(2), 211-20.

Au, R.W., Ungvari, G.S., Lee, E., Man, D., Shum, D.H., Xiang, Y.T., Tang, W.K., 2013. Prospective memory impairment and its implications for community living skills in bipolar disorder. Bipolar Disord, 15(8), 885-92.

Barch, D.M., Ceaser, A., 2012. Cognition in schizophrenia: core psychological and neural mechanisms. Trends Cogn. Sci., 16(1), 27-34.

Barlati, S., Deste, G., De Peri, L., Ariu, C., Vita, A., 2013. Cognitive Remediation in Schizophrenia: Current Status and Future Perspectives. Schizophr Res Treatment, 2013:156084 [Epub ahead of print].

Bell, M.D., Choi, K.H., Dyer, C., Wexler, B.E., 2014. Benefits of Cognitive Remediation and Supported Employment for Schizophrenia Patients With Poor Community Functioning. Psychiatr Serv., Jan 2. [Epub ahead of print]

Benedict, R.H.B., Schretlen, D., Groninger, L., Brandt, J., 1998. Hopkins Verbal Learning Test – Revised: Normative Data and Analysis of Inter-Form and Test-Retest Reliability. The Clinical Neuropsychologist, 12(1), 43-55.

Berk, M., Hallam, K.T., McGorry, P.D., 2007. The potential utility of a staging model as a course specifier: a bipolar disorder perspective. Journal of Affective Disorders, 100(3), 279-81.

Berk, M., Malhi, G.S., Hallam, K., Gama, C.S., Dodd, S., Andreazza, A.C., et al., 2009. Early intervention in bipolar disorders: clinical, biochemical and neuroimaging imperatives. Journal of Affective Disorders, 114(1-3), 1-13.

Berk, M., Conus, P., Kapczinski, F., Andreazza, A.C., Yücel, M., Wood, S.J., et al., 2010. From neuroprogression to neuroprotection: implications for clinical care. Medical Journal of Australia, 193(4 Suppl.), S36-40.

Berk, M., Kapczinski, F., Andreazza, A.C., Dean, O.M., Giorlando, F., Maes, M., et al, 2011. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neuroscience Biobehavior Review, 35(3), 804-17.

Bowie, C.R., Grossman, M., Gupta, M., Oyewumi, L.K., Harvey, P.D., 2014. Cognitive remediation in schizophrenia: efficacy and effectiveness in patients with early versus long-term course of illness. Early Interv. Psychiatry, 8(1), 32-8.

Danion, J. M., Huron, C., Vidailhet, P., Berna, F., 2007. Functional mechanisms of episodic memory impairment in schizophrenia. Can. J. Psychiatry, 52, 693–701.

Deckersbach, T., McMurrich, S., Ogutha, J., Savage, C.R., Sachs, G., Rauch, S.L., 2004. Characteristics of non-verbal memory impairment in bipolar disorder: the role of encoding strategies. Psychol. Med., 34(5), 823-32.

Delaloye, C., Moy, G., de Bilbao, F., Weber, K., Baudois, S., Haller, S., Xekardaki, A., Canuto, A., Giardini, U., Lövblad, K.O., Gold, G., Giannakopoulos, P., 2011. Longitudinal analysis of cognitive performances and structural brain changes in late-life bipolar disorder. Int. J. Geriatr. Psychiatry, 26(12), 1309-18.

Demant, K.M., Almer, G.M., Vinberg, M., Kessing, L.V., Miskowiak, K.W., 2013. Effects of cognitive remediation on cognitive dysfunction in partially or fully remitted patients with bipolar disorder: study protocol for a randomized controlled trial. Trials, 14, 378.

Francey, S.M., Nelson, B., Thompson, A., Parker, A.G., Kerr, M., Macneil, C., et al., 2010. Who needs antipsychotic medication in the earliest stages of psychosis? A reconsideration of benefits, risks, neurobiology and ethics in the era of early intervention. Schizophrenia Research, 119(1-3), 1-10.

Gama, C.S., Kunz, M., Magalhães, P.V., Kapczinski, F., 2013. Staging and neuroprogression in bipolar disorder: a systematic review of the literature. Rev Bras Psiquiatr., 35(1), 70-4.

Gilbert, E., Marwaha, S., 2013. Predictors of employment in bipolar disorder: a systematic review. J. Affect. Disord., 145(2), 156-64.

Grande, I., Magalhães, P.V., Chendo, I., Stertz, L., Panizutti, B., Colpo, G.D., Rosa, A.R., Gama, C.S., Kapczinski, F., Vieta, E., 2014. Staging bipolar disorder: clinical, biochemical, and functional correlates. Acta Psychiatr Scand., Mar 15 [Epub ahead of print].

Grillon, M.L., Krebs, M.O., Gourevitch, R., Giersch, A., Huron, C., 2010. Episodic memory and impairment of an early encoding process in schizophrenia. Neuropsychology, 24(1), 101-8.

Hamilton, M., 1960. A rating scale for depression. J. Neurol. Neurosurg. Psychiatry, 23, 56-62.

Hellvin, T., Sundet, K., Simonsen, C., Aminoff, S.R., Lagerberg, T.V., Andreassen, O.A., Melle, I., 2012. Neurocognitive functioning in patients recently diagnosed with bipolar disorder. Bipolar Disord., 14(3), 227-38.

Kapczinski, F., Dias, V.V., Kauer-Sant'Anna, M., Brietzke, E., Vázquez, G.H., Vieta, E., et al., 2009a. The potential use of biomarkers as an adjunctive tool for staging bipolardisorder. Progress in Neuropsychopharmacology an Biological Psychiatry, 33(8), 1366-71.

Kapczinski, F., Dias, V.V., Kauer-Sant'Anna, M., Frey, B.N., Grassi-Oliveira, R., Colom, F., et al., 2009b. Clinical implications of a staging model for bipolar disorders. Expert Review of Neurotherapeutics, 9(7), 957-66.

Kopald, B.E., Mirra, K.M., Egan, M.F., Weinberger, D.R., Goldberg, T.E., 2012. Magnitude of impact of executive functioning and IQ on episodic memory in Schizophrenia. Biol. Psychiatry, 71(6), 545-51.

Kuswanto, C.N., Sum, M.Y., Sim, K., 2013. Neurocognitive Functioning in Schizophrenia and Bipolar Disorder: Clarifying Concepts of Diagnostic Dichotomy vs. Continuum. Front. Psychiatry, 4, 162.

McGorry, P.D., Nelson, B., Goldstone, S., Yung, A.R., 2010. Clinical staging: a heuristic and practical strategy for new research and better health and social outcomes for psychotic and related mood disorders. Canadian Journal of Psychiatry, 55(8), 486-97.

Maekawa, T., Katsuki, S., Kishimoto, J., Onitsuka, T., Ogata, K., Yamasaki, T., Ueno, T., Tobimatsu, S., Kanba, S., 2013. Altered visual information processing systems in bipolar disorder: evidence from visual MMN and P3. Front. Hum. Neurosci., 7, 403.

Meyer, E.C., Carrión, R.E., Cornblatt, B.A., Addington, J., Cadenhead, K.S., Cannon, T.D., McGlashan, T.H., Perkins, D.O., Tsuang, M.T., Walker, E.F., Woods, S.W., Heinssen, R., Seidman, the NAPLS group, 2014. The Relationship of Neurocognition and Negative Symptoms to Social and Role Functioning Over Time in Individuals at Clinical High Risk in the First Phase of the North American Prodrome Longitudinal Study. Schizophr Bull., Feb 18 [Epub ahead of print].

Nuechterlein, K.H., Green, M.F., Kern, R.S., Baade, L.E., Barch, D.M., Cohen, J.D., Essock, S., Fenton, W.S., Frese, F.J.3rd., Gold, J.M., Goldberg, T., Heaton, R.K., Keefe, R.S., Kraemer, H., Mesholam-Gately, R., Seidman, L.J., Stover, E., Weinberger, D.R., Young, A.S., Zalcman, S., Marder, S.R., 2008. The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity. Am. J. Psychiatry, 165(2), 203-13.

Oertel-Knöchel, V., Reinke, B., Feddern, R., Knake, A., Knöchel, C., Prvulovic, D., Fußer, F., Karakaya, T., Loellgen, D., Freitag, C., Pantel, J., Linden, D.E., 2013. Verbal episodic memory deficits in remitted bipolar patients: a combined behavioural and fMRI study. J. Affect. Disord., 150(2), 430-40.

Olvet, D.M., Burdick, K.E., Cornblatt, B.A., 2013. Assessing the potential to use neurocognition to predict who is at risk for developing bipolar disorder: a review of the literature. Cogn. Neuropsychiatry. 18(1-2), 129-45.

Pause, B.M., Zlomuzica, A., Kinugawa, K., Mariani, J., Pietrowsky, R., Dere, E., 2013.
Perspectives on episodic-like and episodic memory. Front. Behav. Neurosci., 18, 7-33.

Pedrini, M., Massuda, R., de Lucena, D., Macêdo, D., Paz, A.V., Lobato, M.I., Belmonte-de-Abreu, P.S., Ceresér, K.M., Rocha, N.P., Curra, M.D., Panizzutti, B.S., Teixeira, A.L., Gama, C.S., 2014. Differences in eotaxin serum levels patients with recent onset and in chronic stable schizophrenia: a clue for understanding accelerating aging profile. Schizophr Res., 152(2-3), 528-9.

Radanovic, M., Nunes, P.V., Forlenza, O.V., Braga Ladeira, R., Gattaz, W.F., 2013. Cognitive-linguistic deficits in euthymic elderly patients with bipolar disorder. J Affect Disord, 5, 150(2), 691-4.

Rodríguez-Sánchez, J.M., Ayesa-Arriola, R., Pérez-Iglesias, R., Periañez, J.A., Martinez-Garcia, O., Gomez-Ruiz, E., Tabares-Seisdedos, R., Crespo-Facorro, B.,

2013. Course of cognitive deficits in first episode of non-affective psychosis: a 3-year follow-up study. Schizophr. Res., 150(1), 121-8.

Romano, F., Elkis, H., 1996. Tradução e Adaptação de um Instrumento de Avaliação Psico-patológica das Psicoses: Escala Breve de Avaliação Psiquiátrica. Versão Ancorada (BPRS-A). Jornal Brasileiro de Psiquiatria, 45, 43-9.

Rosa, A.R., Magalhães, P.V., Czepielewski, L., Sulzbach, M.V., Goi, P.D., Vieta, E., Gama, C.S., Kapczinski, F., 2014. Clinical staging in bipolar disorder: focus on cognition and functioning. J Clin Psychiatry, 75(5), e450-6.

Sánchez-Morla, E.M., Barabash, A., Martínez-Vizcaíno, V., Tabarés-Seisdedos, R., Balanzá-Martínez, V., Cabranes-Díaz, J.A., Baca-Baldomero, E., Gómez, J.L., 2009. Comparative study of neurocognitive function in euthymic bipolar patients and stabilized schizophrenic patients. Psychiatry Res., 169(3), 220-8.

Schaefer, J., Giangrande, E., Weinberger, D.R., Dickinson, D., 2013. The global cognitive impairment in schizophrenia: consistent over decades and around the world. Schizophr. Res., 150(1), 42-50.

Schretlen, D.J., Peña, J., Aretouli, E., Orue, I., Cascella, N.G., Pearlson, G.D., Ojeda, N., 2013. Confirmatory factor analysis reveals a latent cognitive structure common to bipolar disorder, schizophrenia, and normal controls. Bipolar Disord., May 9.

Talamini, L.M., de Haan, L., Nieman, D.H., Linszen, D.H., Meeter, M., 2010. Reduced context effects on retrieval in first-episode schizophrenia. PLoS One, 5(4), e10356.

Tsai, P.C., McDowd, J., Tang, T.C., Su, C.Y., 2012. Processing speed mediates gender differences in memory in schizophrenia. Clin. Neuropsychol., 26(4), 626-40.

Torrent, C., Bonnin Cdel, M., Martínez-Arán, A., Valle, J., Amann, B.L., González-Pinto, A., Crespo, J.M., Ibáñez, Á., Garcia-Portilla, M.P., Tabarés-Seisdedos, R., Arango, C., Colom, F., Solé, B., Pacchiarotti, I., Rosa, A.R., Ayuso-Mateos, J.L.,

Anaya, C., Fernández, P., Landín-Romero, R., Alonso-Lana, S., Ortiz-Gil, J., Segura, B., Barbeito, S., Vega, P., Fernández, M., Ugarte, A., Subirà, M., Cerrillo, E., Custal, N., Menchón, J.M., Saiz-Ruiz, J., Rodao, J.M., Isella, S., Alegría, A., Al-Halabi, S., Bobes, J., Galván, G., Saiz, P.A., Balanzá-Martínez, V., Selva, G., Fuentes-Durá, I., Correa, P., Mayoral, M., Chiclana, G., Merchan-Naranjo, J., Rapado-Castro, M., Salamero, M., Vieta, E., 2013. Efficacy of functional remediation in bipolar disorder: a multicenter randomized controlled study. Am J Psychiatry, 170(8), 852-9.

Tse, S., Chan, S., Ng, K.L., Yatham, L.N., 2013. Meta-analysis of predictors of favorable employment outcomes among individuals with bipolar disorder. Bipolar Disord., Nov 13.

Tulving, E., 2002. Episodic memory: From mind to brain. Annual review of psychology, 53(1), 1-25.

Valli, I., Tognin, S., Fusar-Poli, P., Mechelli, A., 2012. Episodic memory dysfunction in individuals at high-risk of psychosis: a systematic review of neuropsychological and neurofunctional studies. Curr. Pharm. Des., 18(4), 443-58.

Vieta, E., Reinares, M., Rosa, A.R., 2011. Staging bipolar disorder. Neurotoxicity Research, 19(2), 279-85.

Vöhringer, P.A., Barroilhet, S.A., Amerio, A., Reale, M.L., Alvear, K., Vergne, D., Ghaemi, S.N., 2013. Cognitive impairment in bipolar disorder and schizophrenia: a systematic review. Front. Psychiatry, 4, 87.

Yatham, L.N., Torres, I.J., Malhi, G.S., Frangou, S., Glahn, D.C., Bearden, C.E., Burdick, K.E., Martínez-Arán, A., Dittmann, S., Goldberg, J.F., Ozerdem, A., Aydemir, O., Chengappa, K.N., 2010. The International Society for Bipolar Disorders-Battery for Assessment of Neurocognition (ISBD-BANC). Bipolar Disord., 12(4), 351-63.

Young, R.C., Biggs, J.T., Ziegler, V.E., Meyer, D.A., 1978. A rating scale for mania: reliability, validity and sensitivity. Br. J. Psychiatry, 133, 429-35.

Wood, S.J., Yung, A.R., McGorry, P.D., Pantelis, C., 2011. Neuroimaging and treatment evidence for clinical staging in psychotic disorders: from the at-risk mental state to chronic schizophrenia. Biological Psychiatry, 70(7), 619-25.

Zhou, F.C., Xiang, Y.T., Wang, C.Y., Dickerson, F., Au, R.W., Zhou, J.J., Zhou, Y., Shum, D.H., Chiu, H.F., Man, D., Lee, E.H., Yu, X., Chan, R.C., Ungvari, G.S., 2012. Characteristics and clinical correlates of prospective memory performance in first-episode schizophrenia. Schizophr Res., 135(1-3), 34-9.

Table 1. Sociodemographic and clinical data

	Early Stage				Late Stage			
	SZ (n=23)	BD (n=17)	Controls (n=28)	P-Value	SZ (n=20)	BD (n=14)	Controls (n=26)	P-Value
Gender								
male/female	13/10	4/13	14/14	0.097 [b]	15/4	13/5	13/13	0.026 [b]
Age in years [a]	24.70 (4.78)	40.65 (14.03)	32.54 (11.86)	<0.001 [c]	47.90 (3.82)	52.07 (11.64)	49.73 (8.73)	0.361 [c]
Years of education [a]	9.75 (3.73)	9.76 (3.40)	11.39 (2.84)	0.144 [c]	9.81 (3.15)	8.31 (2.02)	8.62 (3.19)	0.360 [c]
Years of disease [a]	2.52 (1.83)	10.88 (8.21)	---	---	24.95 (3.23)	21.00 (12.97)	---	---
BPRS score [a]	15.61 (8.09)	---	---	---	13.58 (5.19)	---	---	---
HAM-D score [a]	---	1.12 (1.58)	---	---	---	4.38 (2.87)	---	---
YMRS score [a]	---	1.00 (1.66)	---	---	---	1.54 (2.02)	---	---
Medication , n (%)								
Lithium	---	9 (56.3)	---	---	---	5 (35.7)	---	---
Anticonvulsants	---	10 (62.5)	---	---	---	7 (50.0)	---	---
Atypical antipsychotics	10 (43.50)	5 (31.3)	---	---	3 (15.0)	6 (42.85)	---	---
Typical antipsychotics	7 (30.43)	0	---	---	1 (5.0)	2 (14.3)	---	---
Clozapine	6 (26.07)	0	---	---	16 (80.0)	4 (28.57)	---	---
Antidepressants	---	4 (15.4)	---	---	---	2 (14.3)	---	---
Benzodiazepines	---	0	---	---	---	3 (21.4)	---	---

BD, Bipolar Disorder; SZ, Schizophrenia; BPRS, Brief Psychiatric Rating Scale; HAM-D, Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale

[a] Shown as mean (SD); [b] Chi-square, [c] Analysis of variance

Table 2. Hopkins Verbal Learning Test-Revised (HVLT-R) variables

	Early Controls (EC)		Late Controls (LC)		Early-stage BD (EBD)		Recent-onset SZ (RSZ)		Late-stage BD (LBD)		SZ Chronic Patients (CSZ)	F	p
Immediate Free Recall – 1st trial	6.26 (1.79)	=	6.15 (1.83)	=	5.56 (1.82)	>	4.52 (1.75)	=	4.21 (1.12)	=	3.40 (1.79)	9.611	.000 *
Immediate Free Recall – 2nd trial	8.74 (2.09)	=	7.96 (1.95)	=	6.94 (1.73)	>	6.26 (2.03)	=	5.86 (1.96)	=	5.55 (2.21)	8.795	.000 *
Immediate Free Recall – 3rd trial	9.15 (1.95)	=	9.19 (2.00)	=	7.69 (2.24)	>	6.83 (2.29)	=	6.50 (1.65)	=	6.10 (2.43)	9.057	.000 *
Immediate Free Recall – total recall	24.15 (4.92)	=	23.31 (4.99)	=	20.19 (4.92)	>	17.52 (5.39)	=	16.57 (4.09)	=	15.05 (5.64)	12.060	.000 *
Delayed Free Recall	8.70 (1.90)	=	7.50 (2.76)	=	6.25 (2.35)	>	5.09 (2.09)	=	4.36 (2.34)	=	4.20 (2.31)	13.914	.000 *
% Retention	49.31 (10.26)	=	42.88 (12.46)	=	42.07 (9.61)	=	39.75 (14.48)	>	34,27 (14.46)	=	35.47 (16.22)	3.814	0.03 *
Recognition	11.44 (1.01)	=	11.00 (1.36)	=	10.62 (1.31)	>	10.09 (1.53)	=	9.79 (2.32)	=	10.55 (2.06)	3.033	.013 *

EC, controls matched with the recent-onset/early-stage patients; LC, controls matched with chronic/late-stage patients; EBD, patients with early-stage bipolar disorder; RSZ, patients with recent-onset schizophrenia; LBD, patients with late-stage bipolar disorder; CSZ, patients with chronic schizophrenia

* Test of between subjects effects

Multivariate test (Pillai trace): p=0.343 F=1.141 , for gender; P=0.115 , F=1.707 , for age; P<0.0001, F=2.483 , for group.

Figure 1.

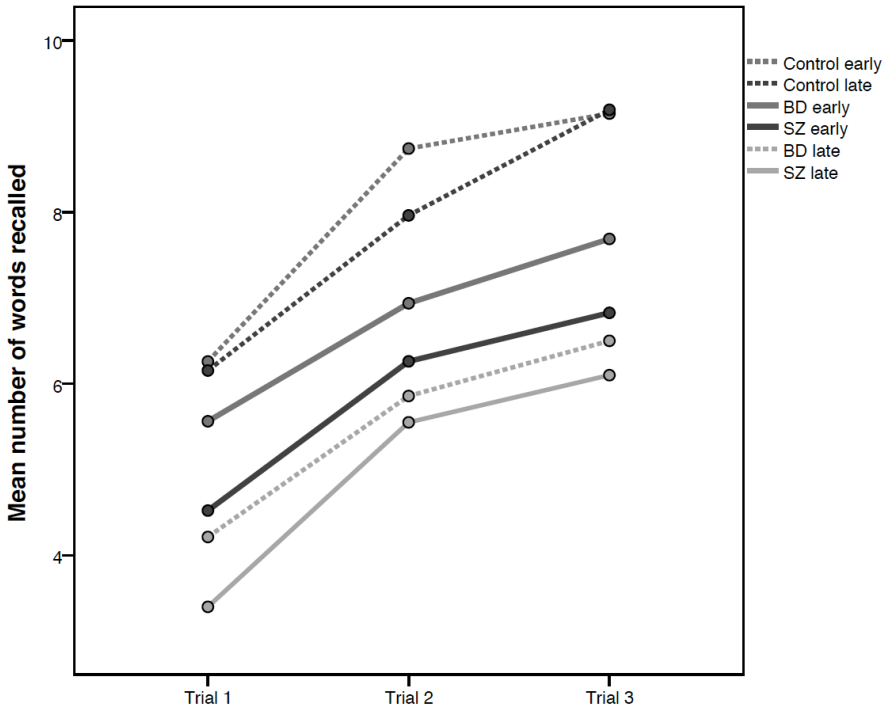

Figure 1. The variation of learning along the 3 trials of immediate free recall between groups footnote: General linear model for repeated measures (p=0.497, F=0.879).

5 ARTIGO #2

Publicado no ***Schizophrenia Bulletin***

Fator de Impacto (2015): 7.757

Versão do manuscrito aceita:

The Relationship of Intellectual Functioning and Cognitive Performance to Brain Structure in Schizophrenia

Letícia S Czepielewski[1,2], Lei Wang[3], Clarissa S Gama[2], Deanna M Barch[1,4]

[1] Department of Psychological & Brain Sciences, Washington University in St. Louis, MO, USA

[2] Laboratório de Psiquiatria Molecular, Hospital de Clínicas de Porto Alegre, Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

[3] Departments of Psychiatry and Behavioral Sciences, Radiology, Northwestern University Feinberg School of Medicine, Chicago, IL, USA

[4] Departments of Psychiatry & Radiology, Washington University in St. Louis, MO, USA

Corresponding Author:

Leticia Sanguinetti Czepielewski

Washington University in St. Louis, Department of Psychology

Box 1125, One Brookings Drive, St. Louis, MO, USA 63130

Phone: 314-935-8729, Fax: 314-935-8790, Email: czepielewski@gmail.com

Word count Abstract: 250

Word count text body: 4803

Abstract

Background: Schizophrenia (SZ) is often characterized by cognitive and intellectual impairment. However, there is much heterogeneity across individuals, suggesting different trajectories of the illness. Recent findings have shown brain volume differences across subgroups of individuals with psychosis (schizophrenia and bipolar disorder), such that those with intellectual and cognitive impairments presented evidence of early cerebral disruption, while those with cognitive but not intellectual impairments showed evidence of progressive brain abnormalities. Our aim was to investigate the relations of cognition and intellectual functioning with brain structure abnormalities in a sample of SZ compared to unaffected individuals.

Methods: 92 individuals with SZ and 94 healthy controls part of the Northwestern University Schizophrenia Data and Software Tool (NUSDAST) underwent neuropsychological assessment and structural MRI. Individuals with SZ were divided into subgroups according their estimated premorbid crystalized intellectual (ePMC-IQ) and cognitive performance. Brain volumes differences were investigated across groups.

Results: SZ with ePMC-IQ and cognitive impairments had reduced total brain volume, intracranial volume (ICV), total brain volume corrected for ICV, and cortical gray matter volume, as well as reduced cortical thickness, and insula volumes. SZ with cognitive impairment but intact ePMC-IQ showed only reduced cortical gray matter volume and cortical thickness.

Conclusions: These data provide additional evidence for heterogeneity in SZ. Impairments in cognition associated with reduced ePMC-IQ were related to evidence of broad brain structural alterations, including suggestion of early cerebral disruption. In contrast, impaired cognitive functioning in the context of more intact intellectual functioning was associated with cortical alterations that may reflect neurodegeneration.

Introduction

Cognitive impairments are a key component of schizophrenia (SZ). Individuals diagnosed with SZ show significant deficits across a number of different cognitive domains, such as sustained attention, executive function, working memory and episodic memory. Further, poor cognitive performance is consistently associated with poorer functional outcomes regardless of age, gender or illness chronicity[1]. Previous studies have suggested some potential common mechanisms that could influence performance across a number of putatively different cognitive domains, such as impairments in structure, function and connectivity of prefrontal, parietal, anterior cingulate and anterior insula[2]. However, while some individuals with SZ show altered cognitive function in the context of reduced premorbid crystalized intellectual functioning (ePMC-IQ), others seem to have more intact ePMC-IQ. This variability seems to suggest potential heterogeneity of neuropathology[3]. As such, the goal of the current study was to extend prior work on cognitive function and IQ in SZ by examining the gray and white matter alterations associated with impaired cognition versus impaired ePMC-IQ.

Individuals with SZ, in addition to the cognitive deficits, show on average a medium-sized deficit in ePMC-IQ compared to healthy controls[4,5]. Not surprisingly, prior research has shown that individuals with SZ who have preserved premorbid crystalized IQ perform better in overall cognitive scores compared to individuals with either a deterioration in crystalized IQ (e.g. normal premorbid IQ, but reduced current IQ) or compromised IQ (e.g. both premorbid and current low IQ)[6], suggesting that the course of intellectual functioning could play a role in the illness progression. One hypothesis has been that reduced ePMC-IQ could be thought as a marker of early neurodevelopmental abnormality. According to the neurodevelopmental hypothesis, the etiology of SZ is influenced by an interaction of genetic and environmental factors that were present before the known onset of illness, affecting the brain before it approaches its adult anatomical state[7]. There is considerable evidence for pathological risk factors that influence early neurodevelopment in SZ, such as both pre- and perinatal (infection, placental pathology, low birth weight), and premorbid (urban environment, childhood trauma, ethnic minority, migrant status) risk factors[8]. Individuals with SZ are more likely to have experienced a combination of these early events during development[9], which in turn could influence brain maturation.

There is strong evidence of reduced brain volume in SZ, including total brain, intracranial and gray matter[10], and these reduced volumes have been associated with impaired cognitive performance[11]. Previous work by Woodward and Heckers[3] showed differences in brain structure among subgroups of individuals with SZ and psychotic bipolar disorder (BD) divided according to ePMC-IQ and current neuropsychological functioning. Cognitively impaired individuals with below-average ePMC-IQ presented evidence of early cerebral hypoplasia demonstrated by reduced intracranial volume. Conversely, both patients with average ePMC-IQ plus current cognitive deficits, and neuropsychologically intact individuals showed reduced total brain volume, which is consistent later cerebral dysmaturation or neurodegeneration. These data provided intriguing evidence about the potential differential relationships of ePMC-IQ versus cognitive function to early neural developmental alterations versus potentially later cerebral changes. However, the Woodward and Heckers[3] study included individuals with diagnoses of both SZ and BD, with a higher percentage of individuals with BD in the neuropsychological intact (32%) versus impaired (19%) groups. Further, the neuropsychologically normal individuals were significantly younger than the impaired individuals. These issues raise the possibility that some of the effects of neuropsychological function could have reflected either diagnosis or age related effects.

Therefore, the current study was designed to investigate the relations between cognition and ePMC-IQ with brain structure abnormalities in a sample of individuals with only diagnoses of SZ compared to healthy controls with similar ages across subgroups. Based on the prior work of Woodward and Heckers[3], we hypothesized that participants with SZ presenting both reduced ePMC-IQ and cognitive deficits would have smaller whole brain and intracranial volumes, as well as reduced gray matter volumes compared to healthy controls. In contrast, we hypothesized that individuals with SZ who had impaired cognition in the context of intact ePMC-IQ would show reduced total brain volume, but only when corrected for intracranial volume. In addition, we were interested in examining whether there might be alterations in cortical thickness or alterations in specific regions related to cognitive performance in SZ in past research, such as hippocampus, anterior cingulate cortex, dorsolateral prefrontal cortex and anterior insula[11,12,13,14] .

Methods

Participants

Participants were part of the Northwestern University Schizophrenia Data and Software Tool (NUSDAST), and the data used in preparation of this article were obtained from XNAT Central (https://central.xnat.org/) and SchizConnect database (http://schizconnect.org). Data collection and sharing was approved by the local institutional review board and was funded by NIMH grant 1R01 MH084803 and by NIMH cooperative agreement 1U01 MH097435, and its procedures were extensively described in previous publication[15]. For the purpose of this study, we considered only participants with SZ and healthy controls (HC) at baseline (some participants had follow up data) who had available cognitive data.

Ninety-four individuals with SZ and 94 unaffected participants were included. The inclusion criteria were: (1) having a diagnosis of schizophrenia or being a healthy control; (2) completing the neuropsychological battery described below; (3) participating in an MRI (magnetic resonance imaging) scanning session that included acquisition of a T1-weighted structural scan. Exclusion criteria were: (1) met DSM-IV criteria for intellectual disability (mild or greater in severity); (2) had a clinically unstable or severe medical disorder, or a medical disorder that confounded the assessment of psychiatric diagnosis or rendered research participation dangerous; (3) had a head injury (past or present) with documented neurological sequelae or loss of consciousness; (4) met DSM-IV criteria for current substance abuse or dependence (i.e., during the month preceding assessment). Informed consent was obtained from each participant after a complete description of the study was given.

Participants with SZ were recruited from local inpatient and outpatient treatment facilities, and were stabilized on antipsychotic medication for at least 2 weeks before participating in the study. Severity of psychotic symptoms was assessed in patients using Scale for the Assessment of Positive Symptoms (SAPS) and Scale for the Assessment of Negative Symptoms (SANS). Healthy controls were recruited using local advertisements in the same community, and were required to have no lifetime history of Axis I psychotic or major mood disorders (e.g., major depressive disorder and bipolar disorder) and no first-degree relatives with a psychotic disorder.

Neuropsychological Assessment

Participants completed a neuropsychological battery to evaluate several of the cognitive domains impaired in SZ. The assessment was comprised of the subtests Logical Memory, Family Pictures, Letter-Number Sequencing, Spatial Span, and Digit Span from the Wechsler Memory Scale (WMS-III). The scaled scores were converted into z scores that were subsequently summed to create a composite score of overall cognitive functioning (COG). For an estimate of crystallized knowledge and premorbid crystalized intellectual functioning (ePMC-IQ), we used the Vocabulary subtest from Wechsler Adult Intelligence Scale (WAIS-III).

Participants with SZ were then divided into groups based on their ePMC-IQ and current cognitive functioning. This classification was made considering the patients' performance above or below the 10^{th} percentile of the healthy control distribution for either the composite cognitive score (COG) or the ePMC-IQ measure, as proposed by Woodward and Heckers[3]. The resulted categories were the following: (1) IQ+/COG+: normal ePMC-IQ and not cognitively impaired (n = 25); (2) IQ+/COG-: normal ePMC-IQ and cognitively impaired (n = 31); (3) IQ-/COG-: lower ePMC-IQ and cognitively impaired (n = 36); (4) IQ-/COG+: lower ePMC-IQ and not cognitively impaired (n = 2). Because the last group had a very small sample size, it was not included in subsequent analyses.

Neuroimaging

NUSDAST provided T1-weighted scans that were acquired on a 1.5 T Vision scanner (Siemens Medical Systems) and collected using an MPRAGE sequence (TR = 10 ms, TE = 4 ms, flip angle = 30° ACQ-1, Matrix = 256 × 256, scanning time = 5.6 min) with 1 mm × 1 mm × 1.25 mm isotropic resolution. Scans were then analyzed and processed using FreeSurfer release 3.0.5 with manual editing in accordance to guidelines provided by FreeSurfer. A 2-dimensional smoothing kernel was applied along the cortical surface with a 20 mm full-width/half-maximum window. Spherical maps for each participant were morphed into a common spherical atlas using a nonlinear surface-registration procedure that allows for high-registration, surface-based averaging, and comparison of cortical measurements across participants[16].

Estimated total intracranial volume (ICV) was defined as the sum of gray matter, white matter, and CSF. Total brain volume (TBV) was defined as the sum of gray matter and white matter. The variables considered in this study were TBV, ICV, whole white and gray matter volumes, cortical white and gray volumes, subcortical gray matter volume, cerebrospinal fluid volume (CSF), cortical thickness, anterior

cingulate cortex volume (ACC), middle frontal gyrus volume, hippocampus volume and anterior insula volume. These regionally specific volumes were taken from the Destrieux parcellation in Freesurfer[17].

Data Analysis

Statistical analyses were completed in SPSS v20. To analyze differences between groups in demographic and clinical data, we used one-way ANOVAs. All brain volumes were analyzed using ANCOVAs with age and gender entered as covariates. For TBV, we repeated the analysis including ICV as an additional covariate. Volumes of specific brain regions were examined also controlling for TBV. Our next level was to compare whole brain volumes for gray and white separately, as well as for cortical and subcortical and cortical thickness. Our last level of analysis was to examine the four specific brain volumes of interest. We used FDR to correct for multiple comparisons across all of these comparisons. In addition, when a brain volume variable showed a significant effect of group, we compared the different subgroups in posthoc analyses.

Results

Demographic, cognitive and clinical data are presented in Table 1, and Supplementary Figures 1 and 2. The groups had similar age, but the gender distribution differed across groups. As expected, healthy controls (HC) had more years in school than cognitively impaired SZ (IQ-/COG- and IQ+/COG-; $p < .001$), and SZ with IQ-/COG- had fewer years in school than SZ with IQ+/COG+ ($p = .001$). SZ with IQ-/COG- had lower SES and had parents with fewer years in school compared to all of the other groups ($p < .005$). All patient groups performed worse than HC on the cognition composite ($p < .015$), even those in the IQ+/COG+ group. The SZ with IQ+/COG+ had fewer negative symptoms than SZ with IQ-/COG- (SANS: $p = .016$), but all groups of SZ were similar regarding positive symptoms (SAPS). As expected, Vocabulary and Cognition Composite were strongly correlated in both participants with SZ ($r = .60$, $p < .001$) and HC ($r = .51$, $p < .001$).

----------Insert Table 1 about here----------

Whole Brain Volume and Intracranial Volume: As shown in Figure 1 and Supplementary Figure 3, there were group differences in TBV, ICV, and TBV corrected for ICV (Table 2), all of which survived multiple comparison correction. SZ with IQ-/COG- had smaller TBV and ICV than HC and IQ+/COG-. SZ with IQ+/COG+

had also smaller TBV than HC, even when we controlled for ICV (Supplementary Figure 1). Thus, overall the SZ with impaired ePMC-IQ differed from the other groups on TBV and ICV, but the SZ with impaired cognition did not differ significantly if their ePMC-IQ was relatively intact.

----------Insert Figure 1 and Table 2 about here----------

White Matter Volume: There were significant group differences in both whole brain white matter volume and cortical white matter volume (Table 2), both of which survived multiple comparison correction. As shown in Supplementary Figure 4, SZ with IQ-/COG- had significantly larger whole white matter volume and total cortical white matter volume than each of the other three groups. Also, SZ with IQ+/COG- had larger white matter volumes than IQ+/COG+ and HC.

Gray Matter Volume and Thickness: There were significant group differences in whole brain gray matter volume and cortical gray matter volume (Table 2), both surviving multiple comparison correction. SZ with IQ-/COG- had smaller total gray matter volume and cortical gray matter volume than each of the other three groups (Figure 2). SZ with IQ+/COG- had smaller total gray matter volume and cortical gray matter volume than HC, and smaller total gray matter volume than SZ with IQ+/COG+. However, there were no differences between groups in subcortical gray matter volume or cerebrospinal fluid (Table 2, Figure 2). In addition, SZ with impaired cognition whether or not they had impaired ePMC-IQ (IQ-/COG- and IQ+/COG-) had significantly thinner cortex than HC, and SZ with IQ-/COG- showed reduced cortical thickness compared to SZ with IQ+/COG+ (Figure 2).

----------Insert Figure 2 about here----------

Specific Brain Regions: We next examined volumes in specific brain regions that had shown evidence of volume reductions in prior research[10]. There were no significant differences between groups in anterior cingulate cortex, middle frontal gyrus or hippocampus volumes (Table 2). However, there was a significant group difference in anterior insula volume (Table 2), which survived multiple comparison correction. Specifically, SZ with impaired cognition (both IQ+/COG- and IQ-/COG-) had smaller anterior insula volume than HC, and SZ with IQ-/COG- also had smaller insula volume than SZ with IQ+/COG+ (Figure 3).

----------Insert Figure 3 about here----------

Discussion

To better understand the relationship between neuropathology and cognitive functioning, we aimed to replicate and extend the work of Woodward and Heckers[3] by examining brain structure in individuals with SZ grouped according their ePMC-IQ and current neuropsychological performance. As predicted, we found that individuals with SZ with cognitive deficits and lower estimated premorbid crystalized intellectual functioning (IQ-/COG-) had smaller intracranial volumes than healthy controls. However, we also found that they had reduced total and cortical gray matter volumes and reduced cortical thickness compared to healthy controls. In contrast, individuals with cognitive impairment in the context of intact ePMC-IQ showed only reduced total and cortical gray matter volume, and cortical thickness. When examining specific structures linked to cognitive function in SZ in prior work, individuals with both IQ+/COG- and IQ-/COG- presented reduced volume compared to controls in the anterior insula, but not in hippocampus, anterior cingulate cortex or dorsolateral prefrontal cortex.

Similar to what was found in Woodward and Heckers[3], individuals with SZ with IQ-/COG- in the current sample had smaller intracranial volumes compared to healthy controls, while individuals with intact ePMC-IQ, regardless of their cognitive function, did not differ from HC in intracranial volume. In typical development, intracranial and whole brain volumes increase between early childhood and early adolescence. Intracranial volume then remains relatively stable across the life course, while total brain volume starts to reduce during adulthood. As such, changes in intracranial volume versus total brain volume relative to intracranial volume can be thought as neurodevelopmental and neurodegenerative impairment indexes, respectively[18]. Thus, like Woodward and Heckers[3], our results suggest a relationship between impaired ePMC-IQ and an early neurodevelopmental process among individuals with SZ. Interestingly, Craddock and Owen[19] hypothesized a model suggesting that the major psychiatric illnesses were part of one spectrum, as they seem to share mechanisms and impairments at the levels of genetic susceptibility, neural systems and cognitive impairment. In this dimensional perspective, SZ would be part of a gradient of psychopathology with neurodevelopmental contribution lesser than intellectual disability and autism, but stronger than bipolar disorder and major depression. Also, there might be different neurobiological biotypes with unique patterns of neurobiological alterations that each contribute to psychosis. This could

explain the heterogeneity seen in the illnesses, and suggests that multiple pathways could lead to similar clinical manifestations of psychosis[20]. However, previous studies have shown that high premorbid intelligence reduced the risk of developing SZ, while individuals with IQ between 70 and 85 had double the risk and individuals with IQ below 70 increased five-fold the risk of being diagnosed with SZ[5]. Such data are consistent with the hypothesis IQ may be associated with early risk factors for psychosis. However, unlike Woodward and Heckers[3], we also found that SZ with impaired ePMC-IQ also showed reduced brain volume relative to intracranial volume, as well as reduced cortical gray matter volume. As such, our results are consistent with the finding that both whole brain and intracranial volumes are positively associated with IQ performance in both clinical and non-clinical populations[21]. Thus, our data suggest that impaired IQ may also be associated with a neurodegenerative process as well as a neurodevelopmental process, though as discussed below, this may relate to mechanisms that impair cognitive function in addition to crystalized IQ.

Unlike Woodward and Heckers[3], we did not find that individuals with SZ who had impaired cognition in the context of intact ePMC-IQ (IQ+/COG-) had reduced total brain volume relative to intracranial volume. However, we did see that these individuals had significantly reduced total and cortical gray matter volume, and cortical thickness compared to healthy controls, as did the individuals with both impaired ePMC-IQ and impaired cognitive function. In SZ, cortical thickness is positively correlated with neuropsychological performance, such as working memory, processing speed, verbal learning and executive functioning[22,23,24], which is consistent with our finding that the participants who had cognitive impairment - regardless of ePMC-IQ - had thinner cortices. There seems to be an important association between cognitive impairment and cortical thinning in SZ, in which the participants with SZ that have near-normal neuropsychological performance show near-normal patterns of cortical thickness, while neuropsychologically impaired individuals have overall reduced cortical thickness[16]. Interestingly, cortical thickness was shown to progressively and widely decrease in SZ after a 5-year period[25]. Nonetheless, there is not yet a consensus as to whether cortical thinning is a progressive pattern in SZ, associated with the duration of illness, or whether it is primarily a neurodevelopmental feature that occurs very early in the disease[26,27]. However, abnormal cortical thinning seems to be associated with the development and conversion to psychosis[28], again suggesting evidence for neurodegeneration. As

such, our findings of reduced cortical thickness SZ with IQ+/COG-, as well as smaller cortical gray matter volume, are consistent with the hypothesis that processes associated with cognitive impairment and cortical thinning may possibly be a “second hit” among individuals with psychosis, potentially bringing an additional and significant neuroprogressive burden.

In addition to examining whole brain metrics of gray matter, we also examined the volume of specific brain regions shown to be reduced in SZ and/or associated cognitive function in previous studies[10,11,12,13,14]. We did not find evidence for reduced volumes of the anterior cingulate cortex, hippocampus or middle frontal gyrus among individuals with SZ who had impaired cognition, either with or without impaired ePMC-IQ. However, we did find evidence of significantly reduced anterior insula volume among individuals with both impaired ePMC-IQ and cognition, compared to both healthy controls and SZ with intact ePMC-IQ and cognitive function. Further, we also saw a reduction in the anterior insula volume of SZ individuals with impaired cognition and intact ePMC-IQ compared to controls. Interestingly, in SZ, meta-analyses showed medium-sized significant reductions in insula volume when compared to healthy controls, regardless of other demographic and clinical variables[29]. Further, a recent meta-analysis identified gray matter loss in the anterior insula as a transdiagnostic neural abnormality across psychiatric diseases[30]. Notably, activation in this area is known to be involved in several processes related to awareness of internal and external emotional and sensory stimuli[31], functions which may be very relevant to understand psychotic processes. Therefore, it is noteworthy that we found reduced anterior insula volume in participants with SZ with impaired cognition, regardless of their ePMC-IQ, and that was present controlling for the differences in TBV.

Our results regarding white matter volumes were unexpected. We found that individuals with impaired cognition and ePMC-IQ had significantly larger white matter volumes than both SZ groups with intact ePMC-IQ, as well as compared to healthy controls. In the literature, findings much more commonly show that individuals with SZ show smaller white matter volumes compared to healthy controls[10]. On the other hand, it could be possible that this may reflect a segmentation issue with Freesurfer, such that poor gray/white discrimination leads to underestimates of gray matter volume and overestimates of white matter volume. However, it is not clear why such a problem would lead to a systematic bias in the direction of white matter versus gray

matter. Further, we did not see any evidence for alterations in CSF, which might have been expected should this have been a methodological issue (though the CSF versus gray/matter white boundary is starker). In general, the literature on schizophrenia has not provided strong evidence for broad reductions in white matter volume. Interestingly, when we considered the individuals with SZ as a single group, there where no white matter volumes differences compared to healthy controls ($p > .145$), which is consistent with the broader literature. Nonetheless, our findings in regards to white matter differences in the individuals with schizophrenia who had both impaired cognition and ePMC-IQ were unexpected and require further investigation potentially through examination of diffusion tensor imaging or white matter hypo or hyper intensities, both of which may be more informative of the WM integrity than volumetric MRI measures of WM[32].

Regarding the individuals with SZ who had both intact cognitive abilities and ePMC-IQ, we found some slight evidence of reduced total brain volume relative to intracranial volume when compared to HC, but no other differences. This finding was quite unexpected given that this group had relatively intact ePMC-IQ and cognitive function. However, this would not have survived multiple comparisons testing of the follow-up group comparisons in the analysis of total brain volume, given that it was not predicted. This subgroup of patients had a relatively higher percentage of females compared to the other two groups of SZ (60% versus 10% and 33% respectively), though all of our analyses controlled for gender. Further, this group had the highest personal SES of any of the three patient groups. We did not measure factors such as height, weight and body size that may have contributed to this unexpected finding, which will be important to do in future studies. Conversely, a previous study found that, compared to healthy individuals, individuals with SZ who were relatively neuropsychological normal had smaller gray matter volume than controls across several regions and no white matter volume abnormalities. However, this group of individuals with SZ was older (39.5y) and their percentage of males was higher (85.7%) than in our sample. Furthermore, in addition to age and gender, this previous study used height instead of total brain volume to control their analysis of brain structure, what could have contributed to their differential findings[33].

One issue that should be mentioned is whether the IQ differences in the groups and consequently their influences to brain structure were due to biological determinants of the disease or to environmental factors. In our sample, subjects with

SZ with lower ePMC-IQ (IQ-/COG-) had parents with lower years of education compared to all the other groups. This could either suggest that the environment to which the SZ patients were exposed had an influence on their IQ development, or it could reflect inherited cognitive function that may be disease relevant. Further work that would allow one to dissociate these environmental versus genetic contributions would be needed to address this important question, such as twin or adoption designs.

Our study had several limitations. First, we did not have records of past or current medication use, although all patients were stably medicated. Further, we did not have data on age of onset or illness duration, both of which are factors that could also influence metrics of structural brain integrity in psychosis. In addition, we only presented results of analyses of brain structure, and did not have data on brain function or connectivity among these individuals, which may also show important relationships to IQ and cognitive function. Also, the 1.5T scanner may have limit performance in the acquisition of quantitative anatomical data. Finally, we only reported an estimated measure of premorbid crystalized intellectual functioning, and not an actual assessment of IQ before the disease onset. Also, the cognitive composite was created considering subtests that include many but not all cognitive domains that may be impaired in psychosis, which may limit our generalization of the results. Further, we recognize that there are important relationships between crystalized IQ and cognitive function, and that they are frequently correlated, as they were in our sample. However, they can also be dissociated and are thought to have differential contributions from various factors.

In summary, our results are consistent with the hypothesis that there may be different neurodevelopmental mechanisms that contribute to different patterns of crystalized IQ and cognitive impairment among individuals with SZ, though these mechanisms may converge to alter common aspects of brain structure. This interpretation is in line with the idea of psychosis as the end result of multiple different pathogenetic paths[9] possibly deriving from interactions between various genes and environmental insults[7]. Intellectual and cognitive performances could also be related to a spectrum of severity of the illness, as our data suggest that that individuals with schizophrenia with both impaired ePMC-IQ and cognitive function had worse brain structure outcomes. In the present study, we saw that ePMC-IQ related in important ways to brain structure in SZ, suggestive of a protective factor for

certain types of brain structural abnormalities even in the context of impaired cognition. However, like Woodward and Heckers[3], our results suggest that there are alterations in cortical volume and thickness, as well as insula volume, that are present among individuals with impaired cognition even in the context of relatively intact ePMC-IQ. Future research considering differences in premorbid intellectual and cognitive performances will hopefully advance our comprehension of the different pathways of SZ, improving our understanding of its neuropathology and potential pathways for treatment.

Acknowledgement

This study was supported by Brazilian Council of Scientific and Technological Development (CNPq/Brazil). Data used in preparation of this article were obtained from the NU Schizophrenia Data and Software Tool (NUSDAST) database (http://central.xnat.org/REST/projects/NUDataSharing) and from the SchizConnect database (http://schizconnect.org). As such, the investigators within NUSDAST and SchizConnect contributed to the design and implementation of NUSDAST and SchizConnect and/or provided data but did not participate in analysis or writing of this report.

References

5. Fett AK, Viechtbauer W, Dominguez MD, Penn DL, van Os J, Krabbendam L. The relationship between neurocognition and social cognition with functional outcomes in schizophrenia: a meta-analysis. *Neurosci Biobehav Rev* 2011;35:573-88.
6. Barch DM, Ceaser A. Cognition in schizophrenia: core psychological and neural mechanisms. *Trends Cogn Sci* 2012;16:27-34.
7. Woodward ND, Heckers S. Brain Structure in Neuropsychologically Defined Subgroups of Schizophrenia and Psychotic Bipolar Disorder. *Schizophr Bull* 2015;41:1349-59.
8. Woodberry KA, Giuliano AJ, Seidman LJ. Premorbid IQ in schizophrenia: a meta-analytic review. *Am J Psychiatry* 2008;165:579-87.

9. Khandaker GM, Barnett JH, White IR, Jones PB. A quantitative meta-analysis of population-based studies of premorbid intelligence and schizophrenia. *Schizophr Res* 2011;132:220-7.
10. Ammari N, Heinrichs RW, Pinnock F, Miles AA, Muharib E, McDermid Vaz S. Preserved, deteriorated, and premorbidly impaired patterns of intellectual ability in schizophrenia. *Neuropsychology* 2014;28:353-8.
11. Fatemi SH, Folsom TD. The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophr Bull* 2009;35:528-48.
12. Rapoport JL, Giedd JN, Gogtay N. Neurodevelopmental model of schizophrenia: update 2012. *Mol Psychiatry* 2012;17:1228-38.
13. Lewis DA, Levitt P. Schizophrenia as a disorder of neurodevelopment. *Annu Rev Neurosci* 2002;25:409-32.
14. Haijma SV, Van Haren N, Cahn W, Koolschijn PC, Hulshoff Pol HE, Kahn RS. Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. *Schizophr Bull* 2013;39:1129-38.
15. Antonova E, Sharma T, Morris R, Kumari V. The relationship between brain structure and neurocognition in schizophrenia: a selective review. *Schizophr Res* 2004;70:117-45.
16. Kubota M, van Haren NE, Haijma SV, et al. Association of IQ Changes and Progressive Brain Changes in Patients With Schizophrenia. *JAMA Psychiatry* 2015;72:803-12.
17. Sheffield JM, Repovs G, Harms MP, et al. Fronto-parietal and cingulo-opercular network integrity and cognition in health and schizophrenia. *Neuropsychologia* 2015;73:82-93.
18. Sigurdsson T, Duvarci S. Hippocampal-Prefrontal Interactions in Cognition, Behavior and Psychiatric Disease. *Front Syst Neurosci* 2016;9:190.
19. Wang L, Kogan A, Cobia D, et al. Northwestern University Schizophrenia Data and Software Tool (NUSDAST). *Front Neuroinform* 2013;7:25.
20. Cobia DJ, Csernansky JG, Wang L. Cortical thickness in neuropsychologically near-normal schizophrenia. *Schizophr Res* 2011;133:68-76.
21. Destrieux C, Fischl B, Dale A, Halgren E. Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. *Neuroimage* 2010;53:1-15.

22. Courchesne E, Chisum HJ, Townsend J, et al. Normal brain development and aging: quantitative analysis at in vivo MR imaging in healthy volunteers. *Radiology* 2000;216:672-82.
23. Craddock N, Owen MJ. The Kraepelinian dichotomy - going, going... but still not gone. *Br J Psychiatry* 2010;196:92-5.
24. Clementz BA, Sweeney JA, Hamm JP, et al. Identification of Distinct Psychosis Biotypes Using Brain-Based Biomarkers. *Am J Psychiatry* 2016;173:373-84.
25. Pietschnig J, Penke L, Wicherts JM, Zeiler M, Voracek M. Meta-analysis of associations between human brain volume and intelligence differences: How strong are they and what do they mean? *Neurosci Biobehav Rev* 2015;57:411-32.
26. Ehrlich S, Brauns S, Yendiki A, et al. Associations of cortical thickness and cognition in patients with schizophrenia and healthy controls. *Schizophr Bull* 2012;38:1050-62.
27. Hartberg CB, Lawyer G, Nyman H, et al. Investigating relationships between cortical thickness and cognitive performance in patients with schizophrenia and healthy adults. *Psychiatry Res* 2010;182:123-33.
28. Hartberg CB, Sundet K, Rimol LM, et al. Brain cortical thickness and surface area correlates of neurocognitive performance in patients with schizophrenia, bipolar disorder, and healthy adults. *J Int Neuropsychol Soc*.2011;17:1080-93.
29. van Haren NE, Schnack HG, Cahn W, et al. Changes in cortical thickness during the course of illness in schizophrenia. *Arch Gen Psychiatry* 2011;68:871-80.
30. Kubota M, Miyata J, Yoshida H, et al. Age-related cortical thinning in schizophrenia. *Schizophr Res* 2011;125:21-9.
31. Assunção Leme IB, Gadelha A, Sato JR, et al. Is there an association between cortical thickness, age of onset, and duration of illness in schizophrenia? *CNS Spectr* 2013;18:315-21.
32. Cannon TD, Chung Y, He G, et al. Progressive reduction in cortical thickness as psychosis develops: a multisite longitudinal neuroimaging study of youth at elevated clinical risk. *Biol Psychiatry* 2015;77:147-57.

33. Shepherd AM, Matheson SL, Laurens KR, Carr VJ, Green MJ. Systematic meta-analysis of insula volume in schizophrenia. *Biol Psychiatry* 2012;72:775-84.
34. Goodkind M, Eickhoff SB, Oathes DJ, et al. Identification of a common neurobiological substrate for mental illness. *JAMA Psychiatry*. 2015;72:305-15.
35. Craig AD. How do you feel--now? The anterior insula and human awareness. *Nat Rev Neurosci* 2009;10:59-70.
36. Kubicki M, Westin CF, McCarley RW, Shenton ME. The application of DTI to investigate white matter abnormalities in schizophrenia. *Ann N Y Acad Sci* 2005;1064:134-48.
37. Wexler BE, Zhu H, Bell MD, et al. Neuropsychological near normality and brain structure abnormality in schizophrenia. *Am J Psychiatry* 2009;166:189-95.

Table 1. Demographic and clinical data as a function of group

Variables, Mean (SD)	**Healthy Controls** n = 94	**Individuals with Schizophrenia**			ANOVA [a]/ Chi-square
		IQ+/COG+ n = 25	**IQ+/COG-** n = 31	**IQ-/COG-** n = 36	
Age	32.22 (13.64)	35.42 (14.09)	32.20 (12.39)	38.44 (12.67)	F (3, 177) = 2.02, *p* = .113
Gender (male/female)	53/41	10/15	28/3	24/12	X^2 = 17.25, df = 3, p = .001*
Subject education (y)	14.38 (2.66)	13.44 (2.62)	12.38 (1.97)	11.00 (1.74)	F (3, 178) = 18.94, *p* < .001*
Parental education (y)	14.13 (2.61)	14.17 (3.71)	15.04 (2.59)	11.50 (2.06)	F (3, 162) = 8.61, *p* < .001*
Personal SES	3.06 (0.88)	2.86 (1.11)	3.28 (1.06)	4.25 (0.94)	F (3, 131) = 10.33, *p* < 001 *
SAPS	-	18.71 (12.66)	21.74 (15.16)	23.15 (18.48)	F (2, 85) = 0.72, *p* = .490
SANS	-	24.39 (17.74)	29.53 (17.58)	38.43 (17.92)	F (2, 80) = 4.31, *p* = .017*
Cognition Composite	3.41 (4.22)	1.03 (2.73)	- 6.12 (1.97)	- 7.79 (2.85)	F (3, 182) = 119.65, *p* = 000*
Vocabulary (WAIS-III)	11.71 (3.16)	11.62 (2.97)	10.00 (2.21)	4.44 (1.27)	F (3, 182) = 65.19, *p* = 000*

SES: socioeconomic status (higher values indicate lower SES); SAPS: Scale for the Assessment of Positive Symptoms; SANS: Scale for the Assessment of Negative Symptoms

[a] ANOVA with Bonferroni correction

Table 2. Results of MANCOVAS comparing group differences in brain structure.

Variables	ANCOVA	η^2_p	FDR adj P-values	HC vs SZ IQ+/COG+ (Intact)	HC vs SZ IQ+/COG- (Deteriorated)	HC vs SZ IQ-/COG- (Compromised)	SZ IQ+/COG+ vs SZ IQ+/COG-	SZ IQ+/COG+ vs SZ IQ-/COG-	SZ IQ+/COG- vs SZ IQ-/COG-
Total Brain Volume (TBV) [a]	F (3, 175) = 9.95, $p < .001$	.146	.002	**	NS	***	NS	NS	***
Intracranial Brain Volume (ICV) [a]	F (3, 175) = 5.44, $p = .001$	.085	.002	NS	NS	***	NS	NS	NS
Total Brain Volume (corrected) [b]	F (3, 174) = 4.57, $p = .004$	.073	.006	*	NS	***	NS	NS	**
Whole Brain White Matter Volume [c]	F (3, 174) = 12.58, $p < .001$	.178	.002	NS	*	***	*	***	**
Cortical White Matter Volume [c]	F (3, 174) = 11.65, $p < .001$	.167	.002	NS	NS	***	*	***	**
Whole Brain Gray Matter Volume [c]	F (3, 174) = 12.58, $p < .001$	.178	.002	NS	*	***	*	***	**
Total Cortical Gray Matter Volume [c]	F (3, 174) = 8.60, $p < .001$	.129	.002	NS	*	***	NS	***	*
Subcortical Gray Matter Volume [c]	F (3, 174) = 1.85, $p = .140$	.031	.19	NS	NS	NS	NS	NS	NS
Cerebrospinal Fluid Volume [c]	F (3, 174) = .06, $p = .979$	.001	.98	NS	NS	NS	NS	NS	NS
Cortical Thickness [c]	F (3, 174) = 4.54, $p = .004$	.073	.006	NS	**	**	NS	*	NS
Anterior Cingulate Cortex Volume [c]	F (3, 174) = 1.80, $p = .149$	.030	.19	NS	NS	NS	NS	NS	NS
Middle Frontal Gyrus Volume [c]	F (3, 174) = 1.50, $p = .218$	.025	.25	NS	NS	NS	NS	NS	NS
Hippocampus Volume [c]	F (3, 174) = .33, $p = .804$	.006	.87	NS	NS	NS	NS	NS	NS
Anterior Insula Volume [c]	F (3, 174) = 5.58, $p < .001$	.088	.002	NS	*	***	NS	**	NS

HC: healthy controls; SZ: subjects with schizophrenia

[a] Covariates: age and sex; [b] Covariates: age, sex and ICV; [c] Covariates: age, sex and TBV

** p < .05; ** p < .01; *** p < .001;*

Figure 1

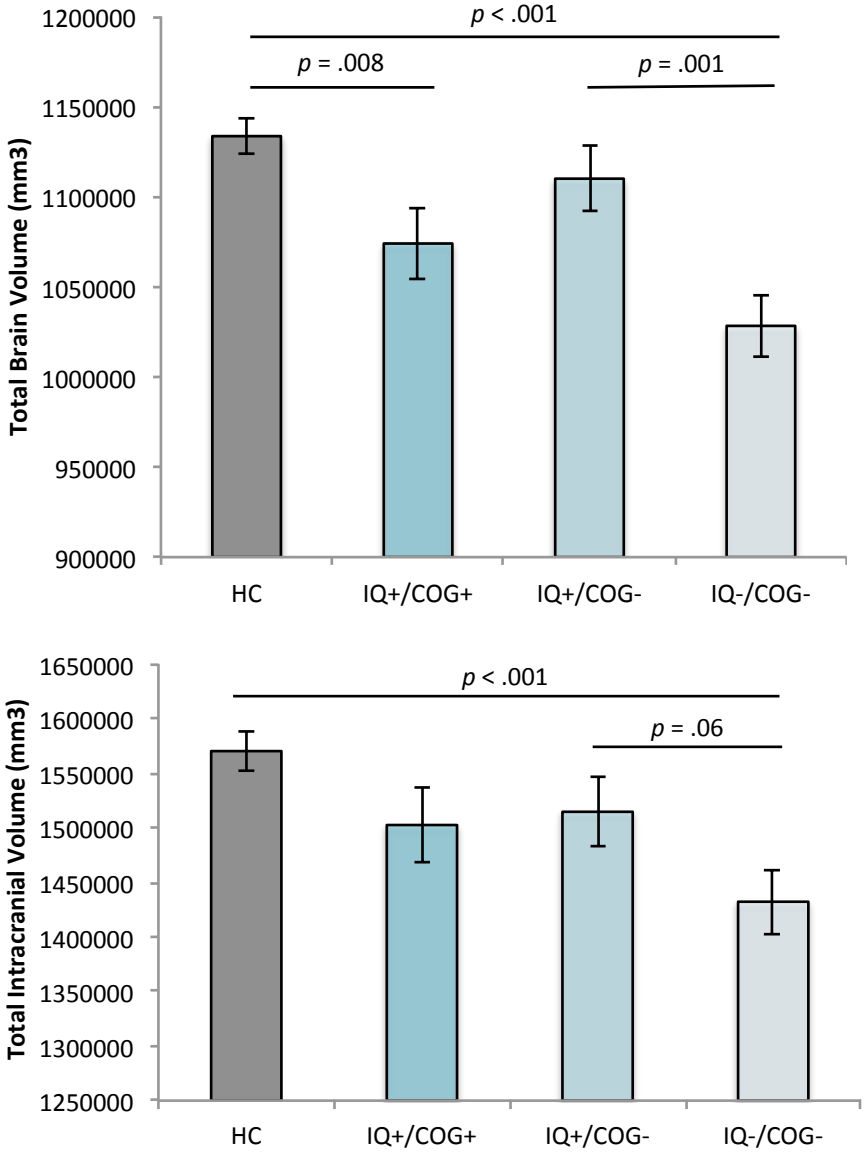

Figure 1. Mean differences in total brain volume and estimated total intracranial volume across healthy controls (**HC**); individuals with schizophrenia (SZ) with normal estimated premorbid crystalized IQ (ePMC-IQ) and not cognitively impaired (**IQ+/COG+**); individuals with SZ with normal ePMC-IQ and cognitively impaired (**IQ+/COG-**); and individuals with SZ with lower ePMC-IQ and cognitively impaired (**IQ-/COG-**). Covariates appearing in the model are evaluated at the following values: age = 33.78, sex = 1.39. Error bars are standard error of the mean (SE).

Figure 2

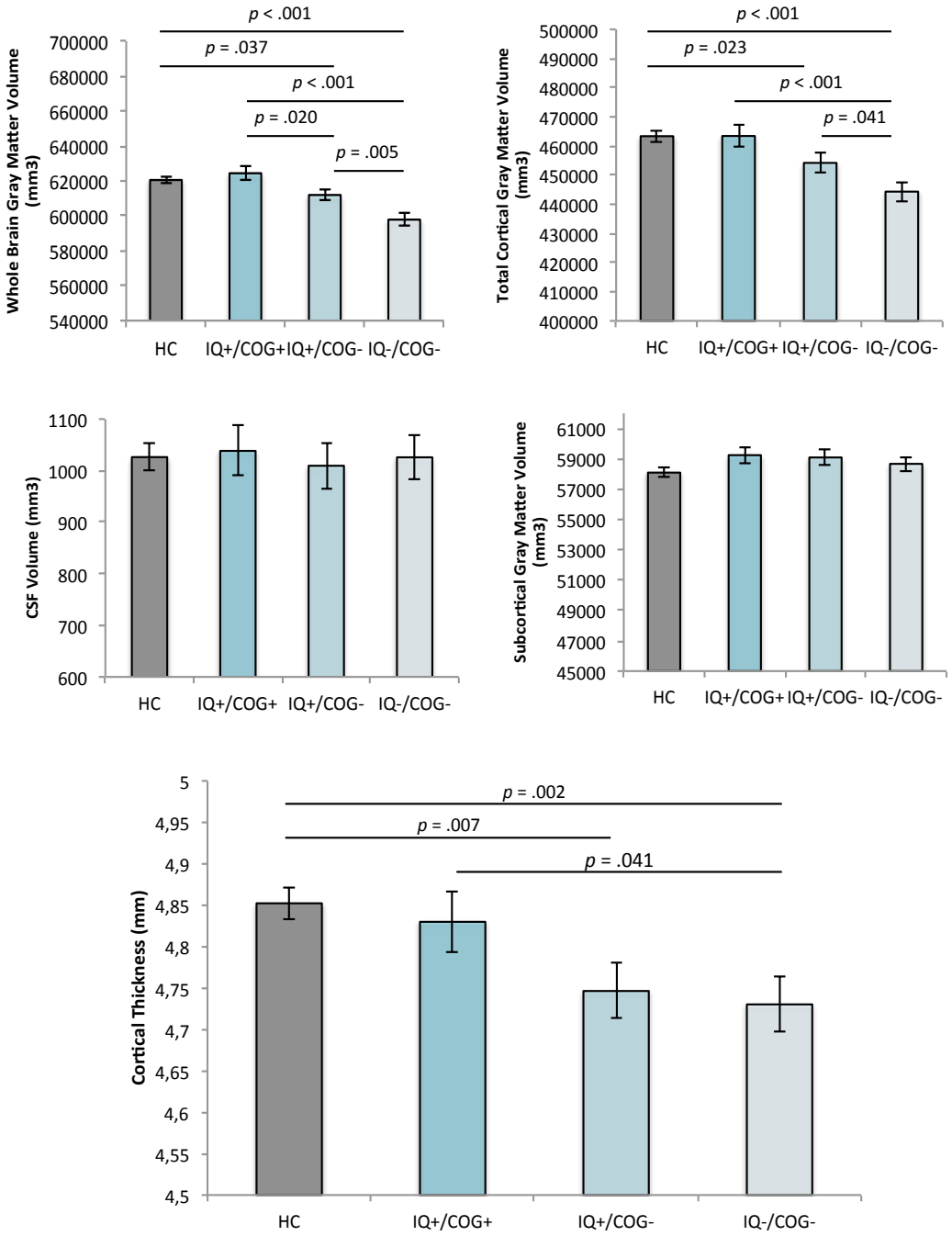

FIgure 2. Mean differences in gray matter volume, cerebrospinal fluid and cortical thickness across healthy controls (**HC**); individuals with schizophrenia (SZ) with normal estimated premorbid crystalized IQ (ePMC-IQ) and not cognitively impaired (**IQ+/COG+**); individuals with SZ with normal ePMC-IQ and cognitively impaired (**IQ+/COG-**); and individuals with SZ with lower ePMC-IQ and cognitively impaired (**IQ-/COG-**). Covariates appearing in the model are evaluated at the following values: age = 33.78, sex = 1.39, TBV = 1101612.03. Error bars are standard error of the mean (SE).

Figure 3

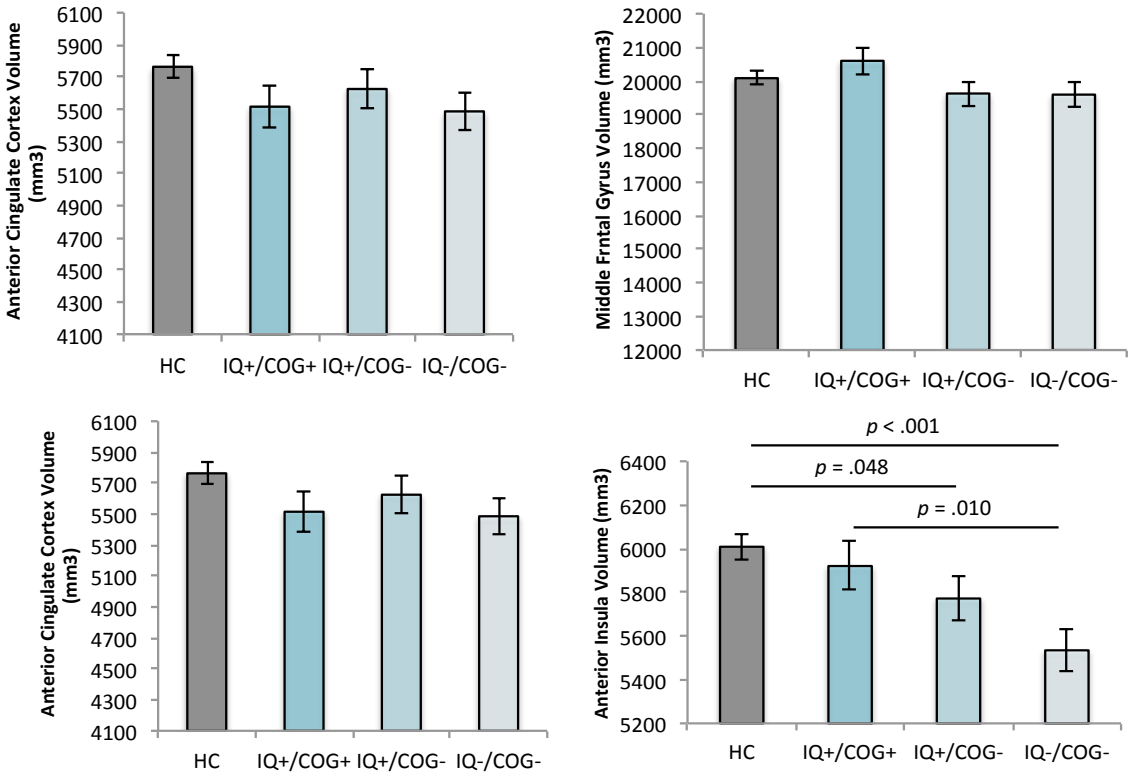

Figure 3. Mean differences in brain volume across healthy controls (**HC**); individuals with schizophrenia (SZ) with normal estimated premorbid crystalized IQ (ePMC-IQ) and not cognitively impaired (**IQ+/COG+**); individuals with SZ with normal ePMC-IQ and cognitively impaired (**IQ+/COG-**); and individuals with SZ with lower ePMC-IQ and cognitively impaired (**IQ-/COG-**). Covariates appearing in the model are evaluated at the following values: age = 33.78, sex = 1.39, TBV = 1101612.03. Error bars are standard error of the mean (SE).

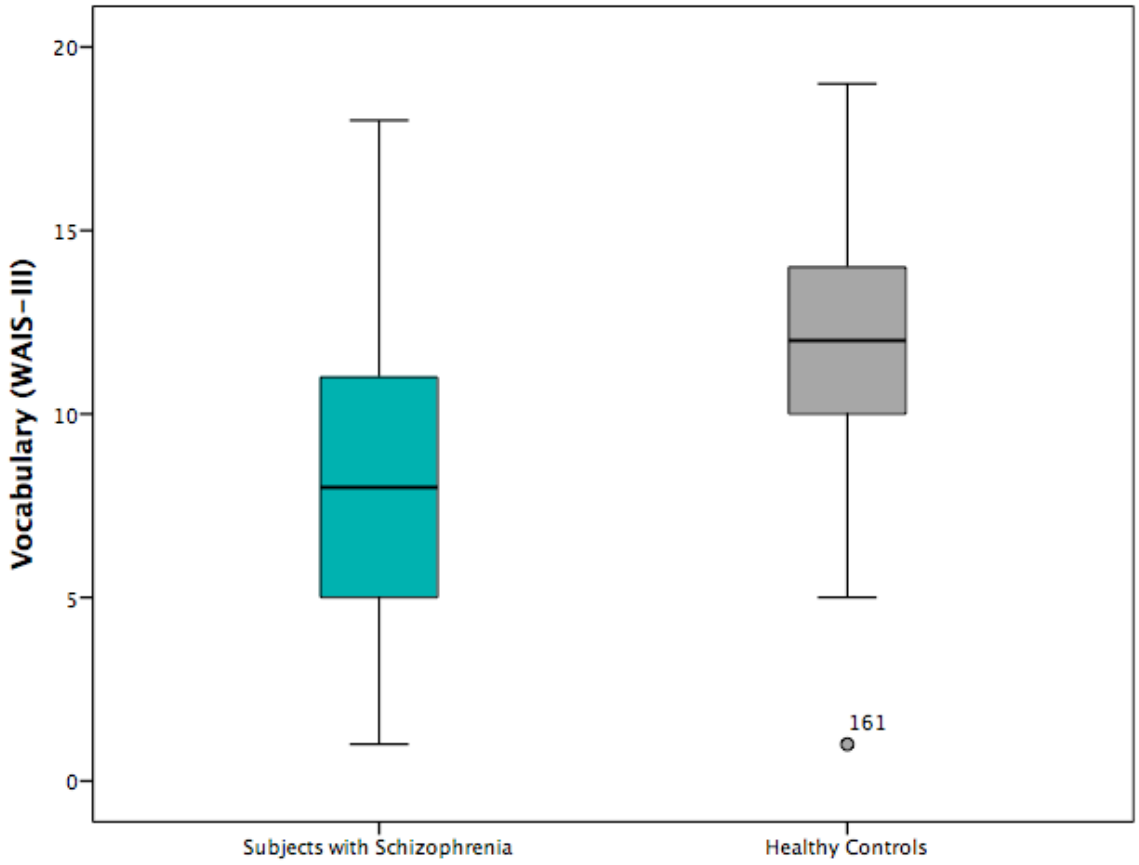

Figure S1. Boxplot of the estimated measure of premorbid crystalized intellectual functioning in subjects with schizophrenia and healthy controls.

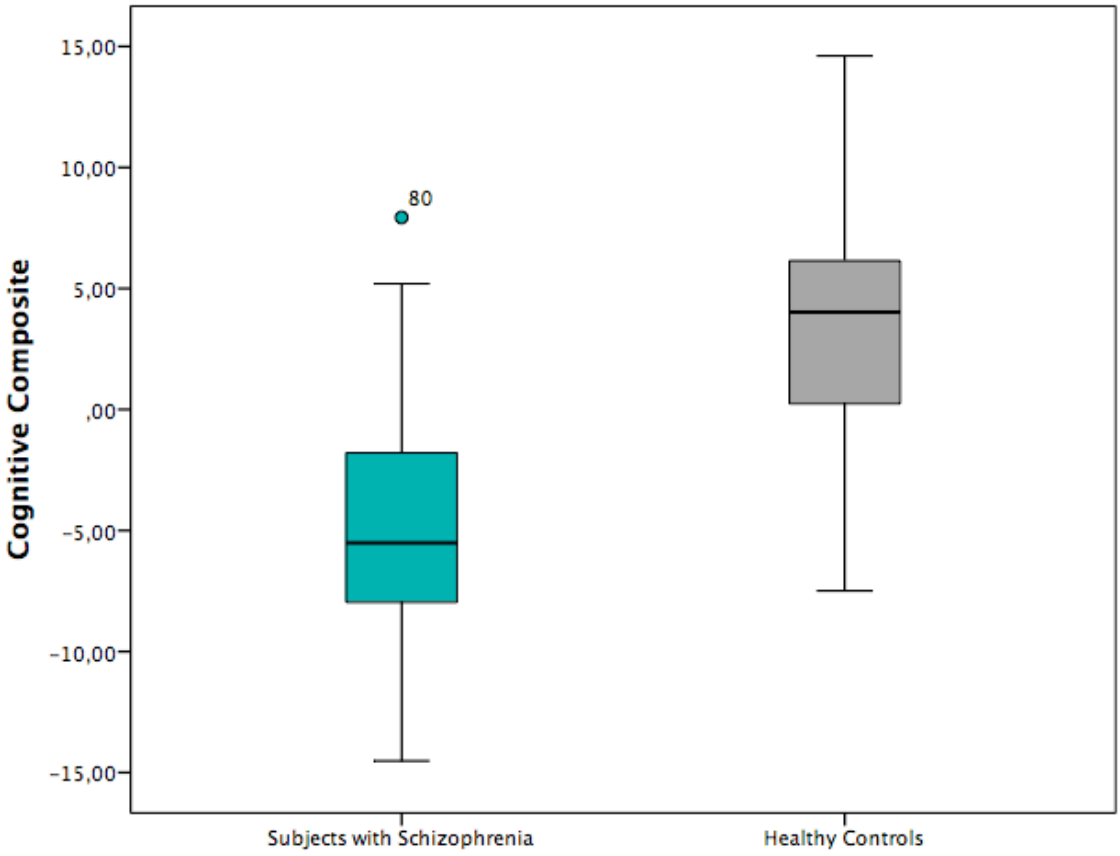

Figure S2. Boxplot of the cognitive composite in subjects with schizophrenia and healthy controls.

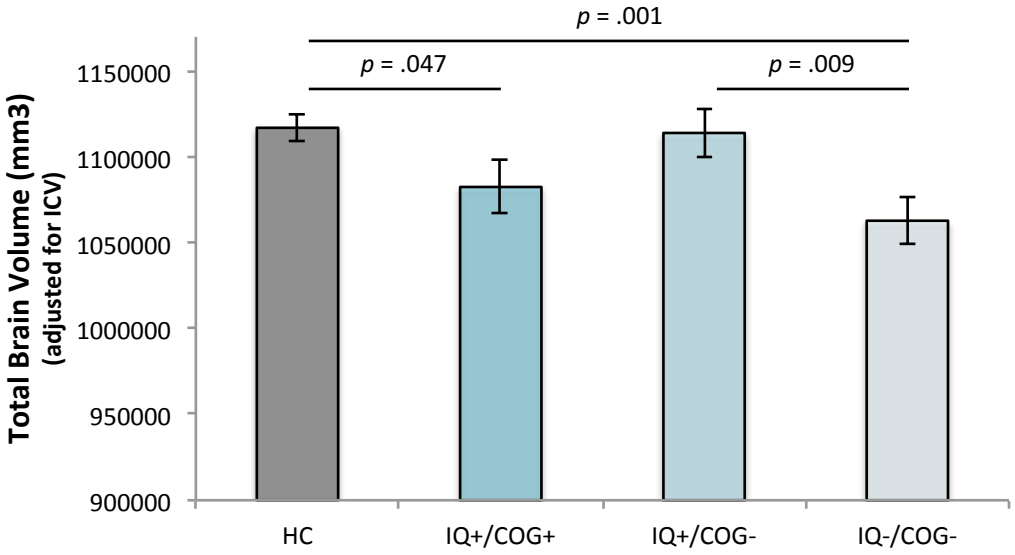

Figure S3. Mean differences in total brain volume across healthy controls (**HC**); individuals with schizophrenia (SZ) with normal estimated premorbid crystalized IQ (ePMC-IQ) and not cognitively impaired (**IQ+/COG+**); individuals with SZ with normal ePMC-IQ and cognitively impaired (**IQ+/COG-**); and individuals with SZ with lower ePMC-IQ and cognitively impaired (**IQ-/COG-**). Covariates appearing in the model are evaluated at the following values: age = 33.78, sex = 1.39, ICV = 1525643.83. Error bars are standard error of the mean (SE).

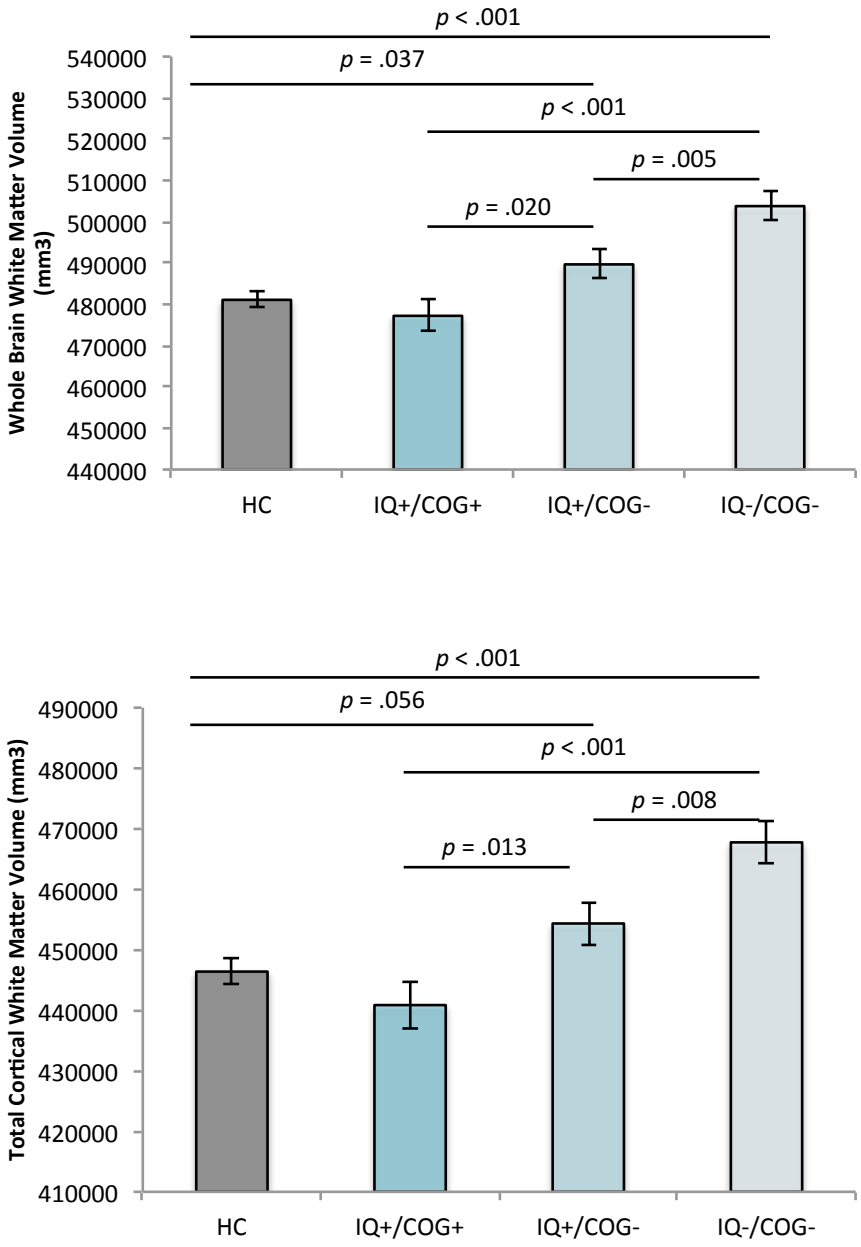

Figure S4. Mean differences in white matter volume across healthy controls (**HC**); individuals with schizophrenia (SZ) with normal estimated premorbid crystalized IQ (ePMC-IQ) and not cognitively impaired (**IQ+/COG+**); individuals with SZ with normal ePMC-IQ and cognitively impaired (**IQ+/COG-**); and individuals with SZ with lower ePMC-IQ and cognitively impaired (**IQ-/COG-**). Covariates appearing in the model are evaluated at the following values: age = 33.78, sex = 1.39, TBV = 1101612.03. Error bars are standard error of the mean (SE).

6 ARTIGO #3

Publicado no ***Schizophrenia Research***

Fator de Impacto (2015): 4.453

Versão do manuscrito aceita:

Telomere length in subjects with schizophrenia, their unaffected siblings and healthy controls: evidence of accelerated aging

Leticia Sanguinetti Czepielewski[1,2], Raffael Massuda[1,3], Bruna Panizzutti[1,2], Eduarda Dias da Rosa[1,4], David de Lucena[5], Danielle Macêdo[5], Lucas Kich Grun[4], Florencia María Barbé-Tuana[4], Clarissa Severino Gama[1,2,]*

[1] Laboratório de Psiquiatria Molecular, Hospital de Clínicas de Porto Alegre, Universidade Federal do Rio Grande do Sul - Porto Alegre, Brazil
[2] Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento, Universidade Federal do Rio Grande do Sul - Porto Alegre, Brazil
[3] Departamento de Psiquiatria, Universidade Federal do Paraná - Curitiba, Brazil
[4] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul - Porto Alegre, Brazil
[5] Núcleo de Pesquisa e Desenvolvimento de Medicamentos, Departamento de Fisiologia e Farmacologia, Universidade Federal do Ceará – Fortaleza, Brasil

*** Corresponding Author:**
Clarissa S Gama
Hospital de Clínicas de Porto Alegre/CPE, Molecular Psychiatry Laboratory,
Rua Ramiro Barcelos, 2350, Prédio Anexo, 90035-903 Porto Alegre, RS, Brazil.
Phone: +55 51 33598845; Fax: +55 51 33598846. E-mail: cgama@hcpa.edu.br

Abstract:

Schizophrenia (SZ) is associated with broad burden. The clinical manifestations of SZ are related to pathophysiological alterations similar to what is seen in normal aging. Our aim was to evaluate the differences in telomere length (TL), a biomarker of cellular aging, in subjects with SZ (n=36), unaffected siblings (SB, n=36) and healthy controls (HC, n=47). SZ had shorter TL compared to HC, but no difference was found in SB comparing to SZ. These findings indicate that a pathological accelerated aging profile could be present in the course of SZ and further studies are needed to confirm TL as potential endophenotype, especially in at risk populations.

Key words: Schizophrenia; telomere length; accelerated aging; endophenotypes; biomarkers.

1. Introduction

Schizophrenia (SZ) has been associated with a broad burden of disease (Whiteford et al., 2013) and increased mortality. The average life span of subjects with SZ is 20-25 years shorter (Jeste et al., 2011). Compared to the general population, the all-cause standardized mortality ratio is approximately 2.5 (Saha et al., 2007). Although death by suicide is extremely prevalent two-thirds of the excess deaths are from other causes (Jeste et al., 2011). In addition to the collateral effects of antipsychotics use, socioeconomic and lifestyle factors play important roles in the adverse physical health outcomes (Saha et al., 2007). However, these are undoubtedly not enough to explain the several other biological associations that are present in SZ, and it is not entirely clear how neurobiological mechanisms involved in psychiatric disorders contribute to the pathogenesis and/or its pathophysiology (Stuart et al., 2015).

An oxidative imbalance (Gama et al., 2006, 2008; Kunz et al., 2008) and increased serum concentrations of several pro-inflammatory cytokines have been implicated in the pathophysiology of SZ (Domenici et al., 2010; Monji et al., 2009; Na et al., 2014; Pedrini et al., 2012, 2014; Watanabe et al., 2010). Observations of an immune dysregulation in SZ overlap with central pathophysiological mechanisms as well as with clinical manifestations of the illness (Kunz et al., 2011). Interestingly, regardless of antipsychotic use, the course of SZ seems to be accompanied by increased physiological changes throughout the body that are usually associated with normal aging, such as insulin resistance, hyperlipidemia, increased blood pressure, decreased bone density, thinning and wrinkling of the skin and hair, decrease in muscle mass and cortical atrophy (Kirkpatrick et al., 2008). These issues raise the hypothesis of an ongoing process of biological accelerated aging to be happening in SZ.

One suggested biomarker of aging is Telomere length (TL). Telomeres are DNA-protein structures that protect chromosome's ends and progressively shorten with each cell division (Armanios, 2009). Shortened TL is related to cell senescence and apoptosis in association with normal aging or illnesses (Armanios, 2013). There is a growing body of evidence linking psychiatric disorders with TL (Lindqvist et al., 2015). In SZ the existing studies are not conclusive, with some showing shortening (Fernandez-Egea et al., 2009; Kao et al., 2008; Kota et al., 2015; Yu et al., 2008), others showing no difference (Malaspina et al., 2014; Mansour et al., 2011) or even

longer TL compared to healthy subjects (Nieratschker et al., 2013). Nevertheless, a recent meta-analysis showed that TL was minor diminished in SZ (Polho et al., 2015).

One interesting way of adding up to this field would be with endophenotype studies, where is possible to identify disease features that help to apprehend the gap between genes and disease. Endophenotypes in SZ are deficits observed when comparing to healthy individuals, that are possibly seen in at least some level in the first-degree relatives of patients that are not affected by the disease itself (Braff, 2015). Therefore, to better understand the possible mechanism of pathological accelerated aging by investigating a potential endophenotype, our aim with the present study was to compare TL of individuals with SZ, their unaffected siblings (SB) and healthy controls (HC).

2. Methods

The Institutional Review Board approved this study protocol. All subjects were advised about the procedures and signed the informed consent prior to participation. We included 119 subjects aged 18 to 60 years divided in the following groups: *a) SZ:* 36 subjects with diagnosis confirmed by the Structured Clinical Interview for DSM-IV (SCID) whose siblings were willing to enroll in the study. The 18-item Brief Psychiatry Rating Scale (BPRS) was used to assess the psychopathological state (Romano and Elkis, 1996); *b) SB:* 36 brothers and sisters of SZ without current or previous history of psychiatric disease confirmed by SCID. *c) HC:* 47 healthy volunteers who had no current or previous history, nor first-degree family history of a major psychiatric disorder, including dementia or intellectual disability. Groups were matched by age.

Additional exclusion criteria were history or presence of neurological disease, actual abuse of drugs, brain tumor, thyroid disease, rheumatological disease, uncontrolled endocrine and cardiac disease, history of autoimmune diseases or chronic infections/inflammatory diseases, severe systemic disease, or having received immunosuppressive therapy. All subjects were non-smokers or smoked up to 10 cigarettes per day.

Subjects underwent a psychiatric evaluation to collect sociodemographic and clinical data. Following, 10 mL of peripheral blood was drawn from all subjects by venipuncture and stored at −80 °C until the experiment. Measurement of relative telomere length was from whole peripheral venous blood that was used for genomic

DNA extraction with a commercial kit (Illustra blood genomicPrep Mini Spin Kit, GE Healthcare) following manufacturers' instructions (the complete description is in the Supplementary Methods).

Analysis was performed using the SPSS version 20.0. Demographic and clinical characteristics were analyzed using the chi-square, Mann–Whitney's or Student's t test, as appropriate. Descriptive data were expressed as mean and standard deviation or as median and interquartile range. p-values < 0.05 were considered significant. To control for possible confounds, we performed an ANCOVA.

3. Results

Subjects with SZ, their unaffected SB and HC did not differ in age and body mass index. There were differences between groups in gender and years of education. Patients were receiving medication according to treatment guidelines (Table 1.).

There were group differences in TL (Table 1.). Individuals with SZ had significantly shorter TL compared to HC (p=0.004), however they were not different than SB (p=0.091) (Figure 1.). In post-hoc analysis with Bonferroni correction, the results were maintained, with a significant difference between SZ and HC (p = .038), and no difference between SB and SZ (p = .766) or HC (p = .545).

To control the effect of possible confounds, we performed ANCOVA with a log10 transformation of TL. We observed a significant main effect of group ($F(2,112) = 3.950$, $p = .022$) and age ($F(1,112) = 6.341$, $p = .013$), but not of gender ($F(1,112) = 2.475$, $p = .119$), body mass index ($F(1,112) = .265$, $p = .608$) and education ($F(1,112) = .436$, $p = .510$). In pairwise comparisons considering the abovementioned variables as covariates, the results remained the same ($F(6,112) = 2.732$, $p = .016$): SZ had reduced TL compared to SZ ($p = .006$); SB was not different to both SZ ($p = .155$) and HC ($p = .227$).

4. Discussion

This is the first study to show that unaffected SB did not differ in TL from subjects with SZ. Moreover, we showed that individuals with SZ had shorter TL compared to HC, which is in accordance with great part of the literature (Polho et al., 2015). This finding suggests that when the clinical syndrome recognized as SZ is present it is possibly associated with an early pathological aging.

The accelerated pathological physical aging is not specific of SZ, but it is often seen in other severe mental disorders (Jeste et al., 2011; Wolkowitz et al., 2011). In addition to TL, telomerase activity seems to be reduced in peripheral blood lymphocyte of subjects with SZ. This enzyme has a role of extending the lifetime of cell by extending and maintaining the telomeres, thereby protecting them and maintaining chromosomal integrity (Porton et al., 2008). The increase of immune cell replication during inflammation and oxidative stress potentially affects the TL (Lindqvist et al., 2015). Accelerated cell aging could be then the consequence of a pathogenic pathway of oxidative stress/pro-inflammatory imbalance (Jeste et al., 2011). Chronic inflammation in SZ leads to increased oxidative stress, which could explain metabolic alterations similar to those seen in elderly (Polho et al., 2015). Accelerated aging in SZ could also occur as a result of smoking, substance use, sedentary lifestyle and poor health care (Jeste et al., 2011). The presence or the risk of metabolic problems, such as glucose intolerance and increased blood pressure, are strongly associated with SZ, independently of antipsychotic use (Kirkpatrick et al., 2008).

It should be mentioned that it is still inconclusive whether TL is an aging biomarker. There is consistent proof that telomeres are implicated in cellular aging and human diseases of premature aging, but it is indubitably not a unique and universal marker (Mather et al., 2011). Important confounds in interpreting TL may be the influence of several variables such as age, sex, education, socioeconomic status, health behaviors, diet, history of early life adversities, latent or active viral infections, psychiatric and medical comorbidities, genetic polymorphisms, methods of DNA extraction, and many other that are impossible to control (Lindqvist et al., 2015). Although we could not consider all the possible confounds in our analysis, our analysis of covariance did not show different results regarding the group differences. Antipsychotic use seems to also have a direct influence in TL (Polho et al., 2015), though previous study showed shorter TL in antipsychotic-naïve patients compared to controls (Fernandez-Egea et al., 2009). We could not evaluate the impact of medication in our study. Furthermore, our sample was not paired by gender. However, although there is evidence of sex differences in TL (Gardner et al., 2014) we did not find an effect of sex, in accordance with previous study (Fernandez-Egea et al., 2009). Our study had important limitations such as small sample size and cross sectional design.

In conclusion, our results showed that participants with SZ had smaller TL compared to controls, while unaffected siblings presented no significant alterations. Although we could not conclude from our results that TL is an endophenotype of the illness, these findings suggest that a pathological accelerated aging profile could be underpinning the pathophysiology of SZ. Furthermore, these results could open a venue for investigation of peripheral aging markers in at risk populations, collaborating for the identification of other health problems that go along with aging and would be more frequent on these populations.

References

Armanios, M., 2009. Syndromes of telomere shortening. Annu Rev Genomics Hum Genet. 10, 45-61.

Armanios, M., 2013. Telomeres and age-related disease: how telomere biology informs clinical paradigms. J. Clin. Invest. 123, 996–1002.

Braff, D.L., 2015. The importance of endophenotypes in schizophrenia research. Schizophr Res. 163 (1-3) 1-8.

Domenici, E., Willé, D.R., Tozzi, F., Prokopenko, I., Miller, S., McKeown, A., Brittain, C., Rujescu, D., Giegling, I., Turck, C.W., Holsboer, F., Bullmore, E.T., Middleton, L., Merlo-Pich, E., Alexander, R.C., Muglia, P., 2010. Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. PLoS One. 5 (2) e9166.

Fernandez-Egea, E., Bernardo, M., Heaphy, C.M., Griffith, J.K., Parellada, E., Esmatjes, E., Conget, I., Nguyen, L., George, V., Stöppler, H., Kirkpatrick B., 2009. Telomere length and pulse pressure in newly diagnosed, antipsychotic-naive patients with nonaffective psychosis. Schizophr Bull. 35 (2) 437-42.

Gama, C.S., Salvador, M., Andreazza, A.C., Kapczinski, F., Silva Belmonte-de-Abreu, P., 2006. Elevated serum superoxide dismutase and thiobarbituric acid

reactive substances in schizophrenia: a study of patients treated with haloperidol or clozapine. Prog Neuropsychopharmacol Biol Psychiatry. 30 (3) 512-5.

Gama, C.S., Salvador, M., Andreazza, A.C., Lobato, M.I., Berk, M., Kapczinski, F., Belmonte-de-Abreu, P.S., 2008. Elevated serum thiobarbituric acid reactive substances in clinically symptomatic schizophrenic males. Neurosci Lett. 433 (3) 270-3.

Gardner, M., Bann, D., Wiley, L., Cooper, R., Hardy, R., Nitsch, D., Martin-Ruiz, C., Shiels, P., Sayer, A.A., Barbieri, M., Bekaert, S., Bischoff, C., Brooks-Wilson, A., Chen, W., Cooper, C., Christensen, K., De Meyer, T., Deary, I., Der, G., Diez Roux, A., Fitzpatrick, A., Hajat, A., Halaschek-Wiener, J., Harris, S., Hunt, S.C., Jagger, C., Jeon, H.S., Kaplan, R., Kimura, M., Lansdorp, P., Li, C., Maeda, T., Mangino, M., Nawrot, T.S., Nilsson, P., Nordfjall, K., Paolisso, G., Ren, F., Riabowol, K., Robertson, T., Roos, G., Staessen, J.A., Spector, T., Tang, N., Unryn, B., van der Harst, P., Woo, J., Xing, C., Yadegarfar, M.E., Park, J.Y., Young, N., Kuh, D., von Zglinicki, T., Ben-Shlomo, Y., Halcyon study team., 2014. Gender and telomere length: systematic review and meta-analysis. Exp Gerontol. 51, 15-27.

Jeste, D.V., Wolkowitz, O.M., Palmer, B.W., 2011. Divergent trajectories of physical, cognitive, and psychosocial aging in schizophrenia. Schizophr Bull. 37 (3) 451-5.

Kao, H.T., Cawthon, R.M., Delisi, L.E., Bertisch, H.C., Ji, F., Gordon, D., Li, P., Benedict, M.M., Greenberg, W.M., Porton, B., 2008. Rapid telomere erosion in schizophrenia. Mol Psychiatry. 13 (2) 118-9.

Kirkpatrick, B., Messias, E., Harvey, P.D., Fernandez-Egea, E., Bowie, C.R., 2008. Is schizophrenia a syndrome of accelerated aging? Schizophr Bull. 34 (6) 1024-32.

Kota, L.N., Purushottam, M., Moily, N.S., Jain, S., 2015. Shortened telomere in unremitted schizophrenia. Psychiatry Clin Neurosci. 69 (5) 292-7.

Kunz, M., Gama, C.S., Andreazza, A.C., Salvador, M., Ceresér, K.M., Gomes, F.A., Belmonte-de-Abreu, P.S., Berk, M., Kapczinski, F., 2008. Elevated serum superoxide

dismutase and thiobarbituric acid reactive substances in different phases of bipolar disorder and in schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry. 32 (7) 1677-81.

Kunz, M., Ceresér, K.M., Goi, P.D., Fries, G.R., Teixeira, A.L., Fernandes, B.S., Belmonte-de-Abreu, P.S., Kauer-Sant'Anna, M., Kapczinski, F., Gama, C.S., 2011. Serum levels of IL-6, IL-10 and TNF-α in patients with bipolar disorder and schizophrenia: differences in pro- and anti-inflammatory balance. Rev Bras Psiquiatr. 33 (3) 268-74.

Lindqvist, D., Epel, E.S., Mellon, S.H., Penninx, B.W., Révész, D., Verhoeven, J.E., Reus, V.I., Lin, J., Mahan, L., Hough, C.M., Rosser, R., Bersani, F.S., Blackburn, E.H., Wolkowitz, O.M., 2015. Psychiatric disorders and leukocyte telomere length: Underlying mechanisms linking mental illness with cellular aging. Neurosci Biobehav Rev. 55, 333-64.

Malaspina, D., Dracxler, R., Walsh-Messinger, J., Harlap, S., Goetz, R.R., Keefe, D., Perrin, M.C., 2014. Telomere length, family history, and paternal age in schizophrenia. Mol Genet Genomic Med. 2 (4) 326-31.

Mansour, H., Chowdari, K., Fathi, W., Elassy, M., Ibrahim, I., Wood, J., Bamne, M., Tobar, S., Yassin, A., Salah, H., Elsayed, H., Eissa, A., El-Boraie, H., Ibrahim, N.E., Elsayed, M., El-Bahaei, W., Gomaa, Z., El-Chennawi, F., Nimgaonkar, V.L., 2011. Does telomere length mediate associations between inbreeding and increased risk for bipolar I disorder andschizophrenia? Psychiatry Res. 188 (1) 129-32.

Mather, K.A., Jorm, A.F., Parslow, R.A., Christensen, H., 2011. Is telomere length a biomarker of aging? A review. J Gerontol A Biol Sci Med Sci. 66 (2) 202-13.

Monji, A., Kato, T., Kanba, S., 2009. Cytokines and schizophrenia: Microglia hypothesis of schizophrenia. Psychiatry Clin Neurosci. 63 (3) 257-65.

Na, K.S., Jung, H.Y., Kim, Y.K., 2014. The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. Prog Neuropsychopharmacol

Biol Psychiatry. 48, 277-86.

Nieratschker, V., Lahtinen, J., Meier, S., Strohmaier, J., Frank, J., Heinrich, A., Breuer, R., Witt, S.H., Nöthen, M.M., Rietschel, M., Hovatta, I., 2013. Longer telomere length in patients with schizophrenia. Schizophr Res. 149 (1-3) 116-20.

Pedrini, M., Massuda, R., Fries, G.R., de Bittencourt Pasquali, M.A., Schnorr, C.E., Moreira, J.C., Teixeira, A.L., Lobato, M.I., Walz, J.C., Belmonte-de-Abreu, P.S., Kauer-Sant'Anna, M., Kapczinski, F., Gama, C.S., 2012. Similarities in serum oxidative stress markers and inflammatory cytokines in patients with overt schizophrenia at early and late stages of chronicity. J Psychiatr Res. 46 (6) 819-24.

Pedrini, M., Massuda, R., de Lucena, D., Macêdo, D., Paz, A.V., Lobato, M.I., Belmonte-de-Abreu, P.S., Ceresér, K.M., Rocha, N.P., Curra, M.D., Panizzutti, B.S., Teixeira, A.L., Gama, C.S., 2014. Differences in eotaxin serum levels patients with recent onset and in chronic stable schizophrenia: a clue for understanding accelerating aging profile. Schizophr Res. 152 (2-3) 528-9.

Polho, G.B., De-Paula, V.J., Cardillo, G., dos Santos, B., Kerr, D.S., 2015. Leukocyte telomere length in patients with schizophrenia: A meta-analysis. Schizophr Res. 165 (2-3) 195-200.

Porton, B., Delisi, L.E., Bertisch, H.C., Ji, F., Gordon, D., Li, P., Benedict, M.M., Greenberg, W.M., Kao, H.T., 2008. Telomerase levels in schizophrenia: a preliminary study. Schizophr Res. 106 (2-3) 242-7.

Romano, F., Elkis, H., 1996. Tradução e adaptação de um instrumento de avaliação psicopatológica das psicoses: A Escala Breve de Avaliação Psiquiátrica - Versão Ancorada (BPRS-A). J Bras Psiquiatr. 45, 43-49.

Saha, S., Chant, D., McGrath, J., 2007. A systematic review of mortality in schizophrenia: is the differential mortality gap worsening over time? Arch Gen Psychiatry. 64 (10) 1123-31.

Stuart, M.J., Singhal, G., Baune, B.T., 2015. Systematic Review of the Neurobiological Relevance of Chemokines to Psychiatric Disorders. Front Cell Neurosci. 10 (9) 357.

Watanabe, Y., Someya, T., Nawa, H., 2010. Cytokine hypothesis of schizophrenia pathogenesis: evidence from human studies and animal models. Psychiatry Clin Neurosci. 64 (3) 217-30.

Wolkowitz, O.M., Mellon, S.H., Epel, E.S., Lin, J., Dhabhar, F.S., Su, Y., Reus, V.I., Rosser, R., Burke, H.M., Kupferman, E., Compagnone, M., Nelson, J.C., Blackburn, E.H., 2011. Leukocyte telomere length in major depression: correlations with chronicity, inflammation and oxidative stress--preliminary findings. PLoS One. 6 (3) e17837.

Yu, W., Chang, H., Lin, C., Chung-Lung, C., 2008. Short telomeres in patients with chronic schizophrenia who show a poor response to treatment. J. Psychiatry Neurosci. 33, 244–247.

Acknowledgment

This study was supported by CAPES and CNPq (Brazil). The authors also thank Fátima Thersinha Costa Rodrigues Guma for the support provided.

Conflict of interest

No conflict of interest.

Contributors

CSG, RM, DL, DM. LKG and FMBT designed the study; RM and LSC collected the data; LSC, BP, RM, LKG, FMBT and CSG performed the analyses and interpreted the results; LSC and CSG wrote the manuscript.

Role of funding sources

This study was supported grants from CNPq (Universal 443526/2014-1, Universal 458294/2014-4 and PQ 304443/2014-0), Brazil. These agencies had no role in the study design, in the acquisition or interpretation of the data, or in writing the report.

Table 1. Descriptive sociodemographic and clinical data of the subjects

	HC (n=47)	**SB (n=36)**	**SZ (n=36)**	Statistical Analysis
Gender (male/female)	21/26	15/21	25/11	X^2 = 6.905, df=2, p = .032* [c]
Age in years [a]	40.00 (13.65)	38.78 (11.88)	36.22 (12.41)	F (2, 116) = .906, p = .407 [d]
Years of education[a]	10.36 (3.16)	12.60 (3.78)	10.36 (3.11)	F (2, 116) = 5.612, p= .005* [d]
BMI [a]	25.96 (4.12)	25.39 (3.57)	25.96 (5.30)	F (2, 116) = .208, p= .812 [d]
BPRS [a]	-	-	14.67 (7.15)	-
Years of disease [a]	-	-	13.58 (11.22)	-
Medication				
Typical Antipsychotics (%)	-	-	22.2	-
Atypical Antipsychotics (%)	-	-	25.0	-
Clozapine (%)	-	-	52.8	-
Telomere length [b]	1.156 (1.207)	.976 (.748)	.873 (.397)	H(2) = 8.886, p < .05* [e]

Abbreviations: SZ: Schizophrenia; SB: Unaffected Siblings; HC: Paired healthy controls;

BMI: Body Mass Index; BPRS: Brief Psychiatry Rating Scale;

[a] Mean (standard deviation); [b] Median (interquartile range)

[c] Chi-square ; [d] ANOVA with Bonferroni correction; [e] Kruskal-Wallis

Figure 1

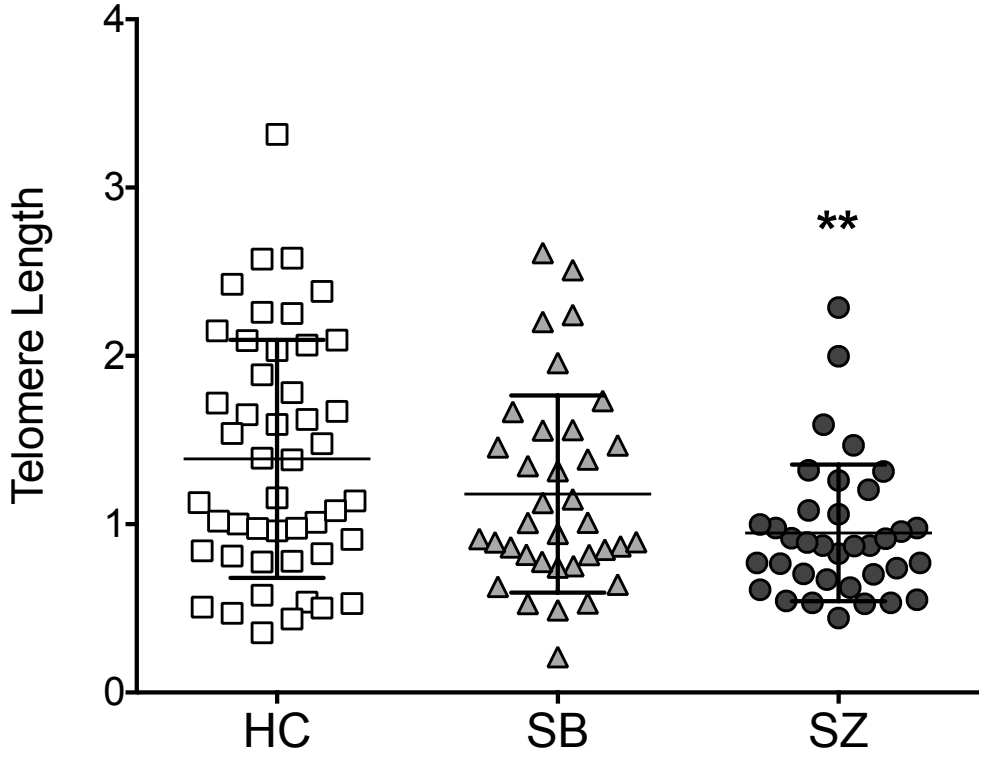

Figure 1. Similar relative telomere length (TL) in subjects with schizophrenia (SZ) and in its unaffected siblings (SB).

Supplementary Methods

Telomere length and CCL11/Eotaxin Measurements

Following psychiatric evaluation, 10 mL of peripheral blood was drawn from all subjects by venipuncture into tubes without anticoagulant. Immediately after withdrawal, the blood was centrifuged at 4000×g for 10 min, and the serum was aliquoted and stored at −80 °C until the experiment.

Measurement of relative telomere length was from whole peripheral venous blood that was used for genomic DNA (gDNA) extraction with a commercial kit (Illustra blood genomicPrep Mini Spin Kit, GE Healthcare) following manufacturers' instructions. Nucleic acid quantification and purity were checked spectrophotometrically (BioPhotometer Plus, Eppendorf, Hamburg, Germany) and samples were stored at -20oC for subsequent analysis. gDNA (25 ng/reaction) was used as template for quantification of relative mean telomere length (T/S) by real time quantitative polymerase chain reaction (qPCR), with minor modifications from previously reported (Cawthon, 2002). In summary, for each sample two separate qPCR were performed in triplicate in separate 96-well plates in the same position. One reaction amplified the telomere (T) repeat sequence while the other amplified a single copy gene, 36B4 (S), which served as a quantitative control. For each participant, relative telomere length was expressed as the T/S ratio. Previously published primer sequences' (26) were (5'→3'): tel 1, GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT; tel 2, TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA and 36B4u, CAGCAAGTGGGAAGGTGTAATCC and 36B4d, CCCATTCTATCATCAACGGGTACAA. T and S master mix reactions were identical in a final volume of 20 µL with 0.1x SYBR® Green (Molecular Probes, CA, USA), 2 mM MgCl2, 0.1 mM each dNTP, 1% DMSO and 0.5 U of Platinum® Taq DNA Polymerase (Invitrogen). Final primer concentrations for telomere amplification were 270 and 1,125 nM for telomeres primers, respectively; and 300 and 500 nM for 36B4u and 36B4d primers. PCR reactions were performed in StepOnePlus™ Real-time PCR system (Applied Biosystems, CA, USA) and analyzed with StepOne™ Software v2.3. (Applied Biosystems). Thermal cycling profile for amplification consisted of an initial incubation step for 2 min at 94oC to activate hot start Platinum

Taq DNA polymerase, followed by 22 cycles of denaturing at 94oC for 15 s and annealing and extension for 2 min at 54oC, for telomere amplification. For 36B4 amplification, amplification consisted of 30 cycles of denaturing at 94oC for 15 s followed by annealing and extension for 2 min at 60oC. The specificity of the amplification was confirmed at the end of each run using melting curve analysis. We additionally confirmed PCR products by agarose gel electrophoresis. In each run, a reference sample was included as a calibrator to normalize the participants' T/S ratio and calculate the final T/S ratio. Finally, in order to check for PCR amplification efficiency, standard curves for telomere and 36B4 amplification were generated from the reference sample, over a 5-fold range by serial dilution from 100 to 0.16 ng of gDNA. Inter-plate variability was 2.7%.

Measurement of eotaxin/CCL11 in the serum of subjects was performed by sandwich-ELISA according to manufacturer instructions (DuoSet, R & D Systems, Minneapolis, MN, USA).

Reference:

Cawthon, R.M., 2002. Telomere measurement by quantitative PCR. Nucleic Acids Res. 30, 10, e47.

7 ARTIGO #4

Submetido ao ***Schizophrenia Bulletin***

Fator de Impacto (2015): 7.757

Versão do manuscrito submetida:

August 4th, 2015

Schizophrenia Bulletin Editorial Office

Dear Editor:

We submit the manuscript entitled "*Telomere Length and CCL11 Levels Are Associated with Gray Matter Volume and Episodic Memory Performance in Schizophrenia: Evidence of Pathological Accelerated Aging*", which we would appreciate being considered for publication in *Schizophrenia Bulletin.* The manuscript contains original work that has not been published or submitted for publication elsewhere and has not been made available on a website.

The goal of the current study was to test hypotheses relevant to the theory of accelerated aging in schizophrenia through the analysis of the relationships of age, aging and inflammatory biomarkers (telomere length and CCL11 levels, respectively), gray matter volume and episodic memory performance in individuals with schizophrenia compared to healthy controls. As such, the current study investigated whether these variables were related differently in individuals with schizophrenia and healthy controls. Also, we wanted to know if age and duration of illness related differently to aging and inflammatory biomarkers, gray matter volume and episodic memory performance in individuals with schizophrenia. Further, we tested whether any relationships to memory performance were mediated by indirect effects of aging and inflammatory biomarkers or gray matter volume.

Participants were 48 individuals with schizophrenia and 64 healthy controls similar in age, sex and education, and originating from the same socioeconomic and educational background. All participants underwent clinical and memory assessment, structural MRI, and had their peripheral blood drawn for biomarkers analysis.

We found associations between increased CCL11, shorter telomere length, reduced gray matter volume and decreased episodic memory in schizophrenia, which were all related to longer duration of illness. Interestingly, telomere length mediated the effect of illness duration to memory performance. In unaffected individuals, there were no significant correlations except between memory and GM. Our results were consistent with the hypothesis of accelerated aging in schizophrenia, and suggest that it is not age itself, but the impact of the disease associated with a pathological aging that might lead to a worse outcome. We believe that these findings provide preliminary relevant evidence for the hypothesis of accelerated aging in schizophrenia. We think that this manuscript will be of broad interest to your readership and believe that it meets your high standards for publication in your journal.

Sincerely,

Leticia Sanguinetti Czepielewski, Raffael Massuda, Bruna Panizzutti, Lucas Kich Grun, Florencia María Barbé-Tuana, Antonio Lucio Teixeira, Deanna M Barch, Clarissa S Gama

Title: Telomere Length and CCL11 Levels Are Associated with Gray Matter Volume and Episodic Memory Performance in Schizophrenia: Evidence of Pathological Accelerated Aging

Running Title: Evidence of Pathological Accelerated Aging in SZ

Leticia Sanguinetti Czepielewski[1], Raffael Massuda[2], Bruna Panizzutti[1], Lucas Kich Grun[3], Florencia María Barbé-Tuana[3], Antonio Lucio Teixeira[4], Deanna M Barch[5], Clarissa S Gama[1]

[1] Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

[2] Departamento de Psiquiatria, Universidade Federal do Paraná, Curitiba, PR, Brazil

[3] Programa de Pós-Graduação em Ciências Biológicas: Bioquímica, Instituto de Ciências Básicas da Saúde, Universidade Federal do Rio Grande do Sul - Porto Alegre, Brazil

[4] Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil

[5] Departments of Psychological & Brain Sciences, Psychiatry and Radiology, Washington University in St. Louis, MO, USA.

Corresponding Author:

Clarissa S Gama

Hospital de Clínicas de Porto Alegre/CPE, Molecular Psychiatry Laboratory,

Rua Ramiro Barcelos, 2350, Prédio Anexo, 90035-903 Porto Alegre, RS, Brazil.

Phone: +55 51 33598845; Fax: +55 51 33598846. E-mail: cgama@hcpa.edu.br

Word count Abstract: 237

Word count text body: 3978

Abstract

Background: Schizophrenia (SZ) is associated with increased somatic morbidity and mortality, in addition to cognitive impairments similar to those seen in normal aging, which may suggest that pathological accelerated aging occurs in SZ. Therefore, we aim to evaluate the relationships of age, telomere length (TL) and CCL11 (aging and inflammatory biomarkers, respectively), and gray matter volume (GM) to episodic memory performance in individuals with SZ compared to healthy controls (HC).

Methods: 112 participants (48 SZ and 64 HC) underwent clinical and memory assessments, structural MRI, and had their peripheral blood drawn for biomarkers analysis. Comparisons of group means and correlations were performed.

Results: Participants with SZ had decreased TL and GM volume, increased CCL11, and worse memory performance compared to HC. In SZ, shorter TL was related to increased CCL11, and both biomarkers were related to reduced GM volume, all of which were related to worse memory performance. Older age was only associated with reduced GM, but longer duration of illness was related with all the aforementioned variables. Younger age of disease onset was associated with increased CCL11 levels and worse memory performance. In HC, there were no significant correlations except between memory and GM.

Conclusion: Our results are consistent with the hypothesis of accelerated aging in SZ. These results may indicate that it is not age itself, but the impact of the disease associated with a pathological accelerated aging that leads to impaired outcomes in SZ.

Keywords: Schizophrenia, pathological accelerated aging, biomarkers, episodic memory, gray matter volume.

Introduction

Schizophrenia (SZ) is associated with strong burden of disease[1] and higher mortality, with a relative risk of all-cause mortality of 2.54[2] and an average life span of 15-25 years shorter than unaffected individuals[3]. Compared to the general population, the median standardized mortality ratio in SZ is 2.41 if only natural causes of death are considered[4]. This is related to the increased risk for several somatic diseases in SZ that are typically associated with the process of aging, such as cardiovascular disease, diabetes and cancer[5]. These observations of increased somatic morbidity and mortality, in addition to cognitive impairments similar to those seen in normal aging, may suggest that a "pathological accelerated aging" occurs in SZ[6]. However, the neurobiological underpinnings and possible progression of SZ are still unclear, and studies are needed to generate evidence in regards to this theory.

A suggested biomarker of aging is telomere length (TL). Telomeres are DNA-protein structures that protect the ends of chromosomes and progressively shorten with each cell division[7]. There is growing evidence linking TL with psychiatric disorders[8], especially showing that TL is shortened in SZ compared to healthy controls[9,10]. Shortened TL is related to more rapid cell senescence and apoptosis in association with aging and disease[11]. More recently, TL was proposed to be a biomarker of "somatic redundancy", which is the body's capacity to absorb damage over time[12]. TL is a heritable trait, but it is also influenced by epigenetic, environmental[8], inflammatory and oxidative stress factors[13,14].

Among inflammatory mediators that could be related to an accelerated aging process, SZ has been consistently associated with abnormalities in cytokines, which are proteins involved in the coordination of immune responses and exertion of neuromodulatory actions[15,16,17,18,19]. A special type of cytokines that regulate the migration of peripheral immune cells directing them to pro-inflammatory activation states is the chemokines, which play a role in the central nervous system by regulating the inflammatory state associated with various pathological conditions[20]. Besides their role in inducing and directing leukocyte migration, chemokines have been implicated in other neurobiological processes. For instance, the binding of a chemokine to its receptor activates signaling cascades that result in increased calcium concentrations and the activation of mitogen-activated protein kinases, important mechanisms for synaptic plasticity[21]. Moreover, the pro-inflammatory chemokine CCL11 was demonstrated to be an age-related systemic factor

associated with decreased neurogenesis in hippocampus and impaired learning and memory in mice[22]. Previous studies showed that CCL11 was increased in chronic, though not in recent onset individuals with SZ, consistent with the hypothesis that it could also be a potential biomarker for a pathological aging process in SZ[23,24].

In normal aging, there is progressive whole brain volume loss in late adulthood[25]. In SZ, brain volumes are consistently decreased[26]. However, a longitudinal study evaluating gray matter density maps showed that this whole brain volume reduction may be due to possible faster aging compared to healthy controls, especially in the first years after disease onset. At baseline, brain age in SZ was 3.36 years greater than chronological age, and SZ showed a further accelerated aging of an additional 4 months in each year after follow-up. The authors proposed that two different processes might influence progressive brain loss in SZ: accelerated aging of the brain and other factors influencing individual variation, such as medication use[27]. Another study revealed a diagnosis by age interaction in the prediction of efficiency of the cingulo-opercular and fronto-parietal networks[28], which are associated with cognitive ability in both health and SZ[29].

Performance across several cognitive domains is also decreased in SZ[30], even in drug-naïve patients[31]. Episodic memory impairments show a particularly large effect size in SZ[32], and have been associated with daily functioning[33,34]. Interestingly, a recent study showed that memory impairment in SZ was similar to the impairment seen in healthy aging, possibly pointing to shared mechanisms[35]. Memory performance clearly reduces with aging, with a particularly strong effect on the ability to learn new associations[36]. Accordingly, episodic memory may be sensitive to the effects of both normal and pathological aging[37], and may be a useful behavioral indicator of aging related outcome.

Given the evidence outlined above, the current study was designed to investigate the following questions: 1) are aging and inflammatory biomarkers more strongly related in SZ than HC?; 2) are aging and inflammatory biomarkers more strongly related to gray matter volume in SZ than HC?; 3) are aging and inflammatory biomarkers and gray matter volume more strongly related to memory performance in SZ than HC?; 4) is age related differently in SZ than HC to aging and inflammatory biomarkers, gray matter volume and memory?; 5) in SZ, are illness duration, age of disease onset and current psychopathological state related to aging and

inflammatory biomarkers, gray matter volume and memory?; and 6) in SZ, are there any indirect effects of aging and inflammatory biomarkers or gray matter volume?

Methods

Participants

There were 112 participants: 48 individuals with schizophrenia (SZ) and 64 unaffected individuals (HC). All participants were between the ages of 18 and 60 years, and were recruited from the same general public hospital in Brazil. The SZ and HC subjects were similar in age, sex and education and all originated from the same socioeconomic and educational background. The Institutional Review Board approved the study protocol. All participants were advised about the procedures and signed informed consent prior to participation.

SZ had their diagnoses confirmed by trained psychiatrists using the Structured Clinical Interview for DSM-IV (SCID). Their psychopathological state was assessed using the 18-item Brief Psychiatry Rating Scale (BPRS)[38]. They had to be stable for at least 6 months and could not be currently in a psychotic episode. Healthy controls (HC) had no current or previous history, nor first-degree family history of a major psychiatric disorder, including dementia or intellectual disability, confirmed by the SCID.

Additional exclusion criteria for the groups were history or presence of neurological disease, abuse or dependence on drugs, brain tumor, thyroid disease, rheumatological disease, uncontrolled endocrine and cardiac disease, history of autoimmune diseases or chronic infections/inflammatory diseases, having any severe systemic disease, or having received immunosuppressive therapy.

Clinical and Memory Assessment

Participants underwent clinical evaluation with trained psychiatrists to collect sociodemographic, clinical and pharmacological data through a structured interview. Furthermore, patient's hospital clinical records were searched to collect supplementary data. All participants were assessed by trained psychologists with the Hopkins Verbal Learning Test-Revised (HVLT-R), which is a word-list task widely used to measure verbal learning and episodic memory[39]. The HVLT-R is part of the MATRICS Consensus Battery for Schizophrenia[40]. It is comprised of three immediate recall trials of 12 words within three categories and a delayed recall followed by a recognition task. We focused our analyses on total immediate recall.

Biomarkers

The complete description is in the Supplementary Methods and it was described elsewhere[10,41].

Neuroimaging

T1-weighted magnetic resonance images were acquired with a Philips Achieva 1.5 T scanner (Amsterdam, the Netherlands). Volumetric segmentations were performed using the Freesurfer image analysis suite software v.5.1.0 (http://surfer.nmr.mgh.harvard.edu/). Technical details are described elsewhere[42,43,44]. All images were processed and checked by the same researcher. Our analyses focused on total gray matter volume (cortical + subcortical).

Data Analysis

Statistical analyses were performed in R (https://www.R-project.org/). We first compared groups by examining demographic, clinical, memory performance, aging and inflammatory biomarkers and gray matter volume variables using Student's t-test or Chi-square test. For the total gray matter volume, intracranial volume was regressed out. Our second level of analysis tested our hypotheses about the relationships between variables within each group using Pearson's correlation coefficient, and Fisher's z transformation to compare the magnitude of correlations across groups. Lastly, we conducted a mediation model using the PROCESS model with aging and inflammatory biomarkers as dependent variables predicting memory performance with total gray matter residual volume as the mediator.

Results

Diagnostic Group Comparisons: HC and SZ (Table 1.) had similar age, gender distribution, education and BMI. SZ had more tobacco use than HC. As expected, SZ had worse episodic memory performance compared to HC. Also as predicted, SZ had increased CCL11 levels and reduced telomere length (TL) compared to HC. After we regressed out intracranial volume from total gray matter volume, SZ showed decreased residuals of total gray matter (GM) volume.

----------Table 1----------

Are aging and inflammatory biomarkers related? As shown in Figure 1., TL and CCL11 were significantly negatively correlated in SZ (r=-.37, p=.032). In HC, this correlation was not significant (r=-.17, p=.27), although its magnitude was not statistically different than SZ (z=-.90, p=.37).

----------Figure 1----------

Are aging and inflammatory biomarkers related to gray matter volume? CCL11 and total GM volume were significantly negatively correlated in SZ (r=-.37, p=.03), but not in HC (r=.14, p=.35), and these relations were statistically different between groups (z=-2.27, p=.02) (Figure 2A.). TL and total GM volume were positively correlated in SZ (r=.39, p=.02). In HC, although the correlation was not statistically significant (r=.18, p=.25), the Fisher transformation showed no significant difference between groups in the magnitude of the correlation (z=.98, p=.33) (Figure 2B.).

Are aging and inflammatory biomarkers and gray matter volume related to memory performance? CCL11 and HVLT-R total immediate recall were negatively correlated in SZ at a trend level (r=-.31, p=.07), but not in HC (r=.17, p=.23), and these relations were statistically different between groups (z=-2.17, p=.03) (Figure 2C.). TL and HVLT-R total immediate recall were positively correlated in SZ (r=.48, p=.003), but not in HC (r=-.12, p=.45), and these relations were also significantly different (z=2.75, p=.006) (Figure 2D.). In both SZ and HC, total GM volume and HVLT-R total immediate recall were positively correlated (r=.41, p=.005 and r=.27, p=.05, respectively).

----------Figure 2----------

What is the relationship of age to biomarkers, gray matter volume and memory? TL and CCL11 were not significantly correlated with age in either SZ (r=-.30, p=.078 and r=.29, p=.092, respectively) or HC (r=-.10, p=.51 and r=-.09, p=.56, respectively). Total GM volume and age were negatively correlated in both SZ (r=-.65, $p < .001$) and HC (r=-.71, $p < .001$). Age and HVLT-R total immediate recall were not significantly correlated in SZ (r=-.23, p=.122), but were negatively correlated in HC (r=-.41, p=.003), although the magnitude of this correlation was not statistically different than SZ (z = .98, p=.33).

What is the relationship of clinical characteristics of SZ to biomarkers, gray matter volume and memory? In SZ, illness duration was significantly correlated with TL (r=-.46, p=.004), CCL11 (r=.45, p=.007) total GM volume (r=-.66, p=.000) and HVLT-R total immediate recall (r=-.44, p=.002). Age of disease onset was only correlated with CCL11 (r=-.34, p=.043) and HVLT-R total immediate recall (r=.39, p=.006). BPRS total score was not significantly correlated with any of the variables ($p > .29$). We used the method of Meng, Rosenthal and Rubin[45] for comparing correlated correlation coefficients to compare the magnitude of the correlations with

age versus illness duration. Not surprisingly, age and illness duration were strongly correlated (*r*=.87*, p*<.001*).* However, illness duration was significantly more strongly correlated than age with TL (*Z*=2.29, *p*=.01), CCL11(*Z*=-.28 *p*=.01), and HVLT-R (*Z*=2.95, *p*=.002), but not for GM volume (*Z*=0.18, *p*=.42) (Figure 3.).

----------Figure 3----------

Does gray matter volume mediate the relationship between aging and inflammatory biomarkers and memory in SZ?

We created two different mediation models. First, we included TL as the independent variable, GM volume as the mediator, and HVLT-R total immediate recall as the dependent variable. There was no significant indirect effect of GM volume on HVLT-R total immediate recall (*p*=.23). We created a second model with CCL11 as the independent variable, GM volume as the mediator, and HVLT-R total immediate recall as the dependent variable. There was a trend indirect effect of GM volume predicting HVLT-R total immediate recall (*p*=.04). The bootstrapped unstandardized indirect effect was -.0006, and the 95% confidence interval ranged from -.0014, .0001, suggesting that CCL11 may predict memory performance in part through GM volume.

Does aging and inflammatory biomarkers or gray matter volume mediate the relationship between illness duration and memory in SZ?

We included illness duration as the independent variable, HVLT-R total immediate recall as the dependent variable and CLL11, TL and GM volume as separate mediators. There were no significant indirect effects of either CCL11 or GM volume on HVLT-R (*p*=.29 and *p*=.14, respectively). However, we found a significant indirect effect of TL on HVLT-R total immediate recall (*p*=.02). The bootstrapped unstandardized indirect effect was -.10, and the 95% confidence interval ranged from -.23, -.03. Therefore, TL mediated the effect of illness duration to memory performance.

Discussion

This study is, to the best of our knowledge, the first to show associations between telomere length (TL), CCL11 levels, gray matter (GM) volume and episodic memory performance in people with schizophrenia (SZ), presenting new evidence relevant to the theory of pathological accelerated aging in SZ. As expected, compared to demographically similar HC, individuals with SZ had worse memory

performance, increased CCL11, reduced TL, and decreased residuals of total GM volume after regressing out intracranial volume. In SZ, shorter TL was related with increased CCL11 levels, and they were both related to reduced GM volume. Shorter TL, increased CCL11 levels and reduced GM volume were all related to worse memory performance. Compared to HC, SZ showed statistically stronger magnitudes of correlations between shorter TL and increased CCL11 levels, and between shorter TL and reduced GM volume. Among SZ, age was significantly correlated only with reduced GM volume. Longer duration of illness was related with all the aforementioned variables. The effects of duration of illness to memory performance were mediated by TL. Younger age of disease onset was related with increased CCL11 levels and worse memory performance. These results may indicate that it is not age itself, but the impact of the disease associated with a pathological aging that leads to worse outcomes among SZ. Each of these sets of findings will be discussed in more detail below.

First, we saw that the aging and inflammatory biomarkers TL and CCL11 were significantly more strongly related in SZ compared to HC. There are several theories that have been proposed to explain normal aging, which is a complex process determined by multiple factors that include genetic, environmental and socioeconomic influences. Aging can be defined as a collection of time-dependent anatomical and physiological changes that reduce functional capacity, physiological and homeostatic reserve and decrease the ability to adjust to stress[46]. There is consistent evidence that associates TL with aging and mortality, although these relationships are not clearly causational[47]. Telomeres are implicated in cellular aging and human diseases of premature aging, but it is not a unique and universal aging biomarker[48]. Diseases of short TL are known to represent premature aging, as they show processes that occur in subjects as they age, including features marked by vascular and degenerative components as well as by cancer predisposition[7]. Hence, TL could be an integrative measure of somatic damage or history of past cell replication[47], representing the pace of biological aging related to the lifespan of cells and the body. The shortening of TL may be accelerated by the cumulative impact of stressors, which in turn may speed the process of biological aging[49]. We saw that SZ had shorter TL compared to HC, what could mean that these individuals had greater impairment related to a faster somatic aging and/or increased damage. Interestingly, TL was significantly correlated to duration of illness and not to age, which is

consistent with the hypothesis of a greater impact of accumulated stress or adversity. Furthermore, TL mediated the effect of illness duration to memory performance, suggesting important functional implications of these processes.

In SZ, shorter TL was associated with increased CCL11 levels, which were significantly increased compared to HC. CCL11 is a chemokine also associated with older age[22]. During inflammatory response and oxidative stress, there is an increased immune cell replication that potentially affects TL[8]. Thus in SZ, CCL11 could be involved in a pathogenic pathway of oxidative stress/pro-inflammatory imbalance that could lead to accelerated cell aging[3]. People with SZ often present a multi-morbidity state, i.e. the presence of two or more chronic conditions in the same individual, which is associated with increased inflammation that contributes to accumulation of a disease burden[50]. As such, the relationship between TL and CCL11 is consistent with the idea that TL may be a biomarker of body's capacity to absorb damage over time[12], with shorter TL reflecting less capacity to do so.

Both shorter TL and increased CCL11 levels were related to reduced total GM volume in SZ, but not HC, and the relationship between CCL11 and GM was statistically stronger in SZ than HC. These relationships are consistent with the idea that chronic pro-inflammatory processes could influence the brain through mechanisms such as neuroinflammation and microglial activation, which in turn could lead to structural and functional consequences, such as loss of gray matter[51]. Alterations in immune function early in life may lead to increased inflammation over time, which in turn can produce brain abnormalities[52]. Interestingly, increased levels of CCL11 were significantly related to longer duration of illness, which in turn was related to reduced GM volumes and worse memory performance in SZ. Additionally, increased CCL11 levels were related to younger age of disease onset. These observations could fit with the neurodevelopmental hypothesis of SZ[53]. There is considerable evidence of pathological risk factors such as early-life infections that influence early neurodevelopment in SZ[54] and affect the brain before it approaches its adult anatomical state[53]. The increased risk of developing SZ associated with the history of varying types of infections may suggest that a pro-inflammatory immune response may contribute to the onset of SZ[55]. Recently, the North American Prodrome Longitudinal Study (NAPLS) identified markers of inflammation, oxidative stress, and dysregulation of hypothalamic-pituitary axis that were able to predict the conversion from clinical high risk to psychosis[56]. Thus, the neurodevelopmental

course of SZ might trigger or be linked to chronic inflammation that in turn may be associated with the onset of the disease and a worse outcome as the illness progresses, producing an accelerated pathological aging.

In addition, aging and inflammatory biomarkers were more strongly related to memory performance in SZ than HC. There is evidence that memory performance shows age-related impairments[37] and vulnerability to different biological processes affecting the brain in illness and healthy aging[37]. Worse memory performance in SZ was associated with increased CCL11, and this relationship was significantly stronger than the relationship in HC. This result is consistent with other studies linking inflammation to cognitive deficits in SZ, although the mechanisms of this relationship are still unclear[57]. One possible explanation would be the aforementioned processes of chronic inflammation influencing the brain[51]. Interestingly, even in our relatively small sample size, we found a small trend effect of GM volume mediating the effect of CCL11 on memory performance, which points toward a possible mechanism to be further investigated.

Worse memory performance was associated with shorter TL in SZ but not in HC. However, better memory performance was correlated with larger GM volume in both groups, even though individuals with SZ had significantly worse scores on HVLT-R. From the mediation model, we saw that TL predicted memory performance not through an indirect effect of GM volume. These results suggest that memory impairment in SZ might have multiple contributing factors, including the impact of stress and other factors associated with pathological aging[12], as indexed by TL and CCL11 levels, though only the latter may be partially mediated by GM reductions.

Lastly, age and illness duration were differently related to aging and inflammatory biomarkers and memory performance in SZ. In SZ, age was only correlated with GM, but duration of illness was associated with TL, CCL11, GM and memory performance, with significantly stronger correlations to all but GM. One might have expected stronger relationships to age given the accelerated aging hypothesis. However, the stronger correlations with illness duration suggest that it is not age itself, but rather the impact of the disease that relates to these impairments. In HC, however, older age was related with worse memory performance in addition to reduced GM volume, which goes in line with what is described in the literature regarding normal aging.

Our study had limitations. It was a cross sectional design with a relatively limited sample size. Further, there are likely confounds that could influence the analysis of aging and inflammatory biomarkers, such as comorbidities or lifestyle factors, which were not able to be addressed in this study. We did not include in the analysis the effect of current medication or history of psychotropic treatment, although all individuals with SZ were stable for at least six months. Also, we only presented results of analyses of brain structure, and we did not have data on brain function or connectivity among these individuals, which may also show important relationships to cognitive function. Finally, we did not have data on the premorbid intellectual functioning, what could have influenced memory performance.

----------Figure 4----------

In summary, our results are consistent with the hypothesis of pathological accelerated aging in SZ. We saw associations between increased CCL11 – an age-related pro-inflammatory marker, shorter telomere length, reduced gray matter volume and decreased episodic memory in SZ, which were all related to longer duration of illness (Figure 4.). These results suggest that it is not age itself, but the impact of the disease associated with a pathological aging that might lead to a worse outcome. Although preliminary, our data point to potentially important mechanisms related to neural and cognitive impairment associated with psychosis. Future steps should focus on longitudinal studies of individuals at high risk to develop psychosis to evaluate the effect of inflammatory and aging biomarkers in the conversion, but also should follow individuals with SZ across their lifespan to confirm the accelerated aging hypothesis.

Acknowledgement

This study was supported by CNPq and CAPES, Brazil. It received grants from CNPq (Universal 443526/2014-1, Universal 458294/2014-4, PQ 304443/2014-0).The authors also thank Fátima Theresinha Costa Rodrigues Guma for the support provided.

References

1. Whiteford HA, Degenhardt L, Rehm J, et al. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet* 2013;382:1575-1586.
2. Walker ER, McGee RE, Druss BG. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. *JAMA Psychiatry* 2015;72:334-341.
3. Jeste DV, Wolkowitz OM, Palmer BW. Divergent trajectories of physical, cognitive, and psychosocial aging in schizophrenia. *Schizophr Bull* 2011;37:451-455.
4. Saha S, Chant D, McGrath J. A systematic review of mortality in schizophrenia: is the differential mortality gap worsening over time? *Arch Gen Psychiatry* 2007;64:1123-1131.
5. Henderson DC, Vincenzi B, Andrea NV, Ulloa M, Copeland PM. Pathophysiological mechanisms of increased cardiometabolic risk in people with schizophrenia and other severe mental illnesses. *Lancet Psychiatry* 2015;2:452-464.
6. Kirkpatrick B, Messias E, Harvey PD, Fernandez-Egea E, Bowie CR. Is schizophrenia a syndrome of accelerated aging? *Schizophr Bull* 2008;34:1024-1032.
7. Armanios M. Syndromes of telomere shortening. *Annu Rev Genomics Hum Genet* 2009;10:45-61.
8. Lindqvist D, Epel ES, Mellon SH, et al. Psychiatric disorders and leukocyte telomere length: Underlying mechanisms linking mental illness with cellular aging. *Neurosci Biobehav Rev* 2015;55:333-364.
9. Polho GB, De-Paula VJ, Cardillo G, dos Santos B, Kerr DS. Leukocyte telomere length in patients with schizophrenia: A meta-analysis. *Schizophr Res* 2015;165:195-200.
10. Czepielewski LS, Massuda R, Panizzutti B, et al. Telomere length in subjects with schizophrenia, their unaffected siblings and healthy controls: Evidence of accelerated aging. *Schizophr Res* 2016;174:39-42.
11. Armanios M. Telomeres and age-related disease: how telomere biology informs clinical paradigms. *J Clin Invest* 2013;123:996-1002.

12. Boonekamp JJ, Simons MJ, Hemerik L, Verhulst S. Telomere length behaves as biomarker of somatic redundancy rather than biological age. *Aging Cell* 2013;12:330-332.
13. Baylis D, Ntani G, Edwards MH, et al. Inflammation, telomere length, and grip strength: a 10-year longitudinal study. *Calcif Tissue Int* 2014;95:54-63.
14. von Zglinicki T. Oxidative stress shortens telomeres. *Trends Biochem Sci* 2002;27:339-344.
15. Monji A, Kato T, Kanba S. Cytokines and schizophrenia: Microglia hypothesis of schizophrenia. *Psychiatry Clin Neurosci* 2009;63:257-265.
16. Watanabe Y, Someya T, Nawa H. Cytokine hypothesis of schizophrenia pathogenesis: evidence from human studies and animal models. *Psychiatry Clin Neurosci* 2010;64:217-230.
17. Domenici E, Willé DR, Tozzi F, et al. Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. *PLoS One* 2010;5:e9166.
18. Na KS, Jung HY, Kim YK. The role of pro-inflammatory cytokines in the neuroinflammation and neurogenesis of schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2014;48:277-286.
19. Pedrini M, Massuda R, Fries GR, et al. Similarities in serum oxidative stress markers and inflammatory cytokines in patients with overt schizophrenia at early and late stages of chronicity. *J Psychiatr Res* 2012;46:819-824.
20. Stuart MJ, Singhal G, Baune BT. Systematic Review of the Neurobiological Relevance of Chemokines to Psychiatric Disorders. *Front Cell Neurosci* 2015;9:357.
21. Rostène W, Kitabgi P, Parsadaniantz SM. Chemokines: a new class of neuromodulator? *Nat Rev Neurosci* 2007;8:895-903.
22. Villeda SA, Luo J, Mosher KI, et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. *Nature* 2011;477:90-94.
23. Teixeira AL, Reis HJ, Nicolato R, et al. Increased serum levels of CCL11/eotaxin in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* 2008;32:710-714.
24. Pedrini M, Massuda R, de Lucena D, et al. Differences in eotaxin serum levels patients with recent onset and in chronic stable schizophrenia: a clue for understanding accelerating aging profile. *Schizophr Res* 2014;152:528-529.

25. Hedman AM, van Haren NE, Schnack HG, Kahn RS, Hulshoff Pol HE. Human brain changes across the life span: a review of 56 longitudinal magnetic resonance imaging studies. *Hum Brain Mapp* 2012;33:1987-2002.
26. Haijma SV, Van Haren N, Cahn W, Koolschijn PC, Hulshoff Pol HE, Kahn RS. Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. *Schizophr Bull* 2013;39:1129-1138.
27. Schnack HG, van Haren NE, Nieuwenhuis M, Hulshoff Pol HE, Cahn W, Kahn RS. Accelerated Brain Aging in Schizophrenia: A Longitudinal Pattern Recognition Study. *Am J Psychiatry* 2016;173:607-616.
28. Sheffield JM, Repovs G, Harms MP, et al. Evidence for Accelerated Decline of Functional Brain Network Efficiency in Schizophrenia. *Schizophr Bull* 2015;42:753-761.
29. Sheffield JM, Repovs G, Harms MP, et al. Fronto-parietal and cingulo-opercular network integrity and cognition in health and schizophrenia. *Neuropsychologia* 2015;73:82-93.
30. Fioravanti M, Bianchi V, Cinti ME. Cognitive deficits in schizophrenia: an updated metanalysis of the scientific evidence. *BMC Psychiatry* 2012;12:64.
31. Fatouros-Bergman H, Cervenka S, Flyckt L, Edman G, Farde L. Meta-analysis of cognitive performance in drug-naïve patients with schizophrenia. *Schizophr Res* 2014;158:156-162.
32. Schaefer J, Giangrande E, Weinberger DR, Dickinson D. The global cognitive impairment in schizophrenia: consistent over decades and around the world. *Schizophr Res* 2013;150:42-50.
33. Green MF. What are the functional consequences of neurocognitive deficits in schizophrenia? *Am J Psychiatry* 1996;153:321-330.
34. Green MF, Kern RS, Braff DL, Mintz J. Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"? *Schizophr Bull* 2000;26:119-136.
35. Silver H, Bilker WB. Similar verbal memory impairments in schizophrenia and healthy aging. Implications for understanding of neural mechanisms. *Psychiatry Res* 2015;226:277-283.
36. Silver H, Goodman C, Bilker WB. Impairment in associative memory in healthy aging is distinct from that in other types of episodic memory. *Psychiatry Res* 2012;197:135-139.

37. Tromp D, Dufour A, Lithfous S, Pebayle T, Després O. Episodic memory in normal aging and Alzheimer disease: Insights from imaging and behavioral studies. *Ageing Res Rev* 2015;24:232-262.
38. Romano F, Elkis H, Tradução e adaptação de um instrumento de avaliação psicopatológica das psicoses: A Escala Breve de Avaliação Psiquiátrica - Versão Ancorada (BPRS-A). *J Bras Psiquiatr* 1996;45:43-49.
39. Benedict RHB, Schretlen D, Groninger L, Brandt J. Hopkins Verbal Learning Test – Revised: Normative Data and Analysis of Inter-Form and Test-Retest Reliability. *The Clinical Neuropsychologist* 1998;12:43-55.
40. Nuechterlein KH, Green MF, Kern RS, et al. The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity. *Am J Psychiatry* 2008;165:203-213.
41. Panizzutti B, Gubert C, Schuh AL, et al. Increased serum levels of eotaxin/CCL11 in late-stage patients with bipolar disorder: An accelerated aging biomarker? *J Affect Disord* 2015;182:64-69.
42. Fischl B, Salat DH, Busa E, et al. Whole brain segmentation: automated labeling of neuroanatomical structures in the human brain. *Neuron* 2002;33:341-355.
43. Fischl B. FreeSurfer. *Neuroimage* 2012;62:774-781.
44. Vianna-Sulzbach M, Rocha NP, Teixeira AL, et al. Right hippocampus size is negatively correlated with leptin serum levels in bipolar disorder. *Psychiatry Res* 2015;230:719-721
45. Meng X, Rosenthal R, Rubin DB. Comparing correlated correlation coefficients. *Psychological Bulletin* 1992;111:172-175.
46. Ahmed A, Tollefsbol T. Telomeres and telomerase: basic science implications for aging. *J Am Geriatr Soc* 2001;49:1105-1109.
47. Simons MJ. Questioning causal involvement of telomeres in aging. *Ageing Res Rev* 2015;24:191-196.
48. Mather KA, Jorm AF, Parslow RA, Christensen H. Is telomere length a biomarker of aging? A review. *J Gerontol A Biol Sci Med Sci* 2011;66:202-213.
49. Reynolds CF 3rd. Telomere Attrition: A Window Into Common Mental Disorders and Cellular Aging. *Am J Psychiatry* 2016;173:556-558.

50. Stepanova M, Rodriguez E, Birerdinc A, Baranova A. Age-independent rise of inflammatory scores may contribute to accelerated aging in multi-morbidity. *Oncotarget* 2015;6:1414-1421.
51. Leza JC, García-Bueno B, Bioque M, et al. Inflammation in schizophrenia: A question of balance. *Neurosci Biobehav Rev* 2015;55:612-626.
52. Fineberg AM, Ellman LM. Inflammatory cytokines and neurological and neurocognitive alterations in the course of schizophrenia. *Biol Psychiatry* 2013;73:951-966.
53. Fatemi SH, Folsom TD. The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophr Bull* 2009;35:528-548.
54. Rapoport JL, Giedd JN, Gogtay N. Neurodevelopmental model of schizophrenia: update 2012. *Mol Psychiatry* 2012;17:1228-1238.
55. Khandaker GM, Cousins L, Deakin J, Lennox BR, Yolken R, Jones PB. Inflammation and immunity in schizophrenia: implications for pathophysiology and treatment. *Lancet Psychiatry* 2015;2:258-270.
56. Perkins DO, Jeffries CD, Addington J, et al. Towards a psychosis risk blood diagnostic for persons experiencing high-risk symptoms: preliminary results from the NAPLS project. *Schizophr Bull* 2015;41:419-428.
57. Ribeiro-Santos A, Lucio Teixeira A, Salgado JV. Evidence for an immune role on cognition in schizophrenia: a systematic review. *Curr Neuropharmacol* 2014;12:273-280.

Table 1. Diagnostic Group Comparisons

Variables, Mean (SD)	**Healthy Controls** n = 60	**Individuals with Schizophrenia** n = 48	t test / Chi-square
Age (y)	36.40 (12.96)	35.12 (12.05)	t (106) = .524, *p* = .601
Gender (male/female)	36/24	33/15	X^2 = .546, df = 1, *p* = .459
Education (y)	11.05 (3.22)	10.31 (3.17)	t (106) = 1.19, *p* = .237
BMI	26.08 (4.40)	25.96 (5.11)	t (90) = .126, *p* = .900
Tobacco use (n/day)	1.68 (5.26)	10.46 (15.11)	t (102) = -4.073, *p* <.001 *
Age of onset (y)	-	22.45 (6.10)	-
Illness Duration (y)	-	12.74 (11.60)	-
BPRS	-	14.88 (12.56)	-
Medication (%)			
Typical Antipsychotics	-	15%	-
Atypical Antipsychotics	-	27%	-
Clozapine	-	58%	-
HVLT-R Total Immediate Recall	24.53 (4.53)	17.12 (5.61)	t (99) = 7.326, *p* < .001*
CCL11 levels	815.51 (592.77)	1531.18 (1251.87)	t (82) = -3.496, *p* < .001*
Telomere length	1.36 (0.65)	0.95 (0.41)	t (79) = 3.345, *p* = .001*
Total Gray Matter Residual Volume [a]	21688.50 (28445.65)	-19137.55 (43403.48)	t (101) = 5.75, *p* < .001*

* Statistically significant; [a] Intracranial volume was regressed out

BMI: Body Mass Index; BPRS: Brief Psychiatric Rating Scale; HVLT-R: Hopkins Verbal Learning Test – Revised

Figure 1.

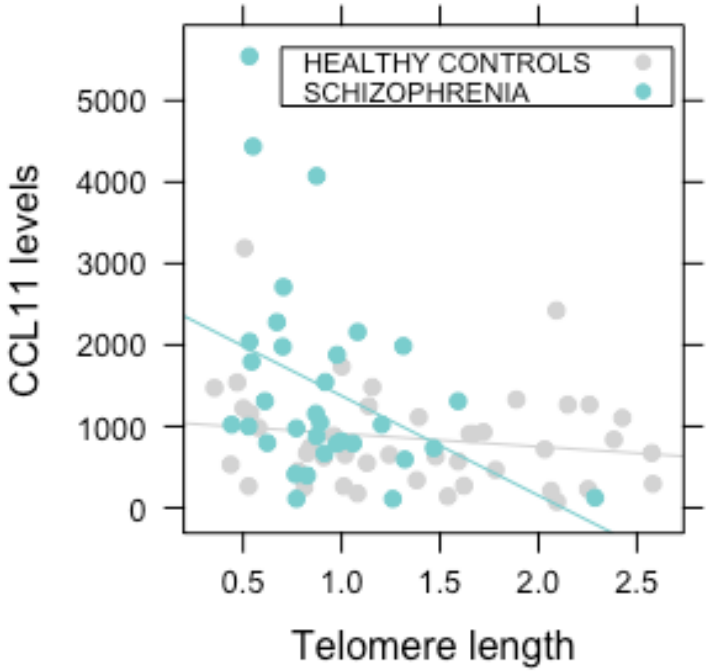

Figure 1. Correlation between CCL11 levels and telomere length in subjects with schizophrenia and healthy controls.

Figure 2.

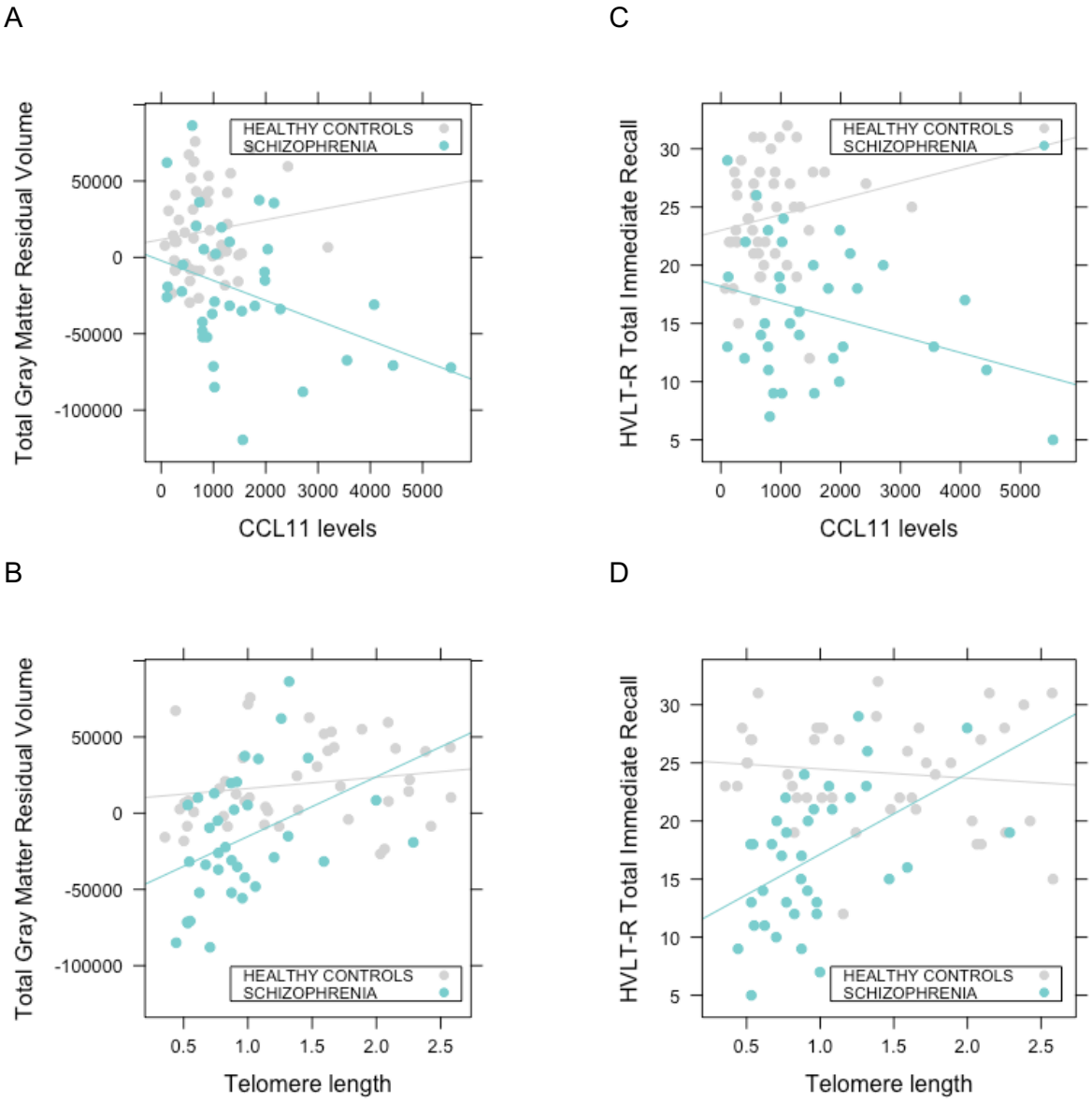

Figure 2. Correlations between CCL11 levels and telomere length to gray matter volume after regressing out intracranial volume (A and B) and to episodic memory performance (C and D) in subjects with schizophrenia and healthy controls.

Figure 3.

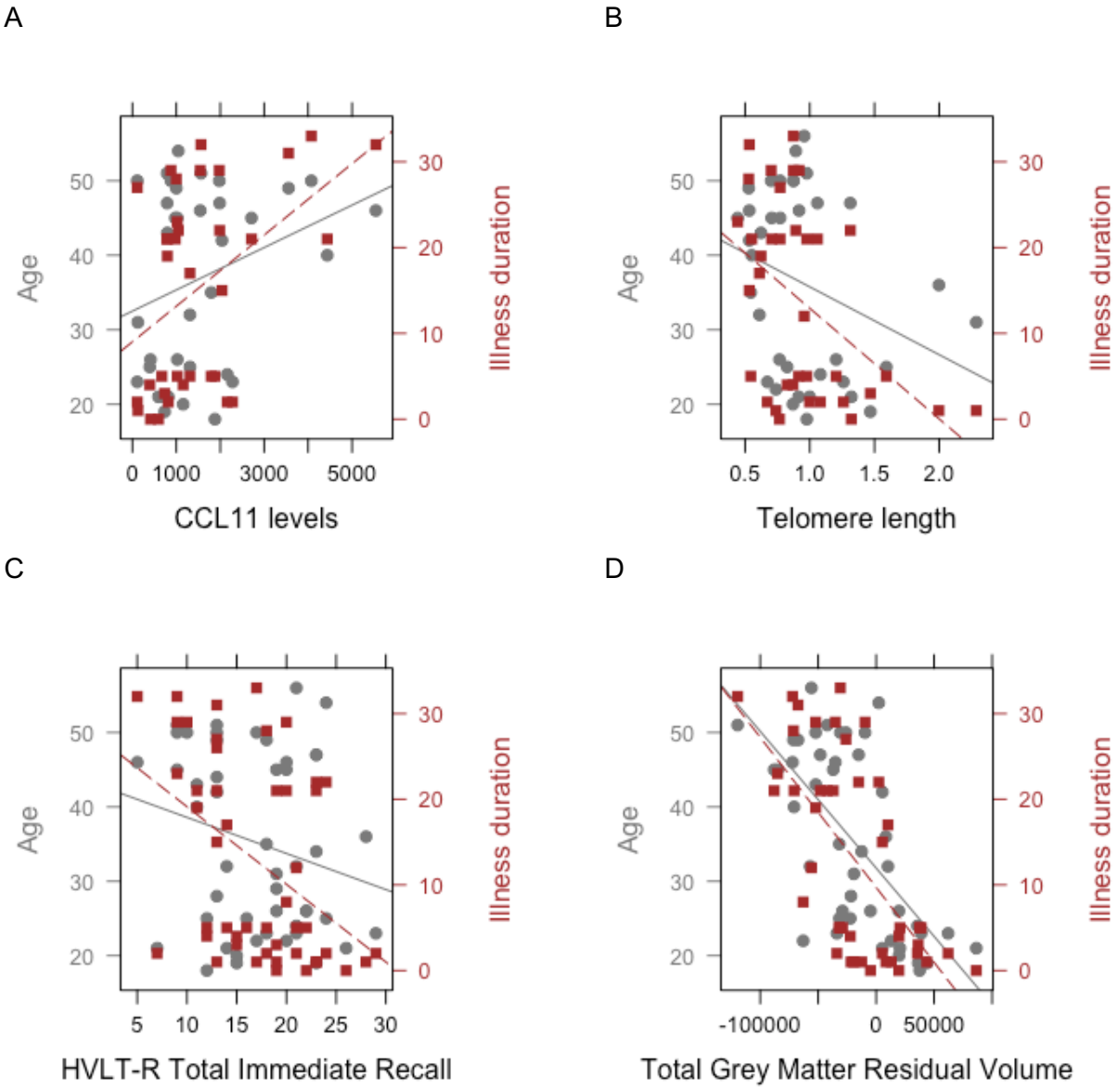

Figure 3. Correlations between age (left y-axis) and illness duration (right y-axis) to CCL11 levels (A), telomere length (B), episodic memory performance (C) and gray matter volume after regressing out intracranial volume (D) in subjects with schizophrenia.

Figure 4.

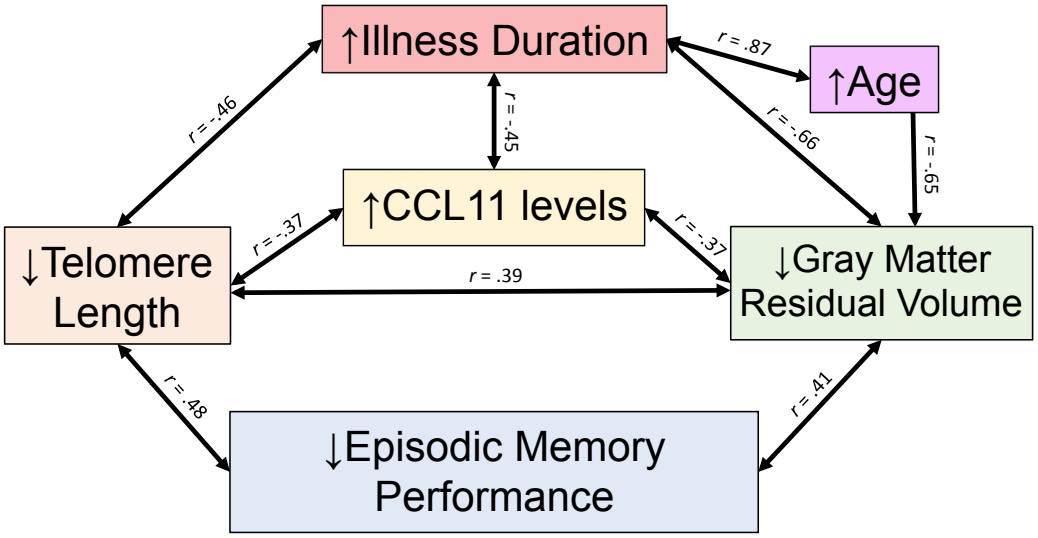

Figure 4. Possible mechanisms involved in a pathological accelerated aging of schizophrenia, based on the relationships observed in this study.

Supplementary Material

Eotaxin/CCL11 levels

5mL of peripheral blood were withdrawn by venipuncture into anticoagulant free tubes. After the blood clot, tubes were centrifuged in 4000 xg by 10 min to separate the serum. For every patient, aliquots of 200uL were done and then stored at -80C. At the day of experiment, one aliquot for each patient were thawed in ice then brought to room temperature, and excess of proteins was removed by acid/salt precipitation, as routinely performed in our laboratory. Briefly, an equal volume of serum and 1.2% trifluoracetic acid/1.35M NaCl were mixed and left at room temperature for 10 min. Samples were then centrifuged for 5 min at 3,000 g and the supernatants adjusted for salt content (0.14M sodium chloride and 0.01 M sodium phosphate) and pH (7.4) for the determination of chemokine levels. Samples were assayed in duplicate by a blinded investigator.

The measurement of CCL11 chemokine in serum was performed by sandwich ELISA according to the procedures supplied by the manufacturer (DuoSet, R &D Systems, Minneapolis, MN, USA). In brief, the capture antibody (concentration provided by the manufacturer) was diluted in phosphate-buffered saline (PBS), added to each well and left overnight at 4°C. The plate was washed four times in PBS with 0.05% Tween 20 (Sigma, St. Louis, MO, USA). The plate was blocked with 1% bovine serum albumin and incubated for 1h at room temperature before washing four times with PBS and 0.05% Tween 20. The samples and standards were added and the plate incubated overnight at 4°C. After washing the plate, detection antibody (concentration provided by the manufacturer) diluted in PBS was added. The plate was incubated for 2h at room temperature. After washing the plate, streptavidin (DuoSet R & D Systems, Minneapolis, MN, USA) was added and the plate incubated for 30 minutes. At last, color reagent o-phenylenediamine (Sigma, St. Louis, MO, USA) was added to each well and the reaction was allowed to develop in the dark for 15 min. The reaction was stopped with the addition of 1M H 2 SO 4 to each well. The absorbance was read on a plate reader at 492 nm wavelength (Emax, Molecular Devices, Minneapolis, MN, USA). All samples were assayed in duplicate. The detection limits for these assays were 5 pg/ml.

Telomere length (TL)

Measurement of relative TL was from whole peripheral venous blood, withdrawn in EDTA tubes and frozen at -80C until further analysis. The genomic DNA (gDNA) extraction was performed using a commercial kit (Illustra blood genomicPrep Mini Spin Kit, GE Healthcare) following manufacturer's instructions. Nucleic acid quantification and purity were checked spectrophotometrically (BioPhotometer Plus, Eppendorf, Hamburg, Germany) and samples were stored at -20oC for subsequent analysis. gDNA (25 ng/reaction) was used as template for quantification of relative mean telomere length by real time quantitative polymerase chain reaction (qPCR), with minor modifications from previously reported (Cawthon, 2002). In summary, for each sample two separate qPCR were performed in triplicate in separate 96-well plates in the same position. One reaction amplified the telomere (T) repeat sequence while the other amplified a single copy gene, 36B4 (S), which served as a quantitative control. For each participant, relative telomere length was expressed as the T/S ratio. Previously published primer sequences' (Cawthon, 2002) were (5'→3'): tel 1, GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT; tel 2, TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA and 36B4u, CAGCAAGTGGGAAGGTGTAATCC and 36B4d, CCCATTCTATCATCAACGGGTACAA. T and S master mix reactions were identical in a final volume of 20 μL with 0.1x SYBR® Green (Molecular Probes, CA, USA), 2 mM MgCl2, 0.1 mM each dNTP, 1% DMSO and 0.5 U of Platinum® Taq DNA Polymerase (Invitrogen). Final primer concentrations for telomere amplification were 270 and 1,125 nM for telomeres primers, respectively; and 300 and 500 nM for 36B4u and 36B4d primers. PCR reactions were performed in StepOnePlus™ Real-time PCR system (Applied Biosystems, CA, USA) and analyzed with StepOne™ Software v2.3. (Applied Biosystems). Thermal cycling profile for amplification consisted of an initial incubation step for 2 min at 94oC to activate hot start Platinum Taq DNA polymerase, followed by 22 cycles of denaturing at 94oC for 15 s and annealing and extension for 2 min at 54oC, for telomere amplification. For 36B4 amplification, amplification consisted of 30 cycles of denaturing at 94oC for 15 s followed by annealing and extension for 2 min at 60oC. The specificity of the amplification was confirmed at the end of each run using melting curve analysis. We additionally confirmed PCR products by agarose gel electrophoresis. In each run, a reference sample was included as a calibrator to normalize the participants' T/S ratio

and calculate the final T/S ratio. Finally, in order to check for PCR amplification efficiency, standard curves for telomere and 36B4 amplification were generated from the reference sample, over a 5-fold range by serial dilution from 100 to 0.16 ng of gDNA. Inter-plate variability was 2.7%.

8 CONSIDERAÇÕES FINAIS

Este trabalho teve como objetivo ampliar a compreensão dos mecanismos cognitivos, intelectuais, biológicos e de estrutura cerebral da esquizofrenia e, por conseguinte, dos transtornos mentais graves. A partir dos dados apresentados, os principais achados desta tese são:

#1: Indivíduos com esquizofrenia apresentaram um prejuízo cognitivo de memória precoce, diferentemente do que ocorre no transtorno bipolar, o que está de acordo com o que está descrito na literatura. Contudo, mesmo os pacientes mais graves mantiveram suas habilidades de aprendizagem, o que abre espaço para intervenções como a reabilitação cognitiva.

#2: Performances cognitiva e intelectual parecem influenciar de forma complementar os achados de estrutura cerebral em indivíduos com esquizofrenia. O funcionamento intelectual estava relacionado aos volumes globais de cérebro, como volume intracranial total e volume total do cérebro. Já o prejuízo cognitivo estava associado ao volume de massa cinzenta e espessura cortical. Os resultados sugerem que a esquizofrenia é simultaneamente uma doença do neurodesenvolvimento e neurodegenerativa. Durante o curso da doença haveriam dois *hits*, sendo o funcionamento intelectual o marcador de alteração do desenvolvimento e a cognição uma marca adicional ao longo da trajetória da doença. Diferentes mecanismos do neurodesenvolvimento e neurodegeneração levariam a diferentes padrões de funcionamento intelectual e performance cognitiva (heterogeneidade).

#3: Indivíduos com esquizofrenia apresentaram encurtamento de telômero quando comparados a controles, e não se observaram diferenças entre o tamanho de telômero de pacientes e seus irmãos não afetados pela doença. Isso sugere que o envelhecimento patológico precoce pode ser um mecanismo associado ao curso da esquizofrenia.

#4: As evidências de encurtamento de telômero estavam associados a níveis elevados de CCL11, um marcador pró-inflamatório também relacionado ao envelhecimento. Além disso, ambas medidas de comprimento de telômero e nível de CCL11 estavam relacionados à performance de memória e volume de massa cinzenta em indivíduos com esquizofrenia, diferentemente do encontrado em controles saudáveis. Interessantemente, essas correlações estavam mais

associadas ao tempo de doença que a idade dos pacientes, o que sugere que seria a carga da doença, e não a idade em si que levaria a um envelhecimento patológico precoce.

Portanto, podemos sugerir que a esquizofrenia é uma doença do neurodesenvolvimento que traz prejuízos precoces. Contudo, parece também existir uma carga adicional ao longo do curso da doença que leva a um aceleramento do processo de envelhecimento dos pacientes. A heterogeneidade amplamente discutida na literatura pode ser explicada pelas diferentes possíveis influências que os indivíduos podem sofrer ao longo de seu desenvolvimento e envelhecimento. Essas diferenças levam a diferentes trajetórias de curso da doença.

Os estudos aqui apresentados procuraram contribuir para o melhor entendimento dos transtornos mentais graves, incluindo variáveis biológicas e cognitivas no estudo dos mecanismos fisiopatológicos destes transtornos. A partir dos achados em esquizofrenia, pode-se ampliar a compreensão de alterações percebidas nas trajetórias de outras psicopatologias, como depressão maior crônica e transtorno bipolar. Com adequado entendimento desses mecanismos, será possível o desenvolvimento de novos tratamentos e intervenções mais efetivas e eficazes.

REFERÊNCIAS

Baylis D, Ntani G, Edwards MH, Syddall HE, Bartlett DB, Dennison EM, Martin-Ruiz C, von Zglinicki T, Kuh D, Lord JM, Aihie Sayer A, Cooper C. Inflammation, telomere length, and grip strength: a 10-year longitudinal study. Calcif Tissue Int 2014 Jul; 95(1):54-63.

Birkenaes AB, Søgaard AJ, Engh JA, Jonsdottir H, Ringen PA, Vaskinn A, Friis S, Sundet K, Opjordsmoen S, Andreassen OA. Sociodemographic characteristics and cardiovascular risk factors in patients with severe mental disorders compared with the general population. J Clin Psychiatry 2006 Mar; 67(3):425-33.

Bloomfield MA, Buck SC, Howes OD. Schizophrenia: inorganic no more. Lancet Psychiatry 2016 Jul; 3(7):600-2.

Boonekamp JJ, Simons MJ, Hemerik L, Verhulst S. Telomere length behaves as biomarker of somatic redundancy rather than biological age. Aging Cell 2013 Apr; 12(2): 330-2.

Bora E. Neurodevelopmental origin of cognitive impairment in schizophrenia. Psychol Med 2015 Jan; 45(1):1-9.

Carbon M, Correll CU. Thinking and acting beyond the positive: the role of the cognitive and negative symptoms in schizophrenia. CNS Spectr 2014 Dec; 19 Suppl 1:38-52.

Chang CK, Hayes RD, Perera G, Broadbent MT, Fernandes AC, Lee WE, Hotopf M, Stewart R. Life expectancy at birth for people with serious mental illness and other major disorders from a secondary mental health care case register in London. PLoS One 2011; 6(5):e19590.

Citrome L. Unmet needs in the treatment of schizophrenia: new targets to help different symptom domains. J Clin Psychiatry 2014; 75 Suppl 1:21-6.

Craddock N, Owen MJ. The Kraepelinian dichotomy - going, going... but still not gone. Br J Psychiatry 2010 Feb; 196(2):92-5.

Davis J, Eyre H, Jacka FN, Dodd S, Dean O, McEwen S, Debnath M, McGrath J, Maes M, Amminger P, McGorry PD, Pantelis C, Berk M. A review of vulnerability and risks for schizophrenia: Beyond the two hit hypothesis. Neurosci Biobehav Rev 2016 Jun; 65:185-94.

Demyttenaere K, Bruffaerts R, Posada-Villa J, Gasquet I, Kovess V, Lepine JP, Angermeyer MC, Bernert S, de Girolamo G, Morosini P, Polidori G, Kikkawa T, Kawakami N, Ono Y, Takeshima T, Uda H, Karam EG, Fayyad JA, Karam AN, Mneimneh ZN, Medina-Mora ME, Borges G, Lara C, de Graaf R, et al. Prevalence, severity, and unmet need for treatment of mental disorders in the World Health Organization World Mental Health Surveys. JAMA 2004 Jun 2; 291(21):2581-90.

Dickerson F, Origoni A, Schroeder J, Schweinfurth LA, Stallings C, Savage CL, Katsafanas E, Banis M, Khushalani S, Yolken R. Mortality in schizophrenia and bipolar disorder: Clinical and serological predictors. Schizophr Res 2016 Jan; 170(1):177-83.

Dieset I, Andreassen OA, Haukvik UK. Somatic Comorbidity in Schizophrenia: Some Possible Biological Mechanisms Across the Life Span. Schizophr Bull 2016 Nov; 42(6):1316-1319.

Fatemi SH, Folsom TD. The neurodevelopmental hypothesis of schizophrenia, revisited. Schizophr Bull 2009 May; 35(3):528-48.

Fatouros-Bergman H, Cervenka S, Flyckt L, Edman G, Farde L. Meta-analysis of cognitive performance in drug-naïve patients with schizophrenia. Schizophr Res 2014 Sep; 158(1-3):156-62.

Fioravanti M, Bianchi V, Cinti ME. Cognitive deficits in schizophrenia: na updated metanalysis of the scientific evidence. BMC Psychiatry 2012 Jun 20; 12:64.

Flatow J, Buckley P, Miller BJ. Meta-analysis of oxidative stress in schizophrenia. Biol Psychiatry 2013 Sep 15; 74(6):400-9.

Fleischhacker WW, Cetkovich-Bakmas M, De Hert M, Hennekens CH, Lambert M, Leucht S, Maj M, McIntyre RS, Naber D, Newcomer JW, Olfson M, Osby U, Sartorius N, Lieberman JA. Comorbid somatic illnesses in patients with severe mental disorders: clinical, policy, and research challenges. J Clin Psychiatry 2008 Apr; 69(4):514-9.

Goff DC, Romero K, Paul J, Mercedes Perez-Rodriguez M, Crandall D, Potkin SG. Biomarkers for drug development in early psychosis: Current issues and promising directions. Eur Neuropsychopharmacol 2016 Jun; 26(6):923-37.

Green MF. What are the functional consequences of neurocognitive deficits in schizophrenia? Am J Psychiatry 1996 Mar; 153(3):321-30.

Green MF, Kern RS, Braff DL, Mintz J. Neurocognitive deficits and functional outcome in schizophrenia: are we measuring the "right stuff"? Schizophr Bull 2000; 26(1):119-36.

Haijma SV, Van Haren N, Cahn W, Koolschijn PC, Hulshoff Pol HE, Kahn RS. Brain volumes in schizophrenia: a meta-analysis in over 18 000 subjects. Schizophr Bull 2013 Sep; 39(5):1129-38.

Harvey PD, Green MF, Keefe RS, Velligan DI. Cognitive functioning in schizophrenia: a consensus statement on its role in the definition and evaluation of effective treatments for the illness. J Clin Psychiatry 2004 Mar; 65(3):361-72.

Harvey PD, Velligan DI, Bellack AS. Performance-based measures of functional skills: usefulness in clinical treatment studies. Schizophr Bull 2007 Sep; 33(5):1138-48.

Harvey PD, McClure MM, Patterson TL, McGrath JA, Pulver AE, Bowie CR, Siever LJ. Impairment in functional capacity as an endophenotype candidate in severe mental illness. Schizophr Bull 2012 Nov; 38(6):1318-26.

Harvey PD, Strassnig M. Predicting the severity of everyday functional disability in people with schizophrenia: cognitive deficits, functional capacity, symptoms, and health status. World Psychiatry 2012 Jun; 11(2):73-9.

Hayes RD, Chang CK, Fernandes A, Begum A, To D, Broadbent M, Hotopf M, Stewart R. Associations between symptoms and all-cause mortality in individuals with serious mental illness. J Psychosom Res 2012 Feb; 72(2):114-9. (a)

Hayes RD, Chang CK, Fernandes AC, Begum A, To D, Broadbent M, Hotopf M, Stewart R. Functional status and all-cause mortality in serious mental illness. PLoS One 2012; 7(9):e44613. (b)

Hughes E, Bassi S, Gilbody S, Bland M, Martin F. Prevalence of HIV, hepatitis B, and hepatitis C in people with severe mental illness: a systematic review and meta-analysis. Lancet Psychiatry 2016 Jan; 3(1):40-8.

Kambeitz J, Kambeitz-Ilankovic L, Cabral C, Dwyer DB, Calhoun VD, van den Heuvel MP, Falkai P, Koutsouleris N, Malchow B. Aberrant Functional Whole-Brain Network Architecture in Patients With Schizophrenia: A Meta-analysis. Schizophr Bull 2016 Jul; 42 Suppl 1:S13-21.

Kendler KS, Ohlsson H, Mezuk B, Sundquist JO, Sundquist K. Observed Cognitive Performance and Deviation From Familial Cognitive Aptitude at Age 16 Years and Ages 18 to 20 Years and Risk for Schizophrenia and Bipolar Illness in a Swedish National Sample. JAMA Psychiatry 2016 May 1; 73(5):465-71.

Kessler RC, Aguilar-Gaxiola S, Alonso J, Chatterji S, Lee S, Ormel J, Ustün TB, Wang PS. The global burden of mental disorders: an update from the WHO World Mental Health (WMH) surveys. Epidemiol Psichiatr Soc 2009 Jan-Mar; 18(1):23-33.

Khandaker GM, Barnett JH, White IR, Jones PB. A quantitative meta-analysis of population-based studies of premorbid intelligence and schizophrenia. Schizophr Res 2011 Nov; 132(2-3):220-7.

Kirkpatrick B, Messias E, Harvey PD, Fernandez-Egea E, Bowie CR. Is schizophrenia a syndrome of accelerated aging? Schizophr Bull 2008 Nov; 34(6):1024-32.

Kreyenbuhl J, Buchanan RW, Dickerson FB, Dixon LB; Schizophrenia Patient Outcomes Research Team (PORT). The Schizophrenia Patient Outcomes Research Team (PORT): updated treatment recommendations 2009. Schizophr Bull 2010 Jan; 36(1):94-103.

Kuswanto C, Chin R, Sum MY, Sengupta S, Fagiolini A, McIntyre RS, Vieta E, Sim K. Shared and divergent neurocognitive impairments in adult patients with schizophrenia and bipolar disorder: Whither the evidence? Neurosci Biobehav Rev 2016 Feb; 61:66-89.

Laursen TM, Munk-Olsen T, Gasse C. Chronic somatic comorbidity and excesso mortality due to natural causes in persons with schizophrenia or bipolar affective disorder. PLoS One 2011; 6(9):e24597.

Laursen TM, Nordentoft M, Mortensen PB. Excess early mortality in schizophrenia. Annu Rev Clin Psychol 2014; 10:425-48.

Leucht S, Burkard T, Henderson J, Maj M, Sartorius N. Physical illness and schizophrenia: a review of the literature. Acta Psychiatr Scand 2007 Nov; 116(5):317-33.

Levinson D, Lakoma MD, Petukhova M, Schoenbaum M, Zaslavsky AM, Angermeyer M, Borges G, Bruffaerts R, de Girolamo G, de Graaf R, Gureje O, Haro JM, Hu C, Karam AN, Kawakami N, Lee S, Lepine JP, Browne MO, Okoliyski M, Posada-Villa J, Sagar R, Viana MC, Williams DR, Kessler RC. Associations of serious mental illness

with earnings: results from the WHO World Mental Health surveys. Br J Psychiatry 2010 Aug; 197(2):114-21.

Lewis DA, Levitt P. Schizophrenia as a disorder of neurodevelopment. Annu Rev Neurosci 2002; 25:409-32.

Lindqvist D, Epel ES, Mellon SH, Penninx BW, Révész D, Verhoeven JE, Reus VI, Lin J, Mahan L, Hough CM, Rosser R, Bersani FS, Blackburn EH, Wolkowitz OM. Psychiatric disorders and leukocyte telomere length: Underlying mechanisms linking mental illness with cellular aging. Neurosci Biobehav Rev 2015 Aug; 55:333-64.

Lumme S, Pirkola S, Manderbacka K, Keskimäki I. Excess Mortality in Patients with Severe Mental Disorders in 1996-2010 in Finland. PLoS One 2016 Mar 24; 11(3):e0152223.

McGinty EE, Baller J, Azrin ST, Juliano-Bult D, Daumit GL. Interventions to Address Medical Conditions and Health-Risk Behaviors Among Persons With Serious Mental Illness: A Comprehensive Review. Schizophr Bull 2016 Jan; 42(1):96-124.

Millan MJ, Andrieux A, Bartzokis G, Cadenhead K, Dazzan P, Fusar-Poli P, Gallinat J, Giedd J, Grayson DR, Heinrichs M, Kahn R, Krebs MO, Leboyer M, Lewis D, Marin O, Marin P, Meyer-Lindenberg A, McGorry P, McGuire P, Owen MJ, Patterson P, Sawa A, Spedding M, Uhlhaas P, et al. Altering the course of schizophrenia: progress and perspectives. Nat Rev Drug Discov. 2016 Jul; 15(7):485-515.

Miller BJ, Buckley P, Seabolt W, Mellor A, Kirkpatrick B. Meta-analysis of cytokine alterations in schizophrenia: clinical status and antipsychotic effects. Biol Psychiatry 2011 Oct 1; 70(7):663-71.

Müller N, Weidinger E, Leitner B, Schwarz MJ. The role of inflammation in schizophrenia. Front Neurosci 2015 Oct 21; 9:372.

Nordentoft M, Wahlbeck K, Hällgren J, Westman J, Osby U, Alinaghizadeh H, Gissler M, Laursen TM. Excess mortality, causes of death and life expectancy in 270,770

patients with recent onset of mental disorders in Denmark, Finland and Sweden. PLoS One 2013; 8(1):e55176.

Okada N, Fukunaga M, Yamashita F, Koshiyama D, Yamamori H, Ohi K, Yasuda Y, Fujimoto M, Watanabe Y, Yahata N, Nemoto K, Hibar DP, van Erp TG, Fujino H, Isobe M, Isomura S, Natsubori T, Narita H, Hashimoto N, Miyata J, Koike S, Takahashi T, Yamasue H, Matsuo K, et al. Abnormal asymmetries in subcortical brain volume in schizophrenia. Mol Psychiatry 2016 Oct; 21(10):1460-6.

Owen MJ, Sawa A, Mortensen PB. Schizophrenia. Lancet 2016 Jul 2; 388(10039):86-97.

Pearlson GD. Etiologic, phenomenologic, and endophenotypic overlap of schizophrenia and bipolar disorder. Annu Rev Clin Psychol 2015; 11:251-81.

Polho GB, De-Paula VJ, Cardillo G, dos Santos B, Kerr DS. Leukocyte telomere length in patients with schizophrenia: A meta-analysis. Schizophr Res 2015 Jul; 165(2-3):195-200.

Rapoport JL, Giedd JN, Gogtay N. Neurodevelopmental model of schizophrenia: update 2012. Mol Psychiatry 2012 Dec; 17(12):1228-38.

Razzano LA, Cook JA, Yost C, Jonikas JA, Swarbrick MA, Carter TM, Santos A. Factors associated with co-occurring medical conditions among adults with serious mental disorders. Schizophr Res 2015 Feb; 161(2-3):458-64.

Reynolds CF 3rd. Telomere Attrition: A Window Into Common Mental Disorders and Cellular Aging. Am J Psychiatry 2016 Jun 1; 173(6):556-8.

Schnack HG, van Haren NE, Nieuwenhuis M, Hulshoff Pol HE, Cahn W, Kahn RS. Accelerated Brain Aging in Schizophrenia: A Longitudinal Pattern Recognition Study. Am J Psychiatry 2016 Jun 1; 173(6):607-16.

Shmukler AB, Gurovich IY, Agius M, Zaytseva Y. Long-term trajectories of cognitive deficits in schizophrenia: A critical overview. Eur Psychiatry 2015 Nov; 30(8):1002-10.

Siskind D, McCartney L, Goldschlager R, Kisely S. Clozapine v. first- and second-generation antipsychotics in treatment-refractory schizophrenia: systematic review and meta-analysis. Br J Psychiatry 2016 Jul 7. [Epub ahead of print]

Sommer IE, Carpenter WT. Schizophrenia: changing the name and broadening the concept is problematic. BMJ 2016 Feb 23; 352:i1080.

Sørensen HJ, Nielsen PR, Benros ME, Pedersen CB, Mortensen PB. Somatic diseases and conditions before the first diagnosis of schizophrenia: a Nationwide population-based cohort study in more than 900 000 individuals. Schizophr Bull 2015 Mar; 41(2):513-21

Ventura J, Hellemann GS, Thames AD, Koellner V, Nuechterlein KH. Symptoms as mediators of the relationship between neurocognition and functional outcome in schizophrenia: a meta-analysis. Schizophr Res 2009 Sep; 113(2-3):189-99.

von Zglinicki T. Oxidative stress shortens telomeres. Trends Biochem Sci 2002 Jul; 27(7):339-44.

Walker ER, McGee RE, Druss BG. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. JAMA Psychiatry 2015 Apr; 72(4):334-41. Review. Erratum in: JAMA Psychiatry 2015 Jul; 72(7):736. JAMA Psychiatry 2015 Dec; 72(12):1259.

Whiteford HA, Degenhardt L, Rehm J, Baxter AJ, Ferrari AJ, Erskine HE, Charlson FJ, Norman RE, Flaxman AD, Johns N, Burstein R, Murray CJ, Vos T. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. Lancet 2013 Nov 9; 382(9904):1575-86.

Woodberry KA, Giuliano AJ, Seidman LJ. Premorbid IQ in schizophrenia: a meta-analytic review. Am J Psychiatry 2008 May; 165(5):579-87.

Woodward ND. The course of neuropsychological impairment and brain structure abnormalities in psychotic disorders. Neurosci Res 2016 Jan; 102:39-46.

Zink M, Englisch S. Schizophrenia treatment: an obstacle course. Lancet Psychiatry 2016 Apr; 3(4):310-2.

Zipursky RB. Why are the outcomes in patients with schizophrenia so poor? J Clin Psychiatry 2014; 75 Suppl 2:20-4.