UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

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ANÁLISE DE SNPs DE COMPONENTES DO SISTEMA CRFÉRGICO EM TRANSTORNOS PSIQUIÁTRICOS RELACIONADAS AO ESTRESSE: UMA REVISÃO DOS ÚLTIMOS CINCO ANOS

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Trabalho de conclusão de curso de graduação apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Bacharela em Biomedicina.

Orientador: Dr. Mailton Vasconcelos

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Mailton Vasconcelos - UFRGS (orientador)

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RESUMO

A manifestação de transtornos mentais associados ao estresse (e.g. transtorno por uso de substâncias, depressão maior, transtorno bipolar, transtornos de ansiedade e transtorno de estresse pós-traumático) estão relacionados à atividade neuronal do sistema de sinalização do *corticotropin-releasing hormone* (CRH, ou *corticotropin-releasing factor* (CRF)) inapropriada. Este sistema de sinalização é composto pelo hormônio/peptídeo CRH, proteínas de estrutura assemelhada ao CRH (urocortinas), seus receptores (CRHR1 e CRHR2), e a proteína de ligação ao CRH - CRHBP (CRH *binding protein*). A função do CRH é amplamente conhecida por modular a resposta ao estresse, tendo função exercida majoritariamente no eixo hipotálamo-pituitária-adrenal (HPA) e, por este motivo, seus genes são investigados com o objetivo de responder se a variabilidade genética é capaz de conferir vulnerabilidade a psicopatologias. A presente revisão sistemática buscou nos estudos epidemiológicos observacionais publicados nos últimos cinco anos quais os principais polimorfismos dos genes do sistema CRH que se associam a fatores ambientais estressores e ao risco para o desenvolvimento de transtornos relacionados ao estresse. A qualidade dos estudos incluídos nesta revisão e quais as principais variáveis de confusão presentes na condução das pesquisas foram avaliadas. Os resultados mostraram que o gene CRHR1 é o maior alvo de investigações devido ao SNP rs110402 ter sido associado a diversos transtornos clínicos em estudos anteriores. A avaliação de qualidades dessas evidências detectou que a ausência de verificação de etnia, sexo e do uso de substância foram os fatores de confusão presentes com maior frequência, e que a delimitação excessiva da região genética de análise contribui para a geração de associações ao acaso. Tais fatores aumentam as chances de resultados falsos e inconsistentes dentro da genética psiquiátrica.

Palavras-chaves: Estresse; SNP; CRH; CRF; Psiquiatria; Genética.

ABSTRACT

The manifestation of mental disorders associated with stress (eg substance use disorder, major depression, bipolar disorder, anxiety disorders and post-traumatic stress disorder) are related to the inappropriate neuronal activity of the corticotropin-releasing hormone (CRH) or corticotropin-releasing factor (CRF). This signaling system is composed of the hormone / peptide CRH, proteins structurally related to CRH (urocortins), its receptors (CRHR1 and CRHR2), and the CRH binding protein (CRHBP) . The function of CRH is widely known for modulating the stress response, having a role exerted mainly on the hypothalamus-pituitary-adrenal axis (HPA) and, for this reason, its genes are investigated with the objective of answering whether genetic variability is capable of confer vulnerability to psychopathologies. This systematic review sought in the observational epidemiological studies published in the last five years which are the main polymorphisms of the CRH system genes that are associated with environmental stressors and the risk for the development of stress-related disorders. The quality of the studies included in this review and which were the main confounding variables present in conducting the research were assessed. The results showed that the *CRHR1* gene is the main target of investigation due to the SNP rs110402 being associated with several clinical disorders in previous studies. The evaluation of the qualities of this evidence found that the absence of verification of ethnicity, sex and substance use were the most frequent confounding factors, and that the excessive delimitation of the genetic region of analysis contributes to the generation of random associations. Such factors increase the chances of false and inconsistent results within psychiatric genetics.

Keywords: Stress; SNP; CRH; CRF; Psychiatry; Genetics.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

ACTH   Hormônio Adrenocorticotrófico, do inglês *adrenocorticotropic hormone*

Eixo HPA   Eixo Hipotálamo-pituitária-adrenal

CRH   Hormônio liberador de corticotrofina, do inglês *Corticotropin Releasing Hormone*

CRHR1   Receptor 1 do hormônio liberador de corticotrofina, do inglês *Corticotropin Releasing Hormone Receptor 1*

CRHR2   Receptor 2 do hormônio liberador de corticotrofina, do inglês *Corticotropin Releasing Hormone Receptor 2*

CRHBP   Proteína de Ligação do Hormônio Liberador da Corticotropina, do inglês *Corticotropin Releasing Hormone Binding Protein*

GWAS   Estudo de Associação Genômica Ampla, do inglês *Genome Wide Association Study*

PVN   Núcleo Paraventricular do Hipotálamo, do inglês *Paraventricular Nucleus*

SNP   Polimorfismo de Nucleotídeo Ùnico, do inglês *Single Nucleotide Polymorphism*

UCN   Urocortina, do inglês *Urocortin*

SUMÁRIO

1. Introdução

1.1 Aplicações dos métodos moleculares à genética psiquiátrica

Grande parte das características de aspecto mendeliano possuem a etiologia genética esclarecida - no ano de 2009, de acordo com a base de dados disponível em *Online Mendelian Inheritance in Man* - OMIM), já se sabia a base molecular de 2517 fenótipos mendelianos enquanto 1741 fenótipos possuíam a base genética ainda desconhecida. Ao comparar as descobertas entre as origens dos fenótipos mendelianos e complexos, percebe-se que o segundo, embora ocorra mais comumente na população, não acompanha o progresso na mesma velocidade por envolver interações complexas (i.e. etiologia multifatorial) (AVRAMOPOULOS, 2010). Este é o caso dos transtornos psiquiátricos que (1) apresentam diferentes níveis de severidade de sintomas, ou seja, não possuem um fenótipo padrão, (2) podem ter fatores etiológicos distintos e, (3) ao contrário de doenças monogênicas, são originados devido à contribuição genética de diversos alelos que exercem pequeno efeito na característica (TABOR; RISCH; MYERS, 2002).

A genética psiquiátrica surgiu a partir da observação da ocorrência recorrente de casos de bipolaridade e esquizofrenia dentro de uma mesma família, e foi através desse padrão de herdabilidade dos fenótipos que a etiologia genética dos transtornos passou a ser questionada. Embora inicialmente os estudos dentro da área envolvendo indivíduos geneticamente relacionados pudessem parecer promissores, durante muito tempo as tentativas de replicação não foram bem-sucedidas. Atualmente, com a contribuição do Projeto Internacional HapMap (*International HapMap Project*), informações em relação à variabilidade genética da população são fornecidas através da identificação de SNPs (polimorfismos de nucleotídeo único, *single-nucleotide polymorphism*) ao longo do genoma, permitindo que estudos de associação genética usem esses dados para comparar grande número de indivíduos afetados e não-afetados (i. e. estudos caso-controle) (COLLINS et al., 2011).

Na investigação da etiologia genética das doenças, diferentes tipos de métodos de associação são utilizados, sendo empregados estudos de todo o genoma (i.e. *Genome-Wide Association Studies* - GWAS) ou estudos de genes candidatos. A escolha de qual método aplicar é feita de acordo com diversos fatores, dentre eles encontram-se o fenótipo que se pretende estudar, a quantidade de genes considerada necessária para o seu aparecimento, o tamanho de efeito de cada gene na população (i.e. penetrância), se se trata de uma doença mendeliana, se é comum na população ou ainda se depende de influências ambientais. Essas

questões informam se a doença é classificada como complexa ou não, e guiam a maneira como as informações genômicas devem ser manuseadas para detectar regiões que comunicam risco.

Os Estudos de Ligação (*linkage studies,* do inglês) utilizam marcadores genéticos como ferramenta de rastreamento, e geralmente são bem-sucedidos quando aplicados em doenças familiares monogênicas e de alta penetrância (i.e. quando a expressão do gene determina fortemente o fenótipo). A observação do padrão de herdabilidade e detecção dos segmentos de DNA que são segregados juntos (i.e. não sofrem recombinação) e, portanto, não obedecem à segunda lei de Mendel, permitem a localização aproximada do bloco genético - frequentemente cobrindo muitas megabases (FARBER et al., 2009) - que carregam a informação molecular relacionada ao surgimento da patologia. Uma vantagem do método é que os polimorfismos detectados são mais propensos a possuírem variantes causais (HIRSCHHORN; DALY, 2005).

O delineamento também pode ser feito comparando o genoma entre portadores da doença e indivíduos saudáveis dentro de uma população e que não são da mesma família. No caso dos estudos GWAS, são detectadas variáveis dentro do genoma (mais comumente SNPs) que possam estar ligados à característica investigada. Neste tipo de estudo observa-se quais variantes (i.e. alelos) são mais frequentes no grupo que carrega a doença. Como se trata de um estudo de ampla associação, ele fornece dados que informam o perfil genético compartilhado entre os afetados, mas não busca responder quais genes contidos no perfil são causais (MANOLIO, 2010). A abordagem de genes candidatos direciona a busca de acordo com o conhecimento prévio do impacto da função do gene nas vias biológicas envolvidas na doença, pré-especificando as variantes analisadas e observando se elas se correlacionam estatisticamente com a presença da patologia quando comparadas com as variantes de indivíduos saudáveis (i.e. estudo caso-controle). Se a frequência alélica de determinado SNP for predominante no grupo dos afetados, associa-se a variação genética como conferidora de risco para a doença.

Essas três metodologias foram utilizadas no campo da genética psiquiátrica em busca da compreensão do padrão de herança e do impacto das variantes genéticas no desenvolvimento de transtornos. Os Estudos de Ligação são eficazes na detecção de poucas variantes que carregam grande efeito e que são responsáveis por desencadear patologias que acometem múltiplos membros de uma família, como os distúrbios monogênicos. Estas particularidades são opostas ao modo de apresentação dos transtornos psiquiátricos, por possuírem grande variedade de loci de pequeno efeito que contribuem para suscetibilidade e

devido ao poder reduzido de detecção da transmissão genética influenciada pela quantidade insuficiente de familiares afetados (MICHELON; VALLADA, 2005; HODGSON; MCGUFFIN, 2012). Esses motivos exemplificam as razões pelas quais os resultados obtidos em Estudos de Ligação envolvendo Transtorno Bipolar e Depressão Maior foram inconsistentes e de difícil replicação.

Os estudos GWAS, ao contrário, associam a patologia com SNPs ao longo de todo o genoma em indivíduos sem relação consanguínea, suprindo as informações necessárias para a investigação dos transtornos psiquiátricos ao identificar variantes comuns (i.e. presentes em menos de 5% da população), que ao contrário do que é detectado pelos Estudos de Ligação, quando individuais, conferem baixo risco (MANOLIO et al., 2008). A maior limitação dessa aplicação está em não buscar relacionar as associações encontradas com os possíveis mecanismos que estejam envolvidos na apresentação do fenótipo. Já a abordagem de gene candidato é conduzida de acordo com a pressuposição da atuação do gene no transtorno por possuir relevância funcional na via biológica afetada. Embora a metodologia de gene candidato seja mais direcionada que os estudos GWAS, ao limitar a hipótese inicial, gera-se um impeditivo na descoberta de genes antes não suspeitados, o que é essencial para impulsionar o surgimento de novas hipóteses a respeito de genes envolvidos em mecanismos biológicos que não foram totalmente esclarecidos, como é o caso da Depressão (HODGSON; MCGUFFIN, 2012).

1.2   Multifatoriedade dos transtornos relacionados ao estresse

A Organização Mundial da Saúde (OMS, 2016) alerta que influências ambientais, tais como traumas infantis, contribuem para o desenvolvimento de depressão, uso indevido de drogas e comportamentos sexuais de alto risco. A associação feita entre pacientes depressivos que passaram por traumas durante a infância e a atividade neuroendócrina relacionada ao estresse revelou a ausência da supressão dos níveis de hormônio adrenocorticotrófico (*Adrenocorticotropic hormone,* ACTH) e cortisol no testes de supressão de hormônio liberador de corticotrofina (*corticotropin-releasing hormone,* CRH) por dexametasona (GILLESPIE et al., 2019; LU et al., 2016). Este teste é uma forma de avaliar a funcionalidade do eixo hipotálamo-pituitária-adrenal (HPA) através de sua capacidade de resposta de *feedback* negativo. A alteração neuroendócrina do eixo HPA também está relacionada a níveis discrepantes de cortisol observados em indivíduos que passaram por situações traumáticas (e.g. diferentes tipos de abusos) durante a infância, apresentando comportamento suicida, transtornos de humor, transtornos de ansiedade e uso de substâncias (PIRNIA et al., 2020;

KELLNER et al., 2018; WIELAARD et al., 2018; GERRA et al., 2016; AMBRUS; WESTLING, 2019). Estes achados contribuem para a noção de que o desenvolvimento de fenótipos de transtornos psiquiátricos pode ser influenciado por fatores extrínsecos associados à disfunção do eixo HPA.

Inúmeras patologias podem se desenvolver a partir de fatores ambientais e/ou genéticos. A determinação desses fatores pode se dar com o auxílio de estudos comparando gêmeos monozigóticos e dizigóticos (i.e. compartilhamento total e compartilhamento de metade do código genético, respectivamente). A comparação do genoma compartilhado desses indivíduos e suas características adquiridas ajuda a estabelecer se o fenótipo é conferido devido à herdabilidade ou a fatores ambientais. No caso das características comportamentais, por exemplo, as diferenças observadas entre gêmeos monozigóticos são atribuídas a fatores ambientais, enquanto que gêmeos dizigóticos têm diferenças explicadas tanto por efeitos genéticos quanto por efeitos ambientais (STRELAU, 1998). No entanto, atualmente sabe-se que muitas condições não são desenvolvidas devido a uma única etiologia, como no caso das doenças complexas (FEERO; GUTTMACHER; COLLINS, 2010). As psicopatologias são exemplos dessas doenças, de forma que não se apresentam em todos os indivíduos que vivenciaram grandes adversidades, justamente porque além de fatores psicológicos e socioculturais, a variabilidade do perfil genético (i. e. polimorfismos genéticos) parecem se relacionar para estabelecer resiliência ou vulnerabilidade (RUTTER, 2013).

1.3 Sistema CRH na modulação ao estresse

Ao investigar fatores de risco genético em transtornos associados ao estresse, os genes candidatos do sistema CRH são uma escolha recorrente nas análises de associação, visto que suas expressões modulam a resposta ao estresse no eixo HPA e esta se associa ao desenvolvimento de tais transtornos. Para elucidar os mecanismos neuroendócrinos brevemente, pode se dizer que em uma dada situação estressante, os neurônios do núcleo paraventricular (PVN) presentes no hipotálamo secretam CRH. O CRH liberado pelo hipotálamo se liga aos receptores 1 e 2 do hormônio liberador de corticotropina (CRHR1 e CRHR2) na hipófise, desencadeando diferentes efeitos. A ativação do CRHR1 gera a secreção de ACTH pela adeno-hipófise, que através da circulação sistêmica estimula a produção de glicocorticoides (e.g. cortisol) no córtex adrenal (HSU; HSUEH, 2001), exercendo importante papel na resposta de "luta ou fuga". O mecanismo de *feedback* negativo cessa a resposta ao estresse com o aumento da ação do cortisol nos receptores de

mineralocorticoides (MR) e glicocorticoides (GR) presentes em diversas regiões encefálicas como o hipocampo, PVN e na glândula pituitária (van EEKELEN et al., 1991).

Figura 1: Modulação Hormonal do eixo HPA.
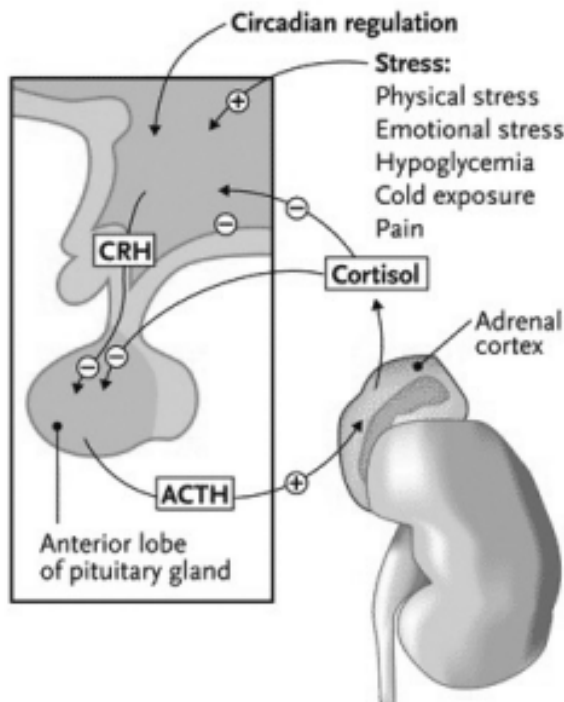
Fonte: KLEMM (2000)

A ativação do CRHR2, em contrapartida, é entendida como envolvida na fase de recuperação do estresse (VASCONCELOS et al., 2020). O CRHR2, além de se ligar ao CRH, liga-se à urocortina (UCN), que apresenta maior afinidade pelo CRHR2 quando comparada ao CRHR1 (HSU; HSUEH, 2001). O sistema de sinalização CRH, conta ainda com uma proteína de ligação. A proteína de ligação ao CRH (CRHBP) possui alta afinidade ao CRH, e por isso os níveis desse hormônio podem ser influenciados por sua ligação com a CRHBP (WESTPHAL; SEASHOLTZ, 2006). Esse mecanismo pode explicar como a disponibilidade de CRH livre diminui à medida que os níveis de CRHBP aumentam. Contudo, as funções específicas de CRHR2, CRHBP e UCNs ainda não foram totalmente esclarecidas (VASCONCELOS et al., 2020).

Figura 2: Interação entre componentes do sistema CRFérgico.
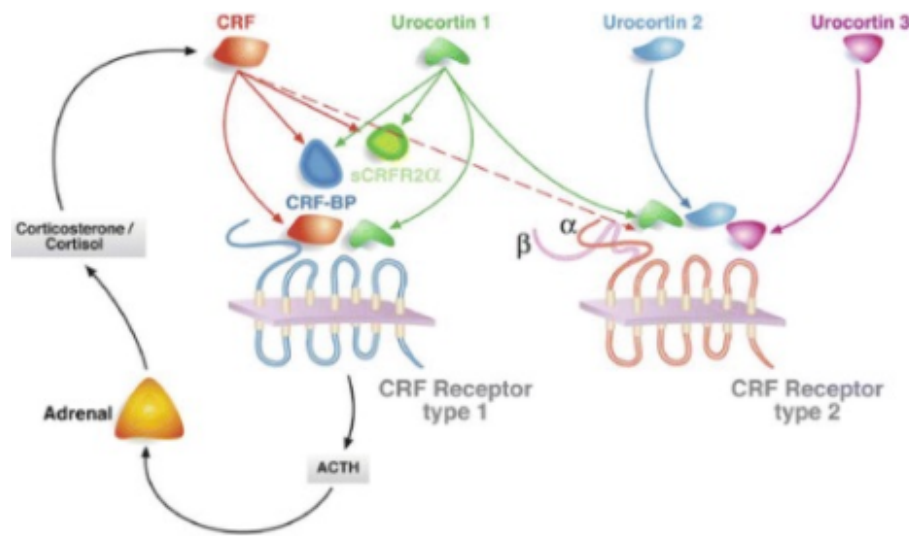
Fonte: CHEN (2016)

Além da sua atuação como hormônio na resposta neuroendócrina ao estresse, o CRH, em particular, também atua como neurotransmissor no sistema nervoso. Uma grande quantidade de estudos sugere que o CRH integra as respostas aos estressores em nível endócrino, imunológico e comportamental dos mamíferos (OWENS; NEMEROFF, 1991). Os estudos apresentados neste Trabalho de Conclusão de Curso baseiam-se na possibilidade de a atividade neuronal inapropriada do sistema CRH poder ser manifestada na forma de transtornos mentais e afetivos, bem como outros transtornos associados ao estresse (BALE; VALE, 2004).

1.4 Justificativa

De acordo com o relatório de "Depressão e outros transtornos mentais comuns: Estimativas da saúde global", há 322 milhões de pessoas vivendo com depressão unipolar ao redor do mundo, além de algumas estimativas apontarem que, até 2017, 1 a cada 7 pessoas no mundo possuía algum problema mental ou relacionado ao uso de substâncias. Embora estas estimativas apontem para uma alta prevalência de tais transtornos, não há hipótese mecanicista etiológica conhecida, impossibilitando tratamentos mais assertivos e dificultando a utilização dos tratamentos de maneira eficaz.

Ao reunir as informações sobre a variabilidade dos genes envolvidos na resposta ao estresse e da contribuição de fatores ambientais (i.e., interação gene $\times$ ambiente) para o aparecimento do desfecho clínico psiquiátrico surgem novas hipóteses a respeito de quais aspectos conferem vulnerabilidade à exposição a eventos adversos. Adicionalmente, para que as hipóteses sejam embasadas em resultados confiáveis, a avaliação de como os estudos epidemiológicos observacionais dentro da psiquiatria genética estão sendo conduzidos contribui para a detecção dos possíveis fatores que contribuem para a geração de resultados inconsistentes e irreprodutíveis dentro da área. Portanto, a revisão da literatura e a avaliação de sua qualidade pode estimular a discussão do que poderia ser feito para o aumento da confiabilidade dos achados na ciência.

1.5 Objetivos gerais

A presente revisão pretende responder se a variabilidade genética de componentes do sistema CRH é capaz de contribuir para o desenvolvimento de transtornos relacionados ao estresse (e.g. transtorno por uso de substâncias, depressão maiorunipolar e transtorno bipolar, transtornos de ansiedade e transtorno de estresse pós-traumático) ao conferir traços de resiliência ou suscetibilidade apresentados em resposta às experiências traumáticas.

1.5.1 Objetivos específicos

- Verificar quais alelos inseridos nos genes do sistema CRFérgico (*CRH, CRHR1, CRHR2, CRHBP, UCN*) foram associados ao aumento do risco para o desenvolvimento de transtornos relacionados ao estresse;
- Verificar quais os SNPs do sistema CRH são mais comumente avaliados dos estudos de psiquiatria genética;
- Analisar qual o impacto das variações genéticas do sistema CRF na predição do sucesso do tratamento psicofarmacológico;
- Observar qual a metodologia molecular mais utilizada na tentativa de responder questões genéticas relacionadas aos transtornos de interesse;
- Detectar no plano de análise dos estudos quais as falhas mais comuns e os passos em que os vieses estão mais presentes;
- Detectar quais os fatores ambientes que mais contribuem para o desencadeamento de transtornos e compreender de que maneira as adversidades com potencial de gerar sofrimento psicológico interagem com o genoma.

2. Artigo científico

Journal of Affective Disorders

Review article

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Title:

CRH signaling system SNPs associated with stress-related disorders: implications of the candidate gene approach

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Abstract

Background: Manifestation of mental disorders associated with stress might be related to inappropriate corticotropin-releasing hormone (CRH) neuronal activity. This system is composed of the CRH hormone/peptide, proteins similar in structure to CRH (urocortins), its receptors (CRHR1 and CRHR2), and the CRH-binding protein (CRHBP). These molecules are responsible for modulating several physiologic responses in the hypothalamic-pituitary-adrenal axis (HPA) stress response. In this review these genes are investigated in an attempt to answer whether genetic variability is capable of conferring vulnerability or resilience to stress-related psychopathologies.

Methods: This systematic review describes observational epidemiological studies published in the last five years on CRH system genes associated with stress-related psychopathologies and assess the quality of these studies.

Results: *CRHR1* gene is the main investigation target due to the SNP rs110402 being recurrently associated with several clinical disorders in the past literature. However, a poor replication of these and associations were observed. The quality assessment of these genetic studies found that the absence of verification of ethnicity, sex and substance use were the most frequent confounding factors. The excessive delimitation of the genetic region of analysis contributes to the generation of random associations.

Limitations: Few studies were selected and the time span was the last five years.

Conclusions: Psychiatric disorders are influenced by many genes with low effect, therefore the association found between only one genetic variable and pathologies, although it may contribute to the appearance of the phenotype, is not enough to determine resilience or vulnerability.

Keywords: Stress, SNP, CRH, CRF, Psychiatry, Genetics.

Introduction

Mental and substance use disorders were not a global health priority until the mid-nineties (Whiteford et al. 2013). This seems to be true when compared with the amount of evidence and research on communicable diseases and non-communicable diseases such as cancer or cardiovascular disease. Therefore, this might as well explain the lack of strong etiological hypotheses for several of the psychiatric conditions. Observable heritability patterns within these diseases encouraged the answers to be sought in genetics, giving rise to psychiatric genetics in the 20th century. Currently, however, with the advancement of genotyping technologies, the search for what predisposes or confers phenotypic characteristics can be sought directly in the genome. Between 2007 and 2017, a ten years interval, more than 200 loci with the ability to confer some genetic risk were described (Smoller, 2017). The hypothalamic-pituitary-adrenal (HPA) axis is involved in the neurobiology of mood disorders and other disabling illnesses, including anxiety disorders, posttraumatic stress disorder (PTSD), and alcoholism (Vasconcelos et al. 2020). The variability of genes relevant to HPA-axis functioning, especially single nucleotide polymorphisms (SNPs), are one of the main targets in the investigation of what confers such susceptibility.

The HPA-axis is a major neuroendocrine system that controls reactions to stress and regulates many body homeostatic processes. The activation mechanism begins with the secretion of the corticotropin-releasing hormone (CRH - or corticotropin-releasing factor (CRF)) by the neurons of the paraventricular nucleus (PVN) in the pituitary, when projecting to the median eminence. In the anterior pituitary, CRH binds to CRHR1 and CRHR2 to regulate adrenocorticotropic hormone (ACTH) secretion, which stimulates the adrenal cortex to release the glucocorticoid cortisol (Hsu and Hsueh, 2001). This glucocorticoid is responsible for the negative feedback mechanism when it exerts an inhibitory action on the secretion of ACTH and CRH by binding mineralocorticoid and glucocorticoid receptors present in the hippocampus, PVN and in the pituitary gland (de Kloet et al., 1991). CRH levels are influenced by their binding with CRH binding protein (CRHBP), as they have high affinity (Westphal and Seasholtz, 2006), which causes their free availability to decrease as CRHBP levels increase. The stress response is modulated differently depending on which CRH receptor binds: while the CRHR1 binding generates flight-or-fight response, the CRHR2 binding is important in the stress recovery phase (Vasconcelos et al. 2020). In addition to binding to CRH, these receptors bind to urocortin (UCN), which have greater affinity for CRHR2 when compared to CRHR1 (Hsu and Hsueh, 2001).

The literature suggests that the CRH integrates responses to stressors at the endocrine, immunological and behavioral levels in mammals, then inappropriate CRH neuronal activity may be related to the manifestation of mental and affective disorders, as well as other disorders associated with stress (Bale and Vale, 2004). This was evidenced in several studies that observed hyperactivity of the HPA axis and increased levels of cortisol in saliva, plasma and urine in a significant portion of depressed patients (Stetler and Miller, 2011). Additionally, these studies reported ACTH and CRH release even after pre-treatment with dexamethasone and the same does not occur after the decrease in symptoms (Holsboer et al., 1987).

The World Health Organization (2016) warned that environmental influences, such as childhood trauma contribute to the development of Major Depressive Disorder (MDD), drug misuse and high-risk sexual behaviors. The presence of childhood trauma in depressed patients was also related to ACTH and cortisol levels in stress and dexamethasone-CRH tests (Gillespie et al., 2019). These data contribute to the notion that the phenotype of psychiatric disorders and HPA axis dysfunction are influenced by extrinsic factors. However, it is not all individuals who have experienced adverse events that develop psychiatric disorders, and this is because in addition to psychological and sociocultural factors, the variability of the genetic profile (i.e. genetic polymorphisms) appears to be related to resilience (Rutter, 2013). Some types of childhood trauma, for example, are associated with the development of depression according to the genetic profile based on the variability of the *CRHR1* gene (Heim, 2009). The present review aims to answer whether the way in which components of the CRH system differ genetically within populations is able to confer resilience or susceptibility in the development of highly prevalent stress-related psychiatric conditions (e.g. substance use disorder, unipolar depression, bipolar disorder, anxiety disorders and post-traumatic stress disorder). For this purpose, we conducted a bibliographic review of experimental studies dealing with the issue published in the last five years. A qualitative assessment of how these observational epidemiological studies were conducted was also carried out, in order to detect which factors may be making it difficult to reproduce studies in the genetic psychiatry field, and, therefore, decreasing the reliability of the results.

Methods

Literature search

A literature search was conducted in three electronic databases: PubMed/MEDLINE, Scopus/Elsevier, PsycINFO/EBSCO. The selected language and publication date filters were: scientific articles published in English from 1/1/2010. A filter was also applied to exclude review articles. The search query consisted of terms considered by the authors to describe the research question. The following terms were used: SNP, haplotype, polymorphism, "genetic variant", epigenetic*, "genetic influence", CRF, CRH, CRFR*, CRHR, CRFBP, CRHBP, "corticotropin releasing factor", "corticotropin releasing hormone", Stresscopin, "CRF receptor*" "CRH receptor*", "CRF binding protein", "CRH binding protein", Urocortin*, resilience, vulnerability, variability, reactivity, susceptibility, coping, "mood disorder", "post-traumatic stress disorders", "psychiatric disorder*", "psychiatric illness", anxiety, depression, abuse, "childhood neglect", "child neglect", PTSD, alcohol, ethanol, drug, suicid*, burnout, adversity, stress. The search query was tailored to the specificities of each database.

Manuscripts were initially screened through title and abstract reading, and later judged for eligibility after full-text assessment. Screening and eligibility procedures were conducted using Rayyan QCRI - a web application to help systematic reviews. Inclusion criteria for the eligibility of the included studies were: (1) original data; (2) human subjects; (3) polymorphism measures of at least one CRH system component and measures of stress-related psychiatric conditions. During screening and eligibility phases, works were excluded for: (1) presenting non-original data (i.e., review articles, conference poster, book chapters); (2) presenting only works on animal models; (3) non-related outcome (i.e., presenting polymorphism measures not related to the CRH system genes or not related to psychiatric conditions); (4) presenting a "mixed effect" in cases where there may be confounding effects contributing to the conditions (i.e., Alzheimer's disease, irritable bowel syndrome, pregnancy, postpartum depression, personality disorder, headache, infection by HIV, subarachnoid aneurysmal haemorrhage, rheumatoid arthritis, asthma, pregnancy).

Authors conducted two training sessions to compare judgment performances using 30 abstracts randomly selected in each round. During these training sessions, inclusion and exclusion criteria were refined by the evaluators (M.V., L.J.). Complete screening of search results was conducted after a high level of agreement among the authors has been obtained. Results were reported and discussed according to the main investigating psychiatric outcomes.

Quality assessment

We used the Quality of Genetic Studies (Q-Genie) tool developed by Sohani et al. (2015) to assess reliability of the evidence gathered in our literature search. The Q-Genie tool contains 11 items (i.e. questions) marked on a 7-point Likert scale covering the following themes related to scientific basis for gene selection: use of comparison groups (i.e. cases and controls), technical and non-technical bias during genetic variant testing, classification of the outcome, diverse sources of bias, sample size and statistical analyses adequacy, evaluation of assumptions in the genetic studies (e.g. agreement with the Hardy Weinberg equilibrium), and adequate interpretation of results. From the original publication, the authors reported psychometric evaluations of the tool and considered that the Q-Genie demonstrates good inter-rater reliability, internal consistency, and overall reliability tested between expert users (i.e. having familiarity with genetic association studies) and non-experienced users. The authors of this review considered themselves non-experienced users and requested guidance of an experienced researcher in the topics covered on the tool.

Results

Literature search

The search was implemented on September 3, 2020, and a total of 494 hits was achieved through search of the three databases. After removal of repeated hits (total = 179), the remaining works were screened for inclusion and exclusion criteria and a small conflict number was obtained (1.5%, 6 articles). Conflicts were solved after complete reading of selected full texts. One work was excluded for not being in English language. One of the databases did not restrict the search for the required time span, so works published before 2010 were excluded during screening of the abstracts. While in the eligibility phase, works published before 2015 were also excluded due to time constraints. This initial version of the manuscript includes only works published in the last five years. After full-text assessment, some articles were excluded according to the aforementioned excluding criteria. Due to low number of hits and/or heterogeneity, articles with outcomes related to epigenetic measures, cognitive and reactivity assessment to stress (mainly concerned with risk for psychiatric conditions instead of clinical diagnosis of psychiatric conditions) were excluded. Figure 1 describes a flow chart of study selection process.

Quality assessment of selected studies

Overall quality of evidence gathered in our literature search was considered high. According to the assessment conducted using the Q-Genie tool, more than 85% of selected studies were of good quality. Two studies presented poor quality and one presented moderate quality (Figure 2), the relevance of these observations will be discussed along with the description of these studies.

We averaged the scores obtained by the included studies and performed a brief analysis of what were the strongest and weakest points of these studies (Figure 3). Overall, the gene candidate studies had adequate rationale to select the CRH system genes for association with the desired outcomes. Outcomes in the psychiatric field have an intrinsic problem to be ascertained, clinical diagnosis rely mainly on the subjective report of participants. In our search of the literature, this topic was considered irrelevant and overall practices for sample selection and use of clinical diagnostic tools was considered as sufficient and of good quality in the screened studies. A third strong point of the selected studies was their adequate draw of conclusions. Whenever the study had a sound methodological planning or a poor one, authors demonstrated care before suggesting conclusions and extrapolation of their results.

Although the majority of studies reported how haplotypes were determined, they performed weakly in reporting testing hypotheses to assure correct haplotype inferences, (e.g., checking for consanguinity, lack of availability of family data). In respect of sample size calculation, *a priori* power analysis and bias during genotyping procedures, the overall studies performed poorly on reporting these procedures. Practices such as: blinded assessment of genotype, reporting whether samples were processed in different batches or different pieces of equipment, and if randomisation was employed balancing randomly selected cases pertaining to each batch, or case and controls whenever the case, were almost never disclosed.

Literature review

Substance use disorder

Su et al. (2020) found no relationship between dependence or relapse for heroin use to any of the individual SNPs evaluated from the *CRHR1* and *CRHBP* genes or to the constructed haplotypes. The authors claim that carriers of the CC genotype of the CRHBP SNP rs3792738 with higher scores on the Perceived Stress Scale (PSS), which measures the degree of stress the individual has experienced in the past year, were more likely to relapse.

Unfortunately, data to prove this last statement were not shown in the study results section or tables.

The relationship between *CRHR1* SNPs, susceptibility to crack addiction and Brain-Derived Neurotrophic Factor (BDNF) levels was investigated in the study conducted by Rovaris et al. (2017). Crack addicted patients showed higher levels of BDNF serum when compared to non-addicted, and three analyzed *CRHR1* SNPs were associated with variations in BDNF levels in all groups (crack addicted at discharge, crack addicted at admission, and non-addicted). The authors reported that rs12944712-A carriers showed lower BDNF levels when compared to G homozygous; rs878886-G carriers showed lower BDNF levels when compared to C homozygous; and rs110402-A carriers showed higher BDNF levels when compared to G homozygous (Rovaris et al., 2017).

In a more embracing assessment of substance use, involving nicotine, alcohol and marijuana, and specific genetic variants, Trucco et al. (2018) verified whether this behavior is related to variables as childhood resilience, behavioral control and depression. The polymorphism rs7728378 of *CRHBP* gene interferes in this association, but the results regarding this polymorphism are not reported. The study described by Trucco et al. (2018) performed poorly on Q-Genie assessment mainly due to non-technical classification of the genetic variant, inappropriateness of comparison groups, inadequately powered and insufficient sample size, poor description of statistical methods and confounding factors, inappropriateness of inferences drawn from result and inadequacy of the presented hypothesis and rationale.

When analyzing the *CRHBP* rs1715749 polymorphism, Goyal et al. (2016) observed in African-American adolescents living in high poverty neighborhoods that there is a significant interaction between *CRHBP* SNP and stressful situations in predicting the susceptibility to alcohol use in C / C homozygotes and C / T heterozygotes, but not in T / T homozygotes. Homozygous participants for minor alleles consumed more alcohol when compared to those who had only one copy or did not have the allele, both having been exposed to a low degree of stressful events during their lives. Interestingly in exposure to a high degree of stress, the opposite occurs - individuals homozygous for the minor allele had greater resilience to alcohol use. The authors propose that the results met the concept of differential susceptibility brought by Belsky and Pluess (2007), in which the most susceptible individuals in disadvantageous conditions are those who perform better in beneficial environments. The significance of the results was lost after the correction for multiple testing.

The impact of *CRH* gene variability related to substance use disorder was investigated by Seeliger et al. (2020). The authors evaluated opioid addicts and healthy volunteers performance in the IOWA gambling task (IGT) - designed to evaluate decision making. The control group performed better and had better performance in the total IGT score, the allelic frequency did not differ according to the SNPs and haplotypes of the groups. The authors could observe that the haplotype seemed to influence the learning curve throughout the test, so that in the group of addicts, TGTCA haplotype carriers had a prolonged learning curve compared to individuals homozygous for the TGTAA haplotype. In the control group, the opposite effect was observed, those with the TGTCA haplotype showed a less pronounced learning curve than those homozygous for TGTAA. As the only variable SNP constructing the haplotype is the one that appears in the fourth position, marked by the A or C allele, rs1870393 SNP was the only one considered to have significant effects. The anxiety state of the participant was also associated with the learning curve; in this case the presence of the C allele was considered a risk for anxiety experience, since it conferred a reduced learning curve to the participants of the control group. In the group of opioid addicts, risk allele carriers had better performance in the task, and therefore, in this case, anxiety could be considered as a trait that benefits IGT performance (Seeliger et al., 2020).

Major Depressive Disorder

CRHR1 was the main candidate gene of CRF system for investigating the impact of SNPs on the predisposition to depressive phenotypes. The study of da Silva et al. (2016) investigated the interaction between the SNPs rs12944712, rs110402, rs878886, smoking status, gender and MDD diagnosis. The authors found an association between depression and the rs878886 SNP in non-smoking males, and in the case of non-smoking females this association was present in rs110402 SNP. This result demonstrates that the effect of the susceptibility to MDD conferred by these *CRHR1* SNPs depends on sex. Within the group of lifetime smokers, no association of SNPs with MDD was found. The authors reinforced the importance of having divided the sample according to sex and smoking status, as its homogeneity contributes to making the investigation of the impacts of SNPs more precise. Sarubin et al. (2017) also raised the question about whether the genetic variability related to MDD operates differently according to gender. These authors reported no associations since the frequency of *CRHR1* SNPs were not significantly different between cases and controls in both genders, and no association was found between the haplotype and depression in both sexes.

Buttenschøn et al. (2017) sought to discover the influence of stressful life events (SLE) on the development of depression and whether this interaction is associated with 239 SNPs of 7 genes important for the functioning of the HPA axis. These authors found a significant association between SLE and depression, but none of the *CRHR1*, *CRHR2* and *CRH* polymorphisms were associated with depression.

Bipolar Disorder

In the study carried out in patients with Bipolar Disorder (BD) conducted by Szczepankiewicz et al., (2018) it was investigated the impact of polymorphisms related to HPA axis regulation in response to lithium long-term treatment (five years). The authors reported no association between *CRHR1* SNPs (rs878886, rs173365, rs242937, rs110402, rs12936511, rs4792887, rs4076452, rs16940655) and response to treatment with lithium.

Suicidality

In studies investigating the association of suicidal behavior with *CRHR1* SNPs, Pawlak et al. (2016) included individuals diagnosed with BD and MDD, divided into two groups (suicide attempters and non-attempters). The authors found an association between the rs16940665 polymorphism and individuals with MDD who attempted suicide vs. non-attempters. In the study conducted by Mirkovic et al. (2017), no association was found between the rs4792887 polymorphism and traits of suicidal behavior or associated with suicidal behavior (e.g. impulsivity and hopelessness) in adolescents.

Investigating the influence of *CRHR1* gene variability on the inflammatory response and suicide risk, Bastos et al. (2016) verified wheter the levels of IL-1β varied according to the SNP allele rs110402. The results show that the distribution of the polymorphism did not differ between controls, suicidal ideation and suicide attempter groups. However, higher levels of IL-1β were observed in suicide attempters A carriers, increasing the risk in 3-fold. The authors suggest that this association occurs due to the effect of the allele in increasing levels of IL-1β, although the mechanisms that explain the impact of IL-1β on suicide behavior are not well understood. It is important to notice that Bastos et al. (2016) work performed poorly on Q-Genie assessment. The study lacks reporting on non-technical classification of the exposure and whether the sample size was sufficient and adequately powered.

Segura et al. (2019) was the only study selected in our search that included *CRHR1*, *CRHR2* and *CRHBP* genes as candidates in the investigation for the association of genetic

variability and suicidality. Analyzing the impact of relevant genes on the activity of the HPA axis and childhood trauma in patients diagnosed with bipolar disorder, the rs7728378 allele of the *CRHBP* gene was associated with suicide. Segura et al. (2019) reported that C allele carriers (TC and CC) were 3.05 times more likely to have suicide-related behaviors than TT-homozygous, but such findings did not survive correction for multiple testing. Sanabrais-Jiménez et al. (2019) searched for associations between SNPs of CRHR1 (rs110402, rs242924, and rs16940665) and *CRHR2* (rs2190242, rs2284217, and rs2014663) in suicide attempt through the genotyping of bipolar and MDD patients and the influence of childhood trauma in this association. There was a Gene × Environment (G × E) interaction in suicide attempters as the SNPs of the *CRHR1* and *CRHR2* genes were associated with childhood trauma. The only association found between the polymorphisms analyzed and suicide attempt was between bipolar disorder suicide attempters and *CRHR1* rs16940665. In the analysis of haplotypes, 3 blocks were constructed, 1 found in the *CRHR1* gene and another 2 in *CRHR2*, but there was no distinction between suicide attempters and non-attempters.

Anxiety

In the analysis of *CRHR1* polymorphisms, Weber et al. (2016) evaluated whether this variability influences neurophysiological, psychological and behavioral mechanisms in panic disorder (PD). In the analysis of haplotypes, 2 blocks were constructed, so that in block 1 (rs7209436 - rs4458044 - rs12936181 - rs3785877 - rs17689918), females carrying the CGTGA haplotype had 3 risk allele SNPs (rs7209436 - rs12936181 - rs17689918), and males carrying the CGCGG haplotype had 1 risk allele SNP (rs7209436) and 2 protective alleles SNPs (rs12936181 and rs17689918). In block 2, consisting of SNPs rs17689966 and rs4792825, the allele A of rs17689966 SNP was considered protective because female homozygous AA carriers for the haplotype had a lower risk of PD, whereas GA haplotype was associated with PD. In order to find out whether SNPs had an effect on fear conditioning in PD patients, functional magnetic resonance imaging (fMRI) was performed. Weber et al. (2016) reported that the risk allele carriers, allele A of the rs17689918 SNP, showed differences in brain activation due to fear processing compared with GG homozygotes. In respect of behavioral effects associated with this genotype, in the Behavioral Avoidance Task (BAT), patients with PD/agoraphobia carrying at least one risk allele (A) of rs17689918 had reduced tendency to escape acute exposure when compared with non-risk allele homozygotes. Finally, analyzing the function of SNPs as Expression quantitative trait loci (eQTLs), *CRHR1*

mRNA levels in human post-mortem brain tissue were influenced by rs17689918 polymorphism genotypes.

Harris et al. (2019) relating the involvement of the endocannabinoid system in amygdala activity regulation and therefore, the level of activity of the HPA axis, assessed the association of *CRHR1* and *FAAH* (Fatty Acid Amide Hydrolase) - genes with cortisol levels in the anxiety state of human subjects. The authors found that the presence of minor alleles of 3 *CRHR1* SNPs (rs7209436 C → T [minor allele]; rs110402, G → A [minor]; and rs242924 G → T [minor]) and *FAAH* homozygous CC (compared with homozygotes for AA and heterozygous AC) was associated with lower levels of cortisol and higher levels of anxiety. These authors observed that the higher the serum cortisol level, the lower the anxiety score and vice versa (Harris et al. 2019).

Womersley et al. (2018) assessed the association between *CRHR1* variation, childhood trauma history and anxiety sensitivity (AS) in two ethnic populations (Black Xhosa Speaking and South Africa Colored). In the colored group, an association was found between the haplotype block (rs7209436 - rs4792887 - rs110402 - rs242924) and AS in males. The A-C-A-G and A-C-A-T haplotypes predicted an increase of 4.72 and 2.27 points, respectively, in the Childhood Anxiety Sensitivity Index (CASI) score when compared with GTGG. Both combinations of alleles increased the risk of AS compared to the CTGG combination. In this study, Womersley et al. (2018) reported that a haplotype block (rs7209436-rs4792887-rs110402) was also detected in the Xhosa participants, but no associations with childhood measures and trauma were found.

PTSD

Jaksic et al. (2019) found no association between *CRHR1* rs17689918 SNP and presence or absence of PTSD symptoms in war veterans of European origin who participated in the Balkan wars during the 90s. Regarding the *CRHR1* SNPs in the success of pharmacological treatment with the CRHR1 antagonist GSK561679, the results presented by Dunlop et al. (2017) demonstrated no benefit for the reduction of PTSD symptoms. However, GG carriers for the rs110402 polymorphism with a history of abuse in childhood, when receiving treatment with the antagonist, declared a decrease in symptoms and presented a higher percentage change in the PSS-SR (PTSD Symptom Scale – Self-Report) (Dunlop et al., 2017).

Genetic Risk Score Approach

Di Iorio et al. (2017) sought to assess whether the multilocus profile of genes related to increased activity on the HPA axis interacts with ELS to predict symptoms of depression and anxiety in young adults through the Biologically-Informed Multilocus Profile Score (BIMPS) (Nikolova et al., 2011). Additionally, they also investigated amygdala reactivity in an emotion face-matching challenge paradigm using fMRI. The multilocus profile score was constructed using 2 SNPs (*FKBP5* rs1360780 and *CRHR1* rs110402) and 1 haplotype (*NR3C2* rs5522 / *NR3C2* rs4635799). Each genotype was considered to have high, intermediate or low adequate functioning of the HPA axis - either due to hyperactivity in the response or due to a deficiency in negative feedback - corresponding to scores of 2, 1 or 0, respectively. Di Iorio et al. (2017) reported that psychological measures of the Mood and Anxiety Symptom Questionnaire (MASQ) symptoms are not associated with the BIMPS x ELS interaction for any measure, but are associated with anxious arousal since individuals with higher BIMPS had more anxious symptoms. The authors do not provide information on the interaction of the activity of the amygdala with the polymorphism of *CRHR1* (rs110402) individually. The BIMPS x ELS interaction in comparison with multilocus profile was associated with reactivity of the right amygdala only, however such reactivity was not associated with symptoms of anxiety or depression, and, as brought by the authors, it is important to consider that the population was young and only a small portion (6.8%) met the criteria for anxiety-related psychiatric disorders (DSM-IV), which differs from clinical samples.

Hu et al. (2020) conducted a study to evaluate the relationship between the severity of symptoms of Posttraumatic Stress Disorder (PTSD) and the genotype of Caucasian war veterans who served in military conflicts. The authors constructed a genetic risk score based on gene markers previously related to PTSD (*CRHR1*, *CHRNA5*, *RORA*, and *FKBP5*). The risk score range covered values from 0 to 8, since each of the four genetic variants could have 0, 1 or 2 risk alleles. The results showed that lifelong trauma and a higher exposure to combat contribute to the increased severity of PTSD symptoms. Hu et al. (2020) demonstrated that veterans diagnosed with PTSD (7.1%), were more exposed to traumatic experiences and had higher genetic risk scores when compared to controls (veterans without PTSD diagnosis). In cases of high exposure to combat and high lifetime traumatic event, there was a significant association between the severity of PTSD symptoms and the genetic risk score, so that the severity of symptoms was lower when the genetic risk was higher, suggesting that genetic variants of risk have less influence on the severity of PTSD symptoms than traumatic

experiences throughout life.

Discussion

From our brief review of the literature, it was possible to overview how genetic association studies elucidate some of the CRH system influence on predisposition to the development of psychiatric disorders associated with stress. The *CRHR1* gene is the main candidate in investigation; the studies demonstrate its association with mood disorders, anxiety, substance use and PTSD. Although some claimings of MDD and BP were already postulated in the literature, in our search the studies aiming to deepen such associations with gender-specific effects, stressful life events and lithium response in BD treatment were not supportive or conclusive. For the presence of suicide thoughts or suicide attempts in patients diagnosed with mood disorders, *CRHR1* polimorphisms were associated with immune modulation and childhood trauma, as these two topics are strongly involved in suicidality. The association of *CRHR1* variation with anxiety was mainly related to the cognitive processing or emotional reaction to anxiogenic stimuli, and this was also seen in a multilocus profile study.

Although CRHR1 was described in the literature as strongly associated with PTSD, in our study *CRHR1* polymorphisms were not associated with the presence of the disease in conflict veterans and were arguably associated with PTSD treatment response. It is plausible that variability in *CRHR1* gene and CRHR1 expression might be associated with PTSD, but not accounting as much as traumatic experiences in risk alleles carriers. Another gene candidate with a more recent attention was the *CRHBP* polymorphisms. Variation in the *CRHBP* gene might underlie how stress confers vulnerability to development of drug dependency but also mediates processes such as relapse and abstinence. The *CRHR1* and *CRH* genes variability were also covered in our search in respect to substance use disorders. However, polymorphisms in these genes were not directly implicated in substance use it is possible that some polymorphisms might interfere with neuroplasticity and anxiety, thus mediating behaviors related to drug use. The candidate gene least evaluated in the five years in search of associations with disorders of this nature was *CRHR2*, probably due to low findings in preview reports.

Although we aimed to review stress-related psychiatric disorders, most articles included in our search were mainly focused on isolating the investigation of the HPA axis and did not consider CRH extrahypothalamic contribution to these disorders. For instance, these

studies have low coverage on mechanisms/regions that modulated HPA-axis activation and inactivation, such as the amygdala and the hippocampus, and their contributions to disease states (Bear et al., 2006; Le Doux, 2001). Weber et al. (2016) were among the few researchers found in this review that explored the effect of psychological stress on brain networks, observing the activity of the amygdala in response to activation of the HPA axis. However, as problematized by Bogdan et al. (2017), it is necessary to recognize that there are still no endocrine measures (e.g. cortisol levels) that actually verify the impact of genetic variation on the HPA axis and changes in amygdala function. Still on inferring axis functionality, many studies attribute associations between polymorphisms of the CRH system genes and HPA axis impairments without having performed tests to determine its activity level. Conclusions based on this reasoning are not strong, mainly because such genes are also expressed in other regions, some of them involved in the emotional, cognitive and homeostatic processing. For instance, CRHR1 and CRHR2 are widely expressed in the neocortex, in limbic regions, and in brain stem regions (Binder et al., 2010; Dautzenberg and Hauger, 2002; Bale T. and Vale W., 2003).

It has also been rare for studies to question the involvement of other systems (e.g. immune system) that are known to influence the neuroendocrine function of the HPA axis, since CRH system polymorphisms could impact these processes. In this sense, Harris et al. (2019) conducted their study considering the interaction of a relevant gene (*FAAH*) of the endocannabinoid system in amygdala activity and its involvement in the HPA axis. Moreover, Bastos et. al (2016), although evaluating the genetic variations of just one gene (*CRHR1*), included the analysis of cytokine levels (IL-1β), considering that the secretory function of the axis interacts with the inflammatory response (Webster et al., 2002).

There were some confounding factors that undermine the reliability of some of the studies selected in our search. The lack of detection of participants who use some substances in studies that do not assess substance use is one of these factors. Obtaining this information at the time of participant selection is important since the deregulation of the HPA axis is influenced by nicotine dependence, for example, which interacts with some genes such as *CRHR1* (Richards et al., 2011; Tang et al., 2015). There were studies in which participants were not divided by gender or ethnicity at the time of the analysis (e.g. Harris et al., 2019); more specifically, the verification of the genetic background was verified in only one study (Di Iorio, 2017). Di Iorio et al. (2017) confirmed the European ancestry of the participants through ancestrally-informative main components - homogeneity within each ethnic group contributes to the strategies of association (Cardon and Bell, 2001) - while in other studies

this determination was made according to the phenotypic characteristics and historical ancestry provided by the participants themselves. The verification of sex was rarely reported, which contributes to the generation of false-positive or false-negative results, especially in studies within neuroscience that are carried out in small samples (Genetics of psychiatric disorders, 2005). Previous studies have already shown gender-specific effects in the relationship of genetic variants with psychosocial stress, MDD, smoking status and anxiety-related disorder (Kumsta et al., 2007; Sarubin et al., 2017; da Silva B., 2016; Seney and Sibille , 2014) and ethnicity-specific effects on the G × E interaction related to anxiety-related disorders (Womersley, 2017).

The presence of inbreeding within the comparison groups was also not verified in any study, and this is another major factor in generating false-positive results, since in non-family based study, individuals can be distantly related. Lastly, the insufficient sample size is another factor widely observed in the selected studies and which contributes to false-positive results as it decreases the power to detect associations between SNPs and phenotypes (Long and Langley, 1999). Few studies had an ideal sample size (e.g. Pawlak et al., 2016; da Silva et al., 2016) in our review, which can be avoided by having knowledge of the preliminary nature of the associations and reaching out for multi-laboratory collaborations. Of all the studies, only two studies performed a previous calculation of power, which demonstrates previous knowledge in estimating the genetic effect. Consulting meta-analysis involving the candidate gene to be analyzed can contribute to a more appropriate analysis plan since they gather data obtained in several association studies and calculate the effect of genetic size (Bertran et al., 2007).

The vast majority of works selected in our review was gene candidate studies that investigated genes chosen according to the relevance of its expression in the HPA system (HPA axis) or according to positive associations already reported in the literature. This follows the opposite premise of studies of broad genomic association (GWAS), carried out through variants of potential genetic risk without previous pathophysiological hypothesis (Hoehe and Morris-Rosendahl, 2018). These study approaches disregard the polygenic architecture of psychiatric diseases, consisting of hundreds or even thousands of small-effect genetic variants and do not have a major loci with genes of great effect. This was exemplified in the study of da Silva et al. (2016), which evaluated only 1 gene (*CRHR1*), and the study by da Bastos et al., (2017), which in addition to the unique *CRHR1* gene evaluated, restricted the analysis to 1 polymorphism (rs110402), basing the entire association observed on the variability within 2 alleles. This excessive delimitation, not only of genes, but also of SNPs,

contributes to controversial and irreproducible results (Hoehe and Morris-Rosendahl, 2018), which could be one of the explanations for some studies treated in this review not having found the same associations as other authors. In this respect, Di Iorio et al. (2017) took into account the impact of genetic interactions when recognizing the limitations of prediction using individual polymorphisms and opted to use a multilocus profile score.

All studies were population-based association studies, which means that they were case-control studies comparing the allele frequency - within a single SNP or a haplotype - between healthy and affected individuals. In some cases, subgroups were added in the comparison within the control group - in this case, individuals who have the same diagnosis but in different degrees of severity of symptoms. Association studies are a widely used epidemiological tool, but it carries the chance of false-positive results, because a statistically significant association does not imply real influence of the allele as a risk aggregator (Cardon and Bell, 2001). The only study that sought to investigate the functionality of the gene effect was carried out by Weber et al. (2016), which analysed the function of a SNP as Expression Quantitative Trait Loci (eQTLs) by associating different alleles with their expression levels (mRNA) in human post-mortem brain. The outcome of the participants in the control group was verified through diagnostic questionnaires that followed Diagnostic and Statistical Manual of Mental Disorders (DSM) based criteria (IV or V), often applied by a psychiatrist. This seems to be an inherent problem in psychiatric research; this methodology might impose restrictions to trace the genetic contribution to the etiology of the disorders, since there is a heterogeneity of phenotypes within the same diagnosis, resulting in several clinical subtypes. This could partially explain the case of the various symptoms that may encompass the clinical condition of individuals diagnosed with depressive disorders having different polygenic loads (Hodgson and McGuffin, 2012). There is no specific genetic profile that is common to all those affected, and the opposite also is true - high degree of symptom overlap between disorders (Hodgson and McGuffin, 2012). In addition, there are candidate genes that are associated with more than one disorder. All of this contributes to the notion that genetic polymorphisms can impact cognitive functions more strongly than within a diagnosis that encompasses stratified clinical presentations (Genetics of psychiatric disorders, 2005).

The G × E relationship was explored in many of the selected studies through the application of the Childhood Trauma Questionnaire (CTQ) - which tracks childhood abuse. The efforts were directed to find a possible association between the history of early life stress, the clinical outcome and the genotypic profile, since several studies have shown that stressful effects during childhood confers vulnerability to the development of PTSD in some

individuals (e. g. Dunlop et al., 2017; Segura et al., 2009). The possible reasons for the associations were not raised in any of the studies. The authors did not discuss or investigate issues related to neurodevelopment, such as the existence of vulnerability windows, which means that during childhood, stressors might have a determining effect on the appearance of symptoms, possibly due to the contribution of epigenetic mechanisms (Heim and Binder, 2012). Detecting periods of sensitivity and being able to identify individuals with genetic risk profiles would be a good way to prevent the onset of disorders throughout development.

Overall, the majority of selected studies were considered of good quality according to the Q-Genie Tool. The use of this tool enabled us to understand whether the findings reported in the studies and especially the discrepancies between findings in the previous literature were due to the poor quality of the studies or because they were genuine findings. We observed that in the selected studies, sample and power calculation procedures, and control for genotyping batches differences were not widely disclosed. However, we acknowledge that the lack of reporting does not signify that such procedures were not implemented. Still, this might represent the view that such procedures are unimportant and might as well be a common malpractice in the field. One of the limitations of the present review relies on the time restriction implemented. Due to that, a deeper analysis was not performed. Including studies published in the past ten years would offer a broader overview of how studies that follow this scientific rationale were conducted and the type of associations that were found. A second limitation was the exclusion of studies with mixed effects from different situations of stress, periods of life or other disorders (e.g., HIV diagnoses, pregnancy, and irritable bowel syndrome). Combining psychiatric genetics studies with the study of comorbidities within psychological disorders can be another source of information to assist in the construction of more robust etiological hypotheses.

Conclusion

The search for understanding stress-related psychiatric disorders through behavioral neurogenetics and behavioral genetics continues to be developed regarding the components of the CRH system. From our recent literature search, little can be said about the role of genes that modulate stress response systems in providing resilience or vulnerability to these disorders. One of the main points observed in this review that may be contributing to the discrepancy and low reproducibility of studies within psychiatric genetics was the use of gene candidates approach. This methodology often excessively delimits the regions of analysis,

reducing the chances of associating a SNP as a genetic marker related to development of psychiatric disorder and increasing the chances of being just a non-causal association. Also, this approach prevents a broader view of what may be generating the effect, that is, the polymorphisms and genes that are around the delimited marker. Although the relentless search for genetic markers is a general feature in the field of genomics that contributes to the detection of genetic risk carriers, the same efforts are not directed towards understanding the mechanisms that confer the susceptibility. The absence of verification of sex and ethnicity effects remains a major source of bias spread across all studies, and because they are confounding factors this contribute to the generation of false-positive and false-negative results.

Finally, the diagnostic tools available in psychiatric practice may be making it difficult to trace the genetic contribution to the etiology of disorders, since polymorphisms may have an effect on a symptom (e.g. alteration of some cognitive function) that not all individuals present. In this sense, advancing the understanding of disorders from a categorical to a dimensional mode can be a good source of contribution to the area. In the G × E interaction, involving early life stress, a more targeted research approach, taking into account the age at which adverse events occurred, could contribute to the identification of vulnerability windows. Moreover, the combination of genetic and epigenetic analysis could bring a better understanding of the mechanisms underlying how the environment interacts with the genome.

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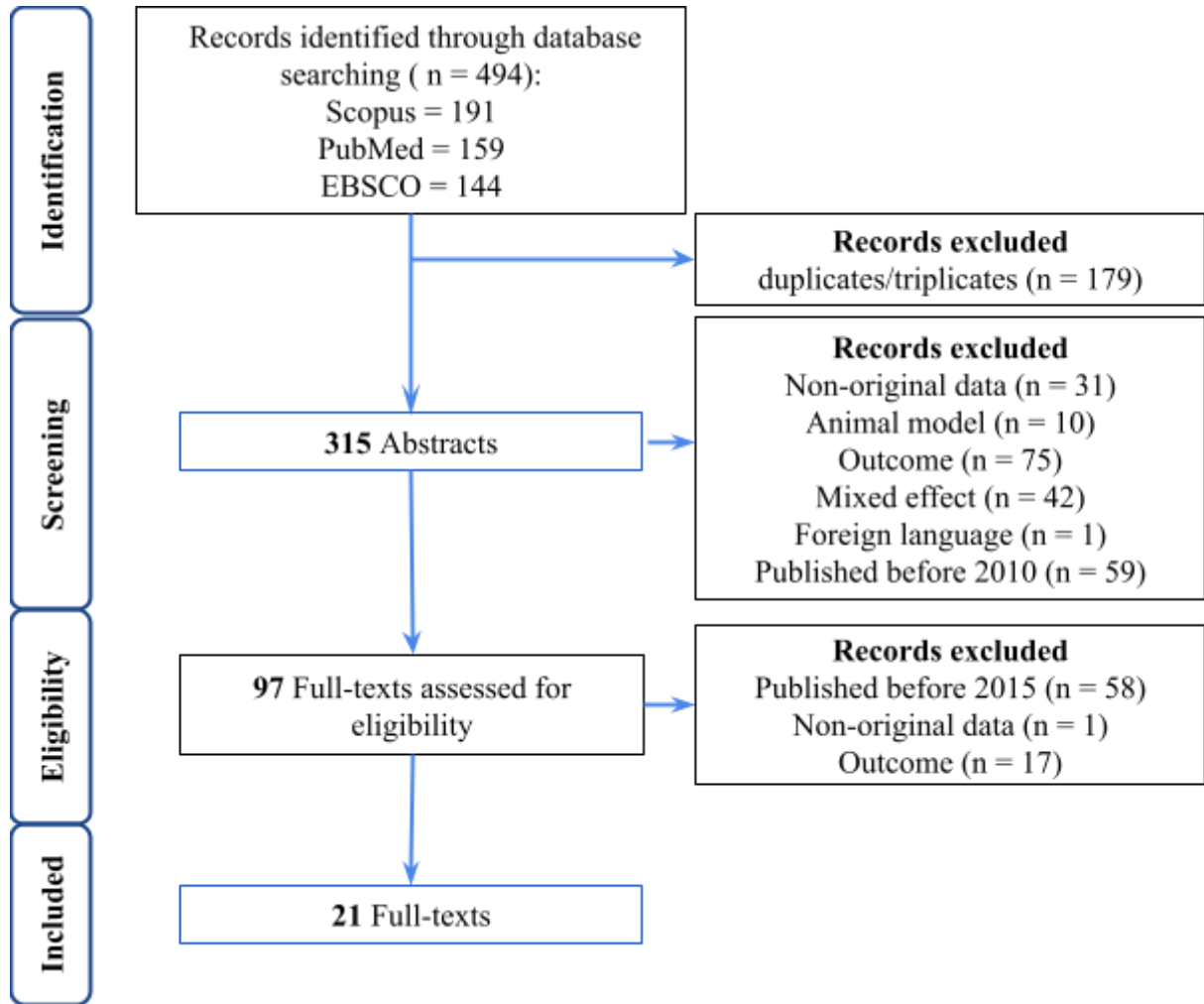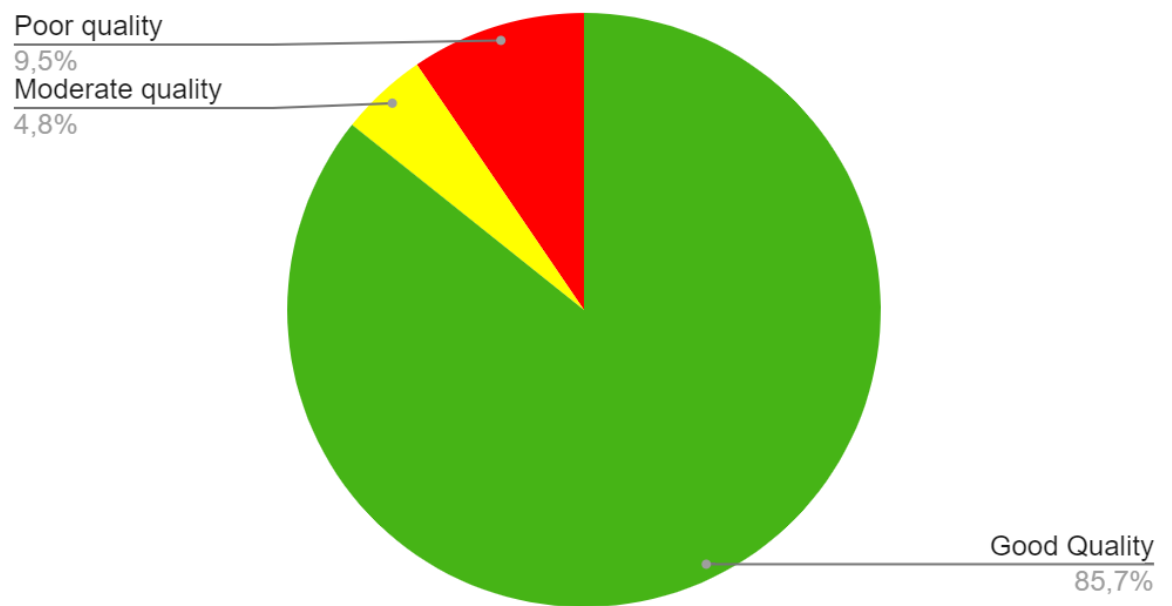
Figure 1 - Flow chart of study selection process

Figure 2 - Quality of studies selected in the literature search and included in the analysis. Quality was assessed using Q-Genie Tool.
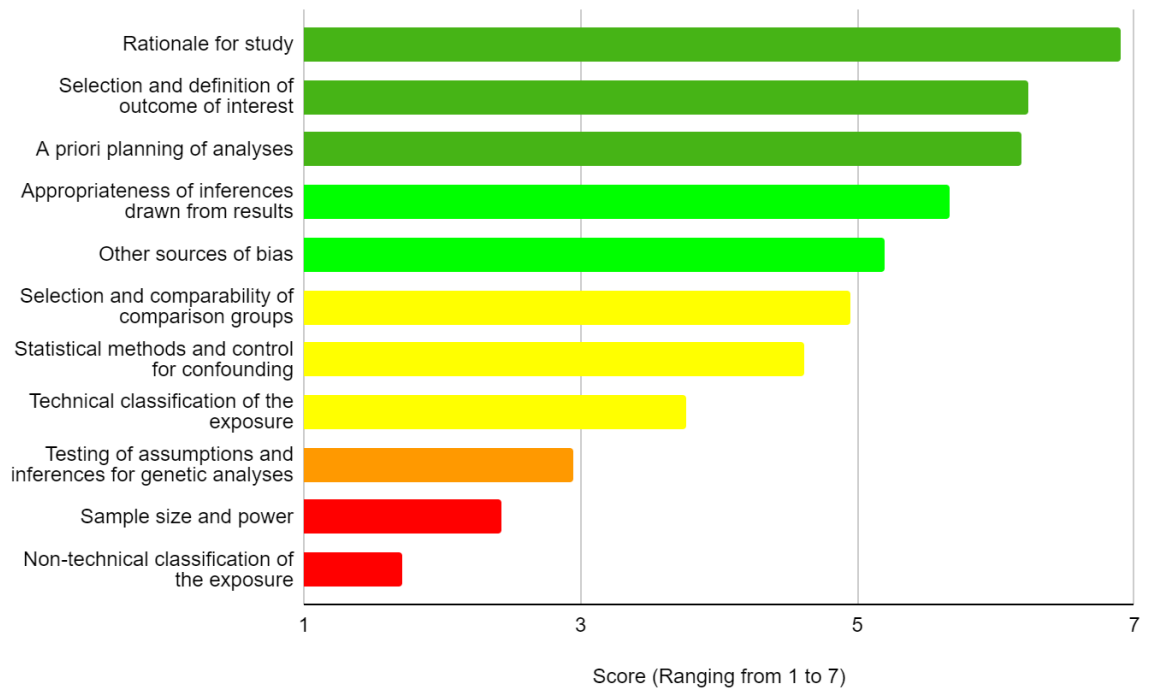

Figure 3 - Mean scores of Q-Genie items obtained by the overall studies included in our literature discussion.

Table 1 - Gene candidate studies results obtained from the literature review involving use substance.

Gene (s) of CRF system	SNP(s)	Association Results
CRHR1	rs12944712, rs110402, rs878886	Associated to BDNF levels regardless of crack use (Rovaris et al., 2017).
CRHBP	rs3792738	Associated to stress scores (PSS) and likelihood of heroin relapse (Su et al., 2018).
CRHBP	rs1715749	Associated to alcohol use in stressful situations (Goyal et al., 2016).
CRH	Haplotype block 'rs6999780 - rs7816410 - rs3176921 - rs1870393 - rs1814583	Associated to IOWA gambling task learning curve in opioid users (Seelinger et al., 2020).

Table 2 - Gene candidate studies results obtained from the literature review involving MDD and BD.

Gene (s) of CRF system	SNP(s)	Association Results
CRHR1, CRHR2, CRH	Not reported	Not associated with stressful events and MDD (Buttenschøn et al., 2017).
CRHR1	rs878886, rs110402	Gender-specific association with MDD in nonsmokers (Silva et al., 2016).
CRHR1	rs7209436, rs4792887, rs110402, rs242924, rs242939	Not gender-specific association with Unipolar Depression (Sarubin et al., 2016).
CRHR1	rs878886, rs173365, rs242937, rs110402, rs12936511, rs4792887, rs4076452, rs16940655	Not associated with lithium treatment response in BD patients (Szczepankiewicz et al., 2018).

MDD = major depressive disorder; BD = Bipolar Disorder.

Table 3 - Gene candidate studies results obtained from the literature review involving suicidality.

Gene (s) of CRF system	SNP(s)	Association Results
CRHR1	rs16940665	Associated with suicide attempt and MDD patients (Pawlak et al., 2016).
CRHR1	rs16940665	Associated with suicide attempt and BP patients (Sanabrais-Jiménez et al., 2019).
CRHR1	rs4792887	Not associated with suicidal behavior in adolescents (Mirkovic et al., 2017).
CRHR1	rs110402	Associated with suicide attempt and IL-1β levels (Bastos et al., 2016).
CRHBP	rs7728378	Not associated with childhood trauma and suicidal behavior after multiple correction (Segura et al., 2019).

MDD = major depressive disorder; BD = Bipolar Disorder.

Table 4 - Gene candidate studies results obtained from the literature review involving anxiety.

Gene (s) of CRF system	SNP (s)	Association Results
CRHR1	Haplotype block rs17689966 - rs4792825	Associated with the risk of panic disorder in women (Weber et al., 2016).
CRHR1	rs110402, rs242924, rs4792887, rs7209436	Ethnicity-specific association with anxiety sensitivity (Womersley et al., 2018).
CRHR1	rs7209436, rs110402, rs242924	Associated with *FAAH* genotype, cortisol level and anxiety (Harris et al., 2019).

Table 5 - Gene candidate studies results obtained from the literature review involving PTSD.

Gene (s) of CRF system	SNP (s)	Association Results
CRHR1	rs17689918	Absence of association with PTSD symptoms in war veterans (Jaksic et al., 2019).
CRHR1	rs110402	Not associated with GSK561679 treatment response in PTSD patients (Dunlop et al., 2017).

PTSD = Post-traumatic stress disorder

Highlights

- CRHR1 was the main target gene in studies involving CRH genes.

- Gene candidate was the only approach used in the surveys.

- The genetic regions (SNPs) of analysis have been excessively delimited.

- Confounding factors that contribute to the generation of false results were detected.

3. Conclusões e perspectivas

Transtornos complexos sofrem influência de muitos genes de pequeno efeito, então a associação encontrada entre apenas uma variante genética e patologias, embora possa contribuir para o aparecimento do fenótipo, não é suficiente para o esclarecimento total de sua etiologia e, além disso, mesmo que todas as variantes fossem estudadas, os cenários ambientais a que indivíduos são expostos são altamente distintos, o que torna difícil estimar a compatibilidade entre eles.. Os transtornos relacionados ao estresse enquadram-se nessa categoria, e ao investigar sua etiologia através da análise do impacto da variabilidade dos genes que compõem o sistema CRH foi possível observar que as associações reportadas possuem baixa reprodutibilidade. Acredita-se que isso se deva, principalmente, à alta chance de associações não-causais devido à região genética alvo ser excessivamente pequena e à presença frequente de viés ao incluir fatores de confusão que contribuem para a geração de resultados falso-positivos e falso-negativos. Além disso, na pesquisa psiquiátrica as ferramentas de diagnóstico utilizadas dificultam o recrutamento apropriado de afetados, uma vez que os indivíduos não deveriam apresentar quadros heterogêneos em estudos caso-controle. Nesse sentido, avançar na compreensão dos transtornos de modo categórico para o dimensional pode ser uma boa fonte de contribuição.

Nos estudos de interação do gene × ambiente envolvendo o impacto do estresse durante o período de desenvolvimento, uma abordagem de pesquisa mais direcionada levando em consideração a idade em que os eventos adversos ocorreram poderia contribuir para a detecção de janelas de vulnerabilidade. Por fim, a combinação da análise genética e epigenética pode trazer um melhor entendimento dos mecanismos subjacentes ao modo de interação entre o genoma e o ambiente.

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ANEXO A – NORMAS DE PUBLICAÇÃO DA REVISTA JOURNAL OF AFFECTIVE DISORDERS

Journal of Affective Disorders - Guide for Authors

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