UNIVERSIDADE DE LISBOA

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DEPARTAMENTO DE BIOLOGIA VEGETAL

Molecular Genetics of Resilience

Inês Rodrigues da Silva Zêzere

Mestrado em Biologia Molecular e Genética

Dissertação orientada por:
Dra. Astrid Moura Vicente

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ACKNOWLEDGMENTS

First and foremost, I want to thank my supervisor Dra. Astrid Moura Vicente for accepting me in the Neurogenetics and Mental Health group at the National Institute of Health Dr. Ricardo Jorge, giving me the opportunity to work in the field that I always wanted to. I want to thank the whole Health Promotion Department who welcomed me this past year and made me feel right at home, but specially the Neurogenetics and Mental Health group, who always encouraged me and never failed to help me when I needed.

This work would not have been possible without Dra. Maria João Heitor, who provided the population in study and the psychosocial data used.

I want to thank João Costa, from the Instituto Gulbenkian de Ciência, for all the help regarding the Sequenom MassARRAY.

To Cláudia Branco, for all her help with the Arlequin software, even at a distance.

To professor Lisete and Cláudia Mendes, who had the patience to explain statistics to a biologist, for their help and availability.

To Carla Feliciano, for all the help in the lab, but mainly for all the patience and friendship throughout this year.

A special thank you to Marta, Célia, Alexandra and João, not only for the coffees and jokes shared, but mainly for the friendship and for making my days much more special.

To Miguel Ramos, whose teachings I still carry to this day.

I want to thank my family, but specially my parents and my brother Ricardo, for the unconditional love and support. For all the times they pretended to understand what I was talking about and for giving me strength when I had none.

A big thank you to Francisco, that despite all these years, never doubted me and still has the patience to endure all my craziness. For always listening no matter the time, the place or the subject.

To my friends, new or old, here or far away, whose support never wavered, whose friendship was always available no matter what. I thank each and every person that ever had to hear me say the words “I cannot do this”. We did it.

2016

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RESUMO ALARGADO

Resiliência é a capacidade de ultrapassar situações de stress e adversidade de modo adaptativo, mantendo um funcionamento psicológico e físico normal. Como característica intrínseca, a resiliência é influenciada por variáveis externas, como a experiência pessoal e o suporte social, mas também por fatores genéticos que conferem suscetibilidade ou resistência, revelando assim a enorme complexidade por detrás da genética das variações comportamentais e doenças do foro psicológico.

Embora os mecanismos subjacentes à resiliência ainda não estejam bem definidos, tudo indica que a predisposição genética do indivíduo juntamente com a interação com fatores ambientais modulam os sistemas neurológicos e neuroquímicos, nomeadamente o eixo hipotálamo-pituitária-adrenal (HPA), o sistema noradrenérgico e os sistemas serotonérgico e dopaminérgico, desta forma levando à variabilidade na resiliência ao stress. Deste modo, a maioria dos estudos de associação genética relativos a este traço psicológico têm recaído sobre os genes relacionados com estes sistemas.

O eixo HPA é o coordenador central dos sistemas neuroendócrinos de resposta ao stress, tal como o sistema nervoso central, o sistema metabólico e o sistema imunitário, através de uma resposta em cascata iniciada no hipotálamo que liberta a hormona libertadora de corticotrofina (CRH), que estimula a libertação da hormona adrenocorticotropica (ACTH) por parte da pituitária, e que consequentemente estimula o córtex adrenal a libertar glucocorticoides para o sistema circulatório, proporcionando assim uma resposta adequado ao estímulo. Genes como *FKBP5* e *CRHR1* influenciam diretamente o eixo HPA ao regular a atividade dos glucocorticoides e de CRH, respetivamente. Por outro lado, genes como *BDNF*, *OXTR* e *NPY* atuam sobre estruturas reguladoras deste eixo, tal como o hipotálamo e a amígdala.

O sistema nervoso central assim como o sistema nervoso simpático são responsáveis pela libertação de epinefrina e norepinefrina, estando envolvidos na regulação dos processos emocionais. Os genes *MAOA* e *COMT* são genes responsáveis pela inativação destes neurotransmissores, e como tal estão bastante envolvidos no bom funcionamento do sistema nervoso central e do sistema nervoso simpático.

Por outro lado, os sistemas dopaminérgico e serotonérgico modulam a atividade do eixo HPA ao projetar tanto para as estruturas que o constituem como para as que o regulam, estando estes sistemas bastante envolvidos no processamento emocional e no controlo do estado de humor. O gene *SLC6A4* é um importante determinante da neurotransmissão da serotonina ao regular o seu término e recaptação, enquanto que o gene *SLC6A3* é responsável pela recaptação da dopamina, regulando assim a sua neurotransmissão. Já o gene *DRD4* apresenta elevada variabilidade e codifica para um recetor de dopamina com expressão em diversas áreas do cérebro, tendo sido implicado em diversos distúrbios psiquiátricos.

O estudo da resiliência e dos mecanismos a ela associados é de enorme relevância, não só porque permitem retratar e compreender melhor a genética dos traços de personalidade e dos distúrbios psiquiátricos, mas também porque a identificação de genes candidatos poderá permitir o desenvolvimento de novos marcadores para exames médicos, e a identificação de fatores protetores poderá prevenir respostas inadaptadas ao stress e assim ajudar a promover a resiliência e a saúde mental.

Assim, este projeto visa compreender a genética molecular da resiliência, identificando os genes e marcadores moleculares que a ela poderão estar associados, assim como outros fatores externos que poderão influenciar este traço.

Para tal, selecionou-se os genes e polimorfismos com maior evidência de estarem associados à resiliência, assim como a distúrbios psiquiátricos, nomeadamente depressão e distúrbios de ansiedade, através de uma extensa revisão bibliográfica, e identificou-se o genótipo, para os marcadores moleculares e genes selecionados, da população em estudo, 261 indivíduos portugueses, cujos componentes psicossociais já tinham sido previamente avaliados, através da aplicação de um questionário que continha a escala de resiliência de Connor-Davidson, entre outras. Para a genotipagem foram utilizados diversos métodos de biologia molecular, nomeadamente Sequenom MassArray, uma tecnologia que permite a genotipagem por espetrometria de massa, e sequenciação por método de Sanger, nos casos em que a primeira genotipagem não foi clara. Foi também utilizado para um dos polimorfismos um TaqMan® 5- nuclease assay, uma tecnologia baseada na técnica de reação em cadeia da polimerase (PCR) em que a região flanqueadora do polimorfismo é amplificada na presença de sondas de fluorescência específicas, assim como PCR seguido de eletroforese em gel de agarose, para analisar os *variable number of tandem repeats* (VNTRs). De seguida, a inferência estatística permitiu avaliar a associação entre o genótipo dos

indivíduos e os valores de resiliência, tendo em consideração outros parâmetros que pudessem influenciar este traço.

Analisámos com sucesso 39 polimorfismos, após termos submetido todos os resultados obtidos pela genotipagem a um controlo de qualidade. Os polimorfismos excluídos da análise foram, portanto os que apresentavam um desvio do equilíbrio de Hardy-Weinberg e/ou os os genótipos dos indivíduos HapMap não correspondiam ao esperado, e como tal não passaram no controlo de qualidade. Os polimorfismos que passaram no controlo de qualidade, mas que se apresentaram como monomórficos também foram excluídos da análise, visto não serem uteis para um estudo de associação. Não nos foi possível genotipar com confiança os indivíduos para os VNTRs do gene *MAOA* e *SLC6A4*, devido a um artefacto visível no que se depreende ser os indivíduos heterozigóticos, possivelmente causado pelo "*reannealing"* de fragmentos complementares com sequências diferentes.

A análise estatística univariada revelou associações entre a existência de depressão e ansiedade e menor resiliência, enquanto que maiores níveis de educação, como estudos pós-graduados e cursos profissionais, indicavam uma maior resiliência. A análise univariada também demonstrou a ausência de associação entre a resiliência e a idade, tomar comprimidos para dormir, o gênero e a tensão arterial.

As outras escalas aplicadas na componente psicossocial eram responsáveis por avaliar o suporte social, a felicidade subjetiva, o estado de saúde mental e a presença de sintomatologia física e psicológica associada a stress. A análise revelou que maiores níveis de felicidade subjetiva, de estado de saúde mental e a ausência de sintomatologia associada a stress sugeriam maior resiliência. Porém, verificou-se que menor suporte social estava correlacionado com maior resiliência, o que vai contra o esperado e poderá ser devido ao tamanho da amostra.

De todos os genes avaliados neste estudo, a análise univariada detetou apenas uma associação entre a resiliência e um polimorfismo do gene *MAOA*. Porém a análise haplotípica envolvendo este polimorfismo não revelou qualquer associação, o que aponta para um falso positivo.

Ao realizar uma análise multivariada, ficou evidente que emoções e humores positivos assim como a ausência de sintomatologia psicológica relacionada a stress moderam a resiliência, demonstrando como um pensamento positivo permite construir melhores mecanismos de defesa

contra situações adversas, assim como é elucidativo da importância de uma resposta biológica ao stress adequada e flexível, de modo a manter um funcionamento físico e psicológico normal em contexto de adversidade.

A análise multivariada também revelou uma associação entre o polimorfismo rs53576 (G>A) pertencente ao gene *OXTR*, demonstrando que indivíduos que possuíam duas cópias do alelo considerado de risco (A) têm menor resiliência, deste modo indicando a influência deste gene no mecanismo biológico de resposta ao stress e na variabilidade deste traço.

Por último, identificou-se também uma associação entre o alelo de risco (9 repetições) do VNTR do gene *SLC6A3* e menor resiliência, o que vai de encontro a estudos anteriores que indicam que este alelo leva a uma menor atividade da proteína, afetando deste modo o mecanismo de resposta ao stress, e podendo assim causar variabilidade na capacidade de ultrapassar adversidades. É de denotar que na análise genotipíca, apenas obtivemos associação com o genótipo heterozigótico, e não para o genótipo homozigótico para o alelo de risco, e que apenas a análise alélica é que nos permite confirmar a associação entre o alelo de risco e o fenótipo de resiliência. Isto terá ocorrido devido à falta de representação deste genótipo na população em estudo.

Este estudo apresenta algumas limitações, nomeadamente o tamanho da amostra, que se revelou pouco representativa em alguns casos. Deverá ter-se em conta que o pretendido era analisar o impacto de cada um dos marcadores em separado, e não obter um único modelo preditivo que explicasse os valores de resiliência, e assim não sentimos necessidade de corrigir os resultados aqui apresentados para testes múltiplos, que devem ser vistos como exploratórios. É de denotar também que a resiliência é um traço extremamente complexo e que não foi possível avaliar todos os genes envolvidos nos sistemas neurológicos e neuroquímicos nem todos os fatores ambientais que poderão influenciar a resiliência, e como tal os resultados descritos aqui poderão não se revelar verdade em estudos de maiores dimensões.

Em conclusão e resumidamente, o presente trabalho fornece fortes evidências da influência da composição genética, bem como outras características pessoais, na resiliência.

Palavras – chave: resiliência, distúrbios psiquiátricos, stress, genética, fatores ambientais

ABSTRACT

Resilience is a personality trait defined as the capacity to adaptively overcome stress and adversity while maintaining normal psychological and physical functioning. The study of resilience is of great interest and promise as it can provide insight on the genetics that underlie some personality traits and psychiatric disorders, like post-traumatic stress disorder (PTSD) and help identify candidate genes that could potentially be used as markers for medical testing, as well as protective factors that can help promote resilience. Although the complex mechanisms that underlie resilient phenotypes are not yet fully understood, evidence suggests that an individual's genetic make-up and the interaction with environmental factors shape the neurochemical and neurological systems, mainly the hypothalamus-pituitary-adrenal (HPA) axis, the noradrenergic system, and the serotonergic and dopaminergic systems, therefore modulating the variability in stress resilience. As such, the vast majority of the association studies relative to resilience have focused on genes linked to these systems. In this study, we genotyped 261 Portuguese individuals for genes and polymorphisms that had been formerly linked to resilience or psychiatric disorders and tested the results for association with the resilience scores previously obtained, considering as well other psychosocial characteristics. The analysis revealed an association between positive emotions and the absence of psychological symptomology associated to stress and higher resilience, which is demonstrative of the impact of a positive mind-set and a flexible biological stress response on resilience variability. After adjusting for all confounding non-genetic variables, it was also noticeable an association between the rs53576 (G>A) polymorphism of the *OXTR* gene, as well as the *SLC6A3* 40 bp VNTR and resilience, with the risk alleles of each polymorphism being associated with lower resilience, therefore indicating a functional impact of these variants on the stress response mechanism and demonstrating their influence on resilience variability. In conclusion, this study points to the influence of genetic factors as well as environmental factors on resilience, and the importance of studying these two components to truly understand this complex trait.

Key words: resilience, psychiatric disorders, stress, genetics, environmental factors

ABBREVIATIONS

5-HTT - serotonin transporter protein

5HTTLPR - serotonin transporter-linked promoter region

ACTH - adrenocorticotropic hormone

ADHD – attention deficit hyperactivity disorder

ASSET – a shortened stress evaluation tool

AVP - arginine vasopressin

BDNF - brain-derived neurotrophic factor

CD-RISC - the Connor-Davidson resilience scale

CNS - central nervous system

COMT - catechol O-methyltransferase

CRH - corticotrophin-releasing hormone

CRHR1 - corticotropin-releasing hormone receptor 1

DAT - dopamine transporter

DRD4 - dopamine receptor D4

FKBP5 - FK506-binding protein 5

HPA axis – hypothalamus – pituitary – adrenal axis

LC - *locus coeruleus*

MAOA - monoamine oxidase A

MHI5 – mental health index

NPY - neuropeptide Y

OXTR - oxytocin receptor

PCR – polymerase chain reaction

PTSD – post-traumatic stress disorder

PVN – paraventricular nucleus

SHS – subjective happiness scale

SLC6A3 - solute carrier family 6 member 3 gene

SLC6A4 - serotonin transporter gene

SNP – single nucleotide polymorphism

SNS – sympathetic nervous system

VNTR – variable number of tandem repeats

1 INTRODUCTION

Most people at different points in their lives will experience distressing, if not debilitating, events but not everyone has the same reaction [1]. Individual differences have been reported in how people respond to trauma, with some people revealing a greater capacity to overcome adversity, whilst others are more vulnerable [1–3].

Resilience can be defined as the capacity to adaptively overcome stress and adversity while maintaining normal psychological and physical functioning, and not merely as the absence of psychopathology [4,5]. Thus a resilient individual is one that has experienced a traumatic event and continues to demonstrate adaptive psychological and physiological stress responses [6]. As a personal characteristic, resilience is likely influenced by external variables, such as adequate social support, that reduces the risk of stress-related mental disorders by buffering the impact of stress [7,8].

Understanding the impact of trauma and how resilience is developed and enhanced is therefore of great relevance in current times, in order to not only promote coping mechanisms but also mitigate maladaptive coping and stress response in psychiatric illnesses, such as depression and post-traumatic stress disorder (PTSD) [4,9,10].

1.1 GENETICS IN MENTAL HEALTH, PSYCHIATRIC TRAITS AND RESILIENCE

It is well known that genetic factors contribute to practically almost every human disease, whether by conferring susceptibility, resistance or by influencing severity and progression, as alterations in the DNA sequence of genes may modify protein expression, which can impact biological functions. Regarding mental health, personality traits and psychiatric disorders, these are extremely complex traits resulting from the intricate wiring of the neurochemical and neurological systems, the interaction between multiple genes linked to these systems and the interplay between multiple genes and environmental factors [11,14].

Character traits are considered to be acquired during development and influenced by sociocultural learning, with evidence indicating a heritable component. This suggests the influence of a genetic element in the individual variability of psychological traits. There are several neurological systems that are assumed to regulate personality, namely the dopamine, serotonin and

noradrenergic systems, and so the study of genes involved in these pathways is of an extreme promise for a better understanding of personality and personality disorders [14].

Resilience, as a personal characteristic, is likely mediated by several environmental factors, as well as genetic and neural mechanisms. Although the range of complex mechanisms that underlie resilient phenotypes is not yet fully understood, evidence suggests that an individual's genetic make-up and the interaction with environmental factors shape the neurochemical and neurological systems, mainly the hypothalamus-pituitary-adrenal (HPA) axis, the noradrenergic system, and the serotonergic and dopaminergic systems, therefore modulating the variability in stress resilience [6].

The study of resilience and the mechanisms that underlie this trait is of great interest, because not only can it provide insight on the genetics behind psychiatric disorders, like PTSD, depression or anxiety disorders, but the identification of candidate genes may provide a starting point for the development of new, useful markers for medical testing, and the identification of protective factors that can help promote resilience and help prevent maladaptive responses to trauma, as well as mitigate mental health issues [6,14,15].

The most common approach to identify the genetic variants underlying a certain phenotype is the genetic association approach, in which a group of unrelated individuals with a certain phenotype is compared for alleles or genotypes, in order to identify candidate genes or genome regions that contribute to disease [14,16]. Regarding resilience, the vast majority of these studies have fallen upon the genes involved in the neurochemical and neurological systems that underlie the stress response mechanism [6].

1.2 THE STRESS RESPONSE

The stress response is understood as the adaptive physiological and psychological processes activated whenever there is a discrepancy between what an organism is expecting and what really exists [17]. The stress response is not harmful in itself but, upon prolonged and demanding stressful situations, homeostasis can be threatened and health may be endangered, since stress can lead to alterations in several neurochemicals that modulate neural circuits, including those involved in the regulation of reward, fear conditioning and social behaviour [17,18].

A suitable stress response is of absolute necessity for sustained health in the face of adversities and for reducing mental health disturbances after exposure to severe adversities. The

major neural systems responsible for the stress response are comprised by the HPA axis, the noradrenergic and sympathetic nervous system (SNS) and the dopaminergic and serotonergic neurotransmitter systems [5].

Resilience has thus been associated with the flexibility of the neurochemical stress response systems as well as the neuronal circuitry involved in the stress response. Therefore, it is possible that genetic make-up can influence resilience through impact on several neurochemical stress pathways [19].

1.3 THE HPA AXIS

The HPA axis is the central coordinator of the mammalian neuroendocrine stress response systems and includes the paraventricular nucleus (PVN) of the hypothalamus, the anterior lobe of the pituitary gland, and an effector organ, the adrenal glands [20,21].

When exposed to stressful stimuli, neurons in the PVN of the hypothalamus release two neurohormones – corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP) – into the blood vessels connecting the hypothalamus and the pituitary. Both these hormones stimulate the anterior pituitary gland to produce and secrete adrenocorticotropic hormone (ACTH) into the general circulation. In turn, the ACTH induces glucocorticoid synthesis and release from the cortex of the adrenal glands. Glucocorticoids modulate metabolism as well as immune and brain function, thereby orchestrating an adequate behavioural response to manage stress [21,22](Fig. 1).

The HPA axis is carefully modulated through elaborate negative feedback systems designed to maintain predetermined hormone levels and homeostasis [5,22]. To this end, secretion of CRH, AVP and ACTH are in part controlled by sensitive feedback exerted by glucocorticoids at the level of the hippocampus, PVN and pituitary gland [22] (Fig. 1).

The HPA axis is also under the inhibitory control of the hippocampus as well as the excitatory control of the amygdala [23]. The hippocampus is implicated in learning and long-term memory formation [2,24] and restrains PVN activity, as well as most aspects of the HPA axis, including the onset and termination of stress responses, through binding of glucocorticoids to hippocampal receptors [20,25,26] (Fig.1).

In contrast, the amygdala, as a part of the limbic system, is associated with processing memories and emotional reactions and appears to be critical in activating the HPA axis in response

to cognitive-emotional challenge and threat [2,24]. Glucocorticoid occupation of the amygdala receptors can facilitate the activity of the HPA axis, often increasing CRH production within the amygdala [24] (Fig.1).

Several genes have been known to have an effect on HPA axis, either by influencing the activity of the hormones involved in this system, like the *FKBP5* and *CRHR1* gene, or by impacting structures, namely the hippocampus and the amygdala, that regulate the HPA axis, as is the case of *BDNF*, *NPY* and *OXTR* gene. Due to their function and impact on biological systems, these genes have been associated not only with resilience but also with many psychiatric disorders, such as depression and PTSD [4,27].

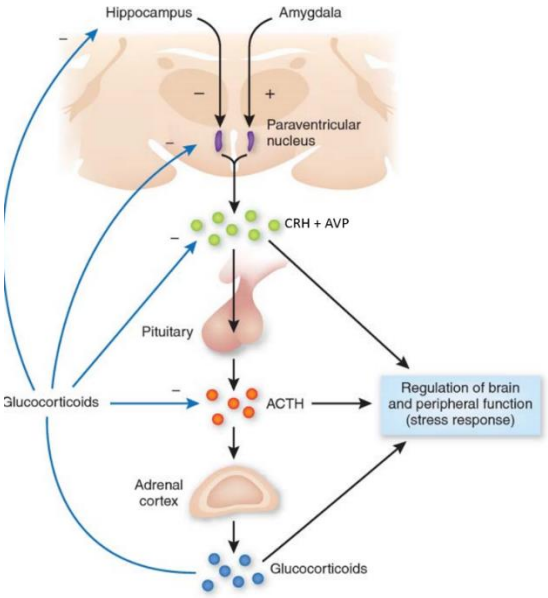

Fig. 1 - The hypothalamic-pituitary-adrenal (HPA) axis. Upon a stressful situation, the paraventricular nucleus (PVN) of the hypothalamus releases corticotrophin-releasing hormone (CRH) and arginine vasopressin (AVP), which stimulate the pituitary to produce adrenocorticotropic hormone (ACTH), which in turn stimulates the release of glucocorticoids from the adrenal cortex, allowing an adequate response. This system is under the inhibitory control of the hippocampus and a sensible negative feedback system, as well as the stimulatory control of the amygdala; (+) – stimulation; (-) – inhibition. Adapted from *Hyman, 2009* [11].

1.3.1 FKBP5 gene

The correct function of glucocorticoid receptors (GR) is dependent of a large molecular complex, necessary for proper ligand binding, receptor activation and transcriptional regulation of target genes [28]. The *FKBP5* gene encodes the FK506-binding protein 5 (FKBP5), a co-chaperone of heat shock protein 90 (hsp90), which binds to the GR. Upon ligand binding, FKBP5 allows the translocation into the nucleus where the complex regulates the expression of glucocorticoid-responsive genes by functioning as a transcription factor [29,30].

1.3.2 CRHR1 gene

The corticotropin-releasing hormone receptor 1 gene (*CRHR1*) encodes the G-protein coupled type 1 CRH receptor (CRHR1) that acts as a key activator of the HPA axis, by binding to receptors that initiate the stress response [31–33]. In addition to its effects on the HPA axis, CRH activity at extra-hypothalamic regions is also thought to produce symptoms of anxiety and depression [34].

1.3.3 BDNF gene

The *BDNF* gene encodes the brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family of polypeptide growth factors that is widely expressed in the mammalian brain and has a crucial role in the regulation of hippocampal plasticity and learning processes dependent of this brain region [35–37]. BDNF activity contributes to various forms of emotional and cognitive learning, as well as spatial and contextual learning, and has been implicated in several psychiatric disorders, including depression, anxiety, and PTSD [35,36].

1.3.4 OXTR gene

The *OXTR* gene encodes the oxytocin receptor (OXTR) by which the hormone oxytocin (OXT) exerts a range of effects throughout the body and brain, with central actions in the limbic system, the forebrain and the automatic centres of the brainstem [38,39]. OXT has widespread receptor-mediated effects on behaviour and physiology, including modulation of HPA axis and amygdala reactivity, as well as attachment processes and social cognition [27,40,41].

1.3.5 NPY gene

The *NPY* gene encodes the neuropeptide Y (NPY), a 36 amino-acid peptide highly conserved among species and with a broad distribution in the central nervous system (CNS) [42,43]. NPY plays a role in the regulation of numerous basic physiological functions, such as circadian rhythm, neuronal excitability, and addictions, as well as modulation of emotional responses to various stressors, and is thought to facilitate the containment of negative consequences following exposure to stress, therefore being recognized as a major neurochemical factor for post-traumatic resilience and recovery in humans [42,43].

1.4 NORADRENERGIC AND SYMPATHETIC NERVOUS SYSTEM

Epinephrine and norepinephrine, of the catecholamine family, have a key role in stress response by being involved in the regulation of emotional processes, acting as a hormone or as a neurotransmitter. Their effects are mediated by adrenergic receptors located in several neurons and glial cells in the brain and they are released in the blood stream by the CNS, mainly the *locus coeruleus* (LC), the primary noradrenergic nucleus in the brain, as well as the SNS [17,44].

Upon a stressful situation, the activation of the noradrenergic system results in increased release of norepinephrine from the LC to its projection sites, which include the amygdala, prefrontal cortex and hippocampus, resulting in the inhibition of the prefrontal cortex, a structure implicated in planning complex cognitive behaviour, thereby favouring instinctive responses [5,45] (fig. 2A). The release of norepinephrine also plays a key role in the consolidation of negative emotional memories and additionally projects the amygdala to further stimulate its activation in a positive feedback fashion [45]. Thus, a hyper response of the noradrenergic system is usually associated with anxiety disorders and cardiovascular problems [46].

The primary role of SNS is establishing a “flight-or-fight” response upon a traumatic or stressful event, preparing the organism for action through the increase of circulating levels of epinephrine and norepinephrine, heart rate, peripheral vasoconstriction and energy mobilization. Stress exposure results in activation of preganglionic sympathetic neurons in the spinal cord, leading to the release of norepinephrine and epinephrine in the blood stream by the postganglionic neurons and the medulla of the adrenal glands, respectively [47] (fig. 2B).

A hyper-responsive SNS can lead to a diminished biological response to stress due to the continuous stimulation of adrenergic receptors, therefore contributing to hypervigilance, fear, intrusive memories and increased risk for hypertension and cardiovascular disease [48,49]. Thus, resilient individuals are those able to maintain SNS activation within a window of adaptive elevation, which would be high enough to ensure an accurate response but no so high as to lead to incapacity, anxiety and fear [48].

The *COMT* and *MAOA* genes have been tested as candidate genes for psychiatric disorders, such as affective disorders and attention deficit hyperactivity disorder (ADHD), as they have been associated with the good functioning of the noradrenergic and sympathetic nervous systems, due

to their regulatory role on the neurotransmission of epinephrine, norepinephrine, among others neurotransmitters [50–53].

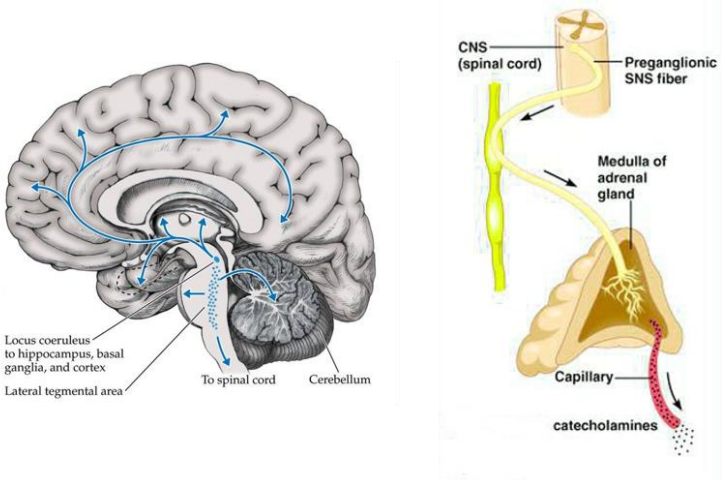

Fig. 2 - (A) Projection sites of the locus coeruleus; (B) – Sympathetic nervous system pathway. (A) The locus coeruleus releases norepinephrine to its projection sites, namely the amygdala, prefrontal cortex and hippocampus. (B) Upon a stressful stimulus, the preganglionic sympathetic neurons are activated and lead to the release of norepinephrine and epinephrine in the blood stream by the postganglionic neurons and the medulla of the adrenal glands, respectively. Adapted from (A) *Rosenzweig, M. R., Breedlove, S.M., & Watson, n. V.* (2005)[54], (B) *Marieb, E.N., & Hoehn, K.* (2013)[56].

1.4.1 COMT gene

The *COMT* gene encodes the catechol O-methyltransferase (COMT) enzyme involved in the inactivation of the catecholamines, including dopamine, epinephrine, and norepinephrine, and is the main factor controlling dopamine levels in the prefrontal cortex [51,52,55]. A reduction in enzyme activity leads to a slower catalysis of catecholamines, and as such it has been implicated in a number of psychiatric disorders, including psychotic, affective and anxiety disorders [51,52,55,57].

1.4.2 MAOA gene

The *MAOA* gene, located in the X chromosome, encodes the monoamine oxidase A (MAOA) enzyme, responsible for breaking down neurotransmitters, such as norepinephrine, serotonin, and dopamine, leading to their inactivation [50,58]. Due to its important role in the serotonergic and dopaminergic pathways, this gene has been implicated in various mental health conditions in both children and adults, including major depressive disorder, autism spectrum disorders, aggressive behaviours, panic disorder, and ADHD [53].

1.5 THE DOPAMINERGIC AND SEROTONERGIC SYSTEMS

The stress-responsive mesocorticolimbic dopaminergic system has an important role in the control of mood, since dopamine is one of the most predominant catecholamine neurotransmitters in the brain, capable of modulating the mechanisms underlying states of fear and anxiety [59,60].

Both the mesocortical and mesolimbic components of the dopaminergic systems are innervated by PVN CRH neurons and the LC - noradrenergic system and are therefore activated during stress [61]. The mesocorticolimbic system consists of dopaminergic neurons of the ventral tegmental area (VTA), which sends projections to the nucleus accumbens as well as the limbic regions, including the amygdala, hippocampus and prefrontal cortex, and is involved in anticipatory phenomena and cognitive functions, as well as being associated with inhibition of the stress system [61,62] (fig. 3).

Serotonin is a neurotransmitter capable of exerting a wide influence over several brain functions. In the brain it is synthesized exclusively in serotonergic neurons located in the raphe nucleus of the brainstem and project to nearly all parts of the CNS, thereby making the serotonergic network one of the most diffused neurochemical systems in the brain [17,63,64] (fig. 3). The widespread distribution of serotonergic fibres accounts for the large variety of functions that are modulated by serotonin, including thermoregulation, emotional processing, and cardiovascular function [17,63,64].

Serotonin plays a regulatory role on stress-induced HPA activity through direct actions at the hypothalamic, pituitary and adrenal level, influencing the secretion of glucocorticoids in a stressor-dependent manner [63,65,66]

Genes like *SLC6A4* and *SLC6A3* are main regulators of the neurotransmission of dopamine and serotonin, and have been known to influence the functioning of the dopaminergic and serotonergic systems and lead to variability in stress sensitivity. On the other hand, the *DRD4* gene has been known to be highly polymorphic and have a wide area of expression in the brain, and so has been linked to several neuropsychiatric disorders [67–69]. These genes have also been targets for drugs used for psychiatric stress-related disorders [70].

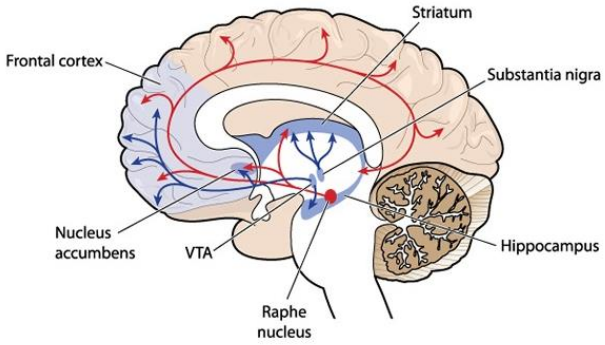

Fig. 3 - Dopaminergic and serotonergic projections. The dopaminergic neurons of the ventral tegmental area (VTA) send projections to the prefrontal cortex, amygdala, hippocampus and the nucleus accumbens. The serotonergic neurons located in the raphe nucleus project to nearly all parts of the central nervous system (CNS), namely the prefrontal cortex, striatum and substantia nigra; blue pathway – dopaminergic pathway; red pathway – serotonergic pathway; Adapted from: Blamb, Image ID: 329843900 via shutterstock.com [71].

1.5.1 SLC6A4 gene

The serotonin transporter gene (*SLC6A4*) encodes the serotonin transporter protein (5-HTT), responsible for terminating serotonergic neurotransmission and recycling supplies of serotonin [67,72]. The serotonin transporter-linked promoter region (5HTTLPR) influences its transcriptional activity, and has been known to moderate psychopathological reactions to stressful experiences, usually being associated with differences in the susceptibility to major depression or depressive symptoms [7,73,74]

1.5.2 SLC6A3 gene

The solute carrier family 6 member 3 (*SLC6A3*) gene encodes a sodium-dependent dopamine transporter (DAT) responsible for the reuptake of dopamine into the presynaptic terminals, therefore playing a key role in the regulation of dopaminergic neurotransmission [68,75,76]. Alterations in gene expression have an impact on the dopamine transporter function, and has thus been associated to PTSD and ADHD [76,77].

1.5.3 DRD4 gene

The human *dopamine receptor D4* gene (*DRD4*) is a highly polymorphic gene with great impact in susceptibility to environmental influences [69,78,80]. The DRD4 protein is expressed in several brain regions, with a high level of expression in the prefrontal cortex, and therefore has received particular attention because of its possible role in neuropsychiatric disorders [79,81].

1.6 THE "IMPACT ASSESSMENT ON EMPLOYMENT STRATEGIES FOR HEALTH - BIOPSYCHOSOCIAL DETERMINANTS IN EMPLOYMENT" PROJECT

The "Impact Assessment on Employment Strategies for Health – biopsychosocial determinants in employment" is a project led by Dra. Maria João Heitor, director of the Department of Psychiatry and Mental Health of the Beatriz Ângelo Hospital, in a partnership between the Institute of Preventive Medicine (IMP) of the Faculty of Medicine of Lisbon (FML) / University of Lisbon (UL), the National Institute of Health Doctor Ricardo Jorge, IP (INSA) and the High Commissioner for Health (ACS).

In this project, an observational study was conducted with a sample of Portuguese workers by applying a psychosocial questionnaire, collecting anthropometric data and blood pressures measurements, as well as blood collections to assess biological parameters. This study was performed to have a better understanding of the work related psychosocial and biological factors that influence the spectrum between health and disease, so that ultimately interventions for health promotion can be developed and applied in the work context [82,83]. As such, the study here presented regarding the molecular genetics of resilience falls within the biological factors studied in this project.

Since the "Impact Assessment on Employment Strategies for Health – biopsychosocial determinants in employment" project studied a population in the context of work environment, where they are frequently exposed to stressful situations, it constitutes a good representation of resilience in the work place, and so can be considered a good model to assess genetic and environmental associations with resilience.

2 OBJECTIVES

Resilience is a personal characteristic described as the capacity to overcome situations of stress that would otherwise compromise the psychological and physical well-being, and so is likely influenced by environmental and genetic factors [4,5,8,84]. The study of resilience is of great importance, not only to have a better understanding of related psychiatric illness, such as post-traumatic stress disorder (PTSD), but also to promote better coping mechanisms and mitigate maladaptive responses [4,9,10].

With this study, we expect to further understand the influence of genetic variants in the response to stressful stimulus in the work environment. We also intended to evaluate how certain factors, such as gender, age, marital status, as well as social support and a positive mind-set, integrated with genetic factors, can modulate how one reacts in a context of adversity. Therefore, the main aims of this work were the identification of genetic markers, as well as other personal characteristics and external factors, that might have an impact on resilience.

For this purpose, we genotyped a population of 261 Portuguese individuals, for which we already had values of resilience and other socio-demographic, lifestyle and clinical parameters in study, for genetic variants selected through an extensive bibliographical review. We evaluated the associations found between our genetic data, the parameters and the resilience scores, searching for significant associations that could potentially explain the individual variability observed in resilience.

3 MATERIAL AND METHODS

3.1 POPULATION IN STUDY

The population in study is part of the sample collected for the project "Impact Assessment on Employment Strategies for Health – biopsychosocial determinants in employment", an observational study carried out with a sample of 400 Portuguese workers, to which a psychosocial questionnaire was applied and blood samples, anthropometric data and blood pressure measurements were collected. The psychosocial questionnaire was applied to the full sample, however blood samples were only collected for 261 individuals, and so these were the ones used for this association study (N = 261) [82,83].

The psychosocial component included self-administered online questionnaire, that comprised the Connor-Davidson resilience scale (CD-RISC), a scale with sound psychometric properties that comprises 25 items, each rated on a 5-point scale, in which higher scores reflect greater resilience [85]. The items evaluated in this scale group into 5 factors: personal competence, high standards and tenacity, trust in one's instincts, tolerance of negative affect and strengthening effects of stress, positive acceptance of change and secure relationships with others, control. spiritual influences. In this case, resilience was also evaluated using the 10 item version of the CD-RISC, obtained from the 25 item based on a psychometric analysis that allowed the identification of the 10 items that best captured the features of resilience with minimal redundancy [86].

The questionnaire also included: 1) a short stress evaluation tool (ASSET), to assess the risk of workplace stress, that comprised two discrete subscales evaluating physical health and mental health. Lower scores indicated less physical and psychological symptomatology related to stress, respectively. 2) The Mental Health Index (MHI-5) scale, a discrete scale of 5 items used for the measurement of mental health status. 3) The Oslo Social Support scale, a discrete scale of 3 items that allows overall assessment of social support. 4) The Subjective Happiness Scale (SHS), a continuous scale comprised of 4 items, which evaluates one's self-assessment of subjective happiness [87–90].

Socio-demographic, lifestyle and clinical data, including age, gender, marital status, level of education, practice of physical activity, suffering from anxiety and/or depression and taking sleeping pills, was also collected.

3.2 BIBLIOGRAPHIC REVISION

For defining the genes and associated polymorphisms to be analysed, a bibliographic revision was carried out. Using keywords, such as “gene”, “resilience”, “polymorphism”, “depression”, “PTSD” in PubMed and google scholar, we were able to retrieve main papers covering these subjects. Several criteria were applied in order to select the more relevant papers, including date of publication (2005 – 2016), targeted population (human adults) and level of evidence ($p<0.05$).

3.3 SAMPLE PREPARATION

DNA was previously extracted from blood samples using a method based on the one previously described by *Lahiri & Nuremberger*, 1991[91]. All the DNAs were quantified using using a NanoDrop 1000 Spectrophotometer, version 3.7.1 (Thermo scientific, USA), at 260 nm and the associated software, and both the ratio of absorbance at 260 nm and 280 nm as well as the ratio of absorbance at 260 nm and 230 nm ratio were evaluated in order to determine the quality of the samples. Subsequently, 3 plates of 96 wells were prepared by diluting all the 261 genomic DNA samples to a final concentration of 50 ng/µL, taking into account the initial concentration of each sample.

3.4 SNP GENOTYPING

Genetic variants can take several forms, including single nucleotide polymorphisms (SNPs), defined as a variation on a single nucleotide that occurs at a specific position in the genome, or variable number of tandem repeats (VNTRs), consisting of a DNA sequence motif that is repeated several times in the genome [11–13].

20 functional SNPs in selected genes were chosen to be genotyped based on the bibliographical review. A further 22 tag SNPs were used to analyse the full *MAOA* and *BDNF* genes, due to their influence in several neuronal pathways, mainly the hypothalamus-pituitary-adrenal (HPA) axis, and the noradrenergic and sympathetic nervous systems. The use of tag SNPs brings us the possibility to identify genetic variation and association to phenotypes without genotyping every SNP in a chromosomal region, for they are representative of a genomic region in which they are in high linkage disequilibrium with[53,92,93]. Tag SNPs were identified using the Haploview software,

version 4.2 (Broad Institute, USA), and the representative SNPs of each haplotype were chosen to be genotyped.

3.4.1 Sequenom MassARRAY

Sequenom's MassARRAY genotyping platform is a multiplex assay, which allows the simultaneous amplification and detection of multiple markers per reaction. Multiplex assay can only occur if the molecular weight of the markers in the same PLEX are not equal. It consists of an initial locus-specific polymerase chain reaction (PCR), followed by an iPLEX assay, in which an oligonucleotide primer and the amplified target DNA are incubated with mass-modified dideoxynucleotide terminators, so that annealing occurs immediately upstream of the polymorphic site. By mass spectrometry (MALDI-TOF) it is possible to distinguish allele-specific primer extension products [94].

3 plates of 96 wells were prepared by diluting all the genomic DNA samples to a final concentration of 15 ng/µL, using the initially prepared plates. Besides the population samples, 6 HapMap individuals (NA07029, NA07357, NA10850, NA12044, NA12146, NA12057) were used as positive controls as well as 5 no template controls as negative controls. We genotyped 42 SNPs using Sequenom MassARRAY genotyping technology, with iPLEX chemistry and analysed the results with the MassARRAY TYPER software (Sequenom, USA), at Instituto Gulbenkian para a Ciência (IGC, Oeiras).

3.4.2 Sanger Sequencing

For the SNPs that were not called correctly through Sequenom MassARRAY, possibly due to less efficient extension reactions, we opted for making user calls. To be confident of the user calls we sequenced those polymorphisms for key individuals that would allow us to make those calls with greater confidence, by the Sanger method [95,96].

For this purpose, primers were designed using Primer3 software, version 0.4.0 (Whitehead Institute for Biomedical Research, USA) and polymerase chain reaction (PCR) amplification was carried out in an Applied Biosystems 2720 thermal cycler (Applied Biosystems, USA), in 25 µL reactions containing 10 pmol of each primer, 2 U of BIOTAQ™ DNA Polymerase (Bioline, UK) enzyme, 25mM of $MgCl_2$ (Bioloine, UK), 2 mM dNTPs, and 25 ng of genomic DNA. Thermal cycling conditions were as follows: an initial denaturation step at 94°C for 5 minutes, followed by 30 cycles of 94°C for 30s, 56°C for 1min 30s and 72°C for 1min, and a last extension step of 72°C for 5min; for 3 SNPs

(rs7124442, rs112592173 and rs1870823) the thermal cycling conditions involved 35 cycles. PCR product analysis was carried out by electrophoresis in 1,5% SeaKem (Lonza, USA) agarose gel, using 3,5 µL of 100 bp ladder and 10 µL of PCR product plus stain. The migration occurred for 45 min at 90 V, after which we proceeded with the visualization of the genotype pattern using a system of image acquisition based on ultraviolet illumination. We followed with the purification of the PCR products, in 7 µL reactions containing 2 µL of illustra ExoProStar 1-Step (GE Healthcare, UK) and 5 µL PCR product, and the conditions were as follows: 37°C for 15 min followed by 15 min at 80°. Lastly, the sequencing reaction occurred in 10 µL reactions containing 2 µL BigDye® Terminator v3.1 Ready Reaction (Applied Biosystems, USA), 2 pmol of primer, forward or reverse according to the SNP, as well as 1 µL of purified PCR product. The sequencing conditions were as follows: an initial step of 1 min at 96°C, followed by 25 cycles of 96°C for 10 secs, 58°C for 5 secs and 55°C for 4 min. Capillary electrophoresis was performed by the Human Genetics Department of the National Institute of Health Dr. Ricardo Jorge and the chromatogram analysis was done with the Staden Package software, version 1.6.0 (Medical Research Council, UK).

3.4.3 TaqMan® 5-nuclease assay

The TaqMan® 5- nuclease assay is a PCR-based assay for genotyping SNPs, in which the region flanking the polymorphism is amplified in the presence of two allele-specific fluorescent probes, each labelled with a fluorescent reporter dye and attached with a fluorescence quencher. During PCR reaction, the 5'-nuclease activity of Taq DNA polymerase cleaves the hybridized probe that is perfectly matched, freeing the reporter dye from the quencher, and therefore generating fluorescence [97,98]. The TaqMan method is high throughput and highly accurate, precise and time-efficient, and so was chosen for genotyping the one SNP (rs27072), that due to molecular weight incompatibility with the other SNPs, was not able to fit in the Sequenom MassARRAY assay.

We carried out a TaqMan® 5-nuclease assay (C___2396868_10) in a 7900 HT fast Real-Time with fast 96-well block module (Applied Biosystems, USA) and the software associated. Reactions were performed in 96-well plates with 20 µL reaction volume containing 0.4 µL of 40x TaqMan® SNP Genotyping Assay (Applied Biosystems, USA), 2.5 µL of TaqMan® Genotyping Master Mix (Applied Biosystems, USA) and 50 ng genomic DNA. 2 Hapmap individuals (NA07029, NA07357, NA10850, NA12044, NA12146, NA12057) were used per 96-well plate as positive controls, as well as 2 no template controls.

3.5 VNTR GENOTYPING

VNTR genotyping was carried out through PCR amplification, followed by electrophoresis in agarose gel, and the genotype of each individual was defined according to the size of the fragments obtained.

3.5.1 SLC6A3 VNTR

For the SLC6A3 40 bp VNTR, the PCR protocol was based on the previously defined by Drury *et al.*, 2013, with some alterations. PCR optimization was carried out by firstly increasing the concentration of the BIOTAQ™ DNA Polymerase (Bioline, UK) enzyme to 2 U, followed by an increase in the annealing time, as well as the extension time, and lastly a decrease in cycles.

In the end, PCR was performed in an Applied Biosystems 2720 thermal cycler (Applied Biosystems, USA), using the 5' primer (forward) and 3' primer (reverse) previously described in Drury *et al.* 2013. PCR was performed in 25 µL reactions with 10 pmol of each primer, 10 xNH_4 (Bioline, UK), 25mM of $MgCl_2$ (Bioloine, UK), 2 mM dNTPs, and 25 ng of genomic DNA. Thermal cycling conditions were as follows: an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of 94°C for 30s, 58°C for 1min 30s and 72°C for 1min, and a last extension step of 72°C for 10min. The analysis of the PCR products was carried out by electrophoresis in 3% NuSieve (Lonza, USA) agarose gel, using 3 µL of 100 bp ladder and 9 µL of PCR product plus stain. The migration occurred for approximately 3 hours at 60 V, after which we proceeded with the visualization of the genotype pattern using a system of image acquisition based on ultraviolet illumination.

3.5.2 DRD4 VNTR

For the DRD4 48 bp VNTR, the amplification primers were designed with the Primer3 software, version 0.4.0 (Whitehead Institute for Biomedical Research, USA), and were as follows: forward 5'-CCGTGTGCTCCTTCTTCCTA-3' and reverse 5'-GTCTGCGGTGGAGTCTGG-3'. Using as a start the procedure described by Hwang *et al.*, 2012, optimization was carried out by firstly increasing the annealing time, followed by an increase in the extension time, and finally a decrease in cycles [99]. As such, PCR was performed in an Applied Biosystems 2720 thermal cycler (Applied Biosystems, USA) in 25 µL reactions, as described above. Thermal cycling conditions were as follows: an initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of 95°C for 20s, 54°C for 1min 30s and 72°C for 45s, and a last extension step of 72°C for 10min. The analysis of the PCR products was carried out by electrophoresis in 4% NuSieve (Lonza, USA) agarose gel, as described above.

3.5.3 MAOA VNTR

For the MAOA 30 bp VNTR, PCR amplification occurred in an Applied Biosystems 2720 thermal cycler (Applied Biosystems, USA) in 25 µL reactions as described above, with 10 pmol of each primer, previously described in Caspi *et al.*, 2002. PCR optimization was carried out by firstly testing the thermal cycling conditions defined by Caspi *et al.*, 2002, followed by trials of increased annealing time, decreased extension time, and fewer cycles. Lastly, we also tested the thermal cycling conditions previously described by Nikulina, Widom and Brzustowicz, 2012 [50,73]. The analysis of the PCR products was carried out by electrophoresis in 4% NuSieve (Lonza, USA) agarose gel, as described above.

3.5.4 5HTTLPR VNTR

Lastly, for the 5HTTLPR 23 bp VNTR, amplification primers were designed using the Primer3 software version 0.4.0 (Whitehead Institute for Biomedical Research, USA), and were as follows: forward 5'-GCCAGCACCTAACCCTAAT-3' and reverse 5'-GTGCCACCTAGACGCCAG-3'. PCR was carried out in 25 µL reactions as described above, in an Applied Biosystems 2720 thermal cycler (Applied Biosystems, USA). PCR optimization was carried out by firstly testing the conditions previously described by Cook Jr *et al.*, 1997, followed by alterations regarding the annealing time, extension time and number of cycles, as well as addition of enhancement agents, specifically DMSO (1%) (Invitrogen, USA) and gelatin from porcine skin (0.025%) (Sigma-Aldrich, USA) [100].The analysis of the PCR products was carried out by electrophoresis in 4% NuSieve (Lonza, USA) agarose gel, as described above.

3.6 Quality Control Analysis

Extensive quality control for all assays was performed by applying several criteria: Call rates of <90%, no correspondence with the positive controls, contamination of negative controls, meaning those whose spectrum peak is equal to the expected for the DNA samples, and deviation from Hardy-Weinberg equilibrium (HWE), calculated with Arlequin software, version 3.5.2.2 (University of Berne, Switzerland) led to polymorphism exclusion from the analysis. Regarding the gene located in the X chromosome, the same criteria was applied but only to the females, as males only have one copy of this gene and therefore HWE cannot be calculated for these cases. Individuals with less than 90% call rates were also excluded from the analysis.

3.7 STATISTICAL ANALYSIS

All statistical inferences were done using SPSS, version 23 (IBM, USA). To test the normality of our CD-RISC data we used the Shapiro-Wilk test, since considering our sample size this is the most potent test [101]. As there were two different versions of the CD-RISC scale, the 25 item and 10 item, we evaluated the correlation between the two scales by applying the Pearson product-moment correlation coefficient, considering a strong relationship for a coefficient higher than 0.8.

Haplotypes were inferred with the Arlequin software, version 3.5.2.2 (University of Berne, Switzerland), for the *BDNF* and *MAOA* tag SNPs, using an expectation-maximization (EM) algorithm to make maximum-likelihood estimates of molecular haplotype frequencies [102].

Univariate analysis was used to assess associations between the resilience scores and socio-demographic, lifestyle and clinical parameters, such as level of education, marital status, blood pressure, practicing physical activity, suffering from depression, suffering from anxiety and taking sleeping pills, and also the scores obtained for selected psychometric scales, namely the MHI-5, SHS, Oslo and the physical and mental health subscale of the ASSET. We considered good to fit the multivariate analysis the parameters that had a significance level <0.15 for the one-way analysis of variance (ANOVA) and were included as co-variants in the multivariate regression analysis. The association between the score values of the CD-RISC scale and the molecular markers at each gene was evaluated by linear regression analysis, considering both genotype and haplotypes for the *BDNF* and *MAOA* genes, which were all dummy coded, and alleles, by analysing the occurrence of 1 or 2 copies of each allele. For the markers located in the X chromosome, the analysis was performed for both genders separately.

Multivariate regression analysis was conducted through forward selection, in which we started by selecting the most significant variables in the univariate analysis, and continued adding other variables until there were no changes to the model [103]. It was defined that age and gender would enter the models, even if not individually associated, as these parameters are important for population context. The effect of several markers from different genes and different chromosomes was tested and the objective was to understand the impact of each marker on resilience, so a different model for each significant marker will be obtained, instead of a single model that would explain overall resilience scores, and as such no correction for multiple testing was applied [104].

4 RESULTS

4.1 BIBLIOGRAPHICAL REVIEW

An extensive bibliographical review allowed us to pinpoint the most relevant genes and polymorphisms associated with resilience, stress and psychiatric disorders, like depression and post-traumatic stress disorder (PTSD), by using the following criteria: date of publication (2005 – 2016), targeted population (human adults) and level of evidence ($p < 0.05$). In total, we selected 10 different genes and 15 associated polymorphisms linked to the stress response mechanism, especially the hypothalamic-pituitary-adrenal (HPA) axis, the noradrenergic and sympathetic nervous systems, as well as the dopaminergic and serotonergic systems (Table 1).

Table 1 - Selected genes and polymorphisms

Gene	Polimorphism	Functional Annotation	References	Genotyping Technique
COMT	rs4680	Missense	[45,51,55,57]	Sequenom MassARRAY
	rs165599	3' - UTR		Sequenom MassARRAY
	rs2097603	Intronic		Sequenom MassARRAY
BDNF	rs6265	Missense	[35,37,105]	Sequenom MassARRAY
NPY	rs16142	5'-UTR	[106–108]	Sequenom MassARRAY
	rs2023890	downstream		Sequenom MassARRAY
SLC6A4	rs25533	5'-UTR	[7,32,109–113]	Sequenom MassARRAY
	rs1042173	3'-UTR		Sequenom MassARRAY
	VNTR	Intronic		PCR and electrophoresis
OXTR	rs53576	Intronic	[27,39,41,114]	Sequenom MassARRAY
	rs2254298	Intronic		Sequenom MassARRAY
CRHR1	rs242924	Intronic	[31–34,115]	Sequenom MassARRAY
	rs4792887	Sinonymous		Sequenom MassARRAY
	rs7209436	Intronic		Sequenom MassARRAY
	rs110402	Intronic		Sequenom MassARRAY
MAOA	VNTR	Upstream	[50,116–118]	PCR and electrophoresis
FKBP5	rs1360780	Intronic	[28,29,33,119–122]	Sequenom MassARRAY
	rs3800373	3'-UTR		Sequenom MassARRAY
	rs4713916	Intronic		Sequenom MassARRAY
	rs9296158	Intronic		Sequenom MassARRAY
	rs9470080	Intronic		Sequenom MassARRAY
DRD4	rs1870723	Intronic	[123–126]	Sequenom MassARRAY
	VNTR	Exonic		PCR and electrophoresis
SLC6A3	rs27072	3'-UTR	[68,75,76,127]	TaqMan® 5- nuclease assay
	VNTR	3'-UTR		PCR and electrophoresis

Due to the big influence of the *MAOA* and *BDNF* genes in the neurologic pathways, mainly the HPA axis, as well as the noradrenergic and sympathetic nervous systems, we used tag single nucleotide polymorphisms (SNPs) in an attempt to scan the full genetic variations for association with resilience [53,92,93]. Regarding the *BDNF* gene, till November 2015 there had been described 424 SNPs in total, which could be tagged with 12 tag SNPs, whilst the *MAOA* gene comprised 3020 SNPs in total that could be tagged with 11 different tag SNPs (Table 2).

Table 2 - Selected tag SNPs for the *MAOA* and *BDNF* genes

Gene	Size	# total SNPs	# tag SNPs	Choosen polymorphisms	Functional annotation	Genotyping Technique
BDNF	67164 bp	424	12	rs6265	Missense	Sequenom MassARRAY
				rs7124442	3'-UTR	Sequenom MassARRAY
				rs11030099	3'-UTR	Sequenom MassARRAY
				rs11030101	5'-UTR	Sequenom MassARRAY
				rs11030102	Intronic	Sequenom MassARRAY
				rs4923464	Intronic	Sequenom MassARRAY
				rs189740576	Intronic	Sequenom MassARRAY
				rs77135086	Intronic	Sequenom MassARRAY
				rs75298795	Intronic	Sequenom MassARRAY
				rs76324918	Sinonymous	Sequenom MassARRAY
				rs66866077	Nonsense	Sequenom MassARRAY
				rs2030324	Intronic	Sequenom MassARRAY
MAOA	91918 bp	3020	11	rs3788862	Intronic	Sequenom MassARRAY
				rs73211189	Intronic	Sequenom MassARRAY
				rs142677545	Intronic	Sequenom MassARRAY
				rs147023114	Intronic	Sequenom MassARRAY
				rs5905809	Intronic	Sequenom MassARRAY
				rs112592173	Intronic	Sequenom MassARRAY
				rs201583370	Intronic	Sequenom MassARRAY
				rs909525	Intronic	Sequenom MassARRAY
				rs5905823	Intronic	Sequenom MassARRAY
				rs142369182	Intronic	Sequenom MassARRAY
				rs140878834	Intronic	Sequenom MassARRAY

4.2 Quality Control Analysis

Altogether, we genotyped a total of 42 SNPs by Sequenom MassARRAY (Fig. 4), 1 SNP by TaqMan® 5- nuclease assay (Fig. 4), and 2 variable number tandem repeats (VNTRs) by polymerase chain reaction (PCR) and electrophoresis.

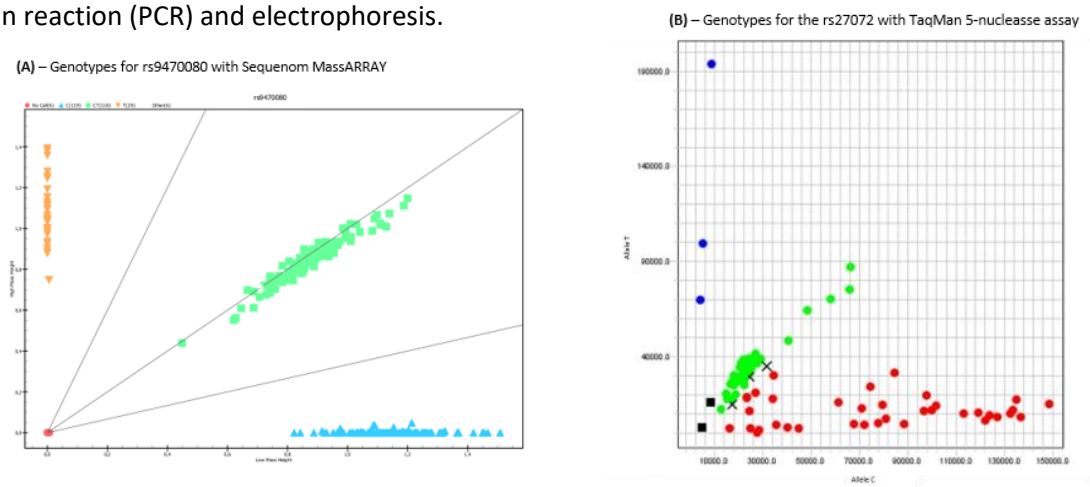

Fig. 4 - Examples of genotyping results obtained with (A) Sequenom MassARRAY (B) TaqMan 5-nuclease assay. (A) – Genotypes obtained for the rs9470080 polymorphism using Sequenom MassARRAY; Orange – TT genotype; green – CT genotype; blue – CC genotype. (B) – Genotypes obtained for the rs27072 polymorphism using TaqMan 5'-nuclease assay; blue – TT genotype; green – CT genotype; red – CC genotype.

We observed that 37 SNPs passed all the quality control criteria. We verified 6 SNPs that did not pass quality control: 1 that showed deviation from Hardy-Weinberg Equilibrium (HWE), 3 whose HapMap individuals did not correspond with the expected, and 2 others that not only showed deviation from HWE, but also whose HapMap individuals did not correspond with the expected. Furtermore, we also observed 3 monomorphic SNPs, but as they are not informative for association studies, we did not proceed with the analysis of these polymorphisms (Table S1). Of all 261 individuals, 2 had call rates <90% (AIS271 and AIS316), 2 had incomplete questionnaires (AIS40 and AIS370), and a final one (AIS334), had genotype calls for some molecular markers that were inconsistent with the gender of the individual, and so they were excluded from the analysis.

In some cases, the MassARRAY TYPER software (Sequenom, USA) did not classify the signal with confidence, and so we classified these genotypes through manual inspection. These user calls were validated by Sanger sequencing in a proportion of key individuals, so that we could have confidence in the genotype calls. For some of these SNPs (rs7124442, rs1870723 and rs11259173), we were not able to confidently assign genotypes. These SNPs presented deviation from HWE, indicating a technical artifact, thus could not be genotyped.

Variable number tandem repeat (VNTR) genotyping was carried out by polymerase chain reaction (PCR) amplification, followed by electrophoresis in agarose gel, and the genotype of each individual was defined according to the size of the fragments obtained. Regarding the 40 base pair (bp) SLC6A3 VNTR, we found the 10 repetition allele (10R) the most frequent, followed by the 9 repetition allele (9R). We also found that the most observed genotype was the homozygous 10R/10R, followed by the heterozygous 10R/9R (Fig. 5).

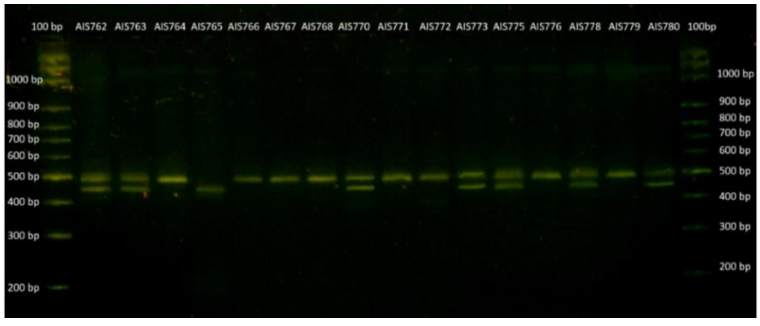

Fig. 5 - Genotype pattern for the 40 bp SLC6A3 VNTR. Lanes 4, 6, 7, 8, 10, 11, 14 and 16 are homozygous for the 10 repetition allele (480 bp), lane 5 is homozygous for the 9 repetition allele (440 bp) and lanes 2, 3, 9, 12, 13, 15 and 17 are heterozygous for the 9 and 10 repetition allele; first and last lane labelled 100 bp as the molecular weight marker, all other lanes are identified with the ID of the individual.

Concerning the 48 bp DRD4 VNTR, we found this population to be monomorphic, with all individuals presenting themselves as homozygous for the 4 repetition allele (540bp) (Fig. S1). The

30 bp MAOA VNTR and the 23 bp 5HTTLPR VNTR presented artifacts caused by the PCR reaction, that despite all efforts of optimization described in methods, were not well resolved. The genotyping of these VNTRs was not carried out (Fig. S2). The same quality control criteria were applied for the VNTRs genotyped, except the HapMap correspondence, as we did not have HapMaps with known genotype calls for these polymorphisms. They both passed all quality control applied. As the 40 bp DRD4 VNTR was monomorphic, and so not informative for an association study, we did not proceed with the analysis of this polymorphism.

4.3 STATISTICAL ANALYSIS

We tested the normal distribution of the CD-RISC data using the Shapiro-Wilk test, since considering our sample size this is the most potent test [101], and established the normality of the distribution. The Pearson correlation coefficient revealed a positive correlation of over 0.8 between the 10 item CD-RISC and the 25 item CD-RISC, and so we opted to only use the 10 item scale, as it is the least redundant.

We detected associations between resilience and several parameters, namely suffering from depression, suffering from anxiety and the level of education. The analysis revealed that individuals who suffered from anxiety, depression or were taking sleeping pills had lower resilience score, whilst individuals who had higher levels of education, namely post-graduate studies and professional courses, had higher resilience scores. We found no association of resilience with gender or age, but as these are important for population context, they were also used for the multivariate analysis. We found no association between marital status, practising physical activity, taking sleeping pills or blood pressure (Table 3).

There were also significant associations between the scores obtained for the Subjective Happiness Scale (SHS), the Mental Health Index (MHI-5), the Oslo Social Support scale and the physical and mental health scales from ASSET, and the resilience scores. Individuals who scored higher in the SHS and MHI-5, indicating individuals who considered themselves happy and had a better mental health status, as well as individuals with lower ASSET physical and mental health scores, meaning those who present less symptomatology, both physical and psychological, associated to stress, revealed higher resilience scores. On the other hand, we also observed that individuals that scored lower in the Oslo social support scale, indicating lower social support, had higher resilience scores (Table 3).

Table 3 - Demographic measures and univariate analysis results for the parameters in study

Parameters	Population in study	β	95% CI	ANOVA p-value
Age, mean ± SD (years)	42,28 ± 0.528	0.085	-0.019 – 0.100	0.177
Gender		-0.026	-1.221 – 0.798	0.680
Gender, male n/N (%)	118/256 (46)			
Gender, female n/N (%)	138/256 (54)			
Marital Status, n/N (%)				0.285
single	53/256 (21)	-0.112	-2.386 – 0.141	
Married/cohabitation	169/256 (66)			
Divorced/seperated	30/256 (12)	0.001	-1.581 – 1.599	
Widow(er)	4/256 (2)	0.042	-2.668 – 5.452	
Level of education, n/N (%)				0.024
Not graduate	68/256 (27)	0.073	-0.542 – 1.882	
Graduate	116/256 (45)			
Post-graduate	68/256 (27)	0.168	0.341 – 2.765	
Others	4/256 (2)	0.130	0.223 – 8.294	
Physical Activity, n/N (%)				0.481
Always	25/256 (10)	0.060	-1.084 – 2.738	
Frequently	41/256 (16)	0.139	-0.082 – 3.168	
When possible	61/256 (24)			
On occasion	43/256 (17)	0.030	-1.281 – 1.924	
Rarely	51/256 (20)	0.029	-1.230 – 1.823	
Never	35/256 (14)	-0.004	-1.748 – 1.665	
Suffer from depression, n/N (%)				0.007
No	177/256 (69)			
Yes	55/256 (21)	-0.176	-2.966 - -0.523	
Does not know/refused to answer	24/256 (9)	-0.120	-3.402 – 0.041	
Suffer from anxiety, n/N (%)				0.000
No	210/256 (82)			
Yes	21/256 (8)	-0.273	-5.802 - -2.302	
Does not know/refused to answer	25/256 (10)	-0.195	-4.301 - -1.065	
Take sleeping pills, n/N (%)				0.096
Yes	33/256 (13)	-0.104	-2.760 – 0.228	
No	223/256 (87)			
Blood pressure, n/N (%)				0.890
Normal	135/256 (53)			
Prehypertension/hypertension/crisis	121/256 (47)	-0.009	-1.079 – 0.937	
Oslo scores, mean ± SD	7.50 ± 1.420	-0.102	-0.645 – 0.062	0.105
SHS scores, mean ± SD	5.30 ± 1.07	0.367	0.960 – 1.835	0.000
MHI-5 scores, n/N (%)				0.000
<=52	28/256 (11)	0.259	1.817 – 4.933	
>52	228/256 (89)			
Physical health scores, mean ± SD	12.25 ± 4.073	-0.230	-0.351 - -0.110	0.000
Mental health scores, mean ± SD	41 ± 6.938	-0.464	-0.337 - -0.209	0.000

Linear regression analysis of each marker with the resilience scores revealed one single SNP in the *MAOA* gene (rs142369182) significantly associated ($p<0.05$) with resilience. However, haplotype analysis did not support these results (Table S2).

To evaluate the influence of genetic polymorphisms on resilience scores, after adjusting for potential confounding variables, comprising age, gender, SHS scores and mental health scores from ASSET, we used a multivariate regression analysis. This multivariable analysis revealed a significant association (p-value<0.05) between resilience scores and the "AA" genotype for the rs53576

polymorphism from the *OXTR* gene (p-value = 0.015; β [95% CI] = -0.130 [-3.447 - -0.271]), after adjusting for confounding variables, with this model explaining almost 27% of the variability on resilience scores observed in our population (R^2=0.269). This model indicates that individuals who possess the "AA" genotype for the *OXTR* polymorphism have lower resilience scores. At the allelic level, the multivariable analysis revealed an association between the "G" allele of this marker (p-value = 0.017; β [95% CI] = 0.137 [0.352 - 3.560]) and higher resilience scores, indicating that individuals with one or two copies of the "G" allele are more resilient than individuals with two copies of the "A" allele (Table 4).

We also identified a significant association (p-value<0.05) between resilience scores and the "9R/10R" genotype for the *SLC6A3* 40 bp VNTR (p-value = 0.014; β [95% CI] = -0.138 [-2.072 - -0.181]), after adjusting for confounding variables, with this model explaining almost 29% of the variability of resilience scores observed in the population in study (R^2=0.289), and revealing that individuals who possess both the 9 repetition allele and 10 repetition allele for the *SLC6A3* 40 bp VNTR have lower resilience scores. At the allelic level, we identified a significant association (p-value<0.05) between the "9R" allele of this marker (p-value = 0.020; β [95% CI] = -0.140 [-2.072 - -0.181]) and lower resilience scores (Table 4).

Table 4 - Multivariate genotypic and allelica analysis results for the polymorphisms associated with CD-RISC 10 scores

Variables	**β**	**95% CI**	**p-value**	**Variables**	**β**	**95% CI**	**p-value**
Age	0.088	-0.010 - 0.095	0.115	**Age**	0.078	-0.015 - 0.090	0.162
Gender	0.087	-0.195 - 1.611	0.124	**Gender**	0.093	-0.144 - 1.647	0.100
SHS scores	0.177	0.203 - 1.147	0.005	**SHS scores**	0.178	0.192 - 1.145	0.006
Mental health scores	-0.389	-0.303 - -0.155	0.000	**Mental health scores**	-0.401	-0.305 - -0.159	0.000
rs53576 (OXTR)				**SLC6A3 40 bp VNTR**			
AA	-0.130	-3.447 - -0.271	0.015	10R/10R			
AG	0.012	-0.827 – 1.020	0.837	10R/9R	-0.138	-2.072 - -0.181	0.014
GG				9R/9R	0.023	-1.606 – 1.240	0.800
A	0.012	-0.827 – 1.020	0.837	10R/11R	0.037	-5.340 – 2.648	0.507
G	0.137	0.352 – 3.560	0.017	10R/3R	0.056	-3.335 – 10.477	0.309
R^2		0.269		10R/8R	.0023	-2.787 – 4.246	0.683
				10R	-0.075	-2.387 – 0.500	0.199
				9R	-0.140	-2.072 - -1.181	0.020
				11R	-0.037	-5.340 – 2.648	0.507
				8R	0.023	-2.787 – 4.246	0.683
				3R	0.056	-3.335 – 10.477	0.309
				R^2		0.289	

No other molecular marker revealed a significant association with the resilience scores during the multivariable analysis, either at the genotypic level nor at the allelic level (Table S3).

5 DISCUSSION

We successfully genotyped 37 single nucleotide polymorphisms (SNPs) and 2 variable number tandem repeats (VNTRs), mapping 10 different genes. Besides these, we found 3 SNPs to be monomorphic, which goes against the expected, as it did not correspond to the minor allele frequency (MAF) described for these SNPs in the European population. We also had to exclude 6 other SNPs for whom the genotype calls of HapMap individuals did not correspond with the expected and/or presented deviation from Hardy-Weinberg Equilibrium (HWE).

In Europe and Middle East populations, the most common allele for the 48 bp DRD4 VNTR is the 4 repetition allele (4R) (~70%), followed by the 7 repetition allele (7R) (~20%), even though in some European populations, namely the Sardinians, these distributions vary, showing no representation of the 7R [128]. Regarding the Portuguese population, studies concerning this polymorphism have mainly included schizophrenic trios. In one of these studies, healthy controls with no history of psychiatric disorders were used, demonstrating the presence of the 7R allele in the healthy Portuguese population, and therefore we would expect to see variability in our population in study as well [129,130]. As we did not, this polymorphism was excluded from the analysis.

Concerning the 30 bp MAOA VNTR and the 23 bp 5HTTLPR VNTR, we were not able to correctly genotype what we can only assume to be the heterozygous individuals, who presented an artifact caused by the polymerase chain reaction (PCR). The artifact present in both VNTRs appeared as a fragment of slower migration than the other two expected fragments, which leads us to believe that it is being caused by the reannealing of complementary fragments that have sequence differences, since the repeats are so similar [131–133]. Despite all efforts of optimization, including many alterations in thermocycling conditions, the use of enhancement agents and other previously described protocols, the full genotyping of these VNTRs was not carried out at this time, since the presence of this artifact did not give us the confidence to genotype with certainty these individuals. Other methods, such as fragment analysis, should be tested.

The absence of association between resilience scores and age or gender in the univariate analysis is indicative that resilience, as an intrinsic characteristic, does not alter with age nor does it vary between genders. The lack of association between blood pressure and resilience is revealing that the cardiovascular problems usually associated to the hyper-response of the noradrenergic and

sympathetic nervous system (SNS), are not indicative of a person's resilience, and there must be other factors influncing these symptoms [46,48,49].

As depression and anxiety are psychiatric disorders related to the imbalance of the stress response systems, the association indicating that individuals that suffer from such disorders are less resilient, reveals the influence of the stress response mechanism on the capability to adaptively overcome adversity [134,135]. In line with this goes the trend for association here found between the consumption of sleeping pills and resilience, indicating that individuals who take sleeping pills are less resilient, as sleep disturbances are considered risk factors for development of depression and anxiety [136].

On another note, assuming that individuals who proceed with their studies have a different life experience than the ones who do not, and possibly encounter different stressful situations, the association observed between individuals with postgraduate studies as well as individuals with professional courses and a greater capacity to overcome stress and adversity, can be seen as the impact of personal experience on the creation of personal resources that allow a more adequate response to stressful stimulus, and ultimately on resilience.

The univariate regression analysis revealed an association between the resilience scores and one single SNP in the *MAOA* gene (rs142369182), but as we did not found any association between the haplotypes involving this polymorphism and resilience, it is possibly a false positive.

The association between the Oslo Social Support scale and resilience revealing that individuals with less social support are more resilient goes against previous studies that identified low social support to be associated with physiological and neuroendocrine indices of heightened stress reactivity. This may be due to the lack of representability of the population in study for this scale, as the mean score is 7.50 ± 1.420 and the range is 4 – 12, with a reduced number of individuals presenting "strong social support" [88,139].

As seen by the results of the multivariable analysis, positive emotions and moods moderate resilience in a way that is demonstrative of how a positive mind-set helps build an individual's personal resources so that he/she has effective coping mechanisms allowing him to adaptively overcome stress and adversity. This result is concordant with the broaden-and-build theory of positive emotions that states that experiences of positive emotions broaden people's momentary thought-action repertoires, which in turn serves to build their enduring personal resources [140–

142]. Furthermore, this finding also falls in line with previous studies that have indicated that certain mind sets can modulate glucocorticoid reactivity, the main hormone of the HPA axis, and in that way possibly impact the stress response mechanism [143] .

The association identified between the absence of stress related symptomatology and higher resilience shows how a flexible and suitable biological stress response is necessary in order to maintain normal physical and psychological functioning in context of adversity, which goes accordingly with previous studies that indicate that a suitable stress response is necessary for sustained health in the face of adversity [4,5]. It is noteworthy that the association between less symptomatology related to stress and higher resilience highlights the importance of the biological component of this trait, mainly the stress response mechanism that is composed by several neurological and neurochemical and is, to a certain extent, modulated by a genetic component.

The polymorphism rs53576 from the *OXRT* gene is an intronic variant of functionality unknown that causes the alteration of a guanine ("G") to an adenine ("A") and has been previously linked to not only alterations in social behaviour, with several studies indicating that carriers of the "G" allele exhibit more empathy, report being less lonely and tend to be more optimistic when compared with "A" allele carriers, but also with alterations in brain structures that compose and modulate the HPA axis, namely the hypothalamus and the amygdala [38,41,144]. The association seen in the multivariable analysis between carriers of the "G" allele and greater resilience, when compared with individuals homozygous for the "A" allele, goes in line with these studies, indicating that this variant impacts the receptor's function, and thus the oxytocin pathway and regulation of the HPA axis, in such a way that it can modulate positive emotions, and consequently resilience.

The 40 bp VNTR localized in the 3' untranslated region of the *SLC6A3* gene is a functional polymorphism that affects protein expression, with most studies stating that carriers of the 9 repetition (9R) allele have a less active dopamine transporter, when compared with the 10 repetition (10R) allele carriers. As such, this 9R allele has been associated with increased risk of lifetime post-traumatic stress disorder (PTSD), substance abuse and cigarette smoking, as well as aggressive and antisocial behaviour [145,146]. The significant association found in the multivariate analysis between carriers of the "9R" allele and lower resilience goes in line with the previous studies, and suggests that this allele is responsible for the decrease in the transporter's expression, thus affecting the stress response mechanism. This effect on the stress response mechanism can possibly be related with amygdala reactivity, as proposed by *Bergman et al*., 2014, as dopaminergic

neurons project to this region, leading to alterations in emotional reactions and memory processing, and causing variability in emotional resilience. However, it should be noted that the genotypic analysis only showed a significant association with the “9R/10R” genotype and not with the “9R/9R”, as was also expected considering the allelic analysis. This was due to the fact that this genotype is poorly represented in our population (12%), when compared to the heterozygous genotype.

6 CONCLUSIONS AND FUTURE PERSPECTIVES

This study provides evidence of the influence of the *SLC6A3* and *OXTR* genes in the capacity to adaptively overcome stress, as we saw that genetic alterations in these genes modulate variability in resilience, possibly by affecting protein expression and causing alterations on the stress response mechanisms.

We also found evidence of the importance of a positive mind-set as well as a suitable stress response mechanism in modulating the capacity to overcome adversity, as we verified an association between positive emotions and moods and the absence of symptomatology associated to stress with higher resilience.

The results here described are consistent with previous literature, which reinforces the role of these genes in resilience and indicates that these must not be false positives.

This study presents some limitations, starting with the size of our sample that was limited and sometimes not representative of certain genotypes and so did not confer sufficient statistical power to detect certain effects, which might lead to false negatives. We also opted for not applying a correction for multiple testing as we wished to analyse the impact of different molecular markers separately, and did not intend to find a single model that would explain overall resilience scores, so results should be seen as exploratory. It should be kept in mind, that resilience is an extremely complex trait that involves several neurological and neurochemical pathways, and so is likely influenced by multiple genes and their interactions, as well as environmental factors and their interactions, and it was not possible to evaluate all possible genes involved in those pathways or all environmental factors that could potentially influence resilience, and so the results here found might not uphold in a wider range association study.

As to future perspectives for this work, we propose that other methods should be tested, in order to correctly genotype the variants not successfully genotyped in this study. Replication of these associations in larger population samples, together with approaches that test gene-gene interaction, will contribute for the validation of these results.

In conclusion, this study points to the influence of genetic factors as well as environmental factors on resilience, and the importance of studying these two components to truly understand this complex trait.

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8 SUPPLEMENTARY MATERIAL

Table S1 - Quality control results for all polymorphisms genotyped

Gene	Polymorphism	MAF	Call Rates (%)	Contaminated Waters	HapMap correspondence	HWE
´**FKBP5**	rs1360780	31% (T)	99%	0%	100%	equilibrium
	rs3800373	29% (C)	100%	0%	100%	equilibrium
	rs4713916	32% (A)	100%	0%	100%	equilibrium
	rs9296158	30% (A)	98%	0%	100%	equilibrium
	rs9470080	33% (T)	100%	0%	100%	equilibrium
CRHR1	rs242924	45% (T)	95%	0%	100%	equilibirum
	rs4792887	8% (T)	99%	0%	100%	equilibrium
	rs7209436	43% (T)	99%	0%	100%	equilibrium
	rs110402	45% (A)	99%	0%	100%	equilibrium
OXTR	rs53576	35% (A)	100%	0%	100%	equilibrium
	rs2254298	11% (A)	99%	0%	100%	equilibrium
BDNF	rs6265	20% (T)	99%	0%	100%	equilibrium
	rs2030324	48% (G)	99%	0%	100%	equilibrium
	rs4923464	22% (T)	100%	0%	100%	- (monomorphic)
	rs7124442	29% (C	99%	0%	33%	deviation (*)
	rs11030099	22% (A)	95%	0%	0%	- (monomorphic)
	rs11030101	46% (T)	99%	0%	100%	equilibrium
	rs11030102	23% (G)	99%	0%	100%	equilibrium
	rs66866077	4% (T)	100%	0%	100%	equilibrium
	rs75298795	12% (T)	99%	0%	100%	equilibrium
	rs76324918	6% (C)	99%	0%	100%	equilibrium
	rs77135086	8% (T)	100%	0%	100%	equilibrium
	rs189740576	11% (G)	100%	0%	100%	- (monomorphic)
NPY	rs16142	26% (G)	99%	0%	100%	equilibrium
	rs2023890	23% (G)	99%	0%	100%	equilibrium
COMT	rs4680	50% (A)	99%	0%	100%	equilibrium
	rs165599	31% (G)	100%	0%	100%	equilibrium
	rs2097603	41% (G)	98%	0%	100%	equilibrium
MAOA	rs909525	34% (C)	100%	0%	100%	equilibrium
	rs3788862	29% (A)	100%	0%	100%	equilibrium
	rs5905809	27% (G)	99%	0%	100%	equilibrium
	rs5905823	25% (G)	100%	0%	100%	equilibrium
	rs73211189	20% (T)	99%	0%	100%	equilibrium
	rs112592173	8% (T)	99%	0%	0%	deviation (*)
	rs140878834	23% (G)	100%	0%	50%	- (monomorphic)
	rs142369182	6% (T)	90%	0%	100%	equilibrium
	rs142677545	5% (A)	99%	0%	100%	equilibrium
	rs147023114	8% (T)	99%	0%	100%	equilibrium
	rs201583370	6% (T)	100%	0%	100%	equilibrium
SLC6A3	rs27072	21% (T)	90%	0%	67%	equilibrium
	VNTR		98%	0%	-	equilibrium
SLC6A4	rs25533	6% (G)	100%	0%	100%	- (monomorphic)
	rs1042173	44% (C)	99%	0%	100%	equilibrium
DRD4	rs1870723	24% (A)	99%	0%	100%	deviation (*)
	VNTR		91%	0%	-	- (monomorphic)

(*) – $p<0.05$

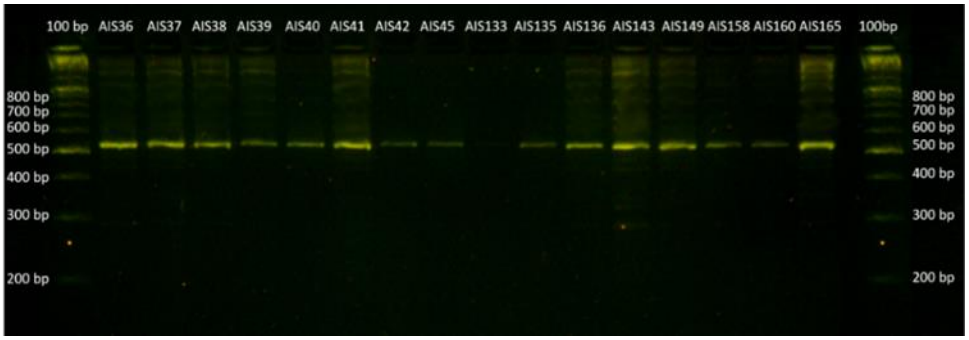

Fig. S1 - Genotype pattern for the 48 bp DRD4 VNTR. All individuals present the 4 repetition allele (540bp); first and last lane labelled 100 bp as the molecular weight marker, all other lanes are identified with the ID of the individual in question.

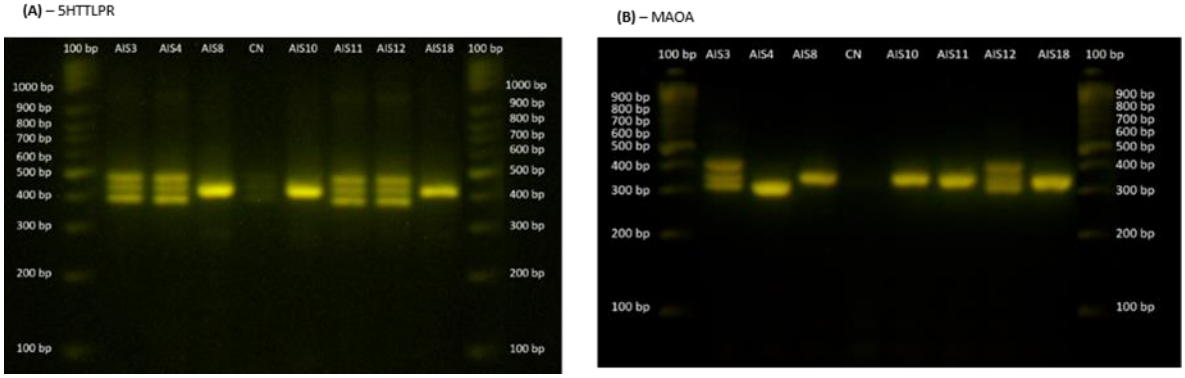

Fig. S2 - Genotype pattern for (A) – 23 bp 5HTTLPR VNTR and (B) – 30 bp MAOA VNTR. (A) – lane 4, 6 and 9 are presumably homozygous for the 16 repetition allele (419 bp), lane 2, 3, 7 and 8 are the presumed heterozygous for the 16 and 14 repetition allele (419 and 375 bp respectively) where the artifact is noticeable; (B) – lane 3 is presumably homozygous for the 3 repetition allele (350 bp), lane 4, 6, 7 and 9 are presumably homozygous for the 4 repetition allele (380 bp), lane 2 and 8 are the presumed heterozygous for the 3 and 4 repetition allele (350 bp and 380 bp respectively) where the artifact is noticeable; first and last lane labelled 100 bp as the molecular weight marker, all other lanes are identified with the ID of the individual in question.

Table S2 - Univariate genotypic, haplotypic and allelic regression analysis results between the molecular markers and CD-RISC 10 scores

Gene	Genetic markers	Population in study, n/N (%)	β	95% CI	ANOVA p-value
FKBP5	**rs1360780**				0.921
	CC	119/256 (46)			
	CT	109/256 (43)	0.021	-0.896 - 1.243	
	TT	28/256 (11)	-0.009	-1.818 - 1.571	
	rs3800373				0.541
	AA	130/256 (51)	-0.047	-2.449 - 1.128	
	CA	102/256 (40)	0.040	-0.730 - 1.400	
	CC	24/256 (9)			
	rs4713916				0.537
	AA	21/256 (8)	0.039	-1.313 - 2.458	
	GA	97/256 (38)	-0.050	-1.1487 - 0.646	
	GG	138/256 (54)			
	rs9296158				0.810
	AA	26/253 (10)	-0.021	-2.038 - 1.463	
	GA	106/253 (42)	0.029	-0.833 - 1.322	
	GG	121/253 (48)			
	rs9470080				0.969
	CC	116/256 (45)			
	CT	111/256 (43)	0.016	-0.937 - 1.206	
	TT	29/256 (11)	0.003	-1.641 - 1.710	
CRHR1	**rs4792887**				0.640
	CC	210/256 (82)			
	CT	44/256 (17)	-0.014	-1.485 - 1.186	
	TT	2/256 (1)	-0.058	-8.419 - 3.028	
	rs110402				0.453
	CC	102/256 (40)	-0.070	-2.138 - 0.981	
	CT	118/256 (46)	0.012	-1.433 - 1.631	
	TT	36/256 (14)			
	rs7209436				0.408
	CC	104/256 (41)	-0.66	-2.154 - 1.059	
	CT	119/256 (46)	0.022	-1.404 - 1.761	
	TT	33/256 (13)			
	rs242924				
	AA	37/256 (14)	0.055	-0.908 - 2.183	0.412
	CA	118/256 (46)	0.087	-0.377 - 1.803	
	CC	101/256 (39)			
OXTR	**rs53576**				0.086
	GG	119/256 (46)			
	AG	114/256 (45)	0.021	-0.875 - 1.220	
	AA	23/256 (9)	-0.131	-3.690 - -0.050	
	rs2254298				0.300
	GG	181/256 (71)			
	AG	70/256 (27)	-0.074	-1.803 - 0.458	
	AA	5/256 (2)	-0.070	-5.714 - 1.568	
BDNF	**rs6265**				0.188
	CC	175/256 (68)			
	CT	68/256 (27)	-0.090	-1.977 - 0.315	
	TT	13/256 (5)	-0.084	-3.869 - 0.741	
	rs2030324				0.978
	AA	79/256 (31)			
	GA	124/256 (48)	0.015	-1.038 - 1.286	
	GG	53/256 (21)	0.009	-1.342 - 1.524	
	rs11030101				0.676
	AA	86/256 (34)			
	TA	118/256 (46)	0.063	-0.630 - 1.655	
	TT	52/256 (20)	0.025	-1.161 - 1.670	
	rs11030102				0.899
	CC	144/256 (56)			
	CG	92/256 (36)	0.028	-0.842 - 1.312	
	GG	20/256 (8)	-0.005	-1.994 - 1.856	
	rs66866077				0.636
	CC	225/256 (88)			
	CT	31/256 (12)	0.030	-1.172 - 1.913	
	TT	0/256 (0)			
	rs75298795				0.124
	CC	201/256 (79)			
	CT	49/256 (19)	-0.78	-2.078 - 0.473	
	TT	6/256 (2)	0.096	-0.724 - 5.908	
	rs76324918				0.755
	TT	227/256 (89)			
	CT	27/256 (11)	0.037	-1.153 - 2.129	
	CC	2/256 (1)	0.031	-4.311 - 7.139	
	rs77135086				0.665
	AA	215/256 (84)			
	TA	41/256 (16)	-0.027	-1.674 - 1.070	
	TT	0/256 (0)			
	Haplotypes				0.531
	C A A C C C T A	27/245 (11)	0.061	-0.957 - 1.059	
	C A A G C C C A	7/245 (3)	0.039	-2.206 - 4.104	
	C A A G C C T A	25/245 (10)	-0.056	-2.555 - 1.059	
	C A A G T C T A	5/245 (2)	-0.094	-2.206 - 4.104	
	C G T C C C T A	98/245 (40)			
	C G T C C C T T	37/245 (15)	-0.013	-1.707 - 1.405	
	C G T C C T T A	33/245 (13)	-0.028	-1.955 - 1.291	
	T A A C C C T A	13/245 (5)	-0.084	-3.904 - 0.857	
NPY	**rs16142**				0.841
	AA	122/256 (48)			
	AG	108/256 (42)	0.025	-0.859 - 1.272	
	GG	26/256 (10)	-0.021	-2.04 - 1.460	
	rs2023890				0.677
	AA	156/256 (61)			
	GA	83/256 (32)	-0.024	-1.304 - 0.885	
	GG	17/256 (7)	-0.055	-2.951 - 1.165	
COMT	**rs4680**				0.125
	AA	45/256 (18)	-0.034	-1.841 - 1.116	
	AG	127/256 (50)	0.108	-0.242 - 2.009	
	GG	84/256 (33)			

Gene	Genetic markers	Allelic Frequency	β	95% CI	ANOVA p-value
FKBP5	**rs1360780**				0.921
	C	68%	0.023	-1.412 - 2.006	
	T	32%	0.021	-0.896 - 1.243	
	rs3800373				0.541
	A	71%	0.071	-0.831 - 2.822	
	C	29%	0.041	-0.730 - 1.400	
	rs4713916				0.537
	A	27%	-0.051	-1.1487 - 0.646	
	G	73%	-0.067	-2.931 - 0-944	
	rs9296158				0.810
	A	31%	0.030	-0.833 - 1.322	
	G	69%	0.039	-1.240 - 2.304	
	rs9470080				0.969
	C	67%	0.008	-1.583 - 1.783	
	T	33%	0.016	-0.931 - 1.206	
CRHR1	**rs4792887**				0.640
	C	91%	0.055	-3.279 - 8.370	
	T	9%	-0.014	-1.485 - 1.186	
	rs110402				0.453
	C	63%	0.008	-1.433 - 1.631	
	T	37%	0.081	-0.410 - 1.765	
	rs7209436				0.408
	C	70%	0.015	-1.404 - 1.761	
	T	36%	0.088	-0.353 - 1.805	
	rs242924				0.412
	A	38%	0.086	-0.377 - 1.803	
	C	63%	0.007	-1.439 - 1.591	
OXTR	**rs53576**				0.086
	G	69%	0.143	0.216 - 3.870	
	A	31%	0.021	-0.875 - 1,220	
	rs2254298				0.300
	G	84%	0.048	-2.318 - 5.118	
	A	16%	-0.075	-1.803 - 0.458	
BDNF	**rs6265**				0.188
	C	82%	0.040	-1.694 - 3.160	
	T	18%	-0.095	-1.977 - 0.315	
	rs2030324				0.978
	A	55%	0.003	-1.291 - 1.357	
	G	45%	0.014	-1.038 - 1.286	
	rs11030101				0.676
	A	57%	0.025	-1.084 - 1.599	
	T	43%	0.059	-0.630 - 1.655	
	rs11030102				0.899
	C	74%	0.020	-1.686 - 2.295	
	G	26%	0.029	-0.841 - 1.312	
	rs66866077				0.636
	C	94%			
	T	6%	-0.030	-1.913 - 1.172	
	rs75298795				0.124
	C	88%	-0.126	-6.856 - 0.067	
	T	12%	-0.081	-2.078 - 0.473	
	rs76324918				0.755
	T	94%	-0.020	-6.833 - 4.982	
	C	6%	0.038	-1.153 - 2.129	
	rs77135086				0.665
	A	92%			
	T	8%	0.027	-1.070 - 1.674	
NPY	**rs16142**				0.841
	A	69%	0.036	-1.273 - 2.250	
	G	31%	0.025	-0.859 - 1.272	
	rs2023890				0.677
	A	77%	0.042	-1.461 - 2.829	
	G	23%	-0.025	-1.304 - 0.885	
COMT	**rs4680**				0.125
	A	42%	0.102	-0.242 - 2.009	
	G	58%	0.116	-0.142 - 2.635	
	rs165599				0.875
	G	39%	-0.031	-1.366 - 0.843	
	A	61%	-0.025	-1.740 - 1.188	
	rs2097603				0.193
	A	66%	-0.078	-2.546 - 0.647	
	G	34%	0.060	-0.586 - 1.564	
MAOA	**rs73211189**				
	C	82%			0.948
	T	18%	-0.006	-1.850 - 1.732	
	C (female)	86%	0.076	-2.953 - 7.498	0.658
	T (female)	14%	0.046	-1.276 - 2.194	
	rs909525				
	C	30%	0.106	-0.626 - 2.357	0.253
	T	70%			
	T (female)	32%	0.200	0.342 - 5.411	0.071
	C (female)	68%	0.113	-0.551 - 2.515	
	rs3788862				
	G	74%			0.424
	A	26%	0.074	-0.924 - 2.180	
	G (female)	73%	0.186	0.168 - 5.818	0.060
	A (female)	27%	0.158	-0.258 - 2.915	
	rs5905809				
	C	79%			0.437
	G	22%	0.072	-0.999 - 2.298	
	C (female)	78%	0.031	-2.770 - 3.906	0.881
	G (female)	22%	0.042	-1.233 - 1.986	
	rs5905823				
	A	76%			0.241
	G	24%	-0.109	-2.554 - 0.647	
	A (female)	76%	0.026	-3.406 - 4.606	0.162
	G (female)	24%	0.167	-0.046 - 2.974	

Table S2 - Univariate genotypic, haplotypic and allelic regression analysis results between the molecular markers and CD-RISC 10 scores (continued)

	rs165599				
	GG	41/256 (16)			
	GA	117/256 (46)	-0.034	-1.740 - 1.188	0.875
	AA	98/256 (38)	-0.002	-1.515 - 1.485	
	rs2097603				
	AA	116/254 (46)			
	AG	105/254 (41)	0.057	-0.604 - 1.547	0.198
	GG	33/254 (13)	0.117	-0.153 - 3.005	
MAOA	**rs73211189**				
	C (male)	97/256 (38)			0.948
	T (male)	21/256 (8)	-0.006	-1.850 - 1.732	
	CC (female)	102/256 (40)			0.658
	CT (female)	33/256 (13)	0.045	-1.276 - 2.194	
	TT (female)	3/256 (1)	-0.061	-6.890 - 3.263	
	rs909525				
	C (male)	35/256 (14)	0.106	-0.626 - 2.357	0.253
	T (male)	83/256 (32)			
	TT (female)	14/256 (5)			0.071
	CT (female)	59/256 (23)	0.112	-0.551 - 2.515	
	CC (female)	65/256 (25)	-0.132	-4.406 - 0.617	
	rs3788862				
	G (male)	87/256 (34)			0.424
	A (male)	31/256 (12)	0.074	-0.924 - 2.180	
	GG (female)	75/256 (29)			0.060
	GA (female)	52/256 (20)	0.154	-0.158 - 2.915	
	AA (female)	11/256 (4)	-0.101	-4.363 - 1.134	
	rs5905809				
	C (male)	93/256 (36)			0.437
	G (male)	26/256 (10)	0.072	-0.999 - 2.298	
	CC (female)	85/256 (33)			0.881
	GC (female)	44/256 (17)	0.040	-1.233 - 1.986	
	GG (female)	8/256 (3)	-0.010	-3.402 - 3.018	
	rs5905823				
	A (male)	90/256 (35)			0.241
	G (male)	28/256 (11)	-0.109	-2.554 - 0.647	
	AA (female)	78/256 (30)			0.162
	GA (female)	55/256 (21)	0.165	-0.046 - 2.974	
	GG (female)	5/256 (2)	0.037	-3.092 - 4.821	
	rs142369182				
	CC (male)	107/229 (47)			
	TT (male)	0/229 (0)			
	CC (female)	120/229 (52)			0.035
	TC (female)	2/229 (1)	-0.192	-12.906 - -0.494	
	TT (female)	0/229 (0)			
	rs142677545				
	C (male)	114/256 (45)			0.898
	A (male)	4/256 (2)	0.012	-3.539 - 4.031	
	CC (female)	126/256 (49)			0.578
	CA (female)	11/256 (4)	0.010	-2.566 - 2.878	
	AA (female)	1/256 (0)	-0.089	-13.263 - 4.120	
	rs147023114				
	C (male)	108/256 (42)			0.276
	T (male)	10/256 (4)	-0.101	-3.799 - 1.095	
	CC (female)	122/256 (48)			0.437
	CT (female)	16/256 (6)	-0.067	-3.203 - 1.392	
	TT (female)	0/256 (0)			
	rs20158837				
	C (male)	107/256 (42)			0.578
	T (male)	11/256 (4)	0.052	-1.690 - 3.016	
	CC (female)	121/256 (57)			0.263
	CT (female)	16/256 (6)	0.108	-0.828 - 3.751	
	TT (female)	1/256 (0)	-0.086	-13.056 - 4.230	
	Haplotypes				0.499
	C?CCCCGTG	12/117 (10)	0.029	-2.658 - 3.552	
	CCCCCCACA	3/117 (3)	-0.072	-7.461 - 3.188	
	CCCCCCGTA	49/117 (42)			
	CCCCCCGTG	35/117 (30)	-0.008	-2.295 - 2.145	
	CCCCGCACA	14/117 (12)	0.169	-0.662 - 4.046	
	CCTCGCACA	3/117 (3)	0.035	-2.451 - 3.464	
	CCTTGCACA	1/117 (1)	-0.049	-6.794 - 3.885	
SLC6A3	**SLC6A3 - VNTR**				0.150
	10R/10R	111/249 (45)			
	10R/9R	101/249 (41)	-0.156	-2.361 - -0.192	
	9R/9R	29/249 (12)	-0.083	-1.714 - 1.576	
	10R/11R	3/249 (1)	-0.042	-6.155 - 3.074	
	10R/8R	4/249 (2)	0.072	-1.721 - 6.307	
	10R/3R	1/249 (0)	0.012	-7.130 - 8.715	
SLC6A4	**rs1042173**				0.880
	AA	86/255 (34)			
	CA	123/255 (48)	0.33	-0.869 - 1.403	
	CC	46/255 (18)	0.028	-1.181 - 1.772	
	rs142369182				
	C	100%	-	-	-
	T	-	-	-	
	C (female)	99%	-	-	0.035
	T (female)	1%	-0.192	-12.906 - 0.494	
	rs142677545				
	C	97%			0.898
	A	3%	0.012	-3.539 - 4.031	
	C (female)	95%	0.092	-4.315 - 13.770	0.578
	A (female)	5%	0.010	-2.566 - 2.876	
	rs147023114				
	C	92%	-	-	0.276
	T	4%	-0.101	-3.799 - 1.095	
	C (female)	94%			
	T (female)	6%	-0.067	-3.203 - 1.392	0.437
	rs201583370				
	C	91%	-	-	0.578
	T	9%	0.052	-1.690 - 3.016	
	C (female)	93%	0.115	-2.997 - 14.747	0.263
	T (female)	7%	0.111	-0.828 - 3.751	
SLC6A3	**SLC6A3 - VNTR**				0.150
	10R	66%	-0.096	-2.869 - 0.454	
	9R	32%	-0.159	-2.361 - -0.192	
	11R	1%	-0.042	-6.155 - 3.074	
	8R	1%	0.072	-1.721 - 6.307	
	3R	<1%	0.012	-7.130 - 8.715	
SLC6A4	**rs1042173**				0.880
	A	58%	-0.003	-1.426 - 1.368	
	C	42%	0.031	-0.869 - 1.403	

BDNF Haplotypes: rs6265-rs2030324-rs11030101-rs11030302-rs66866077-rs75298795-rs76324918-rs77135086

MAOA Haplotypes: rs142677545-rs142369182-rs73211189-rs147023114-rs5905809-rs201583370-rs3788862-rs909525-rs5905823

Table S3 - Multivariate genotypic, haplotypic and allelic regression analysis results for the remaining markers with the CD-RISC 10 scores

Variables		β	95% CI	p-value
Age		0.098	-0.006 - 0.100	0.080
Gender		0.079	-0.266 - 1.550	0.165
SHS scores		0.178	0.198 - 1.157	0.006
Mental Health scores		-0.380	-0.298 - -0.149	0.000
FKBP5	**rs1360780**			
	CC			
	CT	0.33	-0.672 - 1.215	0.572
	TT	0.005	-1.428 - 1.546	0.938
	C	0.016	-1.285 - 1.709	0.780
	T	0.033	-0.672 - 1.215	0.566
	rs3800373			
	AA	-0.044	-2.178 - 0.957	0.444
	CA	0.038	-0.627 - 1.254	0.512
	CC			
	A	0.066	-0.675 - 2.523	0.256
	C	0.039	-0.627 - 1.254	0.512
	rs4713916			
	AA	0.032	-1-184 - 2.136	0.573
	GA	-0.043	-1.300 - 0.582	0.453
	GG			
	A	-0.044	-1.300 - 0.582	0.333
	G	-0.056	-2.528 - 0.859	0.453
	rs9296158			
	AA	-0.001	-1.548 - 1.530	0.991
	GA	0.029	-0.714 - 1.191	0.623
	GG			
	A	0.029	-0.714 - 1.191	0.623
	G	0.028	-1.308 - 1.803	0.755
	rs9470080			
	CC			
	CT	0.024	-0.748 - 1.142	0.682
	TT	0.015	-1.282 - 1.663	0.799
	C	0.001	-1.1469 - 1.482	0.993
	T	0.024	0.748 - 1.142	0.682
CRHR1	**rs4792887**			
	CC			
	CT	-0.001	-1.184 - 1.157	0.982
	TT	-0.075	-8.503 - 1.567	0.176
	C	0.075	-1.652 - 8.561	0.184
	T	-0.001	-1.184 - 1.157	0.982
	rs110402			
	CC			
	CT	0.046	-0.980 - 1.734	0.585
	TT	0.023	-1.188 - 1.573	0.784
	C	0.032	-0.980 - 1.734	0.585
	T	0.022	0.796 - 1.165	0.712
	rs7209436			
	CC	0.017	-1.278 - 1.562	0.844
	CT	0.046	-1.020 - 1.767	0.598
	TT			
	C	0.031	-1.020 - 1.767	0.598
	T	0.028	-0.738 - 1.202	0.638
	rs242924			
	AA	-0.012	-1.512 - 1.227	0.838
	CA	0.024	-0.782 - 1.181	0.689
	CC			
	A	0.024	-0.782 - 1.181	0.689
	C	0.030	-0.997 - 1.681	0.615
OXTR	**rs2254298**			
	GG			
	AG	-0.023	-1.206 - 0.783	0.676
	AA	-0.080	-5.547 - 0.823	0.145
	G	0.073	-1.104 - 5.405	0.194
	A	-0.024	-1.206 - 0.783	0.676
BDNF	**rs6265**			
	CC			
	CT	-0.084	-1.771 - 0.220	0.126
	TT	0.115	-4.137 - 0.120	0.058
	C	0.073	-0.761 - 3.468	0.209
	T	-0.089	-1.771 - 0.220	0.126
	rs2030324			
	AA			
	GA	0.036	-0.725 - 1.316	0.569
	GG	-0.013	-1.390 - 1.130	0.839
	A	0.042	-0.757 - 1.608	0.479
	G	0.033	-0.725 - 1.316	0.569

Variables		β	95% CI	p-value
Age		0.161	0.007 - 0.163	0.033
SHS scores		0.212	0.151 - 1.365	0.015
Mental Health scores		-0.368	-0.328 - -0.123	0.000
MAOA	**rs73211189**			
	C (male)			
	T (male)	-0.031	-1.918 - 1.321	0.716
	CC (female)			
	CT (female)	0.019	-1.289 - 1.675	0.797
	TT (female)	-0.012	-4.735 - 3.992	0.866
	C (female)	0.019	-3.919 - 5.049	0.804
	T (female)	0.020	-1.289 - 1.675	0.797
	rs909525			
	C (male)	0.067	-0.822 - 1.920	0.429
	T (male)			
	TT (female)			
	CT (female)	0.033	1.073 - 1.658	0.672
	CC (female)	-0.077	-3.284 - 1.065	0.315
	C (female)	0.034	-1.073 - 1.658	0.672
	T (female)	0.097	-0.824 - 3.628	0.215
	rs3788862			
	G (male)			
	A (male)	0.057	-0.928 - 1.885	0.502
	GG (female)			
	GA (female)	0.056	-0.866 - 1.879	0.467
	AA (female)	-0.025	-2.807 - 2.005	0.742
	G (female)	0.057	-1.602 - 3.417	0.476
	A (female)	0.058	-0.866 - 1.879	0.467
	rs5905809			
	C (male)			
	G (male)	0.048	-1.067 - 1.921	0.572
	CC (female)			
	GC (female)	0.007	-1.332 - 1.455	0.930
	GG (female)	0.011	-2.548 - 2.940	0.888
	C (female)	-0.007	-2.981 - 2.712	0.926
	G (female)	0.007	-1.332 - 1.455	0.930
	rs5905823			
	A (male)			
	G (male)	-0.109	-3.115 - 0.199	0.056
	AA (female)			
	GA (female)	0.121	-0.214 - 2.371	0.101
	GG (female)	0.024	-2.827 - 3.923	0.749
	A (female)	0.023	-2.88' - 3.941	0.759
	G (female)	0.123	-0.214 - 2.371	0.101
	rs142369182			
	CC (male)			
	TT (male)			
	CC (female)			
	TC (female)	0.111	-9.208 - 1.449	0.152
	TT (female)			
	C (female)			
	T (female)	-0.111	-9.208 - 1.449	0.152
	rs142677545			
	C (male)			
	A (male)	0.051	-2.383 - 4.484	0.546
	CC (female)			
	CA (female)	0.017	-2.076 - 2.609	0.822
	AA (female)	-0.105	-12.829 - 2.073	0.156
	C (female)	0.110	-2.119 - 13.408	0.153
	A (female)	0.017	-2.076 - 2.609	0.822
	rs147023114			
	C (male)	-0.084	-3.350 - 1.100	0.319
	T (male)			
	CC (female)			
	CT (female)	-0.071	-2.917 - 0.989	0.331
	TT (female)			
	C (female)			
	T (female)	-0.071	-2.917 - 0.989	0.331
	rs20158837			
	C (male)			
	T (male)	0.048	-1.514 - 2.740	0.569
	CC (female)			
	CT (female)	0.080	-0.882 - 3.041	0.278
	TT (female)	0.103	-12.722 - 2.116	0.160
	C (female)	0.125	-1.248 - 14.013	0.100
	T (female)	0.082	-0.882 - 3.041	0.278

Table S3 - Multivariate genotypic, haplotypic and allelic regression analysis results for the remaining markers with the CD-RISC 10 scores (continued)

	rs11030101			
	AA			
	TA	0.057	-0.533 - 1.471	0.357
	TT	-0.010	-1.344 - 1.151	0.879
	A	0.056	-0.638 - 1.769	0.355
	T	0.054	-0.533 - 1.471	0.357
	rs11030102			
	CC			
	CG	0.082	-0.243 - 1.644	0.145
	GG	0.012	-1.495 - 1.854	0.833
	C	0.034	-1.213 - 2.253	0.555
	G	0.085	-0.243 - 1.644	0.145
	rs66866077			
	CC			
	CT	-0.22	-1.627 - 1.080	0.691
	TT	-	-	-
	C			
	T	0.022	-1.080 - 1.627	0.691
	rs75298795			
	CC			
	CT	-0.075	-1.889 - 0.346	0.175
	TT	0.055	-1.453 - 4.411	0.321
	C	-0.084	-5.301 - 0.800	0.147
	T	-0.078	-1.889 - 0.346	0.175
	rs76324918			
	TT			
	CT	0.019	-1.190 - 1.681	0.736
	CC	-0.006	-5.307 - 4.761	0.915
	C	0.019	-1.190 - 1.681	0.736
	T	0.011	-4.674 - 5.711	0.844
	rs77135086			
	AA			
	TA	0.037	1.604 - 0.790	0.503
	TT	-	-	-
	A			
	T	0.037	-0.790 - 1.604	0.503
	Haplotypes			
	C A A C C C T A	0.064	-0.706 - 2.382	0.286
	C A A G C C C A	0.010	-2.551 - 3.022	0.868
	C A A G C C T A	-0.015	-1.804 - 1.394	0.801
	C A A G T C T A	-0.077	-5.476 - 1.055	0.184
	C G T C C C T A			
	C G T C C C T T	0.008	-1.278 - 1.462	0.895
	C G T C C T T A	-0.013	-1.600 - 1.280	0.827
	T A A C C C T A	-0.105	-4.012 - 0.189	0.074
NPY	**rs16142**			
	AA			
	AG	0.066	-0.397 - 1.494	0.254
	GG	0.034	-1.979 - 1.067	0.556
	A	0.074	-0.536 - 2.544	
	G	0.067	-0.397 - 1.494	
	rs2023890			
	AA			
	GA	0.004	-0.924 - 0.989	0.946
	GG	-0.049	-2.607 - 1.005	0.383
	A	0.051	-1.045 - 2.713	0.383
	G	0.004	-0.924 - 0.989	0.946
COMT	**rs4680**			
	AA	-0.040	-1.731 - 0.873	0.517
	AG	0.045	-0.633 - 1.365	0.471
	GG			
	A	0.042	-0.633 - 1.365	0.471
	G	0.074	-0.431 - 2.021	0.203
	rs165599			
	GG			
	GA	-0.045	-1.640 - 0.911	0.574
	AA	0.004	-1.275 - 1.345	0.958
	G	-0.048	-1.368 - 0.568	0.416
	A	-0.033	-1.640 - 0.911	0.574
	rs2097603			
	AA			
	AG	0.010	-0.865 - 1.035	0.860
	GG	0.071	-0.523 - 2.255	0.220
	A	-0.061	-2.143 - 0.664	0.300
	G	0.020	-0.783 - 1.114	0.732
SLC6A4	**rs1042173**			
	AA			
	CA	-0.002	-1.013 - 0.974	0.969
	CC	0.033	-0.944 - 1.642	0.595
	C	-0.002	-1.1013 - 0.974	0.969
	A	-0.035	-1.597 - 0.859	0.555
	R^2		0.239	

	Haplotypes			
	C?CCCCGTG	-0.005	-2.785 - 2.620	0.952
	CCCCCCACA	-0.033	-5.611 - 3.651	0.676
	CCCCCCGTA			
	CCCCCCGTG	0.006	-1.873 - 1.982	0.955
	CCCCGCACA	0.144	-0.602 - 3.483	0.165
	CCTCGCACA	0.028	-2.156 - 2.976	0.752
	CCTTGCACA	0.012	-4.299 - 4.998	0.882
R^2			0.297	

BDNF Haplotypes: rs6265-rs2030324-rs11030101-rs11030302-rs66866077-rs75298795-rs76324918-rs77135086

MAOA Haplotypes: rs142677545-rs142369182-rs73211189-rs147023114-rs5905809-rs201583370-rs3788862-rs909525-rs5905823