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TITLE OF THE Ph.D. THESIS

Towards an evolutionary perspective of bipolar disorders: Is there a genetic link between bipolar disorders and non-pathological (adaptive) hyperactivity?

Scientific Disciplinary Sector(s)

MED/50
Regenerative Medicine and Biomedical Applications

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Three years ago, when I started this life chapter, I had no idea how much my life would change. If I had to sum up this whole Ph.D. journey in one word, I would go with one single word: GROWTH. Professional and personal.

I thank my colleagues, assistant professors Alessandra Scano, Giulia Cossu, and Federica Sancassiani, for all the advice they provided during this journey. Thanks also to Professors Germano Orrù and Antonio Preti, who selflessly shared their knowledge and experiences. And finally, thanks to my mentor, Professor Mauro Carta, from whom, in addition to psychiatry and great minds, I also learned about life.

I dedicate my doctorate to my parents, who are my biggest supporters.

Dr. Goce Kalcev

Project development

	I year	II Year	III year
Literature screening			
Insight into susceptibility genes associated with bipolar disorder: a systematic review			
Writing and submitting the protocol for the study			
Research period abroad (Skopje, N. Macedonia)			
Design and administration of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA)			
Administration of other questionnaires			
Blood samples			
DNA extraction			
PCR method + FRET probes			
Sanger sequencing			
Publications			

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List of abbreviations

BD	Bipolar disorders
WHO	World Health Organization
B.C.	Before Christ
A.D.	Anno domini (in the year of the Lord)
DSM	Diagnostic and Statistical Manual of Mental Disorders
TEMPSA	Temperament Evaluation of the Memphis, Pisa, Paris, and San Diego auto-questionnaire
HPA axis	The Hypothalamus-Pituitary-Adrenal Axis
DNA	Deoxyribonucleic acid
GWAS	Genome-wide association studies
SNP	Single nucleotide polymorphism
ANK3	Ankyrin 3
NCAN	Neurocan
TENM4	Teneurin Transmembrane Protein 4
TRANK1	Tetratricopeptide Repeat and Ankyrin Repeat Containing 1
SYNE1	Spectrin Repeat Containing Nuclear Envelope Protein 1
CACNA1C	Calcium Voltage-Gated Channel Subunit Alpha1 C
LTCCs	Cav1.2 L-type calcium channels
CaMKII	Calmodulin-dependent protein kinase II
PKC	Protein kinase C
CREB	cAMP response element-binding protein
CCAT	Calcium channel-associated transcription
LTP	Long-term potentiation
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
NMDA	N-methyl-D-aspartate
CA1	Hippocampal cornu ammonis
Ca^{2+}	Calcium ions

ZNF804A	Zinc Finger Protein 804° gene
DRD 3	Dopamine receptor 3 receptor gene
DRD 4	Dopamine receptor 4 receptor gene
PCA	Principal component analysis
H/NS	Hyperactivity and novelty seeking
BRIAN	Biological Rhythms Interview of Assessment in Neuropsychiatry
SPSS	Statistical Package for Social Sciences
ANOVA	Analysis of variance
MDQ	Mood Disorder Questionnaire
SDS	Sodium dodecyl sulfate
PCR	Polymerase chain reaction
CTAB	Hexadecyltrimethylammonium bromide
NaCl	Sodium chloride
FRET	Fluorescence resonance energy transfer
HCL	Hydrochloric acid
μL	microliter (microlitre)
TRIS	tris(hydroxymethyl)aminomethane
G/G	wild-type sequencing (linked to no risk of bipolar disorders)
G/A	heterozygous profile
A/A	homozygous profile

Introduction

1.1. Bipolar disorders

1.1.1. The definition and burden of bipolar disorders

Bipolar disorders (BD) are recurrent and chronic disorders that affect >1% globally. According to the World Health Organization (WHO), BD has affected 46 million people worldwide in recent years [1]. Following a large cross-sectional survey of 11 countries, the overall lifetime prevalence of bipolar spectrum disorders was 2.4%, with a prevalence of 0.6% for bipolar type I and 0.4% for bipolar type II [2].

Bipolar disorders cause unusual changes in the person's mood, energy, and capacity to function. The clinical history of the disease may present episodes ranging from mild depression and transitory hypomania to critical psychotic mania or depression [3]. The elevated rates of delayed or mistaken diagnosis reported by bipolar patients imply that there is still a huge gap between the available knowledge of BD and its implementation in the clinical setting [4]. There are still controversies related to the identification, classification, and management of this condition. It is widely accepted that bipolar disease is often misdiagnosed. An average of 8.9 years passed between the beginning of symptoms and the appropriate diagnosis of them [5]. The symptoms can also be inconsistent (beginning with impulsive behaviour, fluctuating energy levels, and substance abuse), and they can mask the BD. Accurate and early diagnosis is challenging in clinical practice since the onset of bipolar disorder is frequently characterized by nonspecific symptoms, mood lability, or a depressive episode that can resemble unipolar depression in presentation [6]. Additionally, particularly with hypomanic or manic symptoms, patients and their families frequently do not comprehend the significance of their symptoms [7].

Importantly, this disorder is related to an enhanced risk of mortality. About 25% of patients have attempted suicide, and 11% have died by suicide [8]. All these characteristics suggest that bipolar disorders are a critical public health problem.

1.1.2. Historical overview of bipolar disorders

The history of mood disorders dates back to ancient times. In fact, descriptions of mood swings can be found in every ancient culture, from Babylonian to Egyptian to Hebrew, and are frequently attributed to supernatural or divine forces acting as a form of punishment [9].

The earliest mentions of bipolar disorders in medicine are related to Hippocrates (460-370 B.C.), a well-known physician in ancient Greece, often referred to as “the father of medicine.” He was the first to observe two extreme mental states: feeling extremely depressed (what we now refer to as depression) and feeling extremely stimulated or excited (mania). Interestingly, melancholia was Hippocrates' term for the state of extreme sadness. The phrase directly means "black bile," because "melas" and "chole" both imply black. On the other hand, mania was thought to be brought on by an excess of yellow bile. Hippocrates also presented a cyclical nature to the illness and identified four temperamental subtypes-choleric, melancholic, sanguine, and phlegmatic-as potential risk factors for various mental illnesses. All were produced by an extreme part of the corresponding temper (yellow bile, black bile, blood, and phlegm) [10].

In his writings, the Athenian philosopher Plato (428-348 B.C.) discussed the idea of mania, differentiated into two types: "one involving a mental strain that arises from a bodily cause of origin, the other divine or inspired [11]."

The first-century Greek physician Aretaeus of Cappadocia is recognized as someone who first expressed the idea of a mood spectrum with these extreme moods on each end. He believed that mania and melancholia were linked to brain issues. Being able to separate the two, Hippocrates and Aretaeus each worked to establish the fact that melancholia and

mania were biological disorders and not just psychological responses to certain situations [9].

Later, Galen (131-201 A.D.) evaluated the genetic and environmental factors that contributed to the development of the pathology and linked the onset of melancholia to a cerebral alteration [12].

In the Middle Ages, when all mental disturbances were once more attributed to magical and religious causes, the "humoral" hypothesis was neglected. Depression is once again linked to guilt and sin, which are blamed on demonic possession [9].

At the beginning of the 1500s A.D., there was a comeback to the clinical-scientific classification of mental illnesses: various forms of depression were classified, relating them to organic disorders or to qualitative and quantitative changes in mood [9].

Afterwards, depression and mania were perceived as distinct disorders with different symptoms up until the middle of the 19th century. Around 1850, Jean-Pierre Falret (1794-1870), a French psychiatrist, developed a new and distinct disorder that included both syndromes. He developed the term "folie circulaire," which describes a condition in which a person experiences cycles of mania and depression with various intervals in between [13]. In the same period, another French psychiatrist and neurologist, Jules Baillarger, described a condition called “folie à double forme.” His definition for this status was characterized by periods of mania and depression, but without intervals in between. In time, he said, one extreme would simply turn into the other [14].

Meanwhile, Karl Kahlbaum (1828-1899), a German psychiatrist, divided mental disorders into two categories: those that caused just a slight disturbance of the mind and those that caused a complete disturbance of the mind [15]. A second German psychiatrist,

Emil Kraepelin (1856-1926), who is considered the father of modern psychiatry, combined all types of affective disorders into one disease called manic-depressive disorder. Despite some criticism, Kraepelin's theory was initially accepted [16].

Similar to Kraepelin, Freud developed a psychodynamic theory to explain depression and mania. He came to the conclusion that mania represented the denial of loss and depression was related to grief over the loss of an emotionally significant "object [17]."

Leonhard coined the term "bipolar" to describe mood disorders in which manic and depressive phases alternate, as well as to speculate on the hypotheses for a third type of psychosis known as "cycloid [18]."

In the 1950s, in an effort to standardize and categorize mental illness, experts produced the first Diagnostic and Statistical Manual of Mental Disorders (DSM-I). Manic-depressive insanity, described by Kraepelin as a singular condition, was divided into three categories by the DSM-I: manic, depressive, and other. The category "other" was used to group the bipolar disorder cycle [19]. In 1968, some of the terminology was modified with the publication of the DSM-II. It introduced the term "manic-depressive illness" instead of "manic-depressive insanity." The statement "circular," which was defined as "at least one attack of both a depressive episode and a manic episode," replaced the confusing "other" in the third category. Bipolar disorder was first identified as such in the third edition of the DSM, which was published in 1980. Besides that, it was the first time that the mood disorder was differentiated from generalized depression and the first time that it was defined according to contemporary standards [20].

1.1.3. Etiology and pathogenesis of bipolar disorders

Despite various theories being put forward so far over the years, the origin of BD has always been attributed to organic factors, particularly genetic and neurochemical variations. We can be certain today that a wide range of factors play a role in the formation of mood disorders [21].

In the last decade, there has been an increasing amount of research on the genetics of bipolar disorders, underlying developmental pathways, risk factors, vulnerabilities, interactions between genes and the environment, and putative features of BD [22]. Evidence from twin studies indicates that monozygotic concordance is between 40 and 70 percent, and first-degree relatives have a lifetime risk of 5 to 10 percent, approximately seven times greater than the risk for the general population [23]. Currently, genetic variants that have been linked with BD cannot be applied to predict individual risk, the disorder's course, or the medication results. Additionally, the polygenic identity of these disorders makes it questionable whether a deterministic prognosis will be achievable in the future.

Likewise, a variety of environmental factors, starting with perinatal and prenatal factors, including prenatal viral infections, generally correspond to bipolar disorders [24]. It appears that stressful physical, psychological, or social actions, as well as loss events such as grief and loss, act as triggers for the occurrence of BD. According to some studies, stressful life events like divorce and unemployment, as well as a recent marriage, were tied to the first hospitalization for a manic episode. Such studies also support the concept that life events can end up causing bipolar disorder to progress [25].

With advancements in molecular technologies, our understanding of the pathogenesis and pathophysiology of bipolar disorders has improved. Latest studies have concentrated

more on the modulation of synaptic and neural plasticity in the prefrontal cortex, hippocampus, amygdala, and other limbic system regions in bipolar disorders [26, 27]. In fact, BD patients' post-mortem tissue has shown dendritic spine loss in the prefrontal cortex [28]. BD are also being investigated in terms of the cellular and molecular changes that can affect neuronal interconnectivity, such as mitochondrial dysfunction, endoplasmic reticulum stress, neuroinflammation, oxidation, apoptosis, and epigenetic changes [29]. It is not specified, however, whether the dysfunction of these pathways contributes to the emergence of BD, although this area of study is still in its early stages.

1.1.4. Classification of the bipolar disorders

Currently are recognized four types of bipolar disorder:

Bipolar I: A condition characterized by one or more episodes of mania lasting at least seven days. A depressive episode may or may not occur. This category represents the modern version of manic-depressive disorder (or affective psychosis) previously described in the 19th century [9]. In contrast to the classical description of the disorder, neither psychosis nor having experienced a major depressive episode throughout one's lifetime is a requirement. Depressive episodes typically occur as well and last for at least two weeks. Episodes of depression with mixed features (having depressive symptoms and manic symptoms at the same time) are also seen [30].

A manic episode's mood is frequently described as euphoric, excessively cheery, high, or "feeling on top of the world." In some circumstances, the attitude is of such a highly contagious quality that it can be immediately recognizable as excessive and may be characterized by endless and irregular enthusiasm for interpersonal, sexual, or professional interactions. People with an inflated sense of self-worth, which can range from uncritical self-confidence to explicit grandiosity, may attempt complex tasks such as writing a novel or gaining publicity for an unrealistic discovery. One of the most characteristic features is a decreased need for sleep, which is different from insomnia, in which the person wants to sleep or feels the need to sleep but is unable [31]. The individual’s speech can be rapid, under pressure, loud, and difficult to interrupt. Often, a person's thoughts move faster than they can express them verbally. As well, there is a flight of ideas, including a nearly constant flow of accelerated speech and sudden transitions between topic areas. Speaking may become unorganized, incoherent, and especially disturbing to the individual if there is a severe flight of ideas. Poor judgment,

a lack of insight, and other factors frequently lead to the terrible effects of a manic episode (legal issues and significant financial difficulties) [32].

Bipolar II: a state in which depressive episodes alternate with mild manic episodes but not full-blown manic episodes. This bipolar disorder category is defined by the template of (hypo)manic and depressive episodes, but it lacks the full-blown manic episodes that characterize Bipolar I Disorder. Patients who have had at least one depressive episode followed by at least one spontaneous hypomanic episode are included in this category [33]. The onset age is later, the episodes are shorter, but there are many more relapses overall. The age of onset is later, the episodes last less time, but there are many more relapses overall. Since the patient does not perceive the (hypo)manic episodes as a negative moment but rather as a time of intense activity and energy, identifying them as a diagnosis can be challenging. Commonly, the (hypo)manic episodes do not cause impairment, and are often not viewed as pathological or disadvantageous by the patients. Instead, the major depressive episodes are what really cause the impairment, and people with bipolar II disorder generally see a clinician during a major depressive episode [34]. It is regularly beneficial to get clinical information regarding bipolar II disorder from other informants, such as close friends or relatives. Bipolar II disorder patients are more chronically ill and spend, on average, longer in the depressive phase of their illness than those with bipolar I disorder [35].

Cyclothymic disorder: switching between depressive and manic states for at least 2 years, with periods of normal mood lasting less than 8 weeks. This condition is followed by a sudden and continuous alternation of mild-to-moderately intense depressive and hypomanic phases that do not sufficiently satisfy the full episode [36]. The main attribute of cyclothymic disorder is a persistent, fluctuating mood disturbance with multiple,

distinct episodes of hypomanic symptoms and episodes of depressive symptoms. To completely meet the criteria for a hypomanic episode, the hypomanic symptoms must not be present in sufficient numbers, severity, pervasiveness, or duration. Cyclothymic disorder is diagnosed only when the components of a major depressive, manic, or hypomanic episode have never been met [37].

Unspecified bipolar disorder: this occurs when a person does not meet any of the above criteria but has significant mood elevation [36].

Type of Bipolar Disorder	Characteristics
Bipolar I	A manic episode ± major depressive or mixed episode
Bipolar II	A major depressive episode ± hypomanic episode
Cyclothymic disorder	Chronic fluctuation between subsyndromal and hypomanic episodes (at least 2 years for adults)
Unspecified bipolar disorder	Any bipolar disorder that does not meet criteria for any specific bipolar disorder

Table 1. *Classification of Bipolar Disorders*

1.2. Temperaments

1.2.1. Definition and classification of the temperaments

This term describes the emotional aspect of personality, serving as a link between the psychology and biology of affective disorders. Temperament is now thought to be the temporally stable biological "core" of personality, and it refers to an individual's activity rhythms, levels, moods, and related cognitions, as well as their variability. While personality is a more broad phenotype, it also refers to acquired interpersonal operations [38].

Affective temperament is a collection of five relatively stable, genetically determined forms of emotional response (temperaments), which may be the predisposing component in the development of affective disorders [39]. It is considered that they represent behavioural endophenotypes genetically associated with mood disorders. The idea that various types of temperament are based on constitutional types of behaviour, is linked to Hippocrates' ancient humoral theory. Additionally, he noted that these fundamental affective characteristics were frequently observed in the blood relatives of manic-depressive patients [40].

In their soft, subaffective form, they could be adaptive in an evolutionary context, in particular by subserving superior adjustment to social and professional conditions [40]. In contrast to depressive-like devotion to work, the hyperthymic temperament fosters leadership, excessive involvement, and robustness to stress, making these traits desirable in professional settings. Thus, affective temperaments might indicate a propensity for doing particular jobs [41]. While the hyperthymic temperament encourages robustness to occupational stress, the depressive, cyclothymic, irritable, and anxious temperaments are associated with a poorer response to stress emerging from professional situations [42].

Stable depressive mood, introversion, low energy level, and hypersomnia are characteristics of the depressive temperament [43]. A hyperthymic temperament is characterized by extroversion, a lot of energy, intense emotions, and little need for sleep. A central feature of the cyclothymic temperament is demonstrated by mood swings and emotional instability [44]. The irritable temperament, which is less consistent than the others, is characterized by a propensity for being aggressive and litigious as well as having problems forming close relationships with others [45]. An emerging occurrence is the development of a putative phobic-anxious temperament that is defined by increased sympathetic activity, fear of illness, hypersensitivity to separation, difficulty leaving familiar surroundings, a pronounced need for reassurance, and extreme sensitivity to drugs and substances [46].

Temperament	Characteristics
Hyperthymic	Leadership, hyperactivity, novelty-seeking, exploration, risk-taking, grandiosity, extroversion
Cyclothymic	Inconstant periods of high and low professional and creative productivity, labile self-confidence, social withdrawal followed by unrestrained sociability
Depressive	Pessimistic, preoccupied with failure and negative things, self-disciplining
Anxious	Preoccupied with taking care of others, concern for potential or current external threats to oneself or their relatives
Irritable	Choleric, agitated, dysphoric

Table 2. *Classification of temperaments and their features*

1.2.2. Historical overview of the temperament's development

Kraepelin believed that constitutionally determined personal dispositions-what we now call "temperament"-are the fundamental states from which various affective states arise [47]. Kraepelin's approach was further developed by Kretschmer, who believed that there was a central cyclothymic disposition that, in some individuals, manifested in depressive attributes and, in others, in hypomanic attributes [48]. Another German psychiatrist, Kurt Schneider, who had a profound influence on the field through his writings on what he termed "psychopathic personality," believed that such dispositions as depressive, labile, and hyperthymic had little to do with the "cyclothymic" disorders [49]. Schneider's point is similar to the one taken by DSM-IV. In this pattern, patients presenting with unmistakable affective signs and symptoms, instead of being considered cyclothymic, would receive such labels as "psychopathic," "histrionic," or "borderline." Likewise, patients with double depression could receive personality characterization as "passive," "avoidant," or "obsessoid," rather than being considered "depressive" in temperament. The main problem with the DSM-IV conceptualization is that patients today are at risk of being considered "character-flawed" rather than having constitutionally-based affective disorders [49].

The modern concept of affective temperaments developed by Akiskal and colleagues was largely inspired by the writings of Kraepelin and Kretschmer and was based on both theoretical deliberations and clinical observations [50]. This idea was developed into an instrument known as the Temperament Evaluation of Memphis, Pisa, Paris, and San Diego (TEMPS), which was initially released as a semi-structured test (TEMPS-I, interview version) for determining temperament types such as depressive, cyclothymic, hyperthymic, and irritable [51]. Later, the auto-questionnaire version (TEMPSA) was

created, containing 110 items (109 for men), including the addition of an anxious temperament, and only requiring simple "yes" or "no" responses [52].

Having sub-threshold characteristics or temperament without full-blown disorders can sometimes be maladaptive or present elements of paradoxical adaptivity. However, if we reflect very deeply on the profound implications of these concepts, a fundamentally evolutionary vision of the bipolar spectrum emerges that can predict how the alleged basic vulnerability can also represent a potential advantage in some situations, such as in megacities “in which life is expected to run 24 hours a day for seven days a week” [53].

1.2.3. Hyperthymic temperament and features

The basic components of this type of temperament that stand out are hyperactivity and goal achievement. As previously described, a hyperthymic temperament manifests extroversion, a high energy level, emotional intensity, and a moderate need for sleep. Actually, these kinds of traits seem to provide well-defined advantages in leadership, competition, exploration, and territoriality [54]. The hyperthymic temperament can be classified as abnormal only in the presence of chronic hypomanic symptoms or advanced mood disorders. In general, temperaments have been verified to belong to the domain of normality rather than the sphere of pathology, in accordance with their putative adaptive role [54].

Commonly, achieving success, experiencing excitement and joy, and moving towards core life goals are moments of great importance in life. In this approach, goal and drive achievement demonstrates an adaptive and beneficial side of hyperthymia. On the positive side, setting high goals is one of the strongest predictors of success. It indicates the willingness to set high goals and spend energy pursuing them, which could help clarify the high rates of creative efforts among people with a hyperthymic temperament [55]. However, the pathological scenery appears linked to variability in the adaptiveness with which people follow life goals and accomplishments [56, 57].

1.2.4. The role of affective temperaments in the evolution and course of mood disorders

Psychopathological characteristics and affective temperament are considered to have an effect on how BD develops clinically and over time. This is especially true for putative temperamental traits (cyclothymia and hyperthymia) that may play a predisposing or pathoplastic role in the antidepressant-induced (hypo)manic vulnerability and/or in several co-morbid syndromes [54]. Most of the accessible literature on the pathoplastic role of temperament is concerned with bipolar II or unipolar cases. For instance, unipolar patients with cyclothymia or hyperthymia are reported to have an earlier age at onset, higher levels of education, and higher levels of income [54].

Despite the fact that the relationship between affective temperaments and major mood episodes is quite complex, studies constantly show that hyperthymic and rarely cyclothymic temperaments are characteristic of bipolar I disorder, while a depressive temperament prevails in unipolar major depression [58]. However, hyperthymic temperament is more prevalent in bipolar I patients, who experience more frequent manic episodes than bipolar I patients with a predominant depressive polarity and characteristic depressive temperament [35]. By contract, cyclothymic temperament is highly sensitive (88%) in identifying bipolar II disorder, as the most frequent affective temperament type among patients with bipolar II disorder. Particular affective temperaments may have an impact on the course, clinical expression, and prognosis of mood disorders [35].

Interestingly, an affective temperament profile comes across as an important factor in developing spontaneous or antidepressant-associated hypomania in major depression. For instance, bipolar II depressive subjects with spontaneous hypomania have higher ratings of cyclothymic temperaments and an earlier age of onset [59]. On the other hand, bipolar

II patients with antidepressant-associated hypomania have higher ratings for depressive temperaments. This finding indicates that antidepressant-associated hypomania in major depression is recognised as a genetically less penetrant expression of bipolar II disorder [59].

1.2.5. Affective temperaments as an element of the unipolar and bipolar mood disorder spectrum

Preceding clinical follow-up studies as well as familial-genetic, biological and treatment-response investigations in clinical populations found that there is a continuum between cyclothymic disorder, bipolar II disorder and bipolar I disorder, and also between subsyndromal depression, minor depression/ dysthymia and unipolar major depression, suggesting that patients with milder forms of mood disorders have high risk for progressing its more severe forms [60]. Otherwise, analogous types of studies on affective temperaments, including healthy and nonclinical populations, can provide not only a deeper understanding of the causes of mood disorders but also the potential to identify individuals who are especially vulnerable to developing clinical forms of mood disorders.

The concept of the "Bipolar Spectrum" is a hot topic in both clinical psychiatry and research into the genetic origins of bipolar disorder. H. Akiskal presented the thesis that broad concepts of bipolarity help in identifying patients on the border of bipolar disorder who might be destabilized by antidepressants and could potentially benefit from therapeutic interventions developed for classical bipolar disorder [5]. As far as genetic investigations are concerned, some authorities believe that it would be easier to identify the molecular basis of the illness by focusing on the "hard core" euphoric manic phenotype, while others contend that the most prevalent expressions of bipolar disorder belong to a "soft spectrum," the inclusion of which in genetic investigations would facilitate the discovery of the oligogenic basis for bipolarity. Whether soft manifestations of bipolar disorder can be identified reliably in epidemiologic and clinical populations, and if so, what strategies should be used to validate them [39].

A good starting point for the validation of a spectrum concept is the prevalence of the condition in the general population [61].

1.2.6. Affective temperaments: genetic backgrounds

Generally, the temperament, and affective temperament in particular, is frequently agreed to be biological in nature, determined by genetic factors [39]. They are thought to be mediated by genes involved in central serotonin and dopamine/noradrenaline neurotransmission, which is disrupted in unipolar and bipolar major mood disorders. Some personality traits such as novelty seeking and reward dependence, which strongly relate to hyperthymic and cyclothymic temperament, have been reported to have a significant association with the CACNA1C gene, as well as the dopamine receptor 3 (DRD3) and dopamine receptor 4 (DRD4) receptor genes, which raises the probability that these genes could be effective candidates to describe the potential molecular genetic basis of the hyperthymia-hypomania-mania spectrum [62].

1.3. An evolutionary perspective on bipolar disorders

There has been a lot of interest in the impact of environmental factors, including social life, on brain metabolism and biochemistry, and thus on people's behaviour in recent decades. Nowadays, it is understood that the environment has an impact on our health, and special attention has been given to mental health and mental disorders [65]. It is evident that the neuroendocrine response to environmental stimuli has an impact on brain functions and mental health [66].

It must be highlighted that the interactions between genes and the environment are of particular interest to neurogenesis and neurobiochemical systems development that function as the Hypothalamic-Pituitary-Adrenal (HPA) axis, which is behind the regulation of numerous physiological processes, including digestion, the immune system, mood and emotions, energy, and sexuality, and how they can lead to an emotional response [67]. Actually, the HPA axis regulates cortisol secretion and physiological stress responses in mammals through a feedback-regulated neuroendocrine system, and this activation promotes the organism's effective adaptation to impending threats by shifting the physiological priorities from functions to supporting protective behaviours [68].

Currently, there is evidence for the cumulative impact of social stress and the activation of the HPA axis, which accelerates the development of mental disorders. Indeed, it has been shown that severe and chronic stress can lead to changes in the prefrontal and limbic systems, with lifelong modifications and adjustments in region-specific gene expression, neural plasticity, neuroendocrine function, and behavioural reactions to stressors [65, 68].

Modern city life can have a significant impact on mental health [65, 69]. Certainly, psychiatric disorders tend to be more frequent in urban areas, and their onset, severity, and prognosis are influenced by their exposure to urban environments. The urban specific characteristic and consequent adaption have a high level of variability, and can mediate for a variety of environmental influences [70]. Several factors can affect the regularity of biological rhythms as a part of modern city life, exposing individuals to strong stress but also constituting a potential advantage in terms of adaptation under peculiar circumstances [71]. This potentially adaptive effect might be more evident in the megacities, in which life is supposed to occur 24 hours a day, seven days a week, with complete alterations of sleep-wake cycles [72]. According to this viewpoint, the presence of bipolar genes in a population may be associated with adaptive characteristics in specific circumstances or, conversely, can expose individuals to stress. On one side, they can therefore precede success in relation to the performance that a contemporary urban lifestyle often demands, or, on the other side, they can predispose to the risk of developing bipolar disorders [72]. Cornerstones such as creativity, extraversion, leadership, and openness can spread from adaptive and functional utility to constitute a vulnerability to mental illnesses under specific adverse conditions [71].

From an epidemiological perspective, the hypothesis of adaptive aspects of hyperactivity may explain certain phenomena in modern societies, as witnessed by the continuing increase in the prevalence of mood disorders [71, 72]. Behaviours and reactions as responses to specific demands of the environment might resemble bipolar spectrum disorders, but this could be just a form of adaptive behaviour [73]. This could justify the growing number of overdiagnoses of bipolar disorders [74]. Furthermore, some adaptive

features of hyperactivity, such as those of explorers with hyperthymic temperaments and migrants, may drive people to seek novelty [75].

Also, it has been hypothesized that specific personality traits (or traits of temperament according to Akiskal's definition) associated with a high risk of psychopathology, specifically a high risk of bipolar disorder, could, under defined conditions, produce adaptive behaviours [76, 77]. This theory was supported by observations of how, in the face of rapid social change, people with aptitudes for exploration and hyperactivity could operate a culture "leap," allowing them to acquire "new" and unusual behaviours for their culture of origin while "winning" in terms of new social and economic needs [78]. Such a revolutionary leap would not be without dangers; those who adapt will acquire relevant roles in the new context, while, in contrast, those who try the “leap” without success will be at high risk of psychopathology. This model could explain the epidemic of mood disorders that, according to some lines of research, would have developed in the modern era starting with the "English disease" of the early stages of industrialization [79].

Based on those observations, a study of Sardinian migrants to Latin America was developed [80]. This study showed that migrants who had freely decided to leave for new destinations and second-generation sons of migrants with both Sardinian parents, living in megacities such as Buenos Aires or Sao Paulo, had more frequent episodes of sub-clinical hypomania than the Sardinians who had remained in Sardinia, even if they had an equal frequency of overt mood disorders [80]. This evolutionary/socio-biological vision of the bipolar spectrum could explain how the alleged basic vulnerability linked to a specific pattern of the response of social and biological rhythms can, in contrast,

represent a potential advantage in modern megacities, immersed in light and noise pollution, "in which life runs 24 hours a day for seven days a week." [71, 72]

Another interesting point is the recognition of the component in "new megacity contexts" (apart from migration) that may favour the frequency of a sub-threshold profile of the bipolar pattern. Actually, humans are condensing progressively in huge cities with millions of people. Noise and light pollution are the most reported consequences, with a big impact on sleep patterns and circadian biorhythms [81]. The challenging modern human life has apparently altered, endangering sleep patterns. Although the actual mechanism is unclear, people who have poor sleep quality may be more vulnerable to the negative effects of road traffic noise on their mental health [82]. Otherwise, artificial light alters daily rhythms by allowing activities that are typically carried out during daylight hours to continue during periods of natural darkness. This has a significant impact on the immune-endocrine and circadian mechanisms, as well as other endogenous rhythms that have ensured that human behaviour is more impactful when it is synchronized with variations in light and other environmental factors like seasons and weather [82]. According to some studies, bipolar disorders may be triggered by disruptions of the sleep-wake cycle and artificial light pollution [81, 82].

The suppression of the increase of melatonin at night and the subsequent effect on the methylation of DNA increase the risk of prostate and breast cancer [83, 84]. Although the mechanisms underlying melatonin-steroid-induced mechanisms are generally very complex, the block of melatonin at night due to light pollution causes the estradiol/progesterone ratio to be out of balance in favour of estradiol [83, 84]. As well, the negative impact of light pollution on neurosteroid receptors can also affect mood. Neurosteroids are produced in the brain and have an effect on neuroreceptors in various

brain regions [85]. Furthermore, peripherally synthesized steroids cross the encephalic barrier and cause sequelae matching those of the neurosteroids in modifying brain excitability [85].

Subsequently arise the doubt if the living in the large cities increase the risk of hypomania rather than vice versa [80]. Large cities can provide opportunities and be selective for people with a hyperthymic temperament or who can benefit from concentration skills and the ability to overcome periods of overwork with a little sleep and skipping meals. These changes can also expose people to "new diseases," as in young people coming from traditional communities to large cities. Especially if the new "exaggeration of rhythm" encourages the use of stimulants and anabolic steroids, strong liquors instead of wine and beer, and the media urgently proposes goals that can be a source of frustration (or success) [75]. From a sociobiological point of view, the need for novelty that drives hyperactive and novelty-seeking people towards megacities, the creation of the accelerated milieu in their likeness, and the impact of the new environment on increasing the risk of hypomania act synergic [75, 80]. An ethological perspective shows that genetic predisposition "alone" does not explain how apparently disadvantageous behaviours can become adaptive if modulated by social opportunities.

1.4. Genetic background of bipolar disorders

Large psychiatric disorder cohorts have been used in genome-wide association studies (GWAS), which have produced reproducible common and rare genetic variations that are associated with disease risk. Attempts are currently being made to translate these findings into clinical practice, genetic counselling, and predictive testing [86, 87]. Some experts, however, remain cautious. The associated SNPs are not disease-specific, and the vast majority of people who have a "risk" allele are healthy. Instead, population-based genome-wide studies in psychiatric disorders have rediscovered previously unknown rare structural variants and mutations in genes that cause genetic syndromes [88]. Despite the fact that these conditions do not fit the classic description of any specific psychiatric disorder, they frequently exhibit nonspecific psychiatric symptoms that cross diagnostic boundaries, such as intellectual disability, behavioural abnormalities, mood disorders, anxiety disorders, attention deficit, impulse control deficit, and psychosis.

For many decades, it has been known that genetic factors play a significant role in BD. The heritability of BD has been estimated to be between 80% and 85% [89]. Models of illness are most consistent with multifactorial inheritance. Common variants of a small effect (odds ratio 1.2) have, however, been demonstrated and replicated [90]. Individual gene variant identification has yielded promising results in BD biology, particularly in the identification of genes coding for calcium channel subunits such as CACNA1C [91]. Current methods estimate that the cumulative impact of many frequent alleles with small effects may explain 38% of the BD phenotypic variance [92]. Rare, large-effect variants, such as copy number variants, have been proposed to play a role in BD susceptibility. Calcium channel involvement in the genetic predisposition to BD is the single most consistent finding in BD genetic studies to date [93, 94]. Tremendous efforts have been

made to identify genetic risk factors or biomarkers that would identify individuals at risk and allow for early diagnosis and treatment.

Apart from the CACNA1C (Calcium Voltage-Gated Channel Subunit Alpha1 C) gene, there are also other genes that are linked with BD:

ANK3 (Ankyrin 3)

Ankyrins are a family of proteins that are believed to link the integral membrane proteins to the underlying spectrin-actin cytoskeleton. They play key roles in activities such as cell motility, activation, and proliferation [91].

NCAN (Neurocan)

This gene is involved in the modulation of cell adhesion and migration. A SNP (rs1064395) in the NCAN gene was established to be a risk factor for BD in the European population. The gene is found on chromosome 19 and encodes a protein located in the extracellular space, in the Golgi apparatus, and in the lysosomal cavities [95].

TENM4 (Teneurin Transmembrane Protein 4)

The protein encoded by this gene plays a role in establishing proper neuronal connectivity during development. The hotspot mutations reported in this gene in patients with bipolar spectrum disorders are the variants rs12576775 and rs17138171 [96].

TRANK1 (Tetratricopeptide Repeat and Ankyrin Repeat Containing 1)

Diseases associated with TRANK1 include epileptic encephalopathy and bipolar disorder. The main studied SNP is rs9834970, which is about 12 kb at the 3 ‘UTR of the TRANK1 gene [96].

SYNE1 (Spectrin Repeat Containing Nuclear Envelope Protein 1)

The gene encodes Nesprin-1α, an outer nuclear membrane intracellular protein that connects the nucleus to the cytoskeleton through its N-terminal region. The SNP rs9371601 described in the intergenic region is associated with mood disorders. This gene is also implicated in diseases such as neurological disorders, cancer, myopathies, and arthrogryposis [96].

The features of these genes are listed in *Table 3*.

Gene	Name	Locus	Function	Suspect SNPs associated with BD	Risk Allele
CACNA1C	Calcium Voltage-Gated Channel Subunit Alpha1 C	12p13.33	Encodes an alpha-1 subunit of a voltage-dependent calcium channel	rs1006737	A
ANK3	Ankyrin 3	10q21.2	Cell motility, activation, proliferation	rs10994415	C
TENM4	Teneurin Transmembrane Protein 4	11q14	Establishing proper neuronal connectivity during development8	rs12576775	A
NCAN	Neurocan	19p13.11	Modulation of cell adhesion and migration	rs1064395	A
SYNE1	Synapsin I	6q25	Modulation of neurotransmitter release	rs9371601	T
TRANK1	Tetratricopeptide Repeat and Ankyrin Repeat Containing	3p22	Modulation of synaptic plasticity, neural growth, circadian rhythm	rs9834970	C

Table 3. *Genes and genetic variants associated with bipolar disorders*

1.4.1. CACNA1C gene

CACNA1C is a large gene located on the short arm of chromosome 12p13.3, with over 11,541 established variants [97, 98]. The majority of these variants are found in introns and downstream regions of the gene. Exon missense mutations are extremely rare. CACNA1C encodes the alpha-1 subunit of a voltage-dependent calcium channel. This subunit creates a transmembrane channel that allows calcium ions to enter the cell [99]. Calcium channels are important neuronal regulators of heart muscle contraction, but they also play a role in skeletal muscle contraction. It is supposed that the CACNA1C gene is involved in axon guidance and synaptic transmission in the brain [100]. Recent studies have linked additional intronic SNPs in and around CACNA1C to psychiatric disorders, but replication and functional studies are still lacking [101, 102]. The persistence with which GWAS signals are found within the same general region (intron 3) of CACNA1C indicates the probable significance of the gene in BD psychopathology, despite the fact that some GWAS-identified SNPs cannot be fully replicated across all studies [87]. It appears that CACNA1C has a wider clinical genetic association with mental disorders than BD. Recent research indicates a link between the CACNA1C genotype and schizophrenia and major depressive disorder.

Importantly, marginal modifications in the expression level of this gene have major effects on the intrinsic spontaneous calcium activity of neural progenitors that act in brain development [91]. Hence, the CANCA1C gene acts as a molecular switch, increasing the susceptibility to psychiatric disease.

1.4.2. L-type calcium channels (LTCCs) and pathways

Over the last two decades, work from several laboratories has established Cav1.2 L-type calcium channels (LTCCs) as crucial mediators of experience-dependent brain plasticity [103]. Recently, these channels have been recognized as cornerstones for various neuronal processes essential for normal brain development, connectivity, and function. This is further underscored by the identification of the neuropsychiatric risk genetic variants in the CACNA1C gene, which codes for the Cav1.2 α1 subunit of LTCCs [104]. These variants can modify the levels and functions of the channels, with consequences on neural processing and connectivity indicated by human imaging studies. The LTCCs are included in the family of voltage-gated Ca^{2+} channels, with Cav1.2 and Cav1.3 being the primary LTCC subunits expressed in the brain [105].

Cav1.2 channels are primarily found in postsynaptic dendritic processes and somata, distributed across the dendritic tree in neurons of the central nervous system. This somatodendritic localization of Cav1.2 places it in a key position to integrate neuronal excitation with Cav1.2-mediated Ca^{2+} signalling, modulating gene transcription, and making Cav1.2 a powerful component of signalling pathways [106].

Most of the CACNA1C risk SNPs associated with BD, particularly SNP rs1006737 and those in linkage disequilibrium, are present in a large intron between exons 3 and 4 [107]. Functional studies that evaluate the influence of risk SNPs on gene expression started to establish that these SNPs lie within regions that are under tight transcriptional control, with risk SNPs being able to alter gene expression by differentially binding nuclear proteins and also altering long-range intronic enhancer and promoter interactions within CACNA1C [108]. Biological studies that measure levels of CACNA1C have found both elevated and reduced expression of CACNA1C, depending on the brain region and the

included cellular system [108]. This fact suggests that transcriptional control of CACNA1C is highly complex and, most likely, differentially controlled at the level of brain region and cell type. Nevertheless, these studies provide evidence that intronic CACNA1C risk SNPs can change levels of CACNA1C and that loss or gain of Cav1.2 channels can contribute to the development of the disease symptoms [109].

The electrical activity of neurons and other excitable cells is dependent on a variety of voltage- and ligand-gated ion channels permeable to inorganic ions like sodium, potassium, chloride, and calcium [110, 111]. While the first three ions play primarily an electrogenic role, calcium ions are unique in that they can not only change membrane potential but also act as important signalling entities. The activation of voltage-gated calcium channels causes calcium influx along the electrochemical gradient, resulting in a localized increase in intracellular calcium into the high micromolar range [111]. In turn, it activates a variety of calcium-dependent processes, including gene transcription, neurotransmitter release, neurite outgrowth, and the activation of calcium-dependent enzymes like calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC) [112]. Dysregulation of these processes, as well as alterations in calcium channel activity, have been linked to a wide range of neuropsychiatric disorders, such as epilepsy, bipolar disorder, and migraine [107].

To date, the most credible outcome in BD genetic studies has been the active participation of calcium channels, which is significant for genetic BD susceptibility.

1.4.2.1. Calcium Channels and Gene Transcription

The alpha1c subunit of the Cav 1.2 L-type voltage-gated calcium channel is encoded by the CACNA1C gene [113]. This subunit creates the pore through which calcium enters the cell and transduces downstream signalling cascades. LTCCs play an important role in the regulation of gene expression by coupling membrane depolarization with phosphorylation of cAMP response element-binding protein (CREB) via local $Ca^{2+/}$calmodulin-dependent protein kinase II (CaMKII) signalling [113]. Neuronal depolarization activates CREB gene transcription via two temporally distinct signalling cascades. Brief depolarization (1-10 min) activates CREB via a CaM/CaM kinase-dependent pathway, whereas sustained depolarization (30 min) and extended calcium influx require a secondary Ras/MAPK pathway [114]. The CaM pathway promotes CREB-mediated gene transcription in response to prolonged neuronal activity, but CaM kinase appears to be involved in a faster L-type channel-mediated signalling process. Brief periods of channel activity cause CaM kinase translocation near the calcium channel, where they rapidly signal to the nucleus, activating CREB [115]. This phenomenon appears to be closely linked to the probability of the channel being open rather than a simple increase of intracellular calcium, implying that specific associations with L-type channels are required to support this signalling process. Another occurrence in understanding L-type channel-mediated gene transcription is the observation that the Cav1.2 C terminus region can relocate to the nucleus and serve as a transcription factor known as CCAT (calcium channel-associated transcription). This unexpected function must be taken into account while considering the effects of Cav1.2 channel mutations linked to human disease, like BD [116].

LTP studies in hippocampal neurons provided additional evidence for L-type channel involvement in CREB transcription, demonstrating that CREB transcription was critically dependent on L-type calcium channel activity with relatively little participation from AMPA and NMDA receptors [117]. This was recently confirmed by in vitro studies in which LTCC antagonists reduced the induction of long-term potentiation (LTP) in hippocampal cornu ammonis (CA1) neurons of the rat hippocampus. Cav 1.2 knockdown models have shown decreased CREB transcription and hippocampal LTP, implicating the importance of these channels in gene expression and plasticity. In ciliary ganglion neurons, L-type channels also obstruct nicotinic signalling to CREB, though the underlying mechanism is unknown [118]. This implies that L-type channels can both promote and inhibit gene transcription.

This pathway, specifically CREB, is thought to be essential for learning and memory processes. CREB-activated genes play an important role in synaptic, neuronal, and behavioural plasticity [119]. Synaptic plasticity, which is thought to underlie learning and memory, can be influenced by LTCCs.

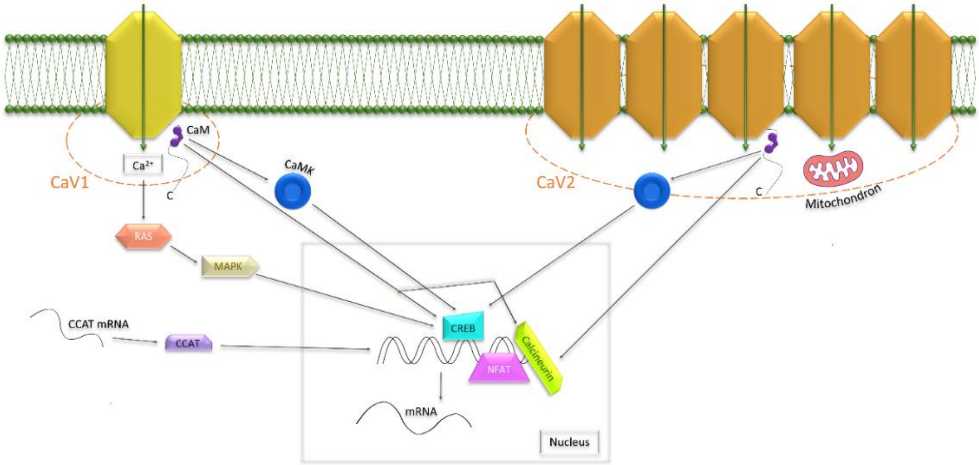

Figure 1. *Pathways included in calcium-channel influenced modification of gene transcription*

1.4.2.2. Calcium Channels and Neuronal firing

Based on the findings on the impact of CACNA1C disease-associated genetic variants on gene expression and channel function, the literature also suggests that higher or lower levels of Ca^{2+} influx in neurons can be damaging. In addition, LTCCs also regulate the process of neuronal firing [120]. For example, LTCCs can instantly produce a depolarizing stimulus; this can stabilize upstates or plateau potentials, thus influencing neuronal firing. LTCCs can also modulate neuronal firing through integration with Ca^{2+}-activated K channels [121]. Therefore, reduced LTCC activity could actually increase firing in some neurons, which may trigger Ca^{2+} influx through other sources (other subtypes of voltage-gated Ca^{2+} channels or glutamate N-methyl-D-aspartate receptors). On the other hand, an increase in LTCC activity as a result of LTCC function could silence neurons [122]. Thus, altered Cav1.2 LTCC levels or activity, in a cell-type-specific manner, can transform neuronal function in different ways that could negatively affect brain function and contribute to neuropsychiatric symptoms in disorders associated with the CACNA1C gene.

1.4.2.3. Oxytocin and dopamine pathways genes

Modifications in the levels of oxytocin have been studied in a variety of neuropsychiatric disorders. Previous studies have found that oxytocin serum levels are significantly higher in BD patients experiencing manic episodes compared to healthy controls, as well as in BD patients experiencing depressive episodes [123]. On the contrary, the evidence for dopamine signalling in BD is discordant. Dopamine transporter up-regulation and down-regulation have been reported in patients with euthymic BD and bipolar depression, respectively [124]. Whether these alterations in the dopamine transporter's availability can be correlated with disease severity or phase has yet to be verified.

In general, the neuropeptides oxytocin and dopamine are known as regulators of socio-emotional behaviour and compassionate states in particular, playing a putative role in modulating social interaction [125]. Neuroimaging studies have revealed that compassion for the pain and suffering of others is associated with increased activity in oxytocinergic and dopaminergic brain regions [124]. A recent genome-wide study suggests that allelic variation in the oxytocin and dopamine signalling pathways might be associated with different personality profiles that include low vs. high compassion [126]. However, it is indicated that that individual differences in compassion are neurologically associated with oxytocinergic and reward mechanisms with dopaminergic systems.

While the role of oxytocin and dopamine is still being investigated in BD, CACNA1C may interact with proteins that play important roles in the ethology of BD [124]. Alterations in CACNA1C gene expression may be unfavourable to the endogenous function of this pathway.

1.4.3. The genetic variant rs1006737

It is known that the intronic genetic variant rs1006737 is recognized as the most cited and studied genetic risk for bipolar disorder. CACNA1C's rs1006737 mutant allele is associated with paranoid ideation, extraversion, trait anxiety, greater harm avoidance, and novelty seeking [127]. This genetic variant is also linked to higher alarm reactivity and also influences semantic language production in conjunction with the underlying neural systems. Also, it is found that CACNA1C rs1006737 is slightly associated with "flight of ideas" (disorganized thought) [128]. This clinical symptom is correlated with DSM-IV-classified manic episodes. It is assumed that this SNP may play a role in altering affective behaviours [129]. Nonetheless, the current assessment of the clinical symptoms associated with manic episodes has some obstructions, including an absence of coherent, quantifiable, and objective categorizations. In the future, a more detailed evaluation of the patient's symptoms in relation to SNPs will be needed.

The A allele, which is thought to be a risk factor for bipolar disorder, is found in 31% of European populations, 6% of Asian populations, and nearly 56% of people of African descent [130]. Because of these variations in allele frequencies, this SNP is susceptible to ethnic admixture confounding effects in GWAS. Actually, the association between the A allele of rs1006737 and bipolar disorders, which was first described in a Caucasian population, could not be replicated with genome-wide implications in African Americans or in some European and Asian studies [131]. Despite the fact that the A allele appears to increase the risk of bipolar disorder in some population subgroups, most people who carry the "minor" allele are healthy [132]. As a result, the question of how rs1006737 might influence disease processes in bipolar disorder remains unanswered completely. Even if this SNP is not in the coding region of the CACNA1C gene, the researchers hypothesized

that it could affect gene expression. The link between CACNA1C risk variants and changes in brain volume is still being debated. According to some reports, this SNP is linked to increased subcortical volume, brainstem alterations, and increased grey matter density [128]. According to the study of Perrier et al., (2011), it was observed that carriers of this SNP had increased grey matter density in the right amygdala and right hypothalamus [133]. A smaller left putamen was detected in BD patients carrying this risk allele in comparison to the healthy controls, suggesting that the rs1006737 polymorphism may impact anatomical variation within subcortical regions incorporated in emotional processing [133]. Also, mutant polymorphism at rs1006737 in the corticolimbic frontotemporal neural system remarkably increased volume of the grey matter and reduced functional connectivity [133]. Taking these facts into consideration, the impact of CACNA1C genetic variation on the functional and structural sides of the corticolimbic system, could be a possible mechanism responsible for the BD neural network.

Brain regional activation has also been investigated in patients who are carriers of this SNP in CACNA1C. Carriers of the risk allele showed decreased activation of the subgenual anterior cingulate cortex region, which is known to regulate adaptive stress-related responses and is linked to affective disorders [134]. The same genetic variant has been linked to changes in brain circuitry, such as elevated hippocampal and prefrontal activity during emotional processing and cognitive function [134]. Regarding emotional processing, the brain imaging studies highlighted the increased amygdala activity involved in this process. Otherwise, decreased bilateral hippocampal activation during episodic memory recall was also noticed [134].

1.5. Preliminary work: Insight into susceptibility genes associated with bipolar disorder: a systematic review (state of the art)

This systematic review [96] identifies a number of potential risk genes associated with bipolar disorders whose mechanisms of action have yet to be confirmed.

They are divided into several groups:

- List of the most significant susceptibility genetic factors associated with BD
- The implication of the ZNF804A gene in BD
- The role of genes involved in calcium signalling in BD
- DNA methylation in BD
- BD and risk suicide genes
- Susceptibility genes for early-onset BD
- Candidate genes common to both BD and schizophrenia
- Genes involved in cognitive status in BD cases
- Genes involved in structural alterations in BD brain tissues
- Genes involved in the lithium response in BD

Future research should concentrate on the molecular mechanisms by which genetic variants play a major role in BD. Supplemental research is needed to replicate the applicable results.

Aims of the study

Aims of the study:

1. The development and validation of a new questionnaire to measure certain adaptive characteristics of hyperactivity and goal pursuit, concerning that emotional dysregulation is a marker of the passage to pathology in older adults.
2. Verifying whether a genetic condition that is associated with bipolar disorders can also be frequent in perfectly adapted elderly people without bipolar disorders, characterized by an aptitude for adaptive hyperactivity and novelty seeking.
3. Confirmation if a genetic feature associated with bipolar disorders can be found in people without bipolar disorders but with characteristics of hyperactivity and novelty seeking. The focus is on the genetic variant RS1006737, identified as a risk SNP for the CACNA1C gene.
4. Evidence about the association of the genetic variant presence (RS1006737) and MDQ / BRIAN scores.
5. Establishment whether the type of biological material (saliva / blood) used for genetic analysis affects the detection of mutations in the CACNA1C gene linked to bipolar disorders.

Materials and Methods

2.1. Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA)

2.1.1. Description of the sample

The investigation of the reliability and factor structure of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA) was carried out through its administration to a sample of 120 older adults selected for a previous randomized controlled trial [135, 136] according to the Declaration of Helsinki and its revisions [137]. The sample had previously been recruited by the Department of Medical Science and Public Health, University of Cagliari, Italy.

2.1.2. Inclusion criteria

- elderly people, both genders, aged 60 or over 60 years
- living at home
- having the capacity to provide informed consent without impairment for severe diseases (e.g., mild cases of hypertension or diabetes were admitted)
- absence of a lifetime history of diagnosed bipolar spectrum disorder conditions

2.1.3. Exclusion criteria

- non-acceptance to participate in the study (non-signing informed consent for accepting participation in the study)
- participants with a diagnosis of bipolar spectrum disorder
- under 60 years of age
- inability to sustain a medium/moderate physical effort

All included subjects provided written informed consent and were invited to fill out the Italian version of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA) [138].

2.1.4. Measures

Socio-demographic information was collected for the following variables: age, sex, socioeconomic status, and civil status. Measures of socioeconomic status were grouped into four categories: elementary school diploma, lower secondary diploma, upper secondary diploma, and university degree [139].

AHGA is a 12-item, five-point Likert scale (from 1 to 5), developed by a team of PhD students, psychotherapists, and psychiatrists with decades of expertise in bipolar spectrum disorders from the University of Cagliari. The final version was reached through a process of item development and selection followed by administering the tool to the same target population of subjects not directly enrolled in the validation phase. This stage was carried out carefully to identify potential issues or unclear terms/items in order to improve the quality and understandability of the questionnaire.

The questions mainly focus on two areas: goal achievement and lifelong adaptive hyperactivity. The tool investigates transfers, employment differences, and economic well-being compared to parents, as well as the perceived speed at which things are done and thoughts flow. Other elements of investigation are related to the usual number of people with whom they have conversations, the amount of physical weekly activity, number of hours spent outside the home and number of used applications or programs during the day. Elements such as how many times a person has been in love in their lifetime, without even being in an official relationship, along with the average number of

hours slept during the night are further components. The original Italian version is included in the current paper as *Appendix A.*

Since this questionnaire has a role to measure principally adaptive hyperactivity, the Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN) serves to indicate particularly pathological hyperactivity and dysregulation of biorhythms, predisposing factors that could suggest the presence of a certain psychiatric disorder [140].

2.1.5. Statistics

Data were imputed in Excel and analysed using the Statistical Package for Social Sciences (SPSS) version 27. Additional analyses were carried out in R. All tests were two-tailed, with alpha set at $p<0.05$.

Means with standard deviation, or counts and percentages, were used to describe the distribution of the data in the sample. An ANOVA, when appropriate, was used to determine whether continuous variables differed among groups of subjects. The Games-Howell test was used to test post-hoc differences in groups, since it does not assume equal variances and sample size. Chi-square tests were used for categorical data. Pearson's correlation coefficient was used for correlations. Reliability was measured as internal coherence using Cronbach's alpha. For group comparisons, a rule of thumb assumes that reliability values of 0.70 are considered acceptable [141].

The data was preliminarily subjected to a Principal Component Analysis (PCA) to establish the spontaneous distribution of the 12 core items of the AHGA into one or more separate dimensions. Parallel analysis was used to determine the optimal number of components. In a parallel analysis, the scree plot of the observed data was compared with

that of a random matrix of the same size as the original. The best solution is based on the number of components with eigenvalues higher than those generated by the random data, either simulated or reassembled by permutation from the original data. The parallel analysis and the subsequent PCA were carried out with the psych package running in R [142, 143]. Both were applied to a matrix of polychoric correlations, since the data were ordinal.

After establishing the factor structure of the AHGA Italian version, the correlation of its measures with those of the BRIAN was assessed to check the convergent validity of the scale and its factors.

2.2. Other administrated tools and data collection

2.2.1. Mood Disorder Questionnaire (MDQ)

This questionnaire is a single-page, self-reported, paper-and-pencil record that can be quickly and simply scored by healthcare professionals *(Appendix B)* [144].

The MDQ is a concise and user-friendly tool that has improved research in the field of bipolar disorders by screening for a lifetime history of a manic or hypomanic syndrome by including 13 yes/no items derived from both the DSM-IV criteria (1994) and clinical experience. Such items assess the macro-area of mood, with a focus on a marked individual variation in the dimensions of irritability, activity, sociability, sleep, libido, thoughts, attention, and energy [144].

Additionally, a yes/no question asks if various reported manic or hypomanic symptoms or behaviours were experienced at the same time. The level of functional impairment brought on by these symptoms is then assessed on a 4-point scale, ranging from "no problem" to "serious problem." [145].

In order to screen positively for possible bipolar disorders, all three successive criteria must be met:

- “YES” to 7 or more of the 13 items in Question number 1 AND
- “YES” to Question number 2 AND
- “Moderate Problem” or “Serious Problem” to Question number 3

2.2.2. Biological Rhythms Interview of Assessment in Neuropsychiatry (BRIAN)

BRIAN (*Appendix C*) is a 21-item interviewer-administered instrument that focuses on evaluating the main areas related to circadian rhythm disturbance: sleep, activities, social rhythms, eating patterns, and the predominant rhythm. Items are estimated using a 4-point scale, (1) = no difficulty, (2) = mild difficulty, (3) = moderate difficulty, and (4) = severe difficulty. The BRIAN scores therefore range from 1 to 72, where higher scores indicate serious circadian rhythm disturbances [140].

This scale was developed by the Bipolar Disorder Program and INTC Translational Medicine and by the Hospital de Clìnicas de Porto Alegre, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil, for the clinical evaluation of sleep and rhythm impairment, focusing on the main problems experienced by people with mental disorders [146]. It is an interviewer-administered instrument, constructed for use by a qualified clinician [140].

The time frame studied refers to the last 15 days before assessment, and the 21 items on the scale are divided into five specific areas related to circadian rhythm disturbances:

- Sleep
- Activities
- Social rhythm
- Eating patterns
- Predominant rhythm

2.2.3. Personal data form

The personal data collection form (*Appendix D*) was designed and created ad hoc for the study by research collaborators. The module made it possible to collect all the personal and biographical data useful for the study, such as age, province of residence, social status, etc., as well as an anamnestic description of the personal and close family mental health status.

Measured variables:

- Age
- Province of origin
- Educational level
- Employment status
- Economic state
- Civil Status
- Number of Children
- Psychiatric diagnosis
- The presence of stressful factors
- Taken therapies for mood disorders

All instruments and questionnaires used in the study have been reported in Italian and are regularly used in clinical practice.

2.3. Laboratory analysis

2.3.1. Design of the study

This is a cross-sectional study in which the frequency of the genetic variant RS1006737 (CACNA1C gene) was measured in a sample of old adults with hyperactivity/novelty-seeking features and compared with the frequency of the same gene and the same variant in a similar group of people with bipolar disorders from the same city and with the control samples identified in literature [147-149]. This study also compared the familial risk for bipolar disorder in the study sample and in the control group (that consisted of individuals with a diagnosis of BD).

2.3.2. Participants and Recruitment

The study sample included older adults (40 participants) in the city area of Cagliari, Italy, who had previously been involved in a clinical trial on the efficacy of middle/moderate exercise [135, 136].

2.3.3. Inclusion criteria

- elderly people, aged 60 or over, of both genders
- living at home
- having the capacity to provide informed consent
- absence of a lifetime history of diagnosed bipolar spectrum disorder conditions

2.3.4. Exclusion criteria

- non-acceptance to participate in the study (not signing the informed consent for the study)
- participants with a diagnosis of bipolar spectrum disorder
- under 60 years of age

- inability to sustain a medium / moderate physical effort

The *control group* (21 participants) involved patients with a formal diagnosis of bipolar spectrum disorders, aged 60 or over, of both genders, from the province of Cagliari. These subjects were identified through the registers of patients attending the Centro di Psichiatria di Consultazione e Psicosomatica of the University Hospital of Cagliari, Italy.

2.3.5. Psychiatric and Hyperactivity/Novelty seeking evaluation

Both groups underwent psychiatric evaluation that included the collection of personal and family history. The measure of hyperactivity was carried out using the "Questionnaire for Adaptive Hyperactivity and Goal Achievement" (AHGA), a tool created and validated specifically for this purpose [138].

2.3.6. Primer design for DNA sequencing

We have investigated the DNA fragment in the CACNA1C gene containing the nucleotide region RS1006737 using Sanger's capillary sequencing method. The molecular approach was developed in accordance with the already published methodology described by Arcadu et al. (2012) for the VCAM1 gene [150]. The CACNA1C gene's fifteen representative sequences, which are listed in the nucleotide NCBI database (GeneBanK), were examined. Clustal-Omega, a program for multiple sequence alignment, was used to align these DNA fragments. The analysis revealed that the mutation spot region was flanked by a 229 bp nucleotide fragment. The two oligonucleotides that were used as PCR primers are shown in *Figure 2,* along with the location of the studied region of the CACN1AC gene.

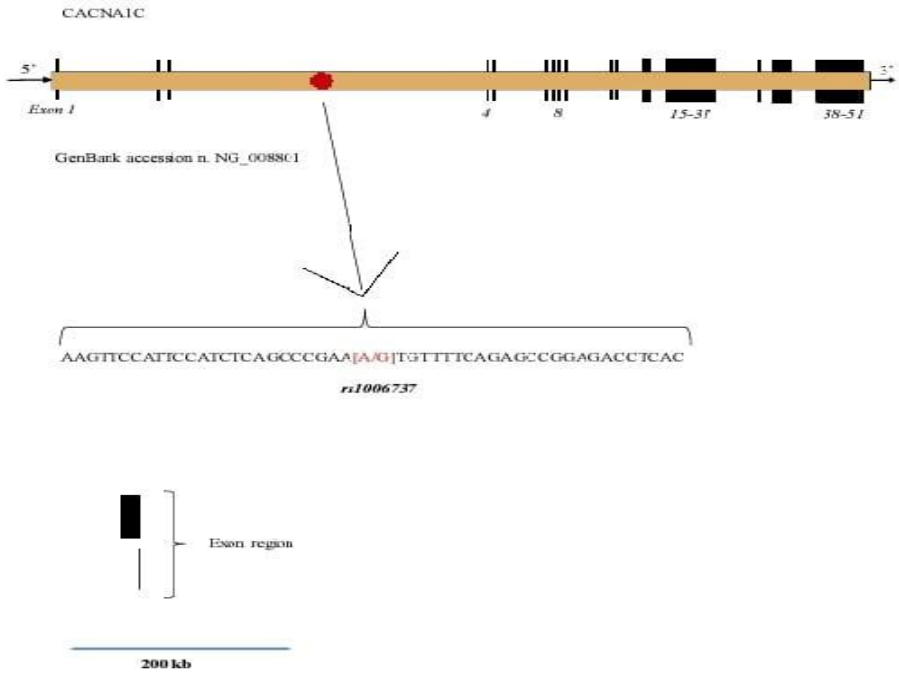

Figure 2. *Location of the hot spot point in the gene CACNA1C including RS 1006737*

Using the Mfold program, the DNA sequence of the 229 bp amplicon was analysed in silico to investigate the secondary structures found along the primers' target area. According to the program's instructions for manufacture, the folding conditions for this amplicon were Na+ 0.05 mol/L, Mg++ 0.002 mol/L, and the hybridization temperature (Ta= 50°C) matched the Sanger PCR annealing temperature.

Between theoretical and measured Tm, there was a difference of 0.7°C. In this study, we first created various sequencing/PCR primers by using conventional techniques described in the literature or by using the Oligo 7 software manufacturer's instructions (MedProbe, Oslo, Norway). These bioinformatic tools advise conducting secondary structure analysis within the boundaries of the primer sequence.

Oligonucleotide primers created in this study were used both in real-time PCR and in Sanger's PCR for sequencing. As indicated, these DNA oligos were formed using a 229 bp sequence fragment of the CACNA1C gene, corresponding to GenBank accession n.

NG_008801 (from nucleotide 270121 to 27600) carrying the wild-type allele (G) often reported in non-diseased patients. The target DNA fragment is shown along with the primer's position and sequence in *Figure 3.*

>NG_008801.2 Homo sapiens calcium voltage-gated channel subunit alpha1 C (CACNA1C), RefSeqGene (LRG_334) on chromosome 12

TCCTCCCATCTGCCTGCCCACTGTGTACAGTGCTTACTACATGCCTTACCTAGTCTAAGTGCTTTACATA
AATGAATTCATTTGACGTTCACAGGACATCACAGATAGGCATTACCATTGTCCCTGTTTTATAAATGAGA
ATACTGAATCATTGAAAGGTTAATAATTTTGCTTATGGACATTTGCTTCTGGAGCTGGACCAGGCAGTTT
GGCTTCAGAGTCCACTTGGCTCTATCAAAGTCTTGCTATCAATTACATAAGTTCCATTCCATCTCAGCCC
GAAGTGTTTTCAGAGCCGGAGACCTCACAGTGTCTCTCAGGACAGTACCTTTCAGGTTTGAATGTGCCCA
AGAGCCATCTGGGGATCCTGGTAAAATGCACATCCTGATTCAGCAGGTTCGGGTTGGGGCCTGAGAATCT
CAGGTCCAGCAAGCTCCCAGGTAATGTCAGTGTGACTGATTGCTGGTCCACACATTGAGCAGCAGTGCTG
TAGTAGACTACTATCATGCCTGTGTACTAATTTCACTGTCAGATTGGAGTTAACTGCATTTGGATAGGGA
GCTCTTTCAGCAATAGAATAATACCAGTAAGAATTTTTAATGAGTGATCAACTATAGTAGCAGTTAGTAA
TCAATTTTTTGTTTAAAAACTAAGTTTCCTAGGACTCTAATGGAAAATGGAAAGGTTATGTCCCCCTTTT
TCGTATCTTTGCCAAGGGTCTTTCCTTTGTTGCTGGGTTTAACTTGGCACTTCTAGGCTACTTTAGTCCT
AACTTTTCTCAGTTACCATTATATGCAGTTCTCCCAGCCACACCCCAGCAGTGTGCAAGGGATCAGACAC
AAGGTTGAATCCATCACAAAAGCAGAATCACCATGGCAACTGCATCCTTTGATTCTTGAGTGTGCCCAGC
AACCTGAGCAGAGGCGATAGTTGAAGTGAACCAAGTTCTCCTGAGAAATGGAGGGGAGTGGTGCCGGGCC
CACACTAGGCTGTGGTATCTTCCTCCCTACAGTGAGGGGCTTCTGTTTACTCTGAAGACTCCAGACACTC
AAAATCTCCTCCCCTCCCTCCAGTCCTGCAGTTAGCCTCCAGGGGTTTCTGGTCCTAAACTTCCACCACC
ATGAGATTACTACAATGCCTTGTGATACTCTTGTTCTTCTGGTTTGAGTTTTGGTAGATAAGCACATCTG
AGTCTTGCTGTGTTAATGTGTCTGTATTTTGGTGTATCTGCTTGCTTGTCGTGTGGGGCATATGCCAAGT
CCAGTAGTGGATGGGCTGGGGAAGACCAGACCTTATCACATGGTGCCCTTGGGGGGAAATCTTAATTCCA
ATGTGTGAAACCAGTGAAAGTATGATTTTCTGGGTCAATTTTAAAAATATACGTTCAAGCAAAAAGCAAC
CTGTTATCTCTTCTCTTTCTGCCTCTGCACACAGCAGCCTCCATTGCCTAGGGTATGATAGTGTGGGTTC
ACTTTGTCCATCTCATTTGGATGACATCAGCGAAGATGCATTCTGTATCTCTCCACTGAGGCCTGTGACA

Figure 3. *The oligonucleotide primers in yellow while the red point represented the mutation zone***.**

Oligo Name	Sequence 5'----3'	Length (nt)	Ta °C
OG650	AATAATTTTGCTTATGGACAT	21	59
OG651	AATCAGGATGTGCATTTTAC	20	60

Table 4. *Used oligonucleotides in the study*

The theoretic melting temperatures of PCR amplicons (Tm) were calculated using module 1 of the DNA hybridization prediction algorithm program DinaMelt together with the subsequent parameter sets: (i) Mg2+ at 0.004 mol/L, (ii) monovalent cation concentration at 0.05 mol/L, (iii) a concentration of PCR products (Top/Bottom strands) at 10 - 7 mol/L, and (iv) hybridization temperature at Ta = 50°C.

2.3.7. Pre-analytical phase

61 blood samples were collected in tubes containing coagulation activators, which have a micronized silica particle coating that activates coagulation when the tube is gently inverted during the mixing process after collection. The samples were transported to the AOU of Cagliari's Molecular Biology Laboratory and immediately centrifuged at 3000 RPM for 10 minutes. All samples were kept at - 20 °C. Each sample arrived at the facility anonymously, accompanied by a specific numerical code to ensure maximum confidentiality of the sensitive data of the participants.

2.3.8. Analytical phase

The CTAB technique protocol was used to extract DNA from blood samples. Several variations were developed in order to adapt the technique to a wide range of organisms. These "homemade approaches" and operational steps are outlined below. 75 µl of SDS/proteinase K were added to 400 µl of biological material. 100 µl of NaCl (5 M) and 100 µl of CTAB/NaCl preheated to 65 °C were added after one hour of incubation at 65 °C. 750 µl of chloroform/isoamyl alcohol (24/1) was added after another hour of incubation. DNA was extracted from the upper liquid phase and transferred to a -20 °C ice-cold tube. After being treated with 450 µL of cold isopropanol, the sample was incubated at -20 °C for 2 hours. A pellet wash with 100 µl of ethanol at 70 °C was performed, and the DNA was resuspended by adding TRIS/HCL at pH > 8 [151]. New biological approaches have expanded the number of molecular diagnostic tests, and new molecular clinical tests are now available.

Polymerase chain reaction (PCR) has been used to detect nucleotide polymorphisms (SNPs) associated with BD at the CACNA1C gene level. The use of bioinformatics tools to design FRET probes was critical in detecting mutations at the gene of interest. For

genotyping rs1006737 with greater specificity/selectivity, we proposed a molecular method based on FRET probes [152]. This method has been shown to be faster, simpler, and more precise than older approaches such as RFLP or DNA sequencing technologies. FRET, in particular, is a probe that uses a dipole-dipole mechanism to transmit energy from an excited donor to an accepting group without the use of ionizing radiation. One of the two probes contains a fluorochrome that can transfer energy to the other probe's fluorochrome, causing it to emit light at a specific wavelength detectable by real-time PCR. As a result, the fluorescence is only detected when the PCR reaction results in the binding of both probes to the DNA. In FRET technologies, the upstream probe is labelled with fluorescein isothiocyanate (F1), while the downstream probe is labelled with the Red 640 fluorophore (acceptor). Nucleotide variation in the target acceptor DNA (Tm) causes melting temperature. This result was observed in the PCR apparatus by observing the F2 fluorescence during sample heating and observing the melting peak. By evaluating melting temperatures, we predicted a high likelihood of allelic diversity in this gene region [153].

We used the Sanger method of sequencing to confirm the detected mutations. It is a four-capillary, fluorescence-based capillary electrophoresis device made adaptable for genetic analysis research to high resolution. This protocol provided 2 PCR reactions, each of which contained a denatured DNA fragment to be sequenced; primers; high processivity DNA polymerase, and 4 triphosphate deoxyribonucleotides (dNTPs). The resulting mixture was divided into four fractions for each nucleotide base (A, T, G, C) to which the corresponding chain terminator was added (ddATP, ddTTP, ddGTP, ddCTP). These chain terminators were added by chance; they formed varying-length fragments with the labeled primer's initial sequence and terminated with dideoxyribonucleotide triphosphate

[154]. Following incubation, the four fractions were heat denatured to separate the paired nucleotide chains and electrophoretically run on a single polyacrylamide gel. The labelled neosynthesized chains migrated in the gel according to their length; the shorter ones moved faster than the longer ones, and they were easily detectable [155]. The electropherogram generated by the sequencer software enabled us to analyze the data using software such as: Multiple Sequence Alignment Program Clustal Omega (ebi.ac.uk/Tools/msa/clustalo/) and Basic Local Alignment Search Tool (blast.ncbi.nlm.nih.gov/Blast.cgi).

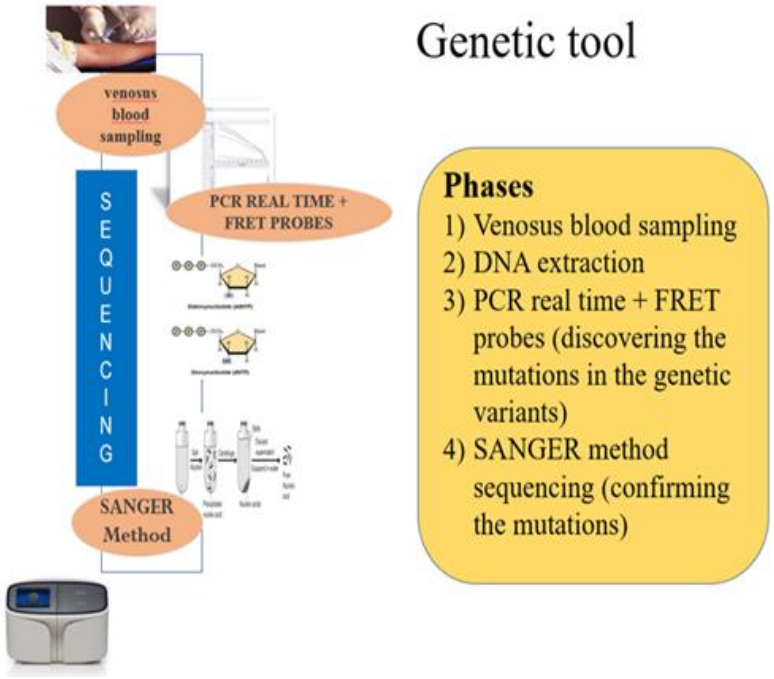

Figure 4. *Visual representation of the genetic tool*

2.3.9. Post-analytical phase

This phase was concerned with the processing and comparison of the data through the application of software to the obtained sequences.

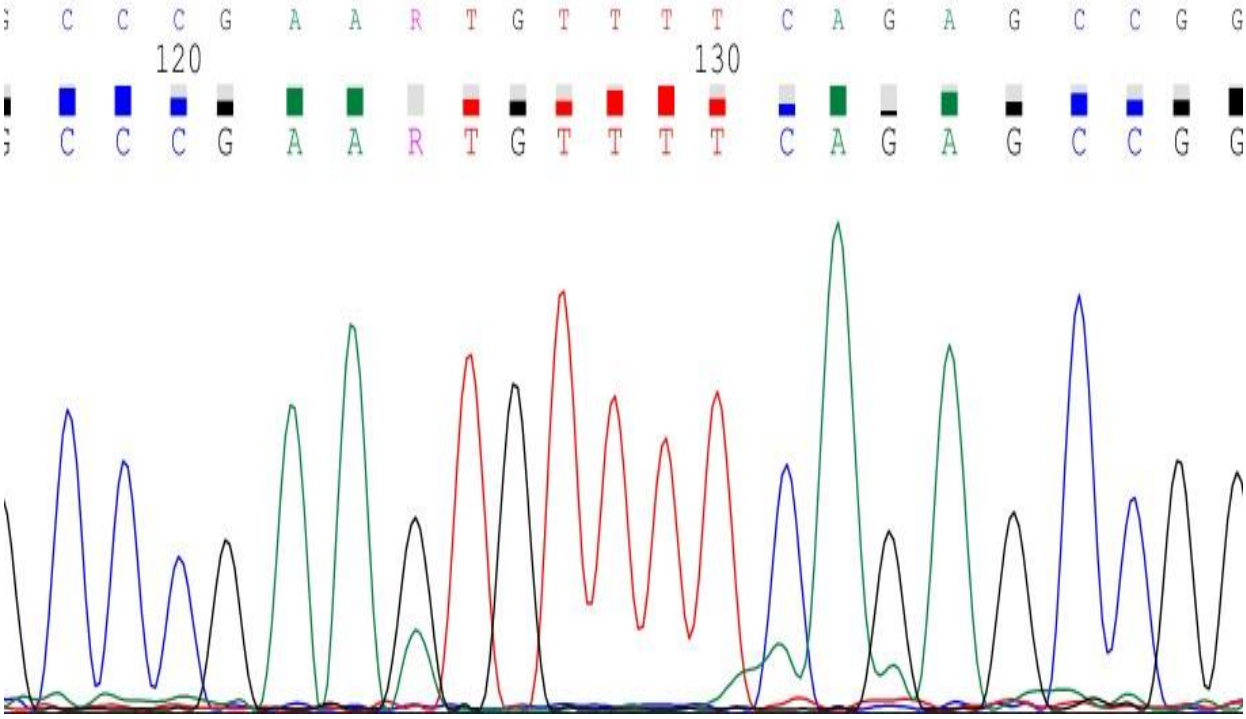

Figure 5. *The presence of the heterozygous mutation (G/A) mutation in genetic variant RS1006737 (CACNA1C gene)*

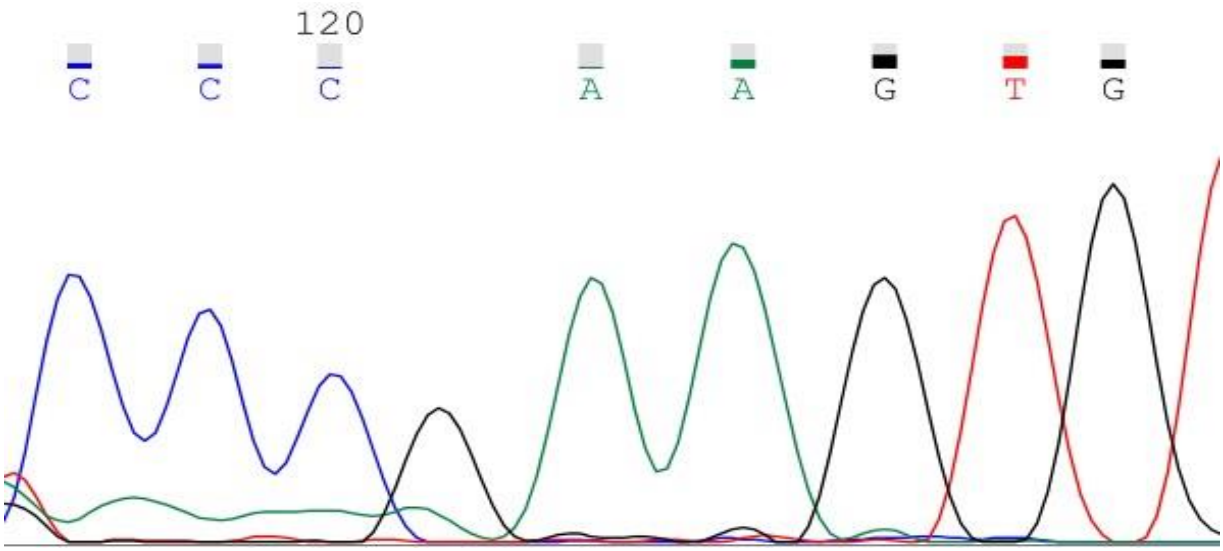

Figure 6*. The wild type profile in genetic variant RS1006737 (CACNA1C gene)*

2.3.10. Statistic

Chi-Square values, P-values, Odds ratios (ORs), and 95% confidence intervals (95% CI) were calculated for the genetic variant RS1006737 regarding its frequency in the group of older adults with hyperactivity/novelty-seeking traits and another similar group of BD subjects. The same statistical measures were also applied when comparing with the control samples found in the literature and concerning the study sample's and the bipolar disorder control group's family risk of developing BD.

Chi-Square values, P-values, Odds ratios (ORs), and 95% confidence intervals (95% CI) were also calculated for the genetic variant RS1006737 regarding its frequency in the three groups of participants: older adults with hyperactivity/exploration traits and another similar group of BD subjects. The same statistical measures were also applied when comparing the genetic profile between the combined groups of participants: the group of people with hyperactivity without bipolar disorder plus people with bipolar disorder, and group of people without either hyperactivity or bipolar disorder.

For the purpose of comparing the MDQ and BRIAN scores between the groups with and without the presence of the analysed genetic variant, the Kruskal-Wallis test was used. To avoid overestimating statistical significance for small data, Yates' correction was applied.

2.3.11. Ethical Considerations

Approval for the study was granted by the Ethical Committee of the Institutional Review Board of the University Hospital of Cagliari, Italy. All included subjects provided written, informed consent. All human procedures followed were in accordance with the guidelines of the Helsinki Declaration of 1975 [137].

Results

3.1. Development of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA)

3.1.1. Preliminary results

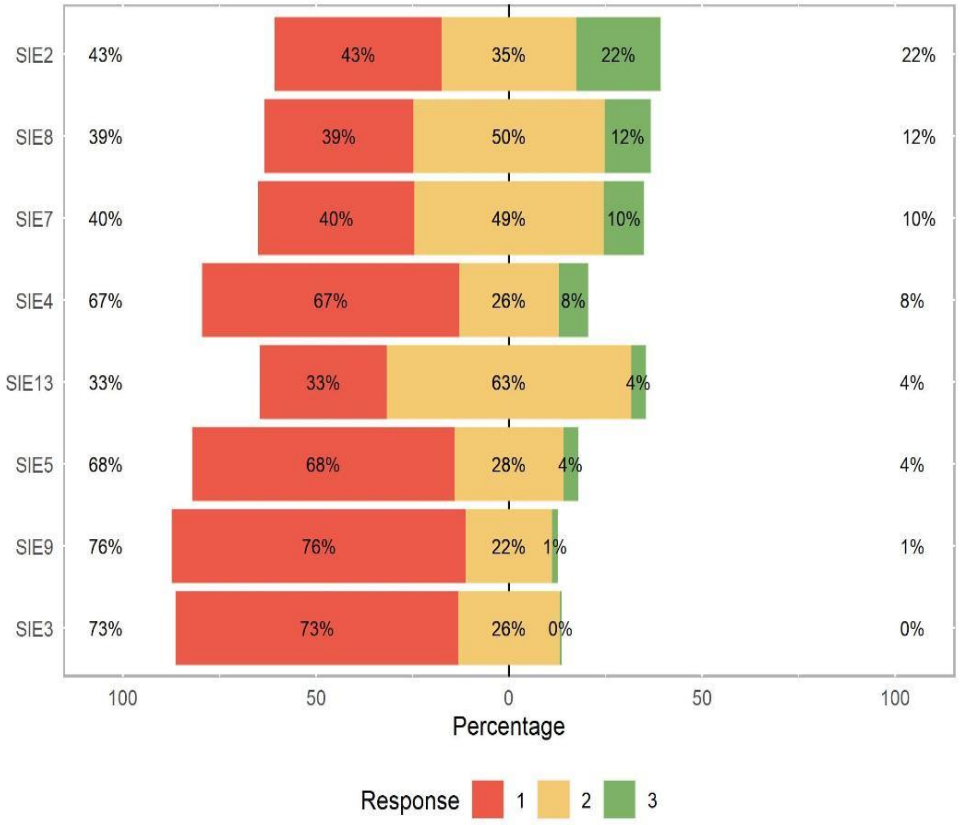

Figure 7a*. Results of the preliminary 21-item questionnaire form of the AHGA*

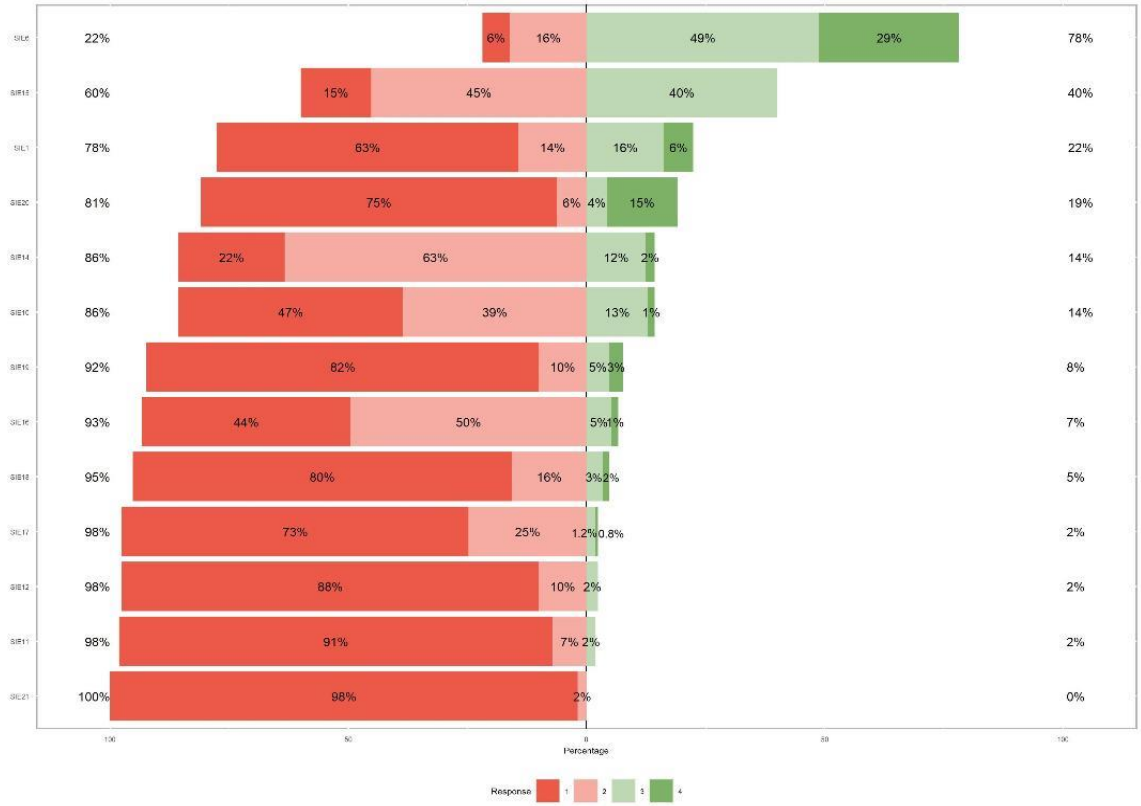

***Figure 7b**. Results of the preliminary 21-item questionnaire form of the AHGA*

The preliminary 21-item questionnaire form of the AHGA (*Appendix E*) was administered to 210 healthy elderly subjects from Sardinia, Italy. The questionnaire consisted of many items, and its preliminary results showed diverse results. Apart from the fact that the sample of 210 people was sufficient, the items showed *low variability.* In addition, we noticed that participants tended to prefer answers 1 and 2 to certain items (*Figures 7a and 7b*). This leads us to the fact that we have a *floor effect* (the minimum level below which the variance of an independent variable is no longer measurable).

According to the preliminary results, this form questionnaire was reformulated in order to have at least the same number of answers for each item (scale Likert), with 12 items in total.

3.1.2. Development and validation of AHGA

The sample was balanced by age and gender *(Table 5).* There was a slight excess of married people among men and widowed people among women (Chi-square = 10.4; df=3; p=0.015).

	Men	Women	Total
Gender	56 (47%)	64 (53%)	120 (100%)
Age	74.5 (5.2)	73.8 (5.1)	74.1 (5.1)
Education level			
elementary	5 (9%)	4 (6%)	9 (7%)
school	10 (18%)	13 (20%)	23 (19%)
lower secondary	23 (41%)	27 (42%)	50 (42%)
upper secondary	18 (32%)	20 (31%)	38 (32%)
university			
Civil status			
unmarried/non	7 (12%)	13 (20%)	20 (17%)
cohabiting	42 (75%)	32 (50%)	74 (61%)
married/cohabiting	6 (11%)	19 (30%)	25 (21%)
widower/widow	1 (2%)	0 (0%)	1 (1%)
non specified			

Table 5. *General epidemiological characteristics of the sample (n=120)*

3.1.3. Reliability

Reliability of the 12-item AHGA, measured as internal consistency, was good: Cronbach's alpha in the sample was 0.713 (95%CI: 0.630 to 0.783).

3.1.4. Factor analysis and extraction of the main components

Bartlett's test of sphericity was 288.7542 ($p < 0.0001$), and Kaiser-Meyer-Olkin's adequacy value was 0.78. The matrix, thus, can be factorized (*Figure 8*).

The parallel analysis suggested a total of two principal components (*Figure 8).* The main components were then analyzed by estimating two factors (*Table 6).*

The separation of the two factors with the varimax technique did not differ from the separation of the two factors with the promax technique, which admits correlation

between the two factors as probable based on their nature. Based on the direction of loading (assigned to the item only if loading is positive) and the relative weight, items 2, 8, 9, 10, 11, and 12 were assigned to the first factor or first component (goal achievement), while items 3, 5, 6, and 7 were assigned to the second factor or second component (hyperactivity), while items 1 and 4 were not clearly separated between the two factors (the two components) (*Figure 9*).

Subscale 1, comprising items 2, 8, 9, 10, 11, and 12, was named "goal achievement" based on the thematic content items. Subscale 2, comprising items 3, 5, 6, and 7, was named "hyperactivity" based on the thematic content of the items. The score of the two subscales for each participant was obtained by adding the values of each item and dividing by the number of items in order to obtain a homogeneous interval from 1 to 5, with scores increasing as the expression of the variable measured by the subscale increases.

Figure 10 summarizes the item distribution in the two components as well as the variance fraction. The solution explained 41% of the total variance.

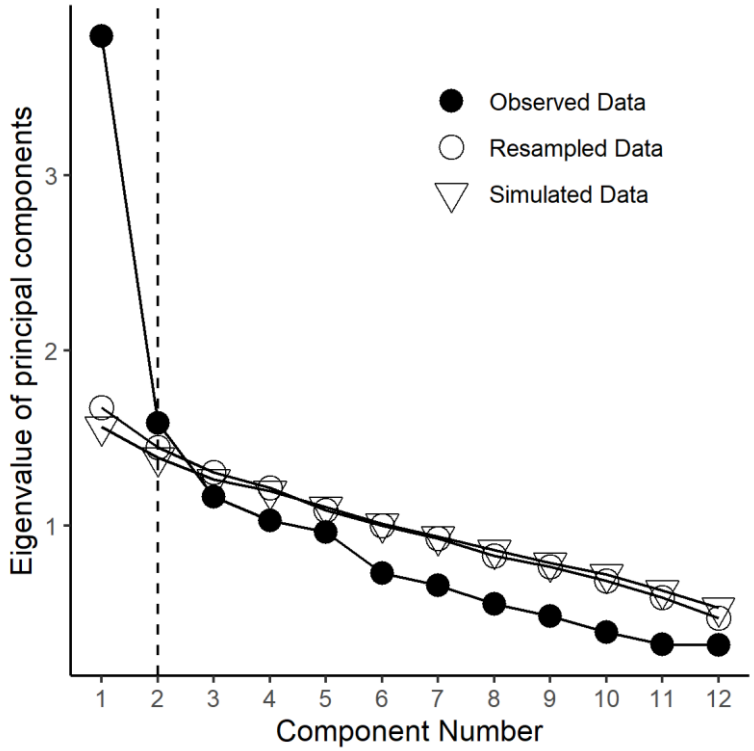

Figure 8. *Parallel analysis was applied to the items of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (n=12). A plot of the eigenvalues calculated on the basis of the actual data and the simulated and resampled data.*

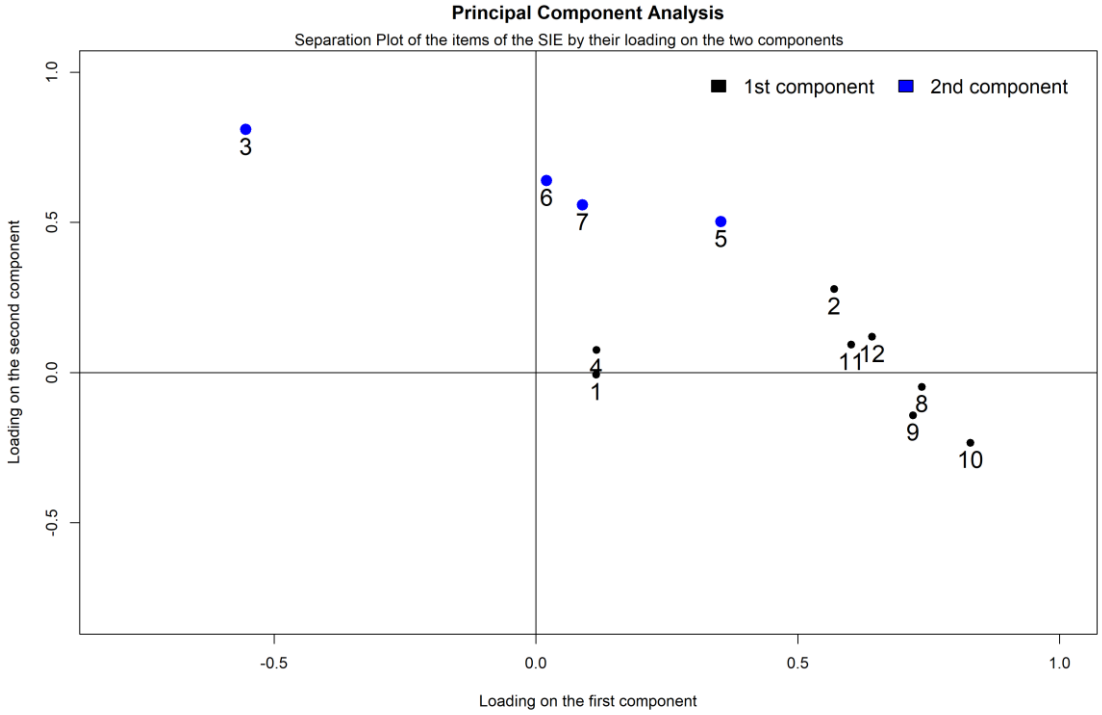

Figure 9*. A separation plot of the results of the principal component analysis is applied to the items of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (n=12). Items are plotted on the basis of their loadings on the two extracted main dimensions.*

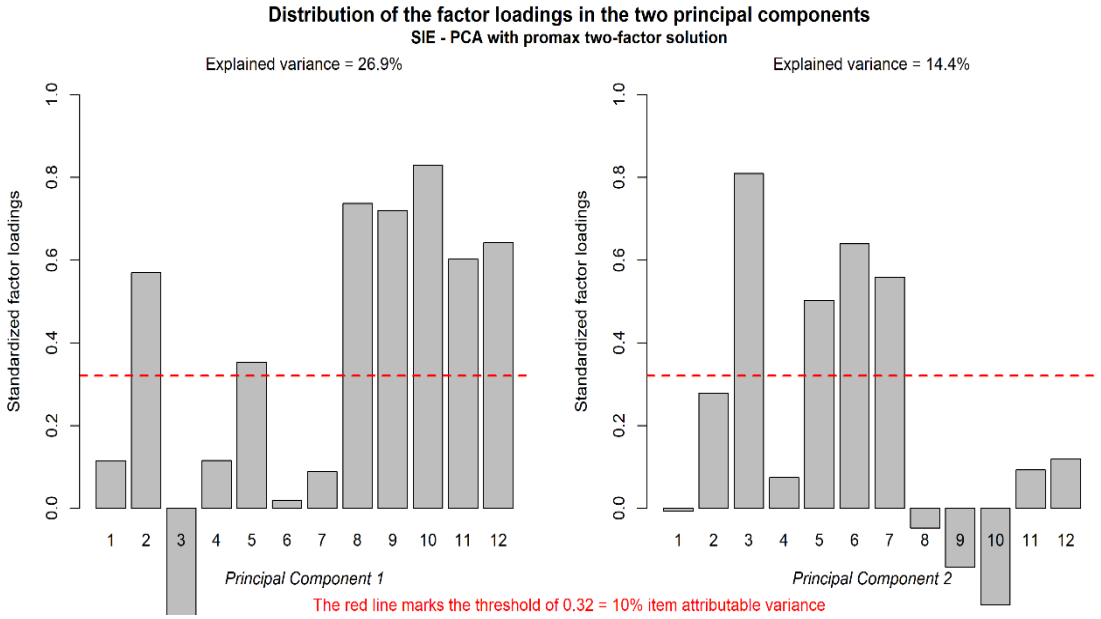

Figure 10. *Distribution of the factor loadings in the two principal components*

Principal component analysis (PCA) of the questionnaire for adaptive hyperactivity and goal achievement		
Item	**First component (goal achievement)**	**Second component (hyperactivity)**
1	0.11	-0.01
2	0.57	0.28
3	-0.55	0.81
4	0.12	0.07
5	0.35	0.50
6	0.02	0.64
7	0.09	0.56
8	0.74	-0.05
9	0.72	-0.14
10	0.83	-0.23
11	0.60	0.09
12	0.64	0.12
SS loadings	3.23	1.73
Proportion of variance	0.27	0.14
Cumulative variance	0.27	0.41

SS (squared loadings)

Table 6. *Principal component analysis (PCA) of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (n=120)*

3.1.5. Relationship of the main components of the AHGA with socio-demographic indicators

The distribution of the score concerning subscale 1 (goal achievement) did not differ among men (mean ± SD: 3.6 ± 0.8) and women (3.4 ± 0.8); F[2;116]= ,683; p=0,057. On the other hand, subscale 2 (hyperactivity) showed higher scores among men (3.1 ± 0.7) than women (2.8 ± 0.9); F[2;116]= ,039; p=0.014.

No relationship was observed in the distribution of the two subscales in relation to age: subscale 1 (Pearson r = 0.081; p = 0.380) and subscale 2 (Pearson r = 0.063; p = 0.493). As well, no difference was observed in the distribution of the scores of the two subscales in relation to the civil status. Instead, there was observed an increase in the scores of the two subscales equivalent with the growth of the level of education. In particular, the subscale 1 showed higher scores among those who had obtained the upper secondary and university diplomas than those with lower educational levels. Subjects with university diplomas appear to be more goal achievers, which may be due to opportunities and resources but also to the type of question (there is a question about school level and one about income, which is influenced by educational level) (*Tables 8a, 8b, and 8c*).

Descriptive analysis (civil status)									
						95% confidence interval for the mean			
		Number	Mean	Standard deviation	Standard error	Lower limit	Upper limit	Minimum	Maximum
Factor 1 (goal achievement)	1 (unmarried/non cohabiting)	20	3,642	,7420	,1659	3,294	3,989	2,0	4,7
	2 (married/cohabiting)	74	3,545	,8405	,0977	3,350	3,740	1,3	4,8
	3 (widower/widow)	25	3,367	,8375	,1675	3,021	3,712	2,0	5,0
	Total	119	3,524	,8223	,0754	3,375	3,673	1,3	5,0
Factor 2 (hyperactivity)	1 (unmarried/non cohabiting)	20	3,000	,8885	,1987	2,584	3,416	1,5	4,5
	2 (married/cohabiting	74	2,953	,8607	,1001	2,753	3,152	1,0	5,0
	3 (widower/widow)	25	2,930	,8085	,1617	2,596	3,264	1,5	4,8

Table 7a*. Descriptive analysis (civil status)*

ANOVA (civil status)						
		sum of squares	Df	quadratic mean	F	Sig.
Factor 1 (goal achievement)	Between the groups	,929	2	,464	,683	,507
	Within the groups	78,865	116	,680		
	Total	79,794	118			
Factor 2 (hyperactivity)	Between the groups	,056	2	,028	,039	,962
	Within the groups	84,774	116	,731		
	Total	84,831	118			

Table 7b. *ANOVA analysis (civil status)*

Descriptive analysis (educational level)									
						95% confidence interval for the mean			
		Number	Mean	Standard deviation	Standard error	Lower limit	Upper limit	Minimum	Maximum
Factor 1 (goal achievement)	2 (elementary school)	9	2,370	,7807	,2602	1,770	2,971	1,3	3,8
	3 (lower secondary)	23	3,043	,6709	,1399	2,753	3,334	1,8	4,0
	4 (upper secondary)	50	3,860	,6532	,0924	3,674	4,046	2,2	4,8
	5 (university)	38	3,632	,7535	,1222	3,384	3,879	2,0	5,0
	Total	120	3,519	,8203	,0749	3,371	3,668	1,3	5,0
Factor 2 (hyperactivity)	2 (elementary school)	9	2,472	,7009	,2336	1,933	3,011	1,5	3,3
	3 (lower secondary)	23	2,804	,8981	,1873	2,416	3,193	1,5	5,0
	4 (upper secondary)	50	3,060	,7963	,1126	2,834	3,286	1,0	4,8
	5 (university)	38	3,046	,8888	,1442	2,754	3,338	1,0	4,8
	Total	120	2,963	,8474	,0774	2,809	3,116	1,0	5,0

Table 8a. *Descriptive analysis (educational level)*

ANOVA (educational level)						
		sum of squares	df	quadratic mean	F	Sig.
Factor 1 (goal achievement)	Between the groups	23,371	3	7,790	15,939	<,001
	Within the groups	56,695	116	,489		
	Total	80,066	119			
Factor 2 (hyperactivity)	Between the groups	3,479	3	1,160	1,641	,184
	Within the groups	81,977	116	,707		
	Total	85,456	119			

Table 8b. *ANOVA analysis (educational level)*

Multiple Comparisons							
Games-Howell							
						95% confidence interval	
Dependent variable	(I) Level of education	(J) Level of education	Difference of the mean (I-J)	Standard error	Sig.	Lower limit	Upper limit
Factor 1 (goal achievement)	2 (elementary school)	3 (lower secondary)	-,6371	,2955	,154	-1,541	,195
		4 (upper secondary)	-1,4896*	,2762	,001	-2,333	-,647
		5 (university)	-1,2612*	,2875	,004	-2,117	-,405
	3 (lower secondary)	2 (elementary school)	,6731	,2955	,154	-,195	1,541
		4 (upper secondary)	-,8165*	,1676	<,001	-1,265	-,368
		5 (university)	-,5881*	,1858	,013	-1,082	-,095
	4 (upper secondary)	2 (elementary school)	1,4896*	,2762	,001	,647	2,333
		3 (lower secondary)	,8165*	,1676	<,001	,368	1,265
		5 (university)	,2284	,1532	,448	-,174	,631
	5(university)	2 (elementary school)	1,2612*	,2875	,004	,405	2,117
		3 (lower secondary)	,5881*	,1858	,013	,095	1,082
		4(upper secondary)	-,2284	,1532	,448	-,631	,174
Factor 2 (hyperactivity)	2 (elementary school)	3 (lower secondary)	-,3321	,2994	,688	-1,175	,511
		4 (upper secondary)	-,5878	,2594	,161	-1,357	,182
		5 (university)	-,5738	,2746	,201	-1,368	,219
	3 (lower secondary)	2 (elementary school)	,3321	,2994	,688	-,511	1,175
		4 (upper secondary)	-,2557	,2185	,649	-,842	,331
		5 (university)	-,2417	,2363	,737	-,872	,388
	4 (upper secondary)	2 (elementary school)	,5878	,2594	,161	-,182	1,357
		3 (lower secondary)	,2557	,2185	,649	-,331	,842
		5(university)	,0139	,1830	1,000	-,467	,495
	5 (university)	2 (elementary school)	,5738	,2746	,201	-,219	1,366

		3 (lower secondary)	,2417	,2363	,737	-,389	,872
		4 (upper secondary)	-,0139	,1830	1.000	-,495	,467

*The difference of the mean is significant at the level of 0.05

***Table 8c**. Multiple comparisons regarding level of education*

3.1.6. Concurrent and divergent validity analysis

We investigated the links between the Adaptive Hyperactivity and Goal Achievement scale and a measure of the rhythms in bipolar disorder, the BRIAN. The analysis was done with Pearson's correlation coefficient (r), using a threshold of $p<0.05$ for statistical significance. We found that the goal achievement subscale of the scale (factor 1) was negatively related to the BRIAN and its subscales, except the rhythm (*Table 9*).

The hyperactivity subscale (factor 2) was less negatively related to the BRIAN and its subscales (*Table 9*). Essentially, greater propensity to goal achievement was related to greater disruption of sleep, general activity, sociality, and eating habits. Greater propensity to hyperactivity only disrupted general activity and sociality. The results support the convergent validity of this new scale, inasmuch as higher the propensity to display symptoms of bipolar disorder, as higher the impact on social rhythms.

	Factor 1 **Goal achievement**	**Factor 2** **Hyperactivity**
BRIAN (Total)	r=-0.368; p<0.0001	r=-0.208; p=0.040
Sleep	r=-0.285; p=0.004	r=-0.153; p=0.132
Activity	r=-0.273; p=0.007	r=-0.221; p=0.029
Sociality	r=-0.246; p=0.015	r=-0.208; p=0.040
Eating	r=-0.255; p=0.011	r=-0.066; p=0.519
Rhythm	r=-0.101; p=0.321	r=-0.116; p=0.257

***Table 9.** Correlation analysis between the adaptive hyperactivity and goal achievement scale and the BRIAN*

3.2. The frequency of a genetic variant (RS1006737) already linked to BD in elderly individuals without BD, but with characteristics of hyperactivity and novelty seeking (H/NS)

Table 10 shows the association of the genetic variant (RS1006737) in older adults with bipolar disorders and old adults without bipolar disorders, but with hyperactivity and novelty seeking features. The comparison profile of the presence of the genetic variant (RS1006737) in our study sample versus control samples identified in the literature studies [147-149] is shown in *Table 11*. Regarding the familiarity of bipolar disorder, *Table 12* shows the frequency of family history for bipolar disorders in older subjects and novelty seekers separately, and *Table 13* presents the frequency of the family history in old adults with or without BD but with the presence of a genetic variant (RS1006737) and in old adults with or without BD without the presence of the same genetic variant.

Groups	Gen+ Variant (RS1006737)	Gen- Variant (RS1006737)	Total	$\chi 2$	P	OR BD	CI 95%
Old Adults With Bipolar Disorder	15 (71%)	6	21	0.486	P=0.487	1.50	0.48-4.70
Old Adults Without Bipolar Disorder (novelty seekers)	25 (62.5%)	15	40				

Table 10. *Gen+ Variant (RS1006737) in old adults with BD and old adults without BD, but with the features of hyperactivity and novelty seeking*

Groups	**Gen+ Variant (RS1006737) (AA/GA)**	**Gen- Variant (RS1006737) (G/G)**	**Total**	**$\chi 2$**	**P**	**OR**	**CI 95%**
Old Adults Without Bipolar Disorder novelty seekers (study sample)	25 (62.5%)	15 (37.5%)	40	Pivot			
Control Group 1 [147]	41 (34.2%)	79 (65.8%)	120	9.938	0.002	3.28	1.5-6.7
Control Group 2 [148]	68 (54.84)	56 (46.67%)	124	0.723	0.395	1.37	0.6-2.8
Control Group 3 [149]	22 (44%)	28 (56%)	50	0.308	0.081	2.12	0.9-4.9
Pooled 3 Control Groups	131(44.55)	163	294	4.553	0.033	2.12	1.1-4.1

Table 11. *Gen+ Variant (RS1006737) in old adults without BD and features of hyperactivity (present sample) and control groups found in literature*

Groups	**With Family History of BD**	**Without Family History of BD**	**Total**	**$\chi 2$**	**P**	**OR +H-BD**	**CI 95%**
Old Adults With Bipolar Disorder	12	9	21	13.652	0.0001	9.33	2.6-33.4
Old Adults Without Bipolar Disorder (novelty seekers)	5	35	40				

Table 12. *Family history for BD in old adults with BD and old adults without BD, but with the features of hyperactivity and novelty seeking*

Groups	With Variant (RS1006737)	Without Variant (RS1006737)	Total	$\chi 2$	P	OR +H-BD	CI 95%
Old Adults With Family History of BD	13	4	17	1.240	0.266	2.04	0.6-7.3
Old Adults Without Family History of BD	27	17	44				

Table 13. *Family history for BD in old adults with or without BD but with the presence of genetic variant (RS1006737) and old adults with or without BD without presence of genetic variant (RS1006737)*

3.3. Bipolar disorder as the consequence of a genetic weakness or not having correctly used a potential adaptive condition

		People with bipolar disorders	People with hyperactivity, but without bipolar disorder	People without hyperactivity, and without bipolar disorders
		N=21	N=25	N=15
Gender	Man	7 (33.4%)	16 (64%)	6 (40%)
	Woman	14 (66.6%)	9 (36%)	9 (60%)

Table 14. *Characteristics of the sample evaluated by gender in the three groups of people: a) with BD b) with hyperactivity but without BD, and c) without hyperactivity and without BD*

Groups	Gen+ Variant (RS1006737)	Gen- Variant (RS1006737)	Total	Homogeneity with +H-BD	P	OR +H-BD	CI 95%
People with bipolar disorder	15 (71%)	6	21	$\chi 2$=0.124	0.725	0.79	0.21-2.95
People with hyperactivity, but without bipolar disorder	19 (76%)	6	25	Pivot			
People without hyperactivity, and without bipolar disorder	6 (40%)	9	15	$\chi 2$=5.184	0.023	4.75	1.19-18.91

Table 15. *People with Hyperactivity and Without Bipolar Disorder (+H-BD) are homogeneous as regards the frequency of variant RS1006737 of the people with Bipolar Disorder (+BD), but don't with the people without Hyperactivity and without Bipolar Disorder (-H-BD)*

Groups	**Gen+ Variant (RS1006737)**	**Gen- Variant (RS1006737)**	**$\chi 2$**	**P**	**OR**	**CI95%**
People with hyperactivity, but without bipolar disorder + people with bipolar disorder	34 (74%)	12	5.763	0.016	4.25	1.24-14.4
People without hyperactivity and without bipolar disorder	6 (40%)	9				

Table 16. *Difference in Genetic Profile (variant RS1006737) in people with Hyperactivity (with or without BD) plus Bipolar Disorders and people without Hyperactivity and without Bipolar Disorder*

Table 14 shows the characteristics of the sample evaluated by gender in the three groups of people:

1) with bipolar disorders
2) with hyperactivity and without bipolar disorders
3) without hyperactivity and without bipolar disorders

The groups were similar in gender distribution.

*Table 1*5 shows that people with hyperactivity and without bipolar disorders (+H-BD) are homogeneous with people with bipolar disorders (+BD) as regard the frequency of the genetic variant RS1006737 (OR=0.79, CI 95% 0.21-2.95), but not with people without hyperactivity and without bipolar disorders (-H-BD) (OR=4.75, CI95% 1.19-18.91). If the group with hyperactivity and without bipolar disorder is added to the group with bipolar disorders (*Table 16*), the set of the two groups has a frequency of the variant RS1006737 that is clearly higher than that of the group without hyperactivity and without bipolar disorder (OR=4.25, CI95% 1.24-14.4).

3.4. The connection of the genetic variant RS1006737 to the MDQ and BRIAN scores

	Gen+ Variant (RS1006737)	Gen- Variant (RS1006737)	Total
0/3 MDQ	24	11	35
1/3 MDQ	5	3	8
2/3 MDQ	6	3	9
3/3 MDQ	5	4	9
Total	40	21	61

Table 17*. The correlation between MDQ scores and the genetic variant RS1006737*

	Gen+ Variant (RS1006737)	Gen+ Variant (RS1006737)	Total	Stat	P
Brian Total Score	36.05±11.33	38.00±13.3	36.72±12.00	F(DF 1,60) 0.362	0.550
-1standard deviation 24.72	4	0	4	Chi Square Yates Correction 0.912	0.340
+1standard deviation 46.72	8	4	12	Chi Square Yates Correction 0.001	0.999

Table 18*. The relation between the presence of the genetic variant RS1006737 and total BRIAN scores*

	Gen+ Variant (RS1006737)	Gen+ Variant (RS1006737)	Total	Stat F(DF 1,60)	P
Brian Total Score	36.05±11.33	38.00±13.3	36.72±12.00	0.362	0.550
Sleep	9.27±3.92	9.18±4.15	9.24±4.00	0.007	0.934
Activities	9.27±4.20	9.76±3.90	9.44±4.10	0.107	0.659
Social rhythms	6.17±2.33	6.46±2.79	6.27±2.49	0.186	0.668
Eating patterns	5.72±2.24	6.47±3.19	5.98±2.57	1.145	0.289
Predominant rhythm	5.60±0.91	6.09±1.20	5.77±1.03	3.193	0.079

Table 19. *Scores on the BRIAN subscales and the presence of the genetic variant RS1006737*

The MDQ scores were compared using the Kruskal-Wallis test based on whether the investigated genetic variant was present. The two categories did not differ significantly between them (H = 0.324; p-value = 0.5692). *(Table 17).*

In terms of the BRIAN questionnaire's total scores, there was no significant difference between the groups in which the genetic variant is present and those in which it is absent. *(Table 18).* Furthermore, the differences in the scores in all of the BRIAN tool's subscales (sleep, activities, social rhythms, eating patterns, and predominant rhythm) between the two groups were statistically not significant *(Table 19).*

3.5. Biological material used for genetic analysis does not affect the detection of mutations in the CACNA1C gene linked to bipolar disorders

	Positive participants (G/A)	Negative participants (G/G)	Total participants
Saliva	28 (59.57%)	19 (40.43%)	47
Blood	40 (65.57%)	21 (34.43%)	61

Table 20. *Total number of positive and negative participants for RS1006737 genetic variant*

Until now, our approach was supported by two complementary studies in which the genetic variant RS1006737 (CACNA1C gene) was investigated. The first experimental group was composed of Sardinian migrants ("volunteers") in the megacities of South America and Europe [99]. Previously, it was found that the frequency of episodes of non-pathological hypomania was double in the Sardinian migrants in the megacities of South America, compared to Sardinians residing in Sardinia, which goes in favour of the adaptive and beneficial side of the hyperthymic temperament [75]. Saliva was collected from them for genetic testing [99]. The second and larger experimental group consisted of older, healthy subjects with characteristics of hyperactivity and novelty seeking in the city area of Cagliari, Italy, previously described in the section on materials and methods (*Table 20*). Although the samples were recruited from different geographical areas and different subjects, the differences between the saliva and blood samples were not significant.

Discussion

4.1. Development and validation of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA)

From an evolutionary point of view, there are many examples that describe conditions that are “maladaptive” in a certain environment but can become adaptive as the context conditions change [156]. The best-known case of such a phenomenon is the evolution of the peppered moth. During the Industrial Revolution, the common white “bladder” peppered moth was replaced by a black “carbonara” type of specimen. This evolution was driven by the interaction between bird predation and coal pollution. Whenever an apparently maladaptive condition inexplicably appears and grows, a similar phenomenon can be found [157]. In humans, some lifestyle circumstances accompanied by the possible dysregulation of biological rhythms, for example those of the urban lifestyle, play a crucial role as triggers of bipolar spectrum disorders, however, hyperactivity and goal pursuit are actually the main drivers of adaptive behaviour in specific living conditions. This could explain the increasing trend in diagnoses of bipolar spectrum disorders in modern societies [158]. Hence, it cannot be excluded that some hypomanic characteristics may be adaptive in some social contexts [159].

Several studies have discovered that certain affective temperaments, such as cyclothymic, hyperthymic, depressive, irritable, and anxious temperaments, are subclinical (trait-related) manifestations and frequently the precursors of mood disorders [38, 40]. However, these premorbid affective temperament types also have a principal role in the clinical growth of major mood disorders, involving the regulation of the polarity and the symptom evolution of the acute mood episodes, so they can also remarkably influence the long-term path and outcome [40].

In fact, Akiskal suggested that mixed affective episodes are the consequence of the fusion of inverse temperamental factors (i.e., depressive or anxious temperament) with a manic

syndrome [40]. This theory also supports the previous findings from Hantouche et al., where the only temperament that significantly differentiated mixed from pure mania in manic patients was the depressive temperament [35].

4.1.1. Hyperthymic temperament and its principal characteristics

Today it is widely debated whether affective temperaments, including hyperthymia, belong to the area of pathology or to that of normality [160]. Hyperthymic temperament is characterized by elevated levels of emotions and feelings, and by the ability to connect to people. This action-oriented temperament, rather than "being lectured to," appeared to improve emotion and life satisfaction, mental health, and social support [161]. This quality is adaptively advantageous when such persons are approached by illness, suffering, or death, which is unavoidable with advancing age. As well, several studies claimed that hyperthymic temperament prevents suicidal ideas and efforts [162].

In an Argentinian study, the quality of life of the hyperthermic individuals was equal to that of controls, qualifying hyperthymia as one of the most adaptive temperamental types in some circumstances and environments [163]. Regarding hyperactivity, males showed a greater inclination towards this trait. Hyperactivity is important for reducing reactivity to life stressors and expanding one's ability to relate to others [164].

Some authors describe this temperament as having overenergetic, upbeat, and overconfident lifelong qualities such as extroversion, cheerfulness, overoptimism, warmth, people-seeking, overconfidence, a high energy level, stimulus-seeking, and habitual short sleep [44]. These attributes are obviously very relevant to territoriality as well as leadership, defending the territory from dangers both inside and outside the social group. Instead, other authors recognized its behavioural properties [51]. Possl and von Zerssen, for instance, described these people's lives in regards to the following features:

extroverted, vivid, active, verbally aggressive, self-assured, self-employed, risk-taking and sensation-seeking, breaking social norms, and generous [165]. This contribution comes from a study of premorbid behaviours in bipolar disorder patients. Exploratory increased activity, unregulated optimism, attachment, certainty, extravagance, independence, vigor, and impulsiveness are all strong positive traits of this temperament. Despite their enormous advantages, hyperthymia's negative correlation with harm avoidance exposes these individuals more vulnerable to the dangers of impulsive action [165].

4.1.2. The role of the novelty-seeking component in hyperthymic temperament

According to Cloninger (1987), novelty-seeking dimension patterns result from multifactorial phylogenetic mechanisms that, at their best, are helpful for an individual's adaptation and survival. It is also probable that each reaction or temperament type may offer advantages during times of newer environmental challenges, giving the social group as a whole a better chance of surviving [160]. In other words, while individual temperamental excesses may not be ideal for a particular person who expresses them during a difficult time, they may, when combined with other extreme temperaments, serve as the best option for society, leading to the evolutionary adaptation of our species as a whole. At the very least, this should encourage people to be more accepting of human diversity since extreme types may possess the genetic information necessary for our adaptation to the planet we all share.

4.1.3. Hyperthymic temperament and professional career

A large number of studies favour the hyperthymic temperament as an important component in the choice and development of a professional career [41, 42]. Hyperthymic temperament appeared to play a central role among managers, self-made businessmen,

and journalists [41]. According to a study conducted regarding the temperamental characteristics of applicants for cadet officer positions in the Italian Air Force, extremely high hyperthymic scores appeared to be correlated with the particular temperamental profile of young applicants and the highest likelihood of success [166]. The findings demonstrate that those who took the entrance exam were more hyperthymic than their peers, and the specificity of this correlation is supported by the finding that those who took the entrance test more than once after failing the first time were more hyperthymic than first-time applicants [166]. This evidence supports the idea that hyperthymic characteristics provide distinct advantages in a profession such as the military, which is directly connected to leadership.

4.1.4. Hyperthymic temperament and somatic diseases

Beyond the sphere of affective illness, affective temperaments have also been reported to have a significant pathoplastic action on a variety of illnesses. Temperament, in particular, has been shown to influence not only psychiatric but also somatic diseases. A temperamental profile suggestive of a hyperthymic temperament is matched to a high capability to cope with somatic obstacles, especially those supposedly related to lifestyle and behavioural habits [167]. For instance, HIV infection represents a condition where behavioural and hyperthymic temperamental characteristics may play a crucial role. The impulsive risk-taking trait associated with a temperamental profile may have had a significant impact on drug use that involved sharing needles and unprotected sexual behaviour, which ultimately resulted in HIV infection [167]. On the other hand, openness and experience seeking are characteristics of hyperthymic HIV-individuals who are interested in seeking new thoughts and ideas and expanding their fund of knowledge. They would be sensitive to their surroundings and the larger environment, but it is very

probable that when facing HIV, these “experience seekers” will be more proactive and seek out information that might afford them some advantage in managing their disease. For hyperthymic HIV-positive subjects openness and experience seeking are significantly vital to expand their knowledge and understanding and seek out new ideas. Regardless of their sensitivity to their surroundings and the larger environment, it is very likely that when confronted with HIV, these "experience and novelty seekers" will be more proactive and seek out information that may provide them with an advantage in managing their disease [168].

4.1.5. Goal achievement as a key constituent of hyperthymic temperament

As presented in the results, higher scores in goal achievement were observed among those who had achieved the highest levels of education (*Tables 8a, 8b*). Goal achievers develop an intense sense of competition and a strong focus on accomplishments, which are valued above rules and circumstances [52].

This temperamental trait appears to include an existential dimension in which the challenge of overcoming boundaries and living emotionally intense experiences is prioritized.

Particularly, individuals with this trait actively accept conflicts with their surroundings rather than follow the prevalent public belief [169]. From a pathological view, people with bipolar disorders report being particularly perfectionists in pursuing goals and seeing goal accomplishment as more important to their self-worth perception [169]. Current evidence indicates that bipolarity lies along a range from extreme temperament to full-blown affective illness. Less research has been done on the continuum of temperaments between average and extreme, although what is established about it does seem to indicate that many, if not all, temperamental traits tend to be continuously distributed.

4.1.6. Strengths and limitations during development and validation of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA)

The sample characteristics were another important aspect of the study in terms of the development and validation of the AHGA; given that the sample is made up of people aged 60 and over with no lifetime psychiatric condition, it is considered quite stable in terms of the possible onset of psychiatric disorders typical of other ages [135, 136]. In fact, due to the pathoplastic effect of age, the older the interviewee gets, the greater the chances of cumulative life events. The seemingly constant number of possible "events" after a certain age represents a predictable scenario.

The preliminary nature of the data represents an obvious limitation of the study; it will be necessary to replicate them to a greater extent. Another limitation lies in the characteristics of the sample. As mentioned above, participants mainly come from the previous study, where different physical and cultural activities are carried out. This factor could define a bias in the sample definition phase, given the presence of a higher frequency of hyperthymic features among the included participants. Further studies, including samples of more diverse socio-cultural and ethnic populations, are needed for a comprehensive understanding of the factorial structure of the AHGA.

4.1.7. Convergent validity of AHGA tool

The results also support the convergent validity of the AHGA tool. The Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA) measures adaptive characteristics of hyperactivity and goal pursuit in contrast to the BRIAN, which measures pathological characteristics (*Table 9*). The results represent a first step in an innovative approach aimed at integrating adaptive and pathological aspects of

hyperthymic features by embracing a broader concept of spectrum that conceptualizes these elements as intrinsically linked.

4.2. The frequency of a genetic variant (RS1006737) already linked to BD in elderly individuals without BD but with characteristics of hyperactivity and novelty seeking (H/NS)

According to the findings of our study, the frequency of the RS1006737 genetic variant in the study group (H/NS) is not higher than in the BD group (*Table 10*), but is statistically significantly higher than in all the control groups of the three studies found in the literature [147-149] (*Table 11*). For example, the first identified study showed a significant association in the Pakistan population for a heterozygous genotype with BD, with 34.2% of control individuals and 50.8% of cases [147]. In the second literature-identified study, 68 subjects out of 124 control subjects were found to have the genetic variant (17 with a homozygous profile and 51 with a heterozygous profile) [148]. As reported by the results of the third study, 22 control individuals out of 50 had the RS1006737 genetic variant (3 homozygous and 19 heterozygous) [149].

However, according to our findings, familiarity with BD is higher in older adults with BD than in the H/NS sample without BD (*Table 12*). The risk of BD in the family (also considering those without BD but with family members with BD) is not associated with the presence of the genetic variant examined (*Table 13*). Several explanations for this outcome are possible: i) The gene may not be associated with the disorder but with some related variable; ii) It would be useful to divide the sample without bipolar disorder (H/NS) into two groups: those who actually have a profile of hyperactivity or novelty seeking and those who don't.

4.2.1. A genetic characteristic considered a "weakness" eventually represents an advantage when used in the appropriate way

The CACNA1C gene that has always been associated with the risk of BD is probably associated with hyperactivity, which might be a determining factor. In other words, there is the possibility that a genetic characteristic considered a "weakness" eventually represents an advantage when used in the appropriate way. The presence of hyperthymic temperament traits in conjunction with a bipolar genetic variant should not be interpreted as a subject's vulnerability. On the contrary, given today's rapid changes and modern lifestyle, if used correctly, they can represent a huge advantage in developing one's own potential and, thus, for society.

The recruitment approach of the sample of older adults with hyperactivity and novelty seeking but without bipolar disorders also needs to be considered. This group, which was recruited through public notices, consisted of participants, particularly the elderly ones, in good health and/or with minor health conditions (diabetes, hypertension, etc.), who were able to perform moderate physical activities, and who did not have any lifetime mood or other mental health disorders. The participants of the other group, on the contrary, suffered from bipolar disorders.

It is well known that this psychiatric disorder has a strong genetic component. As reported by Akiskal et al., the underlying trait being passed on is not the BD syndrome but alterations in temperament [50]. Taking into consideration the results of one of his studies, certain dimensions of temperament are transmitted in families as quantitative traits that are part of a broader bipolar spectrum [39].

However, the consequently high percentage of people with the gene variant RS1006737 found in our study sample (much higher than anticipated in the general population, based on previous literature descriptions) shows that this is a non-representative sample of the general population and that hyperactivity is unbalanced.

4.3. Bipolar disorder as the consequence of a genetic weakness or not having correctly used a potential adaptive condition

The results of this study also found that a genetic variant RS1006737 recognized in the literature as associated with bipolar disorder has a high frequency (76% of individuals) in people without BD but with traits of hyperactivity and novelty seeking; with comparable frequency to those of individuals with bipolar disorders (71%), but with a much higher frequency to individuals without hyperactivity traits and without the bipolar disorders (40% of individuals) (*Table 15*). The positive subjects of the genetic variant RS1006737 identified in our study were all heterozygous (G/A) (*Figure 5*).

Several factors need to be highlighted in considering the results of this study. First, the sample of older adults with hyperactivity without bipolar disorders may have been characterized by a more active social network given that they were recruited through media methods or the territorial health network; second, the individuals in this group were without lifetime mood or other mental health disorders; third, they were in relatively good health or only had ailments that were not uncommon among the elderly (hypertension, diabetes, etc.) and which did not compromise their ability to carry out mild to moderate physical activity; and fourth, they were sufficiently enterprising to start a demanding program of physical exercise. Taking these observations into consideration, our results seem to confirm the hypothesis that some basic characteristics that are typical of bipolar disorder (including genetic characteristics) are not always associated with an increased risk of disease but could also have an adaptive significance in certain circumstances.

4.3.1. Bipolar disorders, Hypothalamic-Pituitary-Adrenal (HPA) axis activity, and environmental factors

The interactions between genetic heritage and environmental factors have aroused specific interest in the neurogenesis of the Hypothalamic-Pituitary-Adrenal (HPA) axis and in its consequences for emotional and behavioural responses [66]. It is known that HPA axis activation by social and psychological stress could increase the risk of the onset of mental conditions. Specifically, bipolar disorder was found to be "associated with dysfunction of HPA axis activity" [67]. Chronic severe stress alters the functioning of the prefrontal and limbic systems, influencing gene expression regulation as well as neuroendocrine, emotional, and behavioural responses [68]. The change or increase in rhythms of modern and urban life could influence the HPA axis in adults and older adults [68]. Inhabitants of cities can access better resources than those living in rural areas, such as education, leisure and cultural activities, and economic and healthcare opportunities [71]. But surprisingly, mood disorders show higher prevalence in urban areas [72], and a dose-dependent association has been found between exposure to the urban environment and the onset of severe mental health episodes as well as a negative prognosis. Therefore, living in cities is associated with more frequent achievement of social goals but also with a higher risk of impaired mental health, including mood disorders. Our group formulated some hypotheses about how cities' noise and light pollution might influence mental health and increase the risk for bipolar disorder [71].

People with novelty-seeking aptitudes and explorers/and "challengers" with hyperthymic temperaments or personalities could have adaptative resources in a new environment, as confirmed in the above-cited studies on voluntary migrants (not refugees) [72]. In fact, if modern life demands that biological rhythms be broken, it could favour people with a basic predisposition to changing and adapting to biological and social rhythms in different ways, i.e., people who can mobilize their energy for some (even limited) periods and with

less need for sleep [81]. The supposed increase in mood disorders would be the pathological side, resulting from the failure of the demands for adaptation, such as when the challenges outweighed the ability to adapt or when the increase in energy and the reduced need for sleep have not been sufficient to achieve the desired goals. Thus, the result can be an adaptation at some times, but at other times, when the demand is too stressful, adaptation fails, and a "new pathology" emerges with features of broken biological and social rhythms.

4.3.2. Bipolar disorders and artificial light pollution

The "English Illness", that is, the mood disorders with new psychopathological characteristics, was born, according to Murphy, in the newly industrialized areas [79]. Supporting this hypothesis, several surveys have found a continuous increase in Western societies of mood and bipolar disorder [73, 74]. Artificial light induces, during natural darkness, activities usually performed in daylight, such as work activities, food intake, or social life [170]. This impacts the immune-endocrine and other biologic rhythms, which evolution finalized to synchronize human behaviour with variations in daylight and changes of season [67]. It was found that the risk of bipolar increased with disorders of the sleep-wake cycle and with artificial light pollution [170]. Melatonin decreases estradiol and increases progesterone levels [83]. Light pollution may lower melatonin secretion and changing the estradiol/progesterone ratio. Thus, the increase of estradiol and other "stimulating" steroid hormones induced by light pollution may have a role in the increasing risk of bipolar disorder [85].

4.3.3. An interpretation of the bipolar disorder not as the simple consequence of a genetic weakness would be an element against stigma and self-stigma

If our hypothesis about the evolutionary genesis of bipolar disorder is confirmed, this will not change much about the pharmacological approach. In other words, if an imbalance in adapting to biological rhythms as we have postulated is central, a mood stabilizer would still be the most appropriate tool based on current knowledge. The socio-biological perspective would, however, change the approach to what until now has been defined as "rehabilitation" and "psycho-social approach." The new interpretation would, in fact, open the way to a new approach to supporting drug therapy in which the rediscovery of the adaptive potential would be central for the individual who has suffered a decompensation. Furthermore, the interpretation of the disorder not as the simple consequence of a genetic weakness would be an element against stigma and self-stigma.

4.3.4. Strengths and limitations of the methodology

The methodology has the following advantages:

1) Working with older people makes it less likely that people identified as "not affected by bipolar disorders" will later develop the disorder. Considering that it is assumed that people with characteristics of hyperactivity may be at high risk for bipolar disorder.

2) Older adults who join an active aging program could more likely have characteristics of hyperactivity, thus facilitating the recruitment of people without lifetime mood disorders but with features of hyperactivity.

3) The fact that the recruited individuals are all from urban areas increases, according to our hypothesis, the probability of a higher frequency of people with hyperactivity.

Also, some evident limits of our study need to be underlined.

First of all, the study is exploratory since it is based on a small sample, and its results need to be confirmed with a much larger sample. The sample of old adults without bipolar

disorders shows a high frequency of people with hyperactivity (25 out of 40 = 62.5%); the mode of recruitment of the sample (old adults who participated in an active aging project lived in an urban area) may have favourably influenced the selection of people with these features. This is not necessarily a cause for bias because this was intended to empower the power of the study. However, the concomitantly high percentage of people with the gene variant found in our sample (much higher than expected in the general population based on previous studies [147-149]) confirms that this is a non-representative sample of the general population and that hyperactivity is overrepresented. This relatively weakens the strength of the association between hyperactivity and the presence of the variant but does not affect the value of the results.

4.4. The connection of the genetic variant RS1006737 to the MDQ and BRIAN scores

4.4.1. The connection between the genetic variant RS1006737 and MDQ scores

Concerning the MDQ scores, the two groups did not differ significantly between them (*Table 17*).

4.4.2. The connection between the genetic variant RS1006737 and BRIAN scores

Our study found no statistically significant difference between the groups in which the genetic variant RS1006737 was present and those in which it was absent regarding the total scores on the BRIAN questionnaire (*Table 18*). Additionally, there was also no statistically significant difference between the two groups' scores on any of the BRIAN tool's subscales (sleep, activities, social rhythms, eating patterns, and predominant rhythm) (*Table 19*).

However, these two validated questionnaires, together with the Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA), will be used further in the later phases of our study, where a larger number of risk genetic variants will be investigated.

4.5. Biological material used for genetic analysis does not affect the detection of mutations in the CACNA1C gene linked to bipolar disorder

Saliva is an extracellular fluid produced and secreted in the mouth by salivary glands. In humans, it contains approximately 99% water, as well as electrolytes, mucus, white blood cells, epithelial cells (from which DNA can be extracted), enzymes (such as lipase and amylase), and antimicrobial agents (such as secretory IgA and lysozymes) [171]. Saliva sampling is a low-cost, non-invasive, and time-saving method. Another significant advantage is that after a brief explanation of the procedure, the subject can take the material on their own. When collecting mucosal salivary samples, the buccal swab should be vigorously rubbed on the inside of the cheek, both right and left. One disadvantage of using saliva as a test material is that it is advised to avoid eating or drinking coloured substances and smoking. It is also necessary to consider the possibility of ongoing oral infections that may result in bleeding or mucus production so that the testing tool can detect these as contaminants. Importantly, in some cases, the reason for re-sampling is a lack of DNA in the salivary sample. The blood is a body fluid in the circulatory system of humans that transports metabolic waste products away from cells while delivering necessary substances such as nutrients and oxygen to them. It is made up of blood cells suspended in plasma [172]. Blood sampling is more expensive than salivary sampling. It is a more invasive method that has the potential to coagulate and hemolyze the sample. As a result, certain temperature ranges associated with sample transport and storage must be respected.

On the other hand, taking a blood sample for genetic analysis always provides a sufficient amount of DNA, and individuals receive no prior recommendations regarding food and

drink restrictions. It is critical that no local or systemic infection poses a threat to providing a viable sample for genetic analysis.

4.5.1. Identified mutations relating to exotic position RS100673

Two different mutations were identified in these analysed samples relating to exotic position RS100673. The first was standard wild-type sequencing (G/G), which was linked to no risk of bipolar disorders. In this work, we have observed a good and interesting association with the heterozygous profile (G/A) (Table 20). At the same time, this investigation demonstrated and suggested the use of capillary sequencing as a fast and reliable method to detect RS100673 mutations in the CACNA1C gene.

4.5.2. The detection of a mutation is unaffected by the sample type

The differences between the saliva and blood samples were not statistically significant, even though the samples were collected from various geographical locations and individuals (*Table 20*). The detection of a mutation in the targeted genetic variant is unaffected by the sample type, despite the differences in composition and biological characteristics. Both blood and saliva were acceptable biological materials.

Conclusions

The reported results for the development and validation of the Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA) as a new tool represent an innovative approach to hyperthymic features by embracing a broader concept of spectrum, which conceptualizes as a continuum the potential transition between pathological and adaptive aspects. This study suggests that the examined genetic variant (RS1006737) is associated with characteristics of hyperactivity rather than just BD. Nevertheless, choosing to participate in an exercise program is an excessively general way to identify hyperactivity/novelty seeking. The next step would be to use an appropriate tool, such as the Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA), to identify older adults with well-defined hyperactivity and novelty-seeking characteristics.

This study also found that a genetic variant RS1006737, recognized in the literature as associated with bipolar disorders, was found in well-adapted older adults without bipolar disorders and high hyperactivity traits with a similar frequency as in older adults with a diagnosis of bipolar disorders but higher than in older adults without bipolar disorders and without hyperactivity. The study involved a very small sample, and its results need to be confirmed. If the results and the hypothesis of an evolutionary genesis of bipolar disorders are confirmed, the new interpretation could open the way to a new approach to supporting drug therapy in which the rediscovery of the adaptive potential resources would be central to the recovery of the individual who has suffered a bipolar disorder onset. Furthermore, the interpretation of the disorder not as the simple consequence of a genetic weakness could be an element against stigma and self-stigma.

Regarding MDQ scores, total scores on the BRIAN questionnaire, and scores on any of the BRIAN tool's subscales (sleep, activities, social rhythms, eating patterns, and

predominant rhythm), a statistically significant difference between the groups in which the genetic variant RS1006737 was present and those in which it was absent was not found. When comparing the biological material, although saliva and blood differ in composition and biological properties, sample type has no effect on the detection of a mutation in the genetic variant of interest. Blood and saliva can both be used as biological materials in later stages of this research.

Future studies need to establish the true frequency of hyperactivity in the general population. The combination of the new questionnaire tool (Questionnaire for Adaptive Hyperactivity and Goal Achievement) with the genetic analysis appears to be an innovative, practicable, and original approach. The following development of this study will include more genetic variables with higher susceptibility for bipolar disorders (ANK3, NCAN, ODZ4, SYNE1, and TRANK1 genes) and obviously more numerous target and control samples.

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APPENDICES

APPENDIX A - Questionnaire for Adaptive Hyperactivity and Goal Achievement (AHGA)

Età:

Genere:

- Maschio
- Femmina
- Preferisco non specificare

Livello di istruzione:

- Non ho frequentato la scuola
- Diploma di Scuola elementare
- Diploma di Scuola media
- Diploma di Scuola superiore
- Laurea

1. Quante volte pensi di esserti innamorato/a (anche senza avere una relazione)?

- Tra Nessuna e 1 volta
- Tra 2 e 3 volte
- Tra 4 e 5 volte
- Tra 6 e 7 volte
- Più di 7 volte

2. Quante volte ti sei spostato in un raggio oltre i 200 km dal luogo in cui vivevi?

- Meno di 3
- Tra 3 e 5
- Tra 6 e 8
- Tra 9 e 11
- Più di 11

3. In media, con quante persone fai una chiacchierata in una settimana?

- Meno di 5
- Tra 5 e 10
- Tra 11 e 15
- Tra 16 e 20
- Più di 20

4. Quante ore dormi in media ogni notte?

- Meno di 4
- Tra 4 e 5
- Tra 6 e 7
- Tra 8 e 9
- Più di 9

5. Quante ore dedichi a settimana all'attività fisica? (es. camminata veloce, tennis, corsa, o sport in generale, attività in campagna, faccende domestiche, ballo)

- Meno di 1
- Tra 1 e 2

□ Tra 3 e 4

□ Tra 5 e 6

□ Più di 6

6. Quante ore al giorno passi fuori casa?

□ Meno di 1

□ Tra 1 e 2

□ Tra 3 e 4

□ Tra 5 e 6

□ Più di 6

7. Quanti programmi/applicazioni usi al giorno? (ad esempio: Posta Elettronica, Skype, Netflix, Facebook, WhatsApp)

□ Tra 0 e 1

□ 2

□ 3

□ 4

□ 5 o più

8. Quanto è diverso il lavoro che hai fatto rispetto a quello del genitore del tuo stesso sesso?

□ Per nulla

□ Poco

□ Abbastanza

□ Molto

□ Moltissimo

9. Il tuo livello scolastico, rispetto a quello del genitore con il livello più alto è:

- Inferiore
- All’incirca uguale (più o meno un anno scolastico di differenza)
- Un poco maggiore (dai due ai cinque anni di differenza)
- Maggiore (dai sei agli otto anni di differenza)
- Di gran lunga superiore (oltre otto anni di differenza)

10. Come valuti il tuo benessere economico/reddito rispetto a quello dei tuoi genitori:

- Inferiore
- All’incirca uguale (tenuto conto anche delle differenze di epoca)
- Un poco maggiore
- Maggiore
- Di gran lunga superiore

11. Mi sembra che gli altri siano:

- Molto più veloci di me nel fare le cose
- Più veloci di me nel fare le cose
- Abbiano la mia stessa velocità nel fare le cose
- Io sia più veloce degli altri nel fare le cose
- Io sia molto più veloce degli altri nel fare le cose

12. Mi sembra che gli altri siano:

- Molto più veloci di me nel pensare
- Più veloci di me nel pensare
- Abbiano la mia stessa velocità nel pensare
- Io sia più veloce degli altri nel pensare
- Io sia molto più veloce degli altri nel pensare

APENDIX B - MDQ

Mood Disorder Questionnaire - item della versione italiana

Questo questionario serve per aiutarci a capire meglio i suoi problemi e a rispondere meglio ai suoi bisogni. Per favore risponda ad ogni domanda facendo un segno sul cerchio accanto alla risposta che più corrisponde alla sua opinione o situazione.

C'è mai stato un periodo di tempo nel quale non si è sentito come il solito e…

1.	stava così bene che gli altri pensavano che fosse troppo su di giri e che rischiava di mettersi nei guai?	○ Si	○ No
2.	era così irritabile che strillava alle persone o litigava facilmente?	○ Si	○ No
3.	si sentiva molto più sicuro di sé del solito?	○ Si	○ No
4.	dormiva molto meno del solito senza sentire la mancanza di sonno?	○ Si	○ No
5.	parlava molto di più o molto più velocemente del solito?	○ Si	○ No
6.	aveva pensieri che si succedevano velocemente nella sua mente o non riusciva a rallentare i suoi pensieri?	○ Si	○ No
7.	era così facilmente distratto dalle altre cose da avere difficoltà a concentrarsi in quello che stava facendo?	○ Si	○ No
8.	aveva molta più energia del solito?	○ Si	○ No
9.	era molto più attivo o faceva molte più cose del solito?	○ Si	○ No
10.	era molto più socievole o espansivo del solito, per esempio poteva telefonare agli amici nel cuore della notte?	○ Si	○ No
11.	era molto più interessato al sesso del solito?	○ Si	○ No
12.	faceva cose che di solito non fa e che altre persone potevano giudicare eccessive, stupide o rischiose?	○ Si	○ No
13.	spendeva troppo tanto da mettere Lei o la sua famiglia nei guai?	○ Si	○ No

Se ha barrato più di una casella della colonna Si nelle domande precedenti:

14.	Molte di queste cose le sono successe mai nello stesso periodo di tempo?	○ Si	○ No
15.	Quanto gravi erano i problemi (sul lavoro, in famiglia, legali, litigi, ecc.) causati dalle cose di cui stiamo parlando? Per favore faccia un segno su un solo cerchio qui sotto: ☐ Nessun problema ☐ Problemi lievi ☐ Problemi medi ☐ Problemi gravi		
16.	Qualcuno dei suoi parenti stretti (cioè figli, fratelli, sorelle, genitori, nonni, zie, zii) ha avuto un disturbo maniaco depressivo o bipolare?	○ Si	○ No
17.	Un medico o uno psicologo le ha mai detto che aveva un disturbo maniaco depressivo o bipolare?	○ Si	○ No

Appendix C - BRIAN

INTERVISTA DI VALUTAZIONE SUI RITMI BIOLOGICI IN NEUROPSICHIATRIA

BIOLOGICAL RYTHMS INTERVIEW OF ASSESSMENT IN NEUROPSYCHIATRY (BRIAN)

Tra gli aspetti riportati di seguito indichi l'opzione che descrive meglio la condotta del paziente negli ultimi 15 giorni

SONNO

1. Quanto ha difficoltà ad addormentarsi alla solita ora?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

2. Quanto ha difficoltà a svegliarsi alla solita ora?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

3. Quanto ha difficoltà ad alzarsi dal letto dopo essersi svegliato?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

4. Quanto ha difficoltà sentirsi riposato con le ore di sonno che sta facendo (questo in riferimento alla sensazione soggettiva di sentirsi riposato per lo svolgimento delle attività quotidiane come guidare, ragionare e lavorare)?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

5. Quanto ha difficoltà a "staccare" nei momenti di riposo?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

ATTIVITÀ

6. Quanto ha difficoltà a portare a termine tutte le attività del suo lavoro?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

7. Quanto ha difficoltà a portare a termine le sue attività abituali (pulizie di casa, fare le spesa)?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

8.Quanto ha difficoltà a mantere il suo ritmo di attività fisica (per esempio: prendere l'autobus/metro, o praticare uno sport - se questo fa parte delle sue abitudini)?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

9. Quanto ha difficoltà a rispettare il programma abituale delle sue attività?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

10. Quanto ha difficoltà a mantenere il suo livello di desiderio/attività sessuale?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

SOCIALE

11. Quanto ha difficoltà a relazionarsi e comunicare con le persone del suo ambiente?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

12. Quanto ha difficoltà a non abusare dell'uso di apparecchi elettronici come TV, internet, ecc. (senza che tale uso alteri il contatto con le persone del suo ambiente o che sottragga troppo tempo ad altre attività)?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

13. Quanto ha difficoltà ad adattare le sue abitudini e il suo sonno a quelli delle persone con cui vive (famigliari, vicini, amici)?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

14. Quanto ha difficoltà a disporre di tempo e attenzione per le persone con cui vive (famigliari, vicini, amici)?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

ALIMENTAZIONE

15. Quanto ha difficoltà a rispettare gli orari dei pasti (colazione, pranzo e cena)?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

16. Quanto ha difficoltà a rispettare le sue abitudini alimentari in termini di non saltare i pasti?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

17. Quanto ha difficoltà a rispettare le sue abitudini alimentari in termini di quantità di cibo?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

18. Quanto ha difficoltà a non consumare in eccesso sostanze stimolanti (come caffé o coca-cola), o cioccolato/dolci?
(1) per nulla
(2) poco
(3) abbastanza
(4) molto

RITMO PREDOMINANTE (serale o mattutino)
Questa parte della scala è opzionale e si riferisce alle sue abitudini. Consideri qui gli ultimi 12 mesi.

19. Ha la tendenza a essere più attivo durante la notte (lavoro, relazioni interpersonali)?
(1) mai
(2) raramente
(3) quasi sempre
(4) sempre

20 Ha la sensazione di essere più produttivo la mattina?
(1) mai
(2) raramente
(3) quasi sempre
(4) sempre

21. Ha la sensazione di scambiare il giorno per la notte?
(1) mai
(2) raramente
(3) quasi sempre
(4) sempre

Appendix D – Personal data form

MODULO DATI ANAGRAFICI

Scheda n° ____

Cognome _____ Nome_____

Luogo di nascita _____ Data di nascita ____/____/_____

1) Età _____

2) Genere

F	M

3) Provincia di origine

CA	NU	OR	SS	SU

4) Livello di scolarizzazione

	SCUOLA ELEMENTARE
	LICENZA MEDIA
	LICENZA MEDIA-SUPERIORE
	LAUREA
	ALTRO: _____

5) Stato occupazionale

	OCCUPATO/A
	DISOCCUPATO/A
	CASALINGO/A
	PENSIONATO/A
	STUDENTE/SSA
	ALTRO: _____

6) Condizione economica

	BUONE (> 2.500€ P/M)
	DISCRETE (1.600€ - 2.500€ P/M)
	SUFFICIENTI (1.000€ - 1.500€ P/M)
	SCADENTI (≤ 700€ P/M)
	NON PRECISATE

7) Stato civile

	NON CONIUGATO/NON CONVIVENTE
	CONIUGATO/CONVIVENTE
	VEDOVO NON CONVIVENTE
	VEDOVO CON NUOVO PARTNER

8) Figli

	SI	QUANTI? _____
	NO	

ANAMNESI INDIVIDUALE/FAMILIARE

1) Ha mai sofferto o soffre di disturbi psichiatrici (depressione, ansia, attacchi di panico, ecc)?

SI	NO

Se si, quale/i?

_____

Se si, quale/i psicofarmaci ha assunto/assume?

_____

_____

2) Ci sono stati nella sua famiglia (genitori, fratelli, figli, zii, nonni) casi di diagnosi di disturbi psichiatrici?

SI	NO

Se si, chi e quale/i?

_____

3) Soffre di altre patologie croniche non psichiatriche?

SI	NO

Se si, quali? _____

Appendix E **- Questionario sull'iperattività e sulla spinta all'esplorazione (First version)**

Età

Sesso

Anni di scuola

1) Quanto lontano da dove sei nato vivi attualmente

Entro 50 Km

Entro 100 Km

Tra 100 e 1000 Km

Oltre 1000 Km

2) In quante città o paesi hai dormito più di 30 volte nella tua vita

2 o meno

da 2 a 10

Più di 10

(Questo indicatore sarà corretto in relazione alla sua età, considera il calcolo dei giorni approssimativamente come lo ricorda)

3) Con quanti partner hai avuto una relazione stabile per più di un anno

2 o meno

da 2 a 10

Più di 10

(Questo indicatore sarà corretto per la tua età)

4) Con quanti partner hai avuto rapporti anche occasionali nell'anno in cui hai cambiato più partner

2 o meno

da 2 a 10

Più di 10

(Questo indicatore sarà corretto per la tua età)

5) Quante volte pensi di esserti innamorato

2 o meno

da 2 a 10

Più di 10

(Questo indicatore sarà corretto per la tua età)

6) Rispetto alla media dei miei genitori

Ho un livello di istruzione inferiore di 2 anni o più

Ho un livello di istruzione simile (lo stesso numero di anni o un anno più o meno)

Ho da 2 a 5 anni in più di istruzione

Ho 5-10 anni in più di istruzione

Ho più di 10 anni di istruzione

7) Considerati anche i periodi in cui hai lavorato in modo autonomo, hai lavorato con contratto o sei stato impiegato da diversi datori di lavoro con retribuzione

Quante condizioni di lavoro hai cambiato

< o 2

da 2 a 10

più di 10

8) Quanti ruoli lavorativi hai cambiato (per funzione e/o mansione) Considerati anche i periodi in cui hai lavorato in modo autonomo, hai lavorato con contratto o sei stato impiegato da diversi datori di lavoro con retribuzione. Considera qualsiasi attività per cui hai ricevuto un compenso

< o 2

da 2 a 10

più di 10

9) Quante lingue parli (abbastanza per poter seguire un programma TV)

2 o meno

2-5

Più di 5

10) Quanti paesi nel mondo hai visitato

5 o meno

da 5 a 10

> 10-50

> 50

11) In quanti paesi nel mondo hai svolto un lavoro per il quale sei stato pagato?

5 o meno

da 5 a 10

> 10-50

> 50

12) Negli ultimi cinque, in quanti paesi del mondo hai scambiato email (almeno una ricevuta e una inviata)

5 o meno

da 5 a 10

> 10-50

> 50

13) Il tuo reddito attuale è

Al di sotto del reddito medio del tuo stato

Tra la media e il doppio della media

Più del doppio della media

(se sei uno studente, punteggio medio)

14) Chi mi conosce dice

Dormo meno della media

Dormo quanto la media

Dormo più della persona media

Dormo molto di più della persona media

15) Chi mi conosce dice

Lavoro meno della persona media

Lavoro tanto quanto la persona media

Lavoro più della persona media

16) In un giorno medio (a metà settimana), quante persone incontri in media (che conosci e che almeno saluti)

<10

10-30

30-50

> 50

17) Con quante persone hai mediamente contatti telefonici ogni giorno?

<10

10-30

30-50

> 50

18) Quante azioni sui social media fai al giorno (es. contatto con persone, pubblicazione di documenti, ecc.)

<10

10-30

30-50

> 50

19) Quante volte hai bevuto tanto alcol da perdere il controllo nella vita

<10

10-30

30-50

> 50

20) Quante volte hai assunto una sostanza proibita dalla legge ad uso edonistico (es marijuana se proibita nel tuo stato all'epoca della assunzione, cocaina ecc=

<10

10-30

30-50

> 50

21) Quante volte hai preso multe per eccesso di velocità o guida pericolosa o hai avuto incidenti della strada con auto o motoveicoli per quali eri responsabile (se non hai la patente codifica il punteggio inferiore)

<10

10-30

30-50

> 50