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THE GENETICS OF BIPOLAR DISORDER AND THE ROLE OF HETEROZYGOSITY FOR NEURONAL CEROID LIPOFUSCINOSIS

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A mia Nonna, da sempre, sempre e per sempre…

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1. Introduction

Bipolar Disorder (BD) is a common and chronic mental disorder causing psychosocial impairment, affecting patients with depressive/manic episodes (Nishioka M, Kazuno AA, et al. 2021; Craddock N, Sklar P. 2013). Bipolar phenotypes are defined only according to clinical features, and, to date, specific diagnostic tests do not yet exist (Craddock N, Sklar P. 2013). Different subtypes of BDs are recognised, including BD type I and BD type II (Goodwin FK, Jameson KR,1990) well described by the Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) (DSM-5. 5th Edition,2013). Bipolar I is defined by the presence of at least one manic episode, which might have been preceded or followed by a hypomanic episode or major depressive disorder (DSM-5. 5th Edition,2013).

Mood in manic episodes is often described as euphoric, excessively cheerful; the individual may engage in multiple overlapping new projects, without any particular experience or talent (DSM-5. 5th Edition,2013). On the contrary, in some cases the predominant mood is irritable rather than elevated, and communication can be marked by complaints, hostilism, and angry tirades. During a manic episode, which generally lasts about a week, individuals often do not perceive that they are ill or in need of treatment (DSM-5. 5th Edition,2013). Mood may shift rapidly to anger or depression, present psychotic features, and is sufficiently severe to cause marked social impairment, necessitating hospitalization to prevent harm to self or others. The lifetime risk of suicide in individuals with bipolar disorder is estimated to be at least 15 times that of the general population. In fact, bipolar disorder may account for one-quarter of all completed suicides (DSM-5. 5th Edition,2013). Bipolar II is instead characterized by the presence of at least one current or past hypomanic episode and a major depressive episode; manic episodes never occur (Craddock N, Sklar P. 2013; Goodwin FK,

Jameson KR,1990; DSM-5. 5th Edition,2013). Hypomanic episodes are defined as distinct periods of about four days of abnormally and persistently elevated or irritable mood, with decreased need for sleep, excessive involvement in activities and disturbance in mood are not severe enough to cause social impairment or necessitate hospitalization.

Recurrent thought of death and suicidal ideation without a specific plan are common DSM-5. 5th Edition,2013).

Bipolar-like phenomena which do not satisfy the criteria for BD I or II, whose symptoms cause clinically significant distress or impairment in social, occupational or other important areas of functioning, are classified as "Unspecified bipolar and related disorders" (Craddock N, Sklar P. 2013; Goodwin FK, Jameson KR,1990; DSM-5. 5th Edition,2013). Several studies demonstrated the BD early age of onset, wherein more than 70% of individuals manifest the typical characteristics of the illness before the age of 25 years (Edvardsen J, Torgersen S, et al.2008; Nowrouzi B, McIntyre RS, et al.2016; McGuffin P, Rijsdijk F, et al. 2003). Multiple evidence have also strongly demonstrated that BD is a highly heritable phenotype (Edvardsen J, Torgersen S, et al.2008; Nowrouzi B, McIntyre RS, et al.2016; McGuffin P, Rijsdijk F, et al. 2003; Stahl EA, Breen G, et al.2019; Goes FS, Pirooznia M, et al. 2016; Rao AR, Yourshaw M, et al. 2017; Toma C, Shaw AD, et al. 2020). In patients with established disease, a family history of mood or psychotic illness is common, with an estimated heritability of approximately 85% (Craddock N, Sklar P. 2013; Edvardsen J, Torgersen S, et al. 2008; Nowrouzi B, McIntyre RS, et al. 2016; McGuffin P, Rijsdijk F, et al. 2003). The familial transmission of BD does not follow any of the simple Mendelian patterns of inheritance, and several analyses show that BD cannot be accounted for as one highly penetrant susceptibility gene (Craddock N, Sklar P. 2013; . Craddock N, Khodel V, et al. 1995). Nevertheless, according to the current knowledge, the fundamental biological

basis of BD still remains unknown Nishioka M, Kazuno AA, et al. 2021. In the past decade, large-scale Genome-Wide Association Studies (GWAS) of Single-Nucleotide Polymorphisms (SNPs) and analyses of Whole-Exome Sequencing (WES) performed on families revealed several tens of genetic loci related to BD, even if these loci explain only 18% of BD susceptibility (Baum AE, Akula N, et al. 2008; Forstner AJ, Fischer SB, et al. 2020; Mullins N, Forstner AJ, et al. 2021). In the present study, we describe a family with twelve affected BD members through five generations, with an apparently Mendelian inheritance pattern. The study was performed on eight subjects: three were affected by BD I or BD II, four without any signs of mental disease, one reported with moderate anxiety and traits of obsessive-compulsive personality. WES was performed in all of them, allowing us to highlight new genes not previously reported in literature in association with BD.

1.1 From DSM to new research challenges

Modern psychiatry recognizes among its founders Emil Kraepelin (1856-1926), a German psychiatrist and psychologist who proposed a new concept in psychiatric illness: a basic biological model to explain the onset of clinical psychiatric phenomenology. It was Kraepelin, in fact, the first to hypothesize that psychiatric pathologies and their symptoms could be strictly correlated to central anatomical-pathological, neuro-physiological and biochemical alterations and marked, we could say, the beginning of contemporary psychiatry in which the pathology was analyzed in its overall picture (descriptive psychopathology), organic etiology and pathological course over time (Salomone & Arnone, 2009). In the second half of the last century, the first standardized diagnostic criteria were developed (Feighner et al., 1972;

Spitzer et.al, 1978), creating a common language among clinicians and allowing to increase the reliability of psychiatric diagnoses, their comparability between professionals and the homogeneity of treatments. Psychiatry, therefore, from its earliest birth to its most modern version, has always seen new classificatory, conceptual and diagnostic advances, adding from one study to another new knowledge essential for its evolution.

1.2 DSM

The 'Diagnostic and Statistical Manual of Mental Disorders (DSM)' was first redacted in 1952 by the American Psychiatric Association (APA), following the drafting of the 1948 ICD classification by the World Health Organization which had extended the classification also to mental disorders. The following versions were the DSM-II of 1968, DSM-III of 1980, DSM-IIIR of 1987 (revision of the previous edition), DSM-IV of 1994, DSM-IV-TR (revised text) of 2000 and finally DSM-5 of 2013 (with transition from Roman to Arabic numbering); each version saw important updates thanks to the expansion of knowledge. Of particular importance was the 1973 reprint of the DSM-II with the exclusion of homosexuality from psychiatric pathologies and the birth of the DSM-III in which standardized diagnostic criteria for clinical practice were developed for the first time. Starting from the third edition, clinical research was also introduced as tools to demonstrate and empirically confirm over time the reliability of the diagnostic criteria outlined. The fifth edition of the DSM was born as a clinical tool useful for mental health professionals to identify different psychiatric disorders through precise and concise descriptions. The diagnostic criteria outlined identify symptoms, personality traits, behaviors, cognitive functions, physical signs capable of describing the most relevant patient manifestations, increasing the validity and accuracy of the diagnoses. In fact, for the

definition of the individual disorders they have been reviewed several times and subjected to "field tests" to verify their diagnostic reliability among the various professionals.

The classification of psychiatric disorders, on the other hand, is not considered scientifically relevant today since the disorders are grouped following a historical cognitive scheme, used in part also in the editions prior to the fourth; it is no coincidence that one of the greatest challenges today is the revision of the structure of the DSM rather than the description of individual disorders. The previous approach considered each disorder as categorically separate from health status and other psychiatric disorders, thus leading to an artificial classification that did not reflect the broad sharing of symptoms, risk factors and other similarities highlighted by numerous comorbid studies. A lot of attention was paid to limiting as much as possible the “false positive” misdiagnoses, consequently outlining very narrow categories which in fact led to a wide use of the term “not otherwise specified”.

The same authors of the DSM-5 state: “[…] DSM needs to evolve in the context of other clinical research initiatives. An important aspect of this transition is the recognition that an excessively rigid categorical system does not capture clinical experience or important scientific observations”.

The new organization of the DSM was conceived as an evolution, a conservative reform imposed by the new scientific researches that have amply verified the copresence of different disorders in patients and demonstrated the overlap of these in the genetic, epidemiological and pathophysiological fields. The multiaxial system of the DSM-IV was not necessary in order to carry out the specific diagnoses and, in parallel with this classification system, a non-axial system was also inserted which limited itself to listing the disorders of axis I-II and III. The multiaxial distinction was purely organizational and did not

determine differences in the definition and conceptualization of the disorders, so in the latest version the non-axial system was chosen with separate notations for some important psychosocial factors and any disabilities (corresponding to the past AXIS IV-V respectively) (APA, DSM-5).

The constant revisions of the DSM make it a “living document” (American Psychiatric Association, 2013), designed to adapt to new discoveries in neurobiology, neuroscience, genetics and epidemiology. The classification of mental disorders in the DSM-5 has, in fact, the purpose of promoting research to increase the understanding of the pathophysiological mechanisms underlying the disorders, of their genetic and symptomatic sharing or divergences, leaving the possibility of re-analyzing the data over time to constantly evaluate its relevance and consequent validity.

1.3 The new challenges of research

The results of numerous studies have shown how the boundaries of the different categories of disorders are actually blurred and how the symptomatic clusters characteristic of some disorders are present in many others, conceptually quite distinct.

The boundaries of some mental disorders, in fact, are often artificial, precisely due to the sharing of clusters of symptoms which in theory should determine the peculiar and distinctive characteristics of each category. To date, scientific evidence now places numerous disorders within well-defined diagnostic categories for clinical use only but, conceptually, identifies them as the evolution of a spectrum of disorders that fade into one another through the sharing of symptoms, genetic and environmental risk factors and in part also pathophysiological substrates. Just thinking about the conceptual evolution that autism has undergone over time, from its discoverer Leo Kanner (1943) who presented in his document 11 case studies of children with "autistic affective contact disorders", which has

become a milestone, "Autistic disturbances of affective contact"(Kanner, 1943), to the current concept of “autism spectrum disorders”. Indeed, numerous subsequent epidemiological, pathophysiological and genetic studies brought new evidence that supported the thesis that autism was actually more like a spectrum of disorders with a varying range of severity (Fombonne et al., 1999; Fombonne et al., 2009; Von dem Hagen et al., 2010; Ronald A et al., 2010). Another striking example is represented by "schizophrenic spectrum disorders", in which different pathological forms (schizophrenia, schizoaffective disorder, schizotypic/paranoid personality disorder, other non-affective psychoses and psychotic affective illness) were identified as manifestations of varying severity of the same spectrum pathological as early as the 1990s (Kenneth et al., 1995). Subsequently, numerous authors used the term "schizophrenic spectrum disorders", including within it shades of disorders characterized by personality and mood alterations, as well as individuals with narrower schizophrenic disorder or "schizotype" (Barrantes-Vidal et al., 2010; Blanchard et al., 2009; Horan et al., 2008). Further debates were also held after the release of DSM-5 which stressed the need to revise the list of diagnoses belonging to the spectrum of schizophrenia and other psychotic disorders, to reflect an even wider psychopathological gradient, from less severe to more severe (Pagsberg, 2012). Currently it is debated whether schizophrenia itself or, better said, the "schizophrenic spectrum" is not itself an integral part of the "autism spectrum", as evidenced by some genetic and clinical studies that have brought to light the sharing by these disorders of clinical and epidemiological risk factors, as well as common genetic variants (Larsson et al., 2005; Daniels et al., 2008; Rapoport et al., 2009). The progress that is being made on autism and schizophrenia reaffirms the need to overcome the limits imposed by the diagnostic categories that define them and to deepen the knowledge on their relationship

(King et al., 2011). Important changes also involved bipolar disorder which, in the third edition of the DSM, had been clearly distinguished from major depressive disorder, while a few years later Akiskal first (1983) proposed to keep the distinction between unipolar and bipolar disorder but expanding the bipolar category in "bipolar spectrum". Subsequently in the DSM-IV it was distinguished in bipolar disorder of type I and II; researches already contemporaneous with the fourth edition had expanded the concept of bipolar II disorder, including within it other pathological conditions characterized by "hyperthymic mood" (irritable mood states that however did not fall within the precise definition of hypomania) (Akiskal et. al, 1999; Cassano GB et al., 1992; Cassano GB et al., 1999; Hantouche EG et al., 1999). Today, even for bipolar spectrum disorders, the discussion has shifted towards what numerous studies have highlighted: the overlap of its spectrum with others, including the schizophrenic spectrum (Potash et al., 2006; Potash & Bienvenu, 2009; Keshavan et al. al., 2011; Yamada et al., 2020) and the autism spectrum (Skokauskas et al., 2010; Joshi et al., 2012; Skoauskas & Frold, 2015;) above. The operative diagnostic criteria present in the DSM and in the 'International Statistical Classification of Psychiatric Pathologies (ICD)' improve the reliability of the diagnosis based on clinical symptoms, also creating a homogeneity of treatment among professionals that otherwise would not have been possible; however, these criteria do not appear to be sufficiently valid when the vision of psychiatric pathologies is broadened, including neurophysiological and genetic knowledge, and they do not solve the problems due to their wide heterogeneity and coexistence (Yamada et al., 2020). Currently, in fact, since pathophysiological mechanisms or irrefutable etiologies are not known, it is not possible to fully validate any disorder or pathological spectrum, so much so that the DSM-5 itself repeatedly affirms its purely clinical utility in the diagnosis and evaluation of the pathological course. and

response to treatments. Therefore, the diagnostic criteria and their relationships within the classification, outlined thanks to current knowledge, are designed to be modified on the basis of the scientific evidence to come. In order to broaden knowledge in the psychiatric field and create an alternative language that can understand various fundamental pathological characteristics not included in the DSM and ICD classification system, two new approaches to psychiatric pathologies have recently been born that aim to overcome those limits that have never been crossed yet. The National Institute of Mental Health (NIHM) has presented an evaluation system to study psychiatric pathologies that excludes classifying diagnoses in favor of a multidimensional evaluation that considers genetic, neurophysiological and behavioral knowledge: the 'Research Domain Criteria (RDoC) system ' (Insel & Cuthbert, 2009; Insel et al., 2010; Cuthbert & Insel, 2013). The model based on HiTOP (Hierarchical Taxonomy of Psycopathology), on the other hand, is a taxonomic classification that derives from the observed covariance between symptoms and maladaptive traits; constructs psychopathological syndromes, their components and subtypes on the basis of symptomatic covariance, grouping together the related symptoms and reducing their heterogeneity. Furthermore, it also combines concomitant syndromes in the spectra, thus mapping the comorbidities (Kotov et al., 2017). In this sense, it considers psychopathology as a single spectrum in which the various syndromes enter by sharing similar or analogous symptomatic clusters. The different psychopathological dimensions are therefore considered in the form of a continuum. These two approaches, born as separate entities, provide new perspectives that are very different from each other, but it is hypothesized that the RDoC-HiTOP interface can provide a synergistic approach that allows to achieve the respective objectives of the two methods: the biobehavioral framework of RDoC can help to clarify the bases

of the clinical dimensions belonging to HiTOP, while the latter can provide new psychometric clinical knowledge on which to base the RDoC research (Michelini et al., 2021).It was once believed that homogeneity of diagnoses could be achieved through the meticulous subclassification of psychiatric disorders within the larger macro categories; today it is evident that this is in reality no longer possible or desirable. The complexity of mental health (as well as physical health) is due to its heterogeneity and scientific research, renewing itself from year to year, provides new tools for understanding and broadening the still unknown genetic, neuropathological and clinical knowledge.

2. Bipolar disorder

American psychiatric association (APA) first 'Diagnostic and Statistical Manual of Mental Disorders (DSM-I)', first introduced by Emil Kraepelin. However, it is necessary to wait until 1959 with Karl Leonhard to reach the modern conception that emphasizes the existence of depressive and bipolar unipolar forms, in which the alternation between depressive, manic or hypomanic episodes and mixed states is pivotal. In 2000 the version of the DSM-IV-TR came out in which the diagnostic criteria of some pathologies were revised and, in particular, a new phenotype of bipolar disorder was introduced: the distinction between bipolar I disorder (which requires a manic episode) and bipolar II (at least one episode of depression and one hypomanic episode) (Dunner et al., 1976).

2.1 Definition

Bipolar disorder is a chronic relapsing psychiatric syndrome affecting approximately 1% of the population, with a widely variable lifetime prevalence (BPD type I: 0.1-1.7% of the population versus 0.1-3% of the population for BPD type II) (Merikangas et al., 2007; 2011). It is characterized by the recurring presence of manic-depressive episodes during the span of life.

There are currently two main phenotypes: bipolar disorder type I and type II, to which are added several other disorders: cyclothymic disorder, bipolar disorder and related disorders induced by substances/drugs, bipolar disorder and related disorders due to other medical condition, bipolar disorder and related disorders with/without other specification. In fact, as previously mentioned, bipolar phenotypes are defined on the basis of exclusively clinical criteria outlined in the DSM-5, for which no specific diagnostic tests exist today to make a diagnosis. The characteristic episodes of bipolar disorder are: manic, hypomanic and major depressive episodes, to which are added the so-called mixed states.

The manic episode is defined in the DSM-5 as: "A period lasting at least one week of abnormally or persistently elevated, expanded or irritable mood and abnormal and persistent increase in purposeful activity or energy, almost all day and most days (can be of any duration if you need hospitalization). During the episode there must be three or more (or four or more if the mood is just irritable) between: hypertrophic self-esteem or grandiosity, decreased need for sleep, increased talkativeness, flight of ideas, distractibility, increased purposeful activity (work, social, scholastic, sexual, etc.), excessive involvement in activities that have a high-risk potential.

Finally, the episode must be severe enough to cause marked impairment in social or occupational functioning or to require hospitalization or with psychotic manifestations. It must not be attributable to the effects of another substance or drug or to any other medical condition. " The hypomanic episode differs from the manic episode in the duration which must be greater than or equal to 4 days (but less than 7).

To diagnose bipolar I disorder, the onset of a manic episode is required over a lifetime without the need for one or more hypomanic or depressive epidoses. The criteria of BPD I are, therefore, the modern evolution of Kraepelin's manic-depressive disorder in which neither psychosis nor the onset of a major depressive episode are needed to make a diagnosis. Bipolar II disorder requires at least one major depressive episode and one hypomanic episode to have occurred over a lifetime. It was once regarded as a "mild" variant of bipolar disorder; however, nowadays it has been seen how important even this nuance of the bipolar spectrum can be for the degree of work and/or social impairment that it determines, due to even very long periods spent with a depressed or unstable mood. The major depressive episode, on the other hand, is characterized by: "A period of at least two weeks in which at least five symptoms are present including: depressed mood, marked decrease in pleasure or interest in doing things, significant weight change (increasing or decreasing, at least 5%), insomnia or hypersomnia, agitation or psychomotor slowdown, fatigue or lack of energy, feelings of excessive self-deprecation or guilt, impaired ability to concentrate, recurrent thoughts of death (not just fear of dying) or recurrent suicidal ideation without a specific plan or suicide attempt.

Among the 5 symptoms at least one must be depressed mood or loss of interest/pleasure in doing things and the symptoms must be present for most of the day, almost every day. The symptoms must then determine a clinically significant occupational or

social discomfort or impairment and must not be related to the use of other substances, drugs or other medical conditions. "The so-called "mixed states" were then introduced which are manic, hypomanic or depressive episodes associated with characteristics belonging to the opposite pole of mood. A manic or hypomanic episode with mixed characteristics is defined as: "A period that fully meets the criteria for a manic or hypomanic episode in which at least three of the following symptoms are present during most days of the current or most recent episode of mania or hypomania: depressed mood, marked decrease in pleasure or interest in doing things, significant weight change (increasing or decreasing, at least 5%), insomnia or hypersomnia, agitation or psychomotor slowdown, fatigue or lack of energy, feelings of excessive self-depreciation or guilt, reduced ability to concentrate, recurring thoughts death (not just fear of dying) or recurrent suicidal ideation without a specific plan or suicide attempt.

Mixed symptoms must be observable by others and represent a change in the person's usual behavior; they must not be attributable to the physiological effects of a substance or drug. "A depressive episode with mixed characteristics differs from the previous one in that the criteria for a major depressive episode and the association of three symptoms belonging to the opposite pole of mood must be met: hypertrophic self-esteem or grandiosity, decreased need for sleep, increased talkativeness, flight of ideas, distractibility, increased purposeful activity (work, social, school, sexual, etc.), excessive involvement in activities that have a high-risk potential. In the fifth edition of the DSM, "bipolar disorder and related disorders" were separated from depressive disorders and placed after "schizophrenia spectrum and other psychotic disorders" disorders.

Their new position between the schizophrenic spectrum and depressive disorders therefore recognizes their role as a "bridge"

between the two diagnostic classes from a symptomatological, genetic and family point of view (American Psychiatric Association; DSM-5).

2.2 The other psychiatric disorders

Psychiatric disorders share symptomatic and substantial clusters of comorbidities, consequently stimulating the debate on their common etiological and clinical nature. Their classification has changed over time and is destined to evolve continuously, so much so that the understanding of the genetic basis is now considered fundamental for the dimensional evolution of disorders, from current categorical distinctions to broader multidimensional approaches that consider the various psychiatric pathologies in the together clinical, etiological and pathophysiological.

2.3 Comorbidities

By comorbidity we mean the co-presence of two or more pathologies characterized by distinct etiologies; comorbidities are common in psychiatric disorders, making it difficult to identify isolated psychiatric disorders.

Bipolar disorder is not an exception, so much so that two thirds up to 99% of the bipolar population can meet the comorbidity criteria (Kessler et al., 2005). The presence of comorbidities in patients with bipolar disorder, in addition to making the diagnostic process more complex, also correlates a worse prognosis and a poor response to therapeutic treatments (Feske et al., 2000). A study conducted in the United States that recruited 43093 patients who were administered structured interviews (AUDADIS-IV) showed that the symptoms of comorbid bipolar patients tend to merge into internalizing

disorders (characterized mainly by anxiety, depressive and somatic) and externalizing (conduct, antisocial, impulse discontrol and substance abuse disorders) (Eisner et al., 2017).

Among the most frequent and comorbidities affecting bipolar patients are therefore anxiety spectrum disorders (74.9%), adolescent or childhood onset impulse discontrol disorders (62.8%) and substance abuse disorders (42, 3%) (Merikangas et al., 2007).

The most frequent anxiety spectrum disorders in bipolar disorder are in particular generalized anxiety disorder (37.8%), specific phobia (35.5%) and social anxiety disorder (37.8%); in addition, panic disorder (20.1%) and obsessive-compulsive disorder (13.6%) are also frequent comorbidities (Merikangas et al., 2007). The high frequency of the symptom cluster of anxiety in patients with bipolar disorder has led some to consider it no longer as a comorbidity, but as a central cluster of the bipolar spectrum (Mcintyre et al., 2006).

While the correlation between anxiety and mania in patients with bipolar disorder appears to be highly confirmed, from another point of view, conflicting results have been obtained regarding the association between anxiety and the severity of bipolar disorder and between anxiety and psychotic disorders in bipolar patients. In particular, a recent study carried out on a sample of 360 patients showed an inverse correlation between anxiety and psychotic disorders, thus underlining how the presence of anxiety disorders in patients with bipolar disorder would seem inversely correlated with the onset of psychotic symptoms (Dell 'Osso et al., 2017; Serafini et al., 2017). Furthermore, some studies have highlighted how the presence of comorbidities of the anxiety spectrum is antithetical to the severity of bipolar disorder, for which the co-presence of anxiety would be found more in patients with a prevalence of

hypomanic or mixed symptoms (Henry et al.., 2003; Serafini et al., 2017; Titone et al., 2018).

Other studies, in contrast to the latter, have not confirmed these types of inverse correlation, instead finding a greater association between anxiety, psychotic symptoms and the severity of bipolar disorder (Altindag et al., 2006; Dilsaver et al., 2008; Saunders et al., al., 2012). The association between bipolar disorder and obsessive-compulsive disorder is also common, with a comorbidity of obsessive-compulsive symptoms in patients with bipolar disorder estimated at around 17% (Amerio et al., 2016). In reality, a question that has not yet been resolved is whether the OCD-BPD association is a finding of obsessive-compulsive symptoms in bipolar patients, or whether it is no longer correct to define it as a comorbidity in the strictest sense of the term with a contemporary diagnosis of disorder. obsessive-compulsive and bipolar disorder; for this reason also in this case the question is whether it is not better to use a dimensional approach to evaluate the obsessive symptoms in bipolar patients, as is done in the evaluation of mood. On the other hand, the use of the dimensional approach has substantially demonstrated a greater analytical power and descriptive subtlety than the categorical method (Jakubovski et al., 2011). The association between the onset of obsessive symptoms and the specific mood phase is interesting: the latest studies suggest that the appearance of the obsessive-compulsive symptom cluster is a state-dependent phenomenon, therefore synchronous with specific phases of the 'humor. There is a strong association demonstrated with depressive symptoms (Amerio et al., 2014), regardless of whether it is a unipolar or bipolar depressive form, to suggest a common genetic substrate (Bolhuis et al., 2014) to the two clusters. Studies on twins have shown, confirming this, that both in the adolescent and adult population obsessive-compulsive and depressive symptoms appear to be moderately heritable (Kendler & Gardner, 2011) in

a manner consistent with the frequent family aggregation between OC disorder and disorder. major depressive, demonstrated in other works (Nestadt et al., 2001; Carter et al., 2004). On the other hand, it seems that OC symptoms are inversely proportional to the development of manic and hypomanic episodes, confirming the hypothesis that this cluster has a trend dependent on the specific mood phase and that there is a common pathogenetic basis between depression and obsession. However, it should be emphasized that the most severe OC symptoms were found in mixed episodes, in which the intensity of the depressive symptomatology did not equal that present in the pure depressive phase; this could be explained by the simultaneous presence of the "depression" cluster, which would favor the onset of repetitive thoughts and behaviors, and the activation of thought associated with the manic and hypomanic phase which would instead favor the perpetuation of obsessive-compulsive symptoms (Tonna et al., 2021). The correlation between bipolar disorder and substance abuse has been well documented. In the adolescent period, patients with bipolar disorder have a 51% risk of developing substance abuse disorders, much higher than the estimated risk of 26% in the non-bipolar adolescent population (Wilens et al., 2016); moreover, the importance of this form of comorbidity is not only relevant in terms of prevalence, but also in terms of pathological course. In fact, substance abuse correlates with a more rapid and severe pathological course, with a higher number of hospitalizations and therefore with a worse prognosis. In particular, the co-presence of a substance abuse disorder has been shown to be a significant predictor of suicide attempt, leading to a risk of 39.5% versus a risk of 23.8% in patients with bipolar disorder. absence of substance abuse (Dalton et al., 2003). Patients characterized by this form of comorbidity are also prone to have less adherence to therapeutic treatment (Weiss et al., 1998; Sollum et al., 2000).

The two forms of disorders, in fact, are united by a series of psychopathological alterations that make this type of patient among the most problematic encountered in clinical practice. Distractibility, impulsivity, irritability, decreased cognitive function, depression, symptoms of helplessness and hopelessness, social isolation and denial are all common factors that contribute to non-adherence to therapeutic treatment (Sollum et al.., 2000). The major risk factors that have been identified for the development of substance abuse disorder in bipolar individuals appear to be: male sex, the high number of manic episodes and suicide attempts; for this reason, closer monitoring and more important therapeutic treatment should be considered in patients with these risk factors (Messer et al., 2017). While in the past much attention has been paid to the study of the more classic comorbidities of bipolar disorder (anxiety spectrum disorders, obsessive-compulsive disorder, depressive disorders and substance abuse), today very interesting new comorbidities have emerged: in particular the frequent co-presence of bipolar spectrum disorders and eating disorders. 27% of the bipolar population is characterized by a co-presence of eating disorders and the most frequent associations are between BPD and bulimia nervosa (with a prevalence of 15%), "binge eating disorder" (12%) and anorexia nervosa (0.2%) (McElroy et al., 2016). The psychopathological similarities shared by eating disorders and bipolar spectrum disorders are manifold; in both categorical disorders, relevant are in fact emotional dysregulation and impulsivity which determine a wide range of manifestations such as emotional lability, with typical alternation over a relatively short time of positive and negative feelings, irritability, the difficulty in concentrating, the use and abuse of alcohol or other substances (such as the abuse of diuretics, laxatives but also stimulants, in an attempt to control weight in anorexia nervosa with purging and bulimia), as well as the high risk of suicide, 15 times higher

than the normal population in bipolar I disorder and approximately 12 per 100,000 cases in anorexia nervosa (Jen et al., 2013; McDonald et al., 2019; McElroy et al., 2005). Weight fluctuations between patients with bipolar disorder and patients with eating disorders are often similar; in fact, manic and hypomanic manifestations, as well as some forms of depression, often present with an increase or decrease in appetite (Lunde et al., 2009). Specifically, the most common manifestations of bipolar disorder have very similar psychopathological correspondences to various eating disorders, consequently also determining similar weight variations. Cyclothymic disorder and bipolar disorder with atypical manifestations, in particular, are characterized by a lack of control of impulses which also manifests itself through binge eating, with a consequent weight gain similar to "binge eating disorder" and bulimia nervosa (Perugi and Akiskal, 2002; Perugi et al., 2006). Anorexia nervosa, on the other hand, has a typical onset in adolescence and the severe malnutrition that characterizes it commonly causes irritability, distractibility, mimicking other adolescent conditions such as ADHD (Moya et al., 2004) and depression with mixed characteristics, instead imitating the classic symptoms of bipolar disorder (Fornaro et al., 2019). The first meta-analysis that analyzed 36 studies focusing on the comorbidity of bipolar spectrum disorders and eating disorders, involving a total of 15,084 patients with primary diagnosis of bipolar disorder, found values rather similar but higher than the aforementioned prevalence found by McElroy in 2016, with a prevalence of BED in bipolar patients of 16.6%, bulimia nervosa of 7.4% and anorexia nervosa of 3.8% (Fornaro et al., 2020).This highlights how even this form of comorbidity, highlighted more recently, is showing an increasing prevalence and how new studies are needed to investigate the clinical impact of eating disorders in bipolar patients. Bipolar disorder therefore shows vast associations with other psychiatric

pathologies that have not yet been completely eviscerated, from a clinical symptomatologic but also a genetic point of view. A Swedish cohort study involving 54,723 patients diagnosed with bipolarity investigated the relative risk of developing bipolar disorder in relatives of affected patients and the co-presence of other psychiatric disorders in bipolar patients and their relatives (schizophrenia, depression, anxiety disorders, ADHD, substance abuse, autism spectrum disorders and personality disorders). The results of the study showed that the risk of developing other psychiatric diseases in bipolar patients increased from 9.7 to 22.9 times (RR) and from 1.7 to 2.8 times in complete siblings. Siblings of single-parent siblings had a relative risk that was always increased, but lower than that of siblings of both paternal and maternal origin; finally, heritability was estimated at around 58% (Song et al., 2015). The wide frequency of the different comorbidities between bipolar disorder and other psychiatric disorders, the important correspondence and overlap between symptomatic clusters and the strong family aggregation all argue in favor of a probable common genetic origin and underlying psychopathological mechanisms not yet known but shared. Known comorbidities between psychiatric disorders are so numerous that it is very rare to identify a single diagnosis over a lifetime in patient follow-up. Anxiety spectrum disorders are the prevalent disorders in the general population and have a documented comorbidity with mood disorders (Angst et al., 2010; Bega et al., 2012; Crump et al., 2013; Hasin et al., 2005; Kessler et al., 2005; Merikangas et al., 2007; Merikangas et al., 2010).

Of particular relevance are some subtypes, such as generalized anxiety disorder and panic disorder, which are typically associated with bipolar disorder and major depressive disorder. The association between depression and anxiety is also characterized by a higher prevalence of chronic diseases, as well as inducing a worse prognosis and a very significant impact on

the quality of life (Liao et al., 2021). Significant comorbidities have also been found with eating disorders (Keel et al., 2005), with typical associations between panic disorder and social anxiety disorder with bulimia nervosa alongside the typical and documented association between anorexia nervosa and obsessive-compulsive disorder (Baker et al., 2010; Buckner et al., 2010; Godart et al.., 2015; Munn-Chernoff et al., 2015).

Finally, comorbidities with neurodevelopmental disorders such as ADHD (Reimherr et al., 2017) and autism spectrum disorders (for a review, see Kent & Simonoff, 2017), as well as substance or alcohol abuse disorders (Buckner et al., 2013; Thomas et al., 1999). Obsessivecompulsive disorder is commonly associated with other psychiatric disorders, so much so that some forms of comorbidities represent its specific associations due to similar symptoms and response to treatments (Stein et al., 2000). Furthermore, some of these disorders frequently occur in the form of family aggregation in relatives of probands with obsessive-compulsive disorder, further demonstrating their likely common etiology. The most frequently occurring comorbidities are: tics (Grandos et al., 2001), anxiety disorders, mood disorders, personality disorders (Samuels et al., 2000), somatoform disorders, anorexia and bulimia nervosa (Bievenue et al., 2000). These specific associations have supported the theory that it is possible to subtype obsessive-compulsive disorder on the basis of comorbidities (Hasler et al., 2005).

By analyzing the specific symptomatology, its severity and associated pathologies, it is therefore possible to identify four classes of obsessive-compulsive disorder: three of which are typically associated with generalized anxiety disorder, obsessive-compulsive personality disorder, eating disorders, major depressant, hyperactivity and body dysmorphic disorder; the fourth is characterized by the co-presence of panic disorder, substance abuse disorder and the absence of depression (Nestadt et al., 2003). Eating disorders are an heterogeneous group of

pathologies in which each disorder has particular comorbidities that on the whole belong to mood, anxiety and substance abuse disorders (Bahji et al., 2019; Hudson et al., 2007; Swanson et al., 2011; Ulfvebrand et al., 2015). Also peculiar is the temporality with which these disorders arise, generally with obsessive-compulsive disorder prior to the development of anorexia nervosa, social anxiety disorder or panic disorder following the onset of bulimia nervosa, variable onset for depression and substance abuse and alcohol (Baker et al., 2010; Buckner et al., 2010; Godart et al., 2015; Munn-Chernoff et al., 2015). These data appear to be important because ideally a better understanding of the temporality and probability of disorders could help practitioners in administering preventive as well as reactive treatments (Alsten & Duncan, 2020).

There are numerous comorbidities of autism spectrum disorders: anxiety, depressive, bipolar, schizophrenic spectrum disorders, ADHD, impulse and conduct dysfunction (for a review, see Hossain et al., 2020). Alcohol and substance abuse disorders showed high rates of comorbidity, affecting over 50% of subjects and showing significant overlap especially with antisocial personality disorder, schizophrenia and bipolar disorder (Regier et al., 1990).

However, the importance of comorbidities in psychiatric patients is not only reflected in the overlapping of disorders categorically distinct from DSM-5, but also in comorbidities with pathologies that are not purely psychiatric; examples are bipolar disorder and migraine (Hirschfeld et al., 2003), major depressive disorder and stroke (Pan et al., 2011), autism spectrum disorders or ADHD and epilepsy (Lo Castro et al., 2014; Bertelsen et al., 2016).

Therefore, the new discoveries highlight the complexity of psychiatric pathologies which is reflected in the recurrence of symptomatic clusters in various pathologies, in the consequent

impossibility in some cases of precisely delineating a disorder or the comorbidities that characterize it, in the need to deepen the investigations. clinical, epidemiological and genetic in order to broaden the vision of disorders thanks to the use of multiparametric models.

2.4 Heritability and family aggregation: studies on families, twins, adoptions

Genetic knowledge of psychiatric pathologies has increased considerably in recent years and fundamental progress has been made thanks to studies on families, twins and adopted children, which represent consolidated methods for estimating what effects family and genetic components have on disturbances. These studies, in fact, have constituted a consolidated model to document the family aggregation and co-aggregation of various psychiatric pathologies, laying the theoretical foundations to hypothesize the heritability of the disorders.

This was a first approach to the study of heritability within families, laying the necessary foundations for a new line of research that aims to identify specific genetic mutations or polymorphisms that induce susceptibility to various psychiatric diseases. Among the most widely investigated pathologies there are certainly bipolar spectrum disorders, whose studies have made it possible to identify a hypothetical genetic epidemiology from which, thanks to the progress made in the technological field, more complex studies have subsequently begun that have allowed to identify the actual susceptibility genes.

Although family, twin and adoption studies use different methods, they have been consistent in documenting the typical family aggregation and heritability of bipolar disorder and that these are influenced or dependent on a common genetic etiology (Smoller et al., 2003). In the first studies, carried out on families,

it was shown that the children of bipolar parents or with major depression had a documented increase in the risk of developing mood disorders; in particular they were predisposed to develop some form of symptomatology due to mood instability during their lifetime, without however always falling within a certain diagnosis (Anderson et al., 1993; Hammen et al., 1987; Hammen et a., 1990). In fact, family studies have largely confirmed the family aggregation and heritability of both bipolar disorder and major depressive disorder (Tsuang et al., 1980; Gershon et al., 1982; Maier et al., 1993); however the extent of these family vulnerabilities to develop these pathologies and the differences between the two have not yet been found, with often conflicting results obtained in different studies.

In particular, the studies on family aggregation that compare these two disorders go in opposite directions: some affirm that the family aggregation of bipolar disorder is greater than that of unipolar disorder, thus noting that the latter has a minor heritable component and likely to be more influenced by environmental factors (Gershon et al., 1982; Aukes et al., 2012; Smoller et al., 2003); other studies, on the other hand, affirm that they have not found differences in heritability and aggregation between the two disorders, underlining that the risk of developing mood disorders in the offspring is equivalent in the children of probands suffering from bipolar disorder and major depressive disorder (Radke- Yallow et al., 1992; Oquendo et al., 2013).

Interesting is the work of Merikangas et al. (2014) who collected 447 probands and their first degree relatives (2082) finding, first of all, a result in line with Gershon, Smoller and Aukes: a family aggregation found in both bipolar disorder and major depressive disorder, but significantly stronger for the former (OR = 8.40 vs OR = 2.26); it also achieved a contrasting result with Anderson and Hammen's previous work. According to Merikangas, in

fact, there is no form of family co-aggregation between bipolar disorder and major depressive disorder, thus suggesting that the heritability of depressive and manic episodes is clearly separated. There would therefore be a specific familial vulnerability for major depressive disorder and for bipolar disorder, despite the strong known comorbidity between these two mood states.

Therefore these two main components of bipolar disorder (manic episode and major depressive episode) could have at the basis of different pathophysiological mechanisms, casting doubt on the classical conception that delineates them as manifestations of variable severity of a common underlying diathesis (Merikangas et al., 2014).

In the 1980s, a study documented the typical family co-aggregation between mood disorders and schizophrenia, highlighting how discordant diagnoses could occur within the same family (children of a bipolar parent diagnosed with schizophrenia, or bipolar children born to a parent with schizophrenic spectrum disorder) and how there was no clear separation between the heritability of unipolar and bipolar disorder (Tsuang et al., 1980).

It should be noted, however, that this result has not always been confirmed by subsequent studies, which instead highlighted a specific family susceptibility to schizophrenia, without increasing the risk of developing other psychiatric pathologies in first degree relatives, such as mood disorders or anxiety (Kendler et al., 1993). These results were later confirmed by Aukes in 2012 (Aukes et al., 2012), in a sibling study in the Dutch population where he investigated sibling family aggregation of patients with schizophrenia, bipolar disorder and major depressive disorder. There was a relevant increased risk for all diseases examined, estimating a three-fold increased risk of developing schizophrenia in siblings of patients with

schizophrenia, a six-fold increased risk of bipolar disorder in siblings of bipolar patients, and a relative cross-diagnosis in siblings by 70% more than in the normal population; however, the latter figure was not statistically significant.

For major depressive disorder, on the other hand, the family vulnerabilities estimated for the single disorder, together with other diagnostic entities, were found to be equivalent, even if small in both cases (Aukes et al., 2012). Therefore, although a shared familial vulnerability of bipolar disorder and schizophrenia appears to be confirmed, this is clearly lower than the inherited vulnerability for disorders of the same spectrum; in other words, siblings of schizophrenic patients or siblings of bipolar patients have a much greater risk of developing the same pathology as their sick sibling than bipolar or schizophrenic disorder respectively.

With regard to the specific heritability of these two disorders, a 27.3% risk of developing schizophrenia was estimated in the offspring of parents both affected by schizophrenia, compared with a risk of 7% when only one parent is affected; in the offspring of bipolar parents a risk of bipolarity was estimated of 24.9%, against a risk of 4.4% when only one parent is sick; finally, a risk of 15.6% for schizophrenia and 11.7% for bipolarity was estimated in the offspring of parents affected by schizophrenia and bipolar disorder (Gottesman et al., 2010). The increase in risk percentages when the parents have different diagnoses than the offspring with a single sick parent appears therefore as a confirmation of the mutual influence in the development of these two psychiatric pathologies, of the possible common genetic etiological basis and of the possible overlap between the two. pathological spectra.

Today, of course, there is a broad consensus that a wide variability of psychiatric disorders coaggregate in families, resulting in an increased risk of mentally ill offspring when one

parent or both are diagnosed with severe disease. psychiatric (Dean et al., 2010; Gottesman et al., 2010). Furthermore, the finding of an increased risk in offspring is important, not only for concordant diagnoses or known pathologies (as emerged from numerous studies), but also for a whole series of other disorders not characterized by a known correlation (Dean et al., 2010). Bipolar disorder, in particular, is related to an increased family risk of developing depression, anxiety disorders, drug abuse, attention deficit hyperactivity disorder, autism spectrum disorders, and schizophrenia (Craddock & Sklar, 2013; Kendler et al., 2020; Song et al., 2015).

The studies on families allow, in particular, to investigate the family co-aggregation and the heritability of psychiatric disorders and their symptomatic clusters, supporting the thesis that a genetic etiological basis contributes to the development of a specific disorder; however, they cannot establish which genes are involved, their role or estimate the extent of their influence on the disorder under analysis.

Studies on twins, on the other hand, compare groups of pairs of individuals matched by environment, degree of kinship and genetics, thus analyzing precisely the extent and role of genetic and environmental components in the onset of a given pathology. In these studies the concordance between monozygotic and dizygotic twins is analyzed, assuming that the former share 100% of the genetic heritage, while the latter 50%, and that in both couples the environmental influences are identical; moreover they can be used to estimate how susceptibility to a given pathology varies on the basis of genetic and environmental factors (Kendler, 2001). Significantly higher concordance rates in monozygotic twins than in dizygotes testify to genetic influence; moreover, concordance rates below 100% in monozygotic twins signal the environmental contribution to the onset of the disease (Smoller et al., 2003).

The first studies on twins, prior to the GWA genomic studies, allowed to further confirm the hypothesis of heritability of psychiatric disorders. In particular, in bipolar disorder, a greater concordance was found between monozygotic twins than between heterozygous twins: Bertelsen et al. (1977), 62% vs 8%; Kendler et al. (1993), 38.5% vs 4.5%; Cardno et al. (1999), 44% vs 9.1%, further confirming the hypothesis that genetic susceptibility contributed significantly to the familiarity of bipolar disorder (Bertelsen et al., 1977; Kendler et al., 1993; Cardo et al., 1999).

More recent studies on twins, similar to the previous ones, which compare the concordance of diagnosis between homozygotes and heterozygotes, have estimated an even greater heritability, i.e. between 60 and 90% (Craddock & Sklar, 2013; Merikangas et al., 2002). These studies also highlighted the fact that family history of bipolar disorder was an important predictor of the onset of mood disorders and that the relative risk of developing them decreased as the degree of kinship (and therefore genetic distance) from the proband increased.

Therefore, if on the one hand the studies on families, twins and adoption have an estimated variable heritability from study to study, on the other hand they have consistently confirmed that the heritability of bipolar disorder is among the greatest of all psychiatric disorders (Bienevenu et al., 2011) and that there are numerous genetic correlations with other disorders, especially with autism and schizophrenic spectrum disorders.

2.5 The genetics of mental disorders: where we are, where we want to go

As previously pointed out, psychiatric disorders are treated in clinical practice as very distinct entities, each characterized by its own diagnostic criteria that allow their identification and

delineation in the main features. However, comorbidity is the rule, so much so that in a study carried out by Kessler et al. (2005) out of a sample of 9,000 US patients with a precise diagnosis, 50% of patients during the year of observation developed symptoms such as to allow the delineation of the diagnostic criteria of another psychiatric disorder (Kessler et al., 2005).

What is found in the clinical setting has a genetic correlation, so much so that 8 of the major psychiatric diseases (schizophrenia, schizoaffective disorder, bipolar disorder, major depressive disorder, anxiety disorders, ADHD, substance and alcohol abuse disorder) have highlighted, through multivariate analyzes, to share in part the same genetic origin in harmony with what has been demonstrated by other studies (Smoller et al., 2013). Furthermore, regardless of the shared genetic origin, specific genetic pathways have been identified that influence either psychotic disorders or disorders not characterized by psychosis (Pettersson et al., 2016).

GWAs studies (Genome-Wide Association Studies) are genetic epidemiological investigations that investigate most of the genes of different individuals belonging to a population, to identify the common gene variations between them; it is therefore a technique suitable for identifying the genetic polymorphisms that unite subjects affected by a specific pathology under investigation.

The samples used for these genetic epidemiology investigations generally collect thousands of people in order to characterize a wide range of single nucleotide polymorphisms (SNPs) related to the pathology under investigation. In these studies the Polygenic Risk Score (PRS) is also used, which consists of a weighted sum of all the genetic variants found for a given pathology from antecedent GWAs. These studies were the most successful investigation strategy for identifying the specific

genetic variants associated with various psychiatric disorders, including bipolar disorder and its comorbidities.

In particular, single nucleotide polymorphisms were found common among bipolar disorder, ADHD, autism spectrum disorders, major depressive disorder and schizophrenia, in 4 specific loci: 3p21, 10q24 and in two voltage-dependent calcium channels of type L (CANA1C and CACNB2). Furthermore, summing up the polygenic risk given by a large part of the common genetic variants, cross effects were highlighted on the adult onset disorders analyzed (bipolar disorder, major depressive disorder and schizophrenia) and between autism spectrum disorder with bipolar disorder and schizophrenia (Smoller et al., 2013).

Disturbance-specific GWAs analyzes partially confirm the data found by Smoller et al. (2013), also identifying single nucleotide polymorphisms belonging to CACNA1C, implicated in the development of susceptibility to various psychiatric pathologies: bipolar disorder, schizophrenia and major depressive disorder (Ferreira et al., 2008; Green et al., 2010). CACNA1C is a centrally expressed L-type calcium channel and neuroimaging studies have shown that its variants are involved in a wide range of brain functions, being involved in circuits for the processing of emotions, executive functions, attention and memory. (Bigos et al., 2010; Thimm et al., 2011; Erk et al., 2010). The CACNB2 channel is a channel involved in membrane trafficking and was instead found in a Chinese GWAs that analyzed patients with bipolar disorder (Lee et al, 2011; Mullins et al., 2020).

The 2020 meta-analysis by Mullins et al. represents one of the biggest breakthroughs to date, gathering 57 case-control cohorts of European, North American and Australian origin, for a total of 41,917 cases affected by bipolar disorder and 371,549 controls. It identified 64 genomic loci associated with bipolar

disorder including 33 newly discovered, associated with calcium (CACNB2) and potassium (KCNB1) channels. Seventeen of these loci found had already been previously highlighted in schizophrenia (Pardinas et al., 2018), while another 7 in major depressive disorder (Howard et al., 2019), demonstrating genetic overlap among mood disorders. of the schizophrenic spectrum. The meta-analysis also revealed a genetic correlation of bipolar disorder with other psychiatric disorders, including sleep disorders and substance abuse, in line with previous reports (Lewis et al., 2020; Zhou et al., 2020; Okbay et al., 2020; al., 2016).

The findings found in this meta-analysis therefore confirm on a large scale the polygenic origin of bipolar disorder and the overlap that associates it with other psychiatric disorders (Mullins et al., 2020). In addition to single nucleotide polymorphisms, rare variants influence the appearance and heritability of a disorder, i.e. single nucleotide variants (VNS) found in less than 1% of the population, sometimes of family origin, sometimes are variations of novo (Goes et al., 2019). In particular, they would appear disruptive in early onset cases (Toma et al., 2018). Copy number variants (CNVs) are DNA length polymorphisms due to the repetition of copies of a gene in abnormal numbers compared to the range present in most of the population. Offspring of older fathers have an increased risk of developing neurodevelopmental disorders such as schizophrenia and autism spectrum disorders; this would appear to be a consequence of the effects of aging on spermatogenesis.

Male aging determines, in fact, an increase in the variants of the number of copies of novo in spermatogenesis (Flatscher-Bader et al., 2011). In bipolar disorder, however, the importance of NVCs appears to be reduced; in fact, their frequency is lower than that observed in schizophrenia and in neruro-development disorders, such as autism spectrum disorders (Kirov et al., 2015). The CNVs found to be correlated to bipolar disorder are

also reduced in number and among these is the duplication in the 16p11.2 region (Green et al., 2016), also implicated in schizophrenia, autism and intellectual disabilities (Kirov et al.., 2015), further demonstrating the genetic overlap that characterizes these pathologies. Furthermore, new scientific evidences are signaling that the genetic overlap among psychiatric disorders does not simply concern the single entities but that in reality we can also speak of the heritability of symptomatic clusters, of clinical traits that unite the various psychiatric pathologies.

The genetic overlap highlighted would therefore have a precise symptomatic phenotype which then, in clinical practice, would result in the known and discussed comorbidities between different disorders. Bipolar disorder has shown, in particular, a common genetic basis with some symptomatic traits: sleep disturbances (divided into daytime sleepiness, terminal insomnia, initial insomnia and intermittent insomnia), mood instability, alcohol abuse, drug abuse, low academic achievement, smoking addiction. Furthermore, more than 90% of the estimated genetic variants correlated bipolar disorder with low school achievement (Mullins et al., 2020).

Understanding of the genetic architecture of bipolar disorder has therefore been significantly expanded by family, twin and adoption studies and by current large consortium-led genetic research. The heritability of bipolar disorder is now widely demonstrated, as well as its polygenic and heterogeneous nature, as well as its substantial overlap with other psychiatric disorders: from the most well-known disorders of the schizophrenic spectrum, to autism spectrum disorders, anxiety disorders, obsessive-compulsive disorder, substance abuse disorder, and attention deficit hyperactivity disorder.

To deepen the role of genetics in the onset of bipolar disorder, it will be necessary to integrate current knowledge with other

genomic and imaging data and it is expected that, thanks to the continuous advancement in technology, the number of loci associated with the disorder will increase in substantially. It will be possible to better identify the genetic variants responsible for the disorder and also to use the clinical applications of genetics aimed at predicting the risk and identifying the most appropriate therapeutic interventions (O'Connel et al., 2020).

2.6 Heritability and family aggregation: studies on families, siblings, adoptions

Studies on families and twins have variously demonstrated the substantial heritability of psychiatric disorders, underlining how genetic factors contribute to the etiology of psychiatric traits. The impact of family history in the onset of psychiatric pathologies appears to be important and, specifically, the presence of parents with psychiatric pathology determines an increased risk of morbidity in the offspring.

Furthermore, this increased risk is not confined to the parent's pathology, but exceeds the diagnostic limits, exposing the subject to a wide range of pathologies, demonstrating that the increased risk is determined by both genetic and environmental factors. which induce a general vulnerability to the development of psychiatric pathologies. Furthermore, this is a further confirmation that the current diagnostic criteria that outline the various pathologies lack validity. The increased risk is particularly high if both parents have a diagnosis (Dean et al., 2010).

The estimates of heritability found are different between the different studies and for the different disorders; it is in fact recognized that the different survey methodologies are not able to capture all the genetic factors that contribute to the variance of the trait. For this reason the heritability estimates are

considered as lower limits. It should also be considered that any form of estimation strictly depends on the analysis sample, the study design, the diagnostic tools and also on the environment or changes in society (Kendler et al., 2000). Furthermore, by changing and evolving over time the diagnostic criteria, the conceptualization of the different pathologies and the diagnostic methods, in a similar way, the values of heritability obtained change (Zablotsky et al., 2015; Thomas et al., 2015).

A further method for estimating the heritability of disorders and their family aggregation is the use of family trees (grandparents, parents, siblings and children) from national registries which have the advantage of giving access to a number of unobtainable data or difficult to find with other methods (Pettersson et al., 2016).

This analysis methodology was used by Pettersson et al. (2019) and allowed to estimate the heritability of eight psychiatric pathologies, using data from the Swedish National Patient Register for a total of 4,408,646 individuals collected, therefore over 2 million pairs of complete siblings and half-siblings of maternal origin. The study also used a further analysis methodology, collecting the h2-SNP estimates obtained from the Psychiatric Genomics Consortium.

The pathologies analyzed were: alcohol dependence, anorexia nervosa, ADHD, autism spectrum disorder, bipolar disorder, major depressive disorder, obsessive-compulsive disorder, schizophrenia. Estimates derived from data collected on families and single nucleotide polymorphisms have shown a varying heritability from high to moderate, albeit with an important difference between the different disorders. However, the estimated heritabilities were all positive, thus suggesting that there is a correlation between the greater heritability or familial aggregation of a disorder and a larger effect given by the complex of its single nucleotide polymorphisms.

Overall, sibling heritability obtained ranged from 0.30 for major depression to 0.80 for ADHD. The h2-SNP estimates, on the other hand, were lower for anxiety disorders (0.10) and obsessive-compulsive disorder (0.28), but still significant and correlated with the estimates obtained in the sibling survey (Pettersson et al., 2019). The study therefore confirms the polygenic nature of psychiatric traits (Visscher et al., 2012) and the previously estimated heritabilities in twin studies (Polderman et al., 2015).

A fundamental meta-analysis carried out on twin studies of the last 50 years has collected over 14 million pairs of twins from 39 different countries and investigated numerous types of traits, of which 51% fell into the psychiatric, metabolic and cognitive domains, while only 1% in the domains of development, connective tissue and infections. This study (Polderman et al., 2015) highlighted how virtually all human traits have a heritable component.

The ten most studied traits were: temperament and personality functions, weight control, height, metabolic disorders, major depressive episode, higher cognitive functions, conduct disorders, anxiety disorders, mental and behavioral disorders related to alcohol, drug and tobacco abuse; overall these represented 59% of the traits analyzed. All traits were inheritable, none reporting zero weighted heritability, and for over two-thirds (69%) the correlations between monozygotic and dizygotic twins followed a simple pattern: the similarity of the traits was solely dependent on additive genetic variation and their causal genetic variants and, therefore, could be studied and detected through a simple additive genetic model.

For the remaining third, however, an additive genetic model was not sufficient to describe the variance of the population under examination, so non-additive genetic models, such as sequencing and GWAS studies, were more suitable for genetic

investigation, despite their potential. very low statistic. The study represents the largest meta-analysis performed to date, providing a comprehensive overview of the genetic and environmental contributions of all human traits studied through twin investigations (Polderman et al., 2015).

Studies are therefore increasingly focusing on investigational purposes that explore the possibility that it is not only psychiatric pathologies as a whole that are inheritable or characterized by peculiar recurrences in families, but also the specific traits that delineate them or that they share with other typically comorbid pathologies.

2.7 Genome-wide association studies: The Brainstorm Consortium

Despite solid evidence of an important genetic contribution in the onset of psychiatric disorders, obtained through family and twin studies, the identification of specific DNA variants has been and still is complex. Genomic sequencing and GWAs studies are confirming the polygenic nature of psychiatric disorders and the latter, in particular, have proved useful in demonstrating the heritability of the various disorders and in the evidence of how much and which genetic variations specifically are decisive in vulnerability. familiar to them.

To obtain reliable results, however, large samples are required, which are also useful for the identification of further genetic variants that constitute the architecture of the disorders. Of particular importance was the Brainstorm Consortium, a collaboration between GWAs meta-analysis consortia joined to perform a comprehensive analysis of the heritability and genetic contributions of numerous psychiatric and neurological disorders.

The study was born from the observation that known pathophysiological mechanisms clearly separate neurological disorders, while psychiatric disorders, of which very little is known about the etiology, are typically characterized by only clinically well-defined limits, for which the better understanding of the genetic basis and related phenotypes would allow to broaden the knowledge on basic biological mechanisms and, consequently, to redefine the respective diagnostic categories. Overall, 265,218 cases and 784,643 controls were collected and 1,191,588 samples were included for 13 brain-related cognitive and behavioral phenotypes, as well as 4 additional phenotypes representing known and well-delineated etiological processes.

Among psychiatric disorders, schizophrenia has shown a significant genetic correlation with numerous other disorders and, in particular, it has been confirmed that there are four psychiatric pathologies with which it is genetically most correlated: bipolar disorder, major depressive disorder, anxiety disorders and ADHD. Each couple of these psychiatric pathologies showed a strong correlation between them and the major depressive disorder, in a peculiar way, showed a positive correlation with all the disorders analyzed, even if not always in a statistically significant way. Anorexia nervosa and obsessive-compulsive disorder also showed a statistically significant positive correlation with schizophrenia, albeit weaker, as well as autism spectrum disorder. Also interesting was the fact that PTSD showed no significant correlation with any psychiatric disorder, potentially representing a disorder well separated from all others. Neurological disorders have shown much more limited genetic correlations than those found for psychiatric disorders, demonstrating that their very distinct etiology determines their clear separation, without consequent shared genetic vulnerability. Parkinson's disease, Alzheimer's disease, epilepsy and multiple sclerosis, for example, have shown

virtually no genetic correlation between them.The correlations between diagnostic categories were also limited, showing a reduced genetic sharing between neurological and psychiatric disorders; specifically, only the cross-correlations between migraine and ADHD, migraine and Tourette's syndrome, migraine and major depressive disorder were significant. This suggests that this neurological disorder may share some genetic basis with the psychiatric disorders mentioned above. The cognitive and behavioral phenotypes considered were: years of education, graduation from college, cognitive performance, intelligence, perceived well-being, depressive symptoms, neurosis, extroversion, pleasantness, conscientiousness, openness and addiction to smoking; in addition to these, 4 additional phenotypes were: height, BMI, coronary heart disease, Chron's disease. Several genetic correlations between brain disorders and cognitive phenotypes have been found and have been found to be positive especially with anorexia nervosa, autism spectrum disorder, bipolar disorder and obsessive-compulsive disorder; they tested negative with ADHD, anxiety disorders, major depressive disorder and Tourette's syndrome. Of particular importance, however, were the correlations observed between psychiatric disorders and personality phenotypes and symptoms; the major positive associations were found between neurosis and anorexia nervosa, anxiety disorders, migraines with or without aura, major depressive disorder, obsessive-compulsive disorder, schizophrenia and Tourette's syndrome, as well as between depressive symptoms and ADHD, anxiety disorders, major depressive disorder and schizophrenia. Furthermore, neurosis showed the greatest correlation with major depressive disorder, and the correlation between these two with anxiety disorders and depressive symptoms suggests a common underlying pathophysiological mechanism. BMI was positively correlated with ADHD and major depressive disorder and, clearly, negatively correlated

with anorexia nervosa. Therefore these results are a sign of the probable genetic architecture shared between psychiatric disorders and the cognitive-behavioral traits that characterize them. Furthermore, the correlations with the phenotypes highlighted give an idea of how important the diagnostic overlap between disorders is, evidenced by the broad nature of the spectra of psychiatric disorders and by how patients over the course of life can fade into different diagnoses (Regier et al., 1998; Laursen et al., 2009). The results of this meta-analysis provide a strong confirmation of the artificiality of the diagnostic criteria that classify the various disorders within excessively limiting diagnostic categories and, above all, confirm the robust correlations between psychiatric disorders, symptomatic clusters and cognitivebehavioral profiles. The high degree of genetic correlation between many of the psychiatric disorders adds further evidence that their current clinical boundaries do not correspond to distinct pathogenic processes. This suggests a common etiopathogenetic basis and underlines the need for further studies aimed at a better diagnostic and therapeutic definition (The Brainstorm Consortium, 2018).

3. The study

The overlap that involves every aspect of psychiatric pathologies still needs to be investigated, not only to obtain diagnostic criteria that allows an increasingly refined clinical practice, but also to understand the biology that determines the onset of the disorders and the genetic susceptibility that they predispose its development. Knowledge of these is, in fact, essential to obtain new therapeutic strategies. On the basis of the literature at our disposal, it is possible to hypothesize that heritability does not concern only psychiatric pathologies in their entirety, but also their symptomatological clusters.In fact,

as previously shown, it was also possible to investigate on a large scale the coaggregation of psychological traits and specific symptoms recurring within families and the results obtained leave room for new in-depth investigations. This pilot study hypothesizes the transmissibility of symptomatic dimensions, understood as a cluster of symptoms within the families of psychiatric patients, rather than of specific diagnoses. What distinguishes this study from previous investigations is that the symptomatological clusters investigated do not look for the symptoms of patients and relatives diagnosed with psychiatric pathology, but investigate the symptoms within the family units, also recruiting the patients' healthy relatives. The study therefore has an even finer objective than the investigations carried out to date in the scientific literature: to investigate the recurrence of symptomatic clusters in the families of patients suffering from psychiatric disease, also interviewing healthy relatives without certain psychiatric diagnosis or treatment. specific. Indeed, it was not of interest to this study to reconfirm the symptoms of known psychiatric pathologies, their family aggregation or the comorbidities that are typically associated in patients and families.Instead, we wanted to see if within family units some symptoms recur among individuals even in the absence of a diagnosis, therefore also without falling within the diagnostic criteria outlined by the DSM-5, very useful in the clinical setting but limiting in the field of research, and subsequently to verify if it was possible to hypothesize the genetic heritability of the symptomatological clusters. It was therefore investigated in the first instance which clusters recurred most in relatives and which symptom profiles could be delineated and, subsequently, what were the possible symptomatic dimensions of in-depth analysis due to inheritability that cannot be discarded.

4. Materials and Method

4.1 Evaluated Individuals

Patients came to the Medical Genetics department after being evaluated by the Unit of Psychiatry, Department of Mental Health (University of Siena, Policlinico "Santa Maria alle Scotte"). For each patient genetic counseling was performed in order to evaluate each individual phenotype. All the subjects involved gave their written informed consent to the study that was carried out according to the Helsinki declaration. Genomic DNA was extracted from EDTA peripheral blood samples using MagCore HF16 (Diatech Lab Line, Jesi, Ancona, Italy) according to the manufacturer's instructions. DNA quantity was estimated using the Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). The pilot study, observational and monocentric, is presented as a preliminary approach to future research, for which the primary objective was the identification of the most appropriate data collection method for carrying out future studies based on psychometric interviews.

The inclusion criteria chosen for the sample collection were:

• subjects belonging to the psychiatric services of the UOC of Psychiatry of the AOUS, hospitalized in the ordinary or Day Hospital regime with a diagnosis of Bipolar Disorder or Major Depressive Disorder;

• subjects belonging to the psychiatric services of the UOC of Psychiatry of the AOUS, hospitalized in the ordinary or Day Hospital regime with a diagnosis of Eating Disorders, Anxiety Disorders, Schizophrenic Spectrum Disorders or Obsessive-Compulsive Disorder, aged between 18 and 65 years;

• first and/or second degree relatives of the patients (fathers, mothers, brothers/sisters, children) whose interviews would have been collected directly or indirectly;

• willingness to provide their free informed consent.

The exclusion criteria were:

• inability to involve the patient's biological family

• severe cognitive impairment

• acute psychosis

• inability to answer questions

• refusal of informed consent.

The patient who met the inclusion criteria was provided with self-administered tests to evaluate different psychopathological dimensions: depression, sleep, anxiety, mania, anger, somatic symptoms, substance use and repetitive thoughts and behaviors.

Other information collected on the probands, extracted from the medical records, were: socio-demographic variables (age, sex, date and place of birth), main psychiatric diagnosis, family history on the maternal line, paternal or both, drug treatment. The psychometric surveys of the relatives, on the other hand, were collected in complete anonymity, indicating only the sex and degree of kinship with the proband. Overall, 46 patients were recruited, of which 26 were females, each with at least one relative, for a total of 15 fathers, 28 mothers, 20 brothers/sisters of which 10 siblings, 9 children of which 3 were girls, with a total of 118 individuals, with 67 girls and 51 boys. In this first study, the search for the heritability and family aggregation of symptomatic clusters in bipolar disorder was of greater interest, for which, although without setting particular limits in the diagnosis of the patients collected, a sample was created consisting of 37 affected patients. from bipolar disorder, 1 patient from major depressive disorder, 2 from anorexia

nervosa, 3 from obsessive-compulsive disorder, 2 from generalized anxiety disorder, 1 from schizoaffective disorder. Psychometric investigations consist of structured interviews that assign a score to the symptomatology reported by the patient, useful for obtaining different types of evaluation. Some interviews are born for diagnostic purposes, with the main objective of diagnosing the disease, as in the case of "The Mood Disorder Questionnaire", a questionnaire that investigates the manic and hypomanic symptoms that occurred during the patient's life, in order to investigate any symptomatology correlated with bipolar spectrum disorders, whose sensitivity is 73% and the excellent specificity of 90% (Hirschfeld et al., 2000). Other interviews, on the other hand, are born with the aim of investigating the extent of the patients' symptoms, not only to obtain a complete picture that goes into deepening various symptomatic nuances, but also to monitor patients over time and evaluate their response to treatments. Specifically, the interviews used in the study are born for this purpose and are interviews designed to be administered both in the form of self-interview, and by the professional himself.

The nine interviews chosen were:

• DSM-5-Self- Rated Cross-Cutting Symptom Measure- Adult Level 1

• DSM-5-Severe-Depression-Adult

• DSM-5 Level 2- Sleep Disturbance- Adult

• DSM-5 Level 2- Mania- Adult

• DSM-5 Level 2- Anxiety- Adult

• DSM-5 Level 2- Anger- Adult

• DSM-5 Level 2- Somatic symptoms- Adult

• DSM-5 Level 2- Substance Abuse- Adult

• DSM-5 Level 2- Repetitive thoughts and Behavior- Adult

These investigations, therefore, are not created to make a diagnosis or as the only tool for evaluating the patient, but are rather devices conceived as an aid to clinical practice and the decision-making process of professionals. They have been made available by the APA (American Psychiatric Association), translated into Italian by 'Raffaello Cortina Editore' and must be administered during the first evaluation of patients or for the monitoring of symptoms and response to ongoing treatments. Although each interview has an apparently precise and precise form of scoring, in reality the APA itself states that for each scale only general instructions are provided for the attribution of the score and its interpretation, so much so that the clinician has the possibility to give his evaluation for each specific item. This form of attribution is necessary, given that the patient is not always able to "self-evaluate" his symptoms and is not always available for a sincere interview in the compilation of psychometric surveys.

Self-administered level 1 cross-sectional symptom rating scale – Adult

	During the past **TWO (2) WEEKS**, how much (or how often) have you been bothered by the following problems?	**None** Not at all	**Slight** Rare, less than a day or two	**Mild** Several days	**Moderate** More than half the days	**Severe** Nearly every day	**Highest Domain Score** (clinician)
I.	1. Little interest or pleasure in doing things?	0	1	2	3	4	
	2. Feeling down, depressed, or hopeless?	0	1	2	3	4	
II.	3. Feeling more irritated, grouchy, or angry than usual?	0	1	2	3	4	
III.	4. Sleeping less than usual, but still have a lot of energy?	0	1	2	3	4	
	5. Starting lots more projects than usual or doing more risky things than usual?	0	1	2	3	4	
IV.	6. Feeling nervous, anxious, frightened, worried, or on edge?	0	1	2	3	4	
	7. Feeling panic or being frightened?	0	1	2	3	4	
	8. Avoiding situations that make you anxious?	0	1	2	3	4	
V.	9. Unexplained aches and pains (e.g., head, back, joints, abdomen, legs)?	0	1	2	3	4	
	10. Feeling that your illnesses are not being taken seriously enough?	0	1	2	3	4	
VI.	11. Thoughts of actually hurting yourself?	0	1	2	3	4	
VII.	12. Hearing things other people couldn't hear, such as voices even when no one was around?	0	1	2	3	4	
	13. Feeling that someone could hear your thoughts, or that you could hear what another person was thinking?	0	1	2	3	4	
VIII.	14. Problems with sleep that affected your sleep quality over all?	0	1	2	3	4	
IX.	15. Problems with memory (e.g., learning new information) or with location (e.g., finding your way home)?	0	1	2	3	4	
X.	16. Unpleasant thoughts, urges, or images that repeatedly enter your mind?	0	1	2	3	4	
	17. Feeling driven to perform certain behaviors or mental acts over and over again?	0	1	2	3	4	
XI.	18. Feeling detached or distant from yourself, your body, your physical surroundings, or your memories?	0	1	2	3	4	
XII.	19. Not knowing who you really are or what you want out of life?	0	1	2	3	4	
	20. Not feeling close to other people or enjoying your relationships with them?	0	1	2	3	4	
XIII.	21. Drinking at least 4 drinks of any kind of alcohol in a single day?	0	1	2	3	4	
	22. Smoking any cigarettes, a cigar, or pipe, or using snuff or chewing tobacco?	0	1	2	3	4	
	23. Using any of the following medicines ON YOUR OWN, that is, without a doctor's prescription, in greater amounts or longer than prescribed [e.g., painkillers (like Vicodin), stimulants (like Ritalin or Adderall), sedatives or tranquilizers (like sleeping pills or Valium), or drugs like marijuana, cocaine or crack, club drugs (like ecstasy), hallucinogens (like LSD), heroin, inhalants or solvents (like glue), or methamphetamine (like speed)]?	0	1	2	3	4	

This survey was created to be completed either directly by the subject under analysis or indirectly by an informant, reporting the symptoms of the last 2 weeks. However, in our study, patients and relatives were asked to report their lifelong symptoms in order to have an initial complete picture of the patient's symptom profile. It is used to assess the domains of mental health that are relevant independently and transversely to the psychiatric diagnosis of the patient and/or relative, and is designed as an aid to clinicians in identifying symptom areas worthy of further study and in monitoring the patient's symptoms over time or response to treatments. It consists of 23 questions aimed at evaluating 13 symptom clusters: depression, anger, mania, anxiety, somatic symptoms, suicidal ideation,

psychosis, sleep disturbances, memory, repetitive thoughts and behaviors, dissociation, personality and substance use.

For each item, the extent or frequency of a certain symptom is assessed, with a rating from 0 to 4. In general, the tool has revealed its clinical usefulness and reliability in empirical studies on DSM-5. The rating scale consists of:

• 0 = Absent or Not at all

• 1 = Very mild or Rarely, less than 1 or 2 days

• 2 = Mild or A few days

• 3 = Moderate or More than half of the days

• 4 = Severe or Most days

In any case, the clinical judgment must guide the decision-making process, so if during the visit any in-depth analyzes come out that would change the score declared by the patient or the professional judges the reported false or irrelevant to reality, he must score the most appropriate score. in the “Highest Domain Score” column. A rating of 2 or higher in any question of the 13 domains (except for "substance use", "suicidal ideation" and "psychosis" for which a "very mild" score of 1 is sufficient) requires further investigation with psychometric interviews specific to the domain or domains in question. The “ Level 2 transversal symptom assessment scales ”, in the table above, are the tools suitable for obtaining the more in-depth information required. The "Self-administered Level 1 Transversal Symptom Assessment Scale - Adult" is therefore a tool that was created as an aid to the clinician and in no way replaces the professional's assessment; moreover, it wants to represent a guide that leaves freedom of use also in the research field. In our specific case, the scale was used for two purposes: to verify the reliability of the answers, being able to see if there was consistency between the answers given in this psychometric

survey and the other specific investigations, and to carry out an initial screening that could verify the general symptomatology of individuals and verify the overall differences between healthy (or apparently healthy) relatives and patients under treatment.

Domain	Domain Name	Threshold to guide further inquiry	DSM-5 Level 2 Cross-Cutting Symptom Measure available online
I.	Depression	Mild or greater	LEVEL 2—Depression—Adult (PROMIS Emotional Distress—Depression—Short Form)[1]
II.	Anger	Mild or greater	LEVEL 2—Anger—Adult (PROMIS Emotional Distress—Anger—Short Form)[1]
III.	Mania	Mild or greater	LEVEL 2—Mania—Adult (Altman Self-Rating Mania Scale)
IV.	Anxiety	Mild or greater	LEVEL 2—Anxiety—Adult (PROMIS Emotional Distress—Anxiety—Short Form)[1]
V.	Somatic Symptoms	Mild or greater	LEVEL 2—Somatic Symptom—Adult (Patient Health Questionnaire 15 Somatic Symptom Severity [PHQ-15])
VI.	Suicidal Ideation	Slight or greater	None
VII.	Psychosis	Slight or greater	None
VIII.	Sleep Problems	Mild or greater	LEVEL 2—Sleep Disturbance - Adult (PROMIS—Sleep Disturbance—Short Form)[1]
IX.	Memory	Mild or greater	None
X.	Repetitive Thoughts and Behaviors	Mild or greater	LEVEL 2—Repetitive Thoughts and Behaviors—Adult (adapted from the Florida Obsessive-Compulsive Inventory [FOCI] Severity Scale [Part B])
XI.	Dissociation	Mild or greater	None
XII.	Personality Functioning	Mild or greater	None
XIII.	Substance Use	Slight or greater	LEVEL 2—Substance Abuse—Adult (adapted from the NIDA-modified ASSIST)

[1]The PROMIS Short Forms have not been validated as an informant report scale by the PROMIS group.

4.2 Depression Severity Rating Scale - Adult

For the evaluation of depression, we opted to use another scale than the one recommended in the previous Table, namely the "Scale of evaluation of the severity of depression - Adult".

The motivation is due to the fact that the interviews would not have been administered only to a population suffering from psychiatric disease, but also to healthy subjects, so the most appropriate survey for our purposes should not have indicated only if in the course of life periods of "important sadness" occurred frequently, since they could still have been within the realm of physiology and responses would have been subject to personal evaluation, but should have required more precise symptoms. The following interview investigates the specific

symptoms that fall within the diagnostic criteria for the definition of major depressive episode, which is therefore useful for the purpose of the study. It was therefore possible to investigate whether any symptoms such as to outline a major depressive episode had ever developed in the relatives of the patients.

Over the last 2 weeks, how often have you been bothered by any of the following problems? *(Use a check mark to indicate your answer)*	**Not at all**	**Several days**	**More than half the days**	**Nearly every day**
1. Little interest or pleasure in doing things	0	1	2	3
2. Feeling down, depressed, or hopeless	0	1	2	3
3. Trouble falling or staying asleep, or sleeping too much	0	1	2	3
4. Feeling tired or having little energy	0	1	2	3
5. Poor appetite or overeating	0	1	2	3
6. Feeling bad about yourself – or that you are a failure or have let yourself or your family down	0	1	2	3
7. Trouble concentrating on things, such as reading the newspaper or watching television	0	1	2	3
8. Moving or speaking so slowly that other people could have noticed? Or the opposite – being so fidgety or restless that you have been moving around a lot more than usual	0	1	2	3
9. Thoughts that you would be better off dead or hurting yourself in some way	0	1	2	3
FOR OFFICE CODING	____ +	____ +	____ +	____
				= Total Score: ____

The scale can be self-administered, reported by the informant or examined by the clinician during the interview, reporting the score of his/her evaluation in the column "by the clinician". The interpretation of the table is as follows:

Severity level of depressive symptoms	PHQ-9 Score
None	0-4
Mild Depression	5-9
Moderate Depression	10-14
Moderate-Severe Depression	15-19
Severe Depression	20-27

Level 2 - Sleep disturbances - Adult

The following rating scale consists of 8 items and is suitable for assessing the domain of sleep disturbances. Requires the symptoms of the last 7 days; however, as mentioned above, in this and all the other scales we have tried to investigate the symptomatology of the domination that has taken place over a lifetime or, in any case, over a longer period of time (last months/years). Each item is rated from 1 to 5 points, for a total score from 8 to 40. Also in this case, the scale can be self-administered, reported by an informant or examined by the clinician during the interview, reporting the score of one's evaluation in the column "a care of the clinician ".

- 1 = Never
- 2 = Rarely
- 3 = Sometimes
- 4 = Often
- 5 = Always

For the calculation of the score it is possible to use the raw score or identify the T-score, (table below)

Sleep Disturbance 8b
Short Form Conversion Table

Raw Score	T-score	SE*
8	28.9	4.8
9	33.1	3.7
10	35.9	3.3
11	38.0	3.0
12	39.8	2.9
13	41.4	2.8
14	42.9	2.7
15	44.2	2.7
16	45.5	2.6
17	46.7	2.6
18	47.9	2.6
19	49.0	2.6
20	50.1	2.5
21	51.2	2.5
22	52.2	2.5
23	53.3	2.5
24	54.3	2.5
25	55.3	2.5
26	56.3	2.5
27	57.3	2.5
28	58.3	2.5
29	59.4	2.5
30	60.4	2.5
31	61.5	2.5
32	62.6	2.5
33	63.7	2.6
34	64.9	2.6
35	66.1	2.7
36	67.5	2.8
37	69.0	3.0
38	70.8	3.2
39	73.0	3.5
40	76.5	4.4

*SE = Standard Error on T-score metric

The interpretation is given by:
• 24 or <55 (T-score) = absent to sporadic symptoms
• 25-29 or 55-59.9 = Mild
• 30-37 or 60-69.9 = Moderate
• 38-40 or> 70 = Severe

Level 2 - Anxiety - Adult

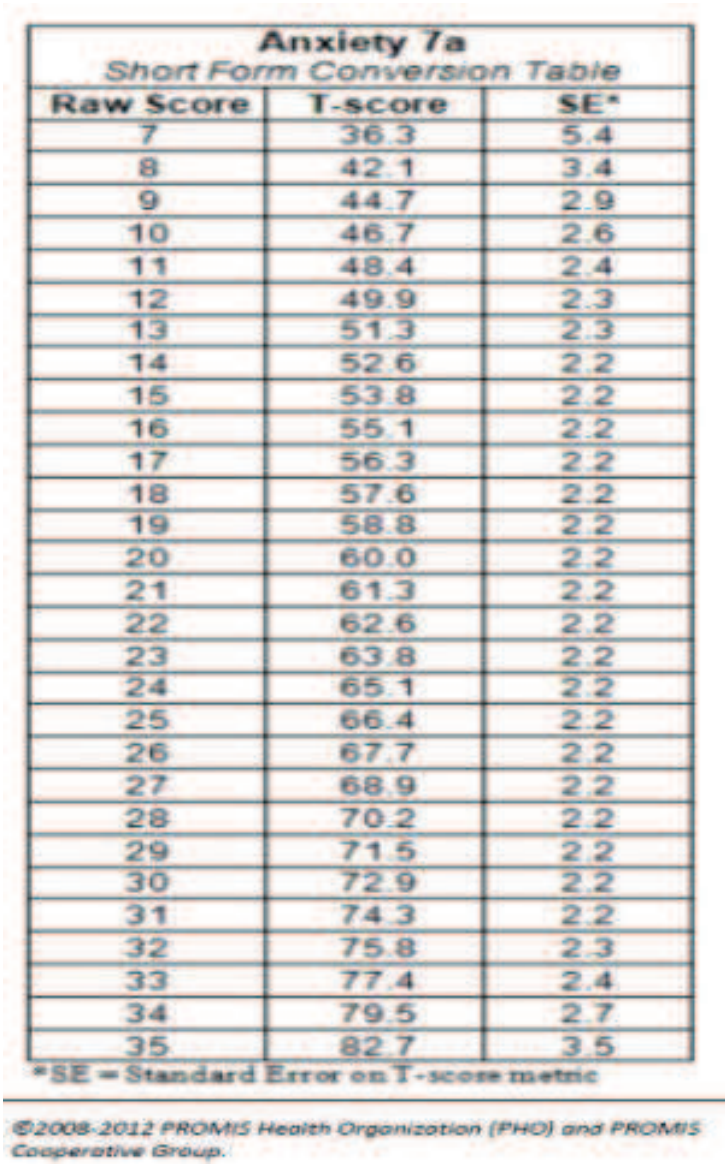
The scale consists of 7 items and was created to evaluate the anxiety domain of the last 7 days, which we adapted to a lifetime symptomatology or a longer period. Each item is rated from 1

to 5 points, for a total score from 7 to 35. The scale can be self-administered, referred by an informant or examined by the clinician during the interview by reporting the score of their assessment in the column "by the clinician". For each question:

- 1 = Never
- 2 = Rarely
- 3 = Sometimes
- 4 = Often
- 5 =Always

							Clinician Use
In the past SEVEN (7) DAYS....							Item Score
		Never	Rarely	Sometimes	Often	Always	
1.	I felt fearful.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
2.	I felt anxious.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
3.	I felt worried.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
4.	I found it hard to focus on anything other than my anxiety.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
5.	I felt nervous.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
6.	I felt uneasy.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
7.	I felt tense.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
						Total/Partial Raw Score:	
						Prorated Total Raw Score:	
						T-Score:	

The assessment of the severity of anxiety can be obtained either by summing the raw scores or by deriving the T-score in the table:



Anxiety 7a
Short Form Conversion Table

Raw Score	T-score	SE*
7	36.3	5.4
8	42.1	3.4
9	44.7	2.9
10	46.7	2.6
11	48.4	2.4
12	49.9	2.3
13	51.3	2.3
14	52.6	2.2
15	53.8	2.2
16	55.1	2.2
17	56.3	2.2
18	57.6	2.2
19	58.8	2.2
20	60.0	2.2
21	61.3	2.2
22	62.6	2.2
23	63.8	2.2
24	65.1	2.2
25	66.4	2.2
26	67.7	2.2
27	68.9	2.2
28	70.2	2.2
29	71.5	2.2
30	72.9	2.2
31	74.3	2.2
32	75.8	2.3
33	77.4	2.4
34	79.5	2.7
35	82.7	3.5

*SE = Standard Error on T-score metric

The interpretation:

- 15 or <55 (T-score) = absent to sporadic symptoms
- 16-19 or 55-59.9 = Mild
- 20-27 or 60-69.9 = Moderate
- 28-35 or> 70 = Severe

Level 2 - Mania - Adult

The “Level 2 Rating Scale - Mania - Adult” consists of Altman Self Rating Mania Stairs. It is a tool consisting of 5 items, outlined for the evaluation of the possible presence of manic symptoms and their severity. Each item is rated from 1 to 5 points for a score ranging from 5 to 25.

The scale, like the others, can be self-administered or referred by an informant and in any case leaves the final evaluation to the clinician, being able to compile the score himself during the interview and report it in the column "By the clinician".

		Clinician Use
Question 1		Item score
❑ 1	I do not feel happier or more cheerful than usual.	
❑ 2	I occasionally feel happier or more cheerful than usual.	
❑ 3	I often feel happier or more cheerful than usual.	
❑ 4	I feel happier or more cheerful than usual most of the time.	
❑ 5	I feel happier of more cheerful than usual all of the time.	
Question 2		
❑ 1	I do not feel more self-confident than usual.	
❑ 2	I occasionally feel more self-confident than usual.	
❑ 3	I often feel more self-confident than usual.	
❑ 4	I frequently feel more self-confident than usual.	
❑ 5	I feel extremely self-confident all of the time.	
Question 3		
❑ 1	I do not need less sleep than usual.	
❑ 2	I occasionally need less sleep than usual.	
❑ 3	I often need less sleep than usual.	
❑ 4	I frequently need less sleep than usual.	
❑ 5	I can go all day and all night without any sleep and still not feel tired.	
Question 4		
❑ 1	I do not talk more than usual.	
❑ 2	I occasionally talk more than usual.	
❑ 3	I often talk more than usual.	
❑ 4	I frequently talk more than usual.	
❑ 5	I talk constantly and cannot be interrupted.	
Question 5		
❑ 1	I have not been more active (either socially, sexually, at work, home, or	
❑ 2	I have occasionally been more active than usual.	
❑ 3	I have often been more active than usual.	
❑ 4	I have frequently been more active than usual.	
❑ 5	I am constantly more active or on the go all the time.	
	Total/Partial Raw Score:	
	Prorated Total Raw Score:	

Reprinted from Altman EG, Hedeker D, Peterson JL, Davis JM: The Altman Self-Rating Mania Scale. Biological Psychiatry 42: 948-955, 1997

The table on the following page shows the interpretation scale:

- a score of 6 or more indicates a high probability of the condition of mania or hypomania
- a score of 6 or more may indicate the need for treatment and / or further diagnostic evaluation
- a score of 5 or less indicates less association with significant symptoms of mania

Level 2 - Anger - Adult

The scale consists of 5 items rated from 1 to 5, for a minimum score of 5 and a maximum of 25: the higher the scores, the greater the severity of the anger; the method of administration is analogous to all other scales. The evaluation:

- 1 = Never
- 2 = Rarely
- 3 = Sometimes
- 4 = Often
- 5 = Always

							Clinician Use
In the past SEVEN (7) DAYS....							Item Score
		Never	Rarely	Sometimes	Often	Always	
1.	I was irritated more than people knew.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
2.	I felt angry.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
3.	I felt like I was ready to explode.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
4.	I was grouchy.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
5.	I felt annoyed.	❑ 1	❑ 2	❑ 3	❑ 4	❑ 5	
						Total/Partial Raw Score:	
						Prorated Total Raw Score:	
						T-Score:	

The interpretation of the severity of anxiety can be obtained either from the sum of the raw scores or from the T-score in the table:

- 13 or <55 (T-score) = absent to sporadic symptoms
- 14-15 or 55-59.9 = Mild
- 16-20 or 60-69.9 = Moderate
- 21-25 or> 70 = Severe

Score	T-score	SE*
5	32.9	5.3
6	38.1	4
7	41.3	3.7
8	44	3.5
9	46.3	3.4
10	48.4	3.3
11	50.5	3.3
12	52.6	3.2
13	54.7	3.2
14	56.7	3.2
15	58.8	3.2
16	60.8	3.2
17	62.9	3.2
18	65	3.2
19	67.2	3.2
20	69.4	3.3
21	71.7	3.3
22	74.1	3.3
23	76.8	3.4
24	79.7	3.5
25	83.3	3.9

*SE = Standard Error on T-score metric

Level 2 – Somatic Symptoms - Adult

The following scale consists of 15 items to assess the domain of somatic symptoms. Each item requires the subject (or informant) to assess the severity of somatic symptoms in the past 7 days but, as in the other scales, the investigation period was extended for the needs of the study.

Each item is rated from 0 to 2 and the total score ranges from 0 to 30. The clinician can review each item during the interview and report the score in the "By the clinician" column.

• 0 = Not at all

• 1 = Little

• 2 = A lot

During the past 7 days, how much have you been bothered by any of the following problems?	Not bothered (0)	Bothered a little (1)	Bothered a lot (2)
a. Stomach pain	○	○	○
b. Back pain	○	○	○
c. Pain in your arms, legs, or joints (knees, hips, etc)	○	○	○
d. Menstrual cramps or other problems with your periods [WOMEN ONLY]	○	○	○
e. Headaches	○	○	○
f. Chest pain	○	○	○
g. Dizziness	○	○	○
h. Fainting spells	○	○	○
i. Feeling your heart pound or race	○	○	○
j. Shortness of breath	○	○	○
k. Pain or problems during sexual intercourse	○	○	○
l. Constipation, loose bowels, or diarrhea	○	○	○
m. Nausea, gas, or indigestion	○	○	○
	Not at all (0)	Several days (1)	>Half the days (2)
n. Feeling tired or having low energy	○	○	○
o. Trouble sleeping	○	○	○
Total Score*:			

The interpretation is carried out following the table below:

Severity level of somatic symptoms	PHQ-15 Score
Minimum	0-4
Low	5-9
Medium	10-14
High	15-19

Level 2 - Substance Use - Adult

The Level 2 Assessment Scale - Substance Use - Adult is an adapted version of NIDA - Modified ASSIST. The scale should be administered to all those subjects who in the self-administered Level 1 Transversal Symptom Assessment Scale -

Adult survey have completed at least one question with a "very mild" or greater answer (equal to 1 point) in the corresponding domain. It consists of 10 items and is used to assess the domain of alcohol, tobacco/nicotine, prescription and illicit drug use. Each item requires the subject to assess the severity of use of various substances over the past 2 weeks. The rating of each question is on a 5-degree scale, from 0 to 4.

- 0 = Not at all
- 1 = 1 or 2 days
- 2 = A few days
- 3 = More than half of the days
- 4 = Most days

In the past two weeks how often have you used drugs on your own or without a prescription, in higher doses or longer than prescribed		Never	1 or 2 days	Some days	More than half of the days	Nearly every day	Clinician Use Item Score
a.	Painkillers	❑0	❑1	❑2	❑3	❑4	
b.	Stimulants	❑0	❑1	❑2	❑3	❑4	
c.	Sedatives or tranquilizers (such as sedatives or anxiety relievers)	❑0	❑1	❑2	❑3	❑4	
Or drugs such as:							
d.	Marjuana	❑0	❑1	❑2	❑3	❑4	
e.	Cocaine or Crack	❑0	❑1	❑2	❑3	❑4	
f.	Discotheque drugs (such as ecstasy)	❑0	❑1	❑2	❑3	❑4	
g.	Hallucinogens (such as LSD)	❑0	❑1	❑2	❑3	❑4	
h.	Heroin	❑0	❑1	❑2	❑3	❑4	
i.	Inhalants or Solvents (such as glue)	❑0	❑1	❑2	❑3	❑4	
j.	Methamphetamine	❑0	❑1	❑2	❑3	❑4	
					Total Score:		

In this case, an interpretative table of scores was not provided as each item must be interpreted individually, as it involves the use of very different and specific substances; clinical judgment therefore must guide the decision making process.

In any case, the attribution of scores above 0 or the positivity of more items indicates a greater seriousness and complexity of the use of substances. This survey can be very useful for monitoring over time patients who use substances and to verify any improvements; for this reason the scale should be compiled at

regular intervals based on the patient's symptomatic stability and the treatment in progress.

Level 2 - Repetitive thoughts and behaviors - Adult

The scale consists of 5 items to evaluate the domain of repetitive thoughts and behaviors. Each item is graduated on a scale from 0 to 4 and evaluates the severity of the repetitive thoughts and behaviors of the subject under analysis, investigating the symptoms of the last 7 days.

Each item is rated on a 5-point scale (from 0 to 4) and the total score ranges from 0 to 20; the higher the scores, the greater the severity of repetitive thoughts and behaviors. If the subject scores 8 or higher, it could be obsessive-compulsive disorder so a more in- depth evaluation should be considered.

						Clinician Use
During the past SEVEN (7) DAYS....						**Item Score**
1. On average, how much *time* is occupied by these thoughts or behaviors each day?	❑ 0—None	❑ 1—Mild (Less than an hour a day)	❑ 2—Moderate (1 to 3 hours a day)	❑ 3—Severe (3 to 8 hours a day)	❑ 4—Extreme (more than 8 hours a day)	
2. How much *distress* do these thoughts or behaviors cause you?	❑ 0—None	❑ 1—Mild (slightly disturbing)	❑ 2—Moderate (disturbing but still manageable)	❑ 3—Severe (very disturbing)	❑ 4—Extreme (overwhelming distress)	
3. How hard is it for you to *control* these thoughts or behaviors?	❑ 0—Complete control	❑ 1—Much control (usually able to control thoughts or behaviors)	❑ 2—Moderate control (sometimes able to control thoughts or behaviors)	❑ 3—Little control (infrequently able to control thoughts or behaviors)	❑ 4—No control (unable to control thoughts or behaviors)	
4. How much do these thoughts or behaviors cause you to *avoid* doing anything, going anyplace, or being with anyone?	❑ 0—No avoidance	❑ 1—Mild (occasional avoidance)	❑ 2—Moderate (regularly avoid doing these things)	❑ 3—Severe (frequent and extensive avoidance)	❑ 4 - Extreme (nearly complete avoidance; house- bound)	
5. How much do these thoughts or behaviors *interfere* with school, work, or your social or family life?	❑ 0—None	❑ 1—Mild (slight interference)	❑ 2— Moderate; (definite interference with functioning, but still manageable)	❑ 3—Severe (substantial interference)	❑ 4—Extreme (near-total interference; incapacitated)	
					Total/Partial Raw Score:	
					Prorated Total Raw Score (if 1 item is left unanswered):	
					Average Total Score:	

4.3 The method of collection

Among the primary objectives of this pilot study was the identification of the most appropriate data collection method for carrying out future studies based on psychometric interviews. This type of surveys have already been used previously in numerous studies on families and the collection methods used were of two main types: direct interviews, carried out on patients and relatives during visits, indirect interviews collecting surveys on relatives from probands and integrating with the history of the information that can be collected from medical records. This important methodological distinction arises from the advantages and limitations that characterize each approach.

The first method, which provides for the collection of all interviews in person or electronically, has the advantage of having direct information even from relatives on their symptoms, ongoing treatments and personal history, making investigations notoriously more sensitive than the indirect method. (Smoller et al., 2003).

However, this method collides with organizational difficulties, relating to the availability of relatives in answering questionnaires and the not always flexible timing of research studies. Relatives, in fact, are often difficult to reach, geographically or electronically, or reluctant to carry out the interviews and the relationship with the proband is not always characterized by close attendance; moreover, with this survey method, valuable information on any deceased relatives is lost and, inevitably, the time elapsing between the delivery of the survey (in person or electronically) and the actual receipt of the interview filled out. The second approach, on the other hand, provides for two methods of collection: direct on probands, indirect on relatives. This methodology allows to overcome almost all the limitations of the first type of approach: it allows

in the time of a single visit to collect both the investigation of the proband and of the relatives, thus shortening the timing, it allows to overcome the reticence that some relatives may have in the conducting the interview; then allows you to retrieve any important information on deceased relatives or geographically distant or difficult to reach. However, it is a method known for its low sensitivity: probands are often unable to report detailed information on the symptoms of relatives and, often, there is a tendency to underestimate the importance of some character traits even in the most relatives. narrow (Smoller et al., 2003). In our case, we also add to these known limitations the narrowness of the study due to the impossibility of having access to large national registers, since it is in fact an observational and monocentric study. For all these reasons, it was decided to launch two investigation arms, with the primary objective of the study precisely identifying the most suitable methodology to allow the use of the most effective approach for expanding the data in future investigations. In the first arm, therefore, probands and relatives both received the interviews to be answered, with the possibility of choosing between four available methods: in presence during the visit of the proband, by telephone, electronically via email with the pdf of the interviews attached and online, by completing the questionnaires transformed into digital form. In the second approach, on the other hand, it provided for the collection of interviews during the visit of the proband, interviewing the same on the known symptoms of the relatives. Overall, 46 patients were collected with at least one relative associated (for a total of 118 individuals), of which the first 23 recruited in the first collection arm, the second 23 in the method that used indirect collection of relatives. The most important difficulties encountered in the first arm were the lengthening of the time between delivering the interviews and receiving the answers and the reluctance of many relatives to answer questions. The

current epidemiological context has certainly influenced in an important way in the verification of these limits, not allowing relatives for almost the entire duration of the study to accompany patients on visits and not giving the possibility to organize unique days in which to bring relatives in small groups for the rapid completion of the questionnaires in person. The reluctance, not always openly manifest, of numerous relatives to respond directly to the questionnaires made it difficult to expand the sample, so much so that for the first 23 patients it was rarely possible to obtain more than one relative willing to answer, with the exception of one the only patient, very young, for whom the whole family unit showed willingness to be recruited into the study. Sometimes the reluctance has manifested itself in a clear refusal to recruit; sometimes, despite the numerous options available, quick and easy to use, for filling in the questionnaires, it has never been possible to obtain the response to the surveys back, contrary to the initial availability provided; sometimes the compilation of the questionnaires was obviously not reliable. This in particular is an interesting fact: 3 relatives of 3 different patients, in fact, had answered the questionnaires giving all interviews scores equal to or equal to zero, however declaring very high scores in the interview on "Mania". In fact, that questionnaire investigates apparently positive symptoms: the patient's happiness or cheerfulness, higher than usual, self-confidence, having a lot of energy despite the poor night's rest, eloquence and sociability, high daily energy levels (social, sexual, or as a physical activity at work, at home or at school); based on the response to this survey, the 3 relatives would have been in a frank manic phase, with scores much higher even than patients with bipolar disorder. Due to these considerations the 3 relatives were excluded from the database. Always considering the epidemiological context in progress, the second method of collection, on the other hand, proved to be much more usable overall, allowing all the

necessary investigations to be carried out during the normal control visit of the probands. The collection times were therefore shortened, also allowing to enlarge the sample substantially (in 6 cases it was possible to collect the symptoms of the entire family unit) and to overcome the reluctance of relatives in answering the questionnaires. However, the limitations of this method that Smoller highlighted were confirmed et al. (2003): often the sensitivity of the questionnaires was partially reduced due to the actual difficulties of the probands in reporting (and knowing) the in-depth symptoms of relatives.

5. Whole Exome Sequencing

Sample preparation was performed following the Illumina DNA Prep with Enrichment manufacturer protocol. The workflow uses a bead-based transposome complex to mediate a uniform tagmentation reaction of genomic DNA, which fragments and then tags DNA with adapter sequences in one step. A subsequent target enrichment workflow is then applied. Following pooling, the double stranded DNA libraries are denatured and biotinylated. Illumina Exome Panel (CEX) probes are hybridized to the denatured library fragments. After hybridization, Streptavidin Magnetic Beads (SMB) then capture the targeted library fragments within the regions of interest. The captured and indexed libraries are eluted from beads and further amplified before sequencing. The whole exome sequencing analysis was performed on the Illumina NovaSeq 6000 (Illumina San Diego, CA, USA) according to the NovaSeq 6000 system guide. Reads were mapped against the hg19 reference genome by using the Burrow-Wheeler aligner BWA (Craddock N, Khodel V, et al., 1995). Variant calling was obtained using an in-house pipeline which takes advantage of the GATK Best Practices workflow (Baum A, Akkula N, et al., 2008). We

obtained mean coverage of 105x for targeted sequenced regions (range, 95- 145x). WES data were analyzed using the eVai (enGenome, (CE-IVD) software. In order to identify any potential pathogenic variants segregating among the family, we firstly approached the concept of "Reverse Phenotyping" on all the affected subjects. In this step, no filters in the prioritization of variants were applied. The aim was to check for the presence of any alterations involved in the manifestation of psychosis, in genes not previously described to be associated with mental disorders. Once excluded this first step, variants' prioritization was carried out and obtained by: selecting rare variants (Minor Allele Frequency, MAF <0,01) found only in BD subjects; evaluating genes previously associated with BD in literature, from GWAS and WES studies (Supplementary, Table 1) (Rao AR, Yourshaw M et al., 2017; Toma C, Shaw AD et al., 2020; Ferreira MA, O' Donovan MC et al., 2008; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011) using HPO terms: bipolar affective disorder (HP:0007302), depression (HP:0000716), obsessive- compulsive behaviour (HP:0000722), mania (HP:0100754), psychosis (HP:0000709), anxiety (HP:0000739), suicide ideation (HP:0031589). Missense variants were predicted to be damaging by CADD-phred prediction tools (Wellcome Trust Case Control Consortium,2011) and splice site variants by four in silico tools: SpliceSiteFinder-like, MaxEntScan, NNSPLICE, GeneSplicer. The UCSC genome browser (https://genome.ucsc.edu;December2021), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/ ; January 2022), Intervar (Clinical Interpretation of genetic variants by ACMG/AMP 2015 guideline, https://wintervar.wglab.org/; December 2021), VarSome (https://varsome.com/ ; January 2022), CADD (Combined Annotation- Dependent Depletion, https://cadd.gs.washington.edu/ ; January 2022), OMIM (Online Mendelian Inheritance in Man- https://www.MIM.org/

; December 2021), BipEx Consortium (Bipolar Exosome, https://bipex.broadinstitute.org/ , February 2022), DECIPHER (DatabasE of Chromosomal Imbalances and Phenotypes using Ensembl Resourceshttps:// www.deciphergenomics.org/, February 2022) databases, and Alamut Visual software application v.2.11 (Jan 2018 Interactive Biosoftware, Rouen, France) were used in the interpretation of the results.

6. Statistic analysis

The statistical objective of the study was the identification of any symptomatic dimensions that were possible for further study due to nondiscardable heritability. To do this, the symptomatic clusters recurring in the relatives of the probands and in the probands themselves were identified; the specific symptom profiles were defined in the different degrees of kinship, relating them to the probands, to understand if there were similar character traits between families and if it was possible to outline symptomatic associations similar to comorbidities and family aggregations, already widely highlighted by the scientific literature; finally, it was possible to hypothesize, on the basis of the aforementioned analyzes, which were the possible symptomatological dimensions of in-depth analysis due to heritability which cannot be discarded.

Studies on families in general, using this type of investigation on probands and relatives, are able to indicate the family aggregation of disorders, but cannot correlate it to the presence of specific genes involved; it is known, in fact, that in the family onset of a disorder non-genetic reasons may also be shared, such as the shared environment (Smoller et al., 2003). Similarly, in the following study it was possible to confirm that within families, also by analyzing the family units as a whole, taking healthy relatives into analysis, there is an aggregation of symptomatic clusters and a specific recurrence of symptoms,

therefore that in this sense there may be a specific heritability of clusters.

R Core Team software package R (R Core Team, 2021) was used to carry out the statistical analyzes of the study. First a separation into groups was carried out, identifying the different degrees of kinship that would be analyzed. We then separated the sample into:

- 46 patients
- 28 mothers
- 15 fathers
- 20 brothers/sisters
- 9 children

The collected data was entered into a database and openxlsx was used to read and use excel files (Schauberger & Walker 2021). Since each scale of the survey had different maximum and minimum values (and therefore the same numerical value had different values between one survey and another), as a second step the standardization of the data was carried out, a procedure that allowed us to homogenize all the data of the various surveys on a graduated scale between 0 and 1. To carry it out, the z-score formula was used: for the graphical representation of our data we used the ggplot2 package (Wickham, 2016) and its ggfortify function (Tang et al., 2016). We then obtained the averages and represented the histogram, with the aim of identifying the general trend of the various psychometric investigations, thus being able to compare the scores of the different groups of family members with each other and the group of patients. This type of initial investigation also made it possible to assess whether the results obtained were reliable and whether the administration and compilation of the psychometric scales had occurred correctly. We then worked on identifying which symptom profiles were relevant and recurring between the different groups, in order to identify any similarities, not only by comparing the symptoms of patients with that of relatives,

but also with data from the scientific literature. So we have done some Corrplots (package ggcorrplot; Kassambara et al., 2019), correlation matrix graphs that made it possible to identify recurrent clusters and symptom profiles.

The statistics continued with the analysis of the main components and related Bplot (package PCAtools, Blighe & Lun, 2021) which made it possible to delineate the symptomatological profiles of the groups with greater precision and to analyze in which symptom dimensions the 2 main components of the samples under analysis were most distributed. Finally, we carried out the Wilcoxon Test to identify those symptomatic clusters that could be investigated for non-discardable heritability.

7. Results

7.1 Clinical Description

Clinical findings of evaluated patients are described as follows. The family pedigree is shown in Figure 1. Psychometric evaluations were carried out for each patient by the Unit of Psychiatry, Department of Mental Health.

Patient 1 (Figure 1; V;5) Patient 1 is a 27 years old woman. She was born at term from spontaneous delivery. She experienced sleep disturbances until the age of six, for which she started therapy with Noprom. She manifested her first psychopathological symptoms when she was eighteen years old, when she began to attend university. She developed symptoms of demoralization, obsessive- compulsive behavior, anxiety, mood swings and irritability. The family doctor prescribed her an unspecified antidepressant. After three days of intaking, her mood expanded, she developed sub-total insomnia, logorrhea,

tangential thought process, ease in relationship with strong behavioral disinhibition, episodes of dysphoria and impulsiveness, mystical and megalomaniac delusions regarding conviction of possessing paranormal powers. Over the years her psychiatric history has been characterized by the alternation of satisfying conditions and symptomatic re-exacerbations. Currently she is in good psychopathological condition. She currently takes Valproic Acid 600 mg per day, Lorazepam 1mg, Cariprazine 1,5mg two per day, Lamotrigine 50 one per day.

Patient 2 (Figure 1; IV;10) Patient 2 is the 55 years old proband's mother affected by Bipolar Disorder II. Her first signs of depression arose following the death of the mother. After being treated with Diamox due to the diagnosis of Meniere Syndrome, she started to experience depression, anxiety, sleep disturbances, labile mood, irritability, inner tense and polarization about her daughter's health problems. Later on, she developed sadness, apathy, and abulia. Over the years her psychiatric history has been characterized by the alternation of satisfying conditions and symptomatic re-exacerbations. Currently she displays significant clinical improvement. She is in therapy with Valproic acid CHR 300 mg, Citalopram 10 mg.

Patient 3 (Figure 1; IV;11)

Patient 3 is the maternal uncle of the index subject. He is a 63 years old male affected by BD type I. He manifested subclinical depression since he was young. He was hospitalized for episodes of depression, lack of volition, insomnia, delusions of failure, partial insight, and further manic episodes and behaviors in line with the DSM-5 criteria for BD I. Over the years, his psychiatric history has been characterized by the alternation of satisfying conditions and symptomatic re-exacerbations. He was released in good psychopathological condition after being treated with: Olanzapine 5 mg, Lamotrigine 150 mg, Sertraline 50 mg, lithium carbonate 900 mg.

Patient 4 (Figure 1; V;4)

Patient 4 is the middle sister of Patient 1. She is 30 years old. Psychiatrists reported moderate anxiety and traits of obsessive-compulsive personality. No other relevant clinical data were described. The girl was referred to be in good health.

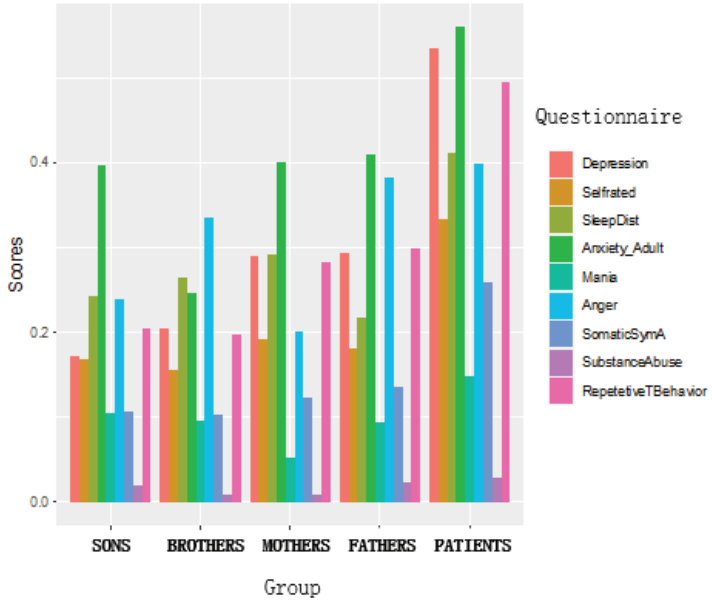
7.2 Molecular Characterization

Results obtained by WES analysis are summarized in Table 1. All variants refer to the Human Genome Assembly GRCh37/hg19. We searched for common variants among the affected subjects, and we found heterozygous missense and synonymous variants in 10 genes: PDE1C, HERC2, CHD7, MRC2, SPON1, ZNF92, TTC19, RBM12, CLN6, WDR62 (Table 1). However, variants in PDE1C, HERC2, CHD7, MRC2 and TTC19 were noticed not only in BD subjects, but also in healthy ones (Supplementary, Figure 1), and were thus excluded from the analysis. Because of their low CADD phred scores (<15) (Wellcome Trust Case Control Consortium,2011), variants in SPON1, RBM12 and WDR62 were excluded too (Table 1). On a first analysis, we therefore considered mutated genes only in BD subjects, and we obtained that the variant in common was found in ZNF92 (Figure 2). As a second step, we considered the mutated genes in common also with the "borderline" subject, and we obtained the missense variant in CLN6 (Figure 2). According to this data, we could hypothesize that the variant in CLN6 alone is responsible for the obsessive-compulsive phenotype, but when combined with the variant in ZNF92 is causative for the complete BD phenotype. Finally, assuming that the effect of some of the identified variants could be aggravated by the concomitant presence of any Structural Variants (SVs), we analyze WES raw data from affected subjects using the read count–based tool EXCAVATOR2. The tool uses BAM files of, respectively, whole genome sequencing

and WES experiments to extract the depth of sequencing coverage and identify regions with altered copy number (Maaser A, Forstner AJ et al, 2018). Then, SVs were annotated through AnnotSV (https://lbgi.fr/AnnotSV; Supplementary, Bipolar Disorder Family SVs). The tool uses BED or CVF SV files to produce a tab-separated values file, scoring and ranking the SVs into five classes from pathogenic to benign (Zhang T, Hou L et al., 2018). Results are summarized in Table 2. The analysis showed the presence of 12 SVs among two out of three BD individuals, plus the borderline one. Probably due to insufficient coverage, WES data from Patient V;5 were excluded from the comparison of the shared SVs in order to prevent this sample leading to biased results. The sample has indeed passed the quality control required by the EXCAVATOR2 pipeline, but it showed a distortion in the results of all the chromosomes that could lead to wrong interpretations (Supplementary, Figure 2). None of the 12 observed SVs has been confirmed among healthy subjects. Some deletions have been scored as Likely Pathogenic (score 4); none of them involves genes previously associated with BD.

7.3 Results of the study hypothesizes the transmissibility of symptomatic dimensions

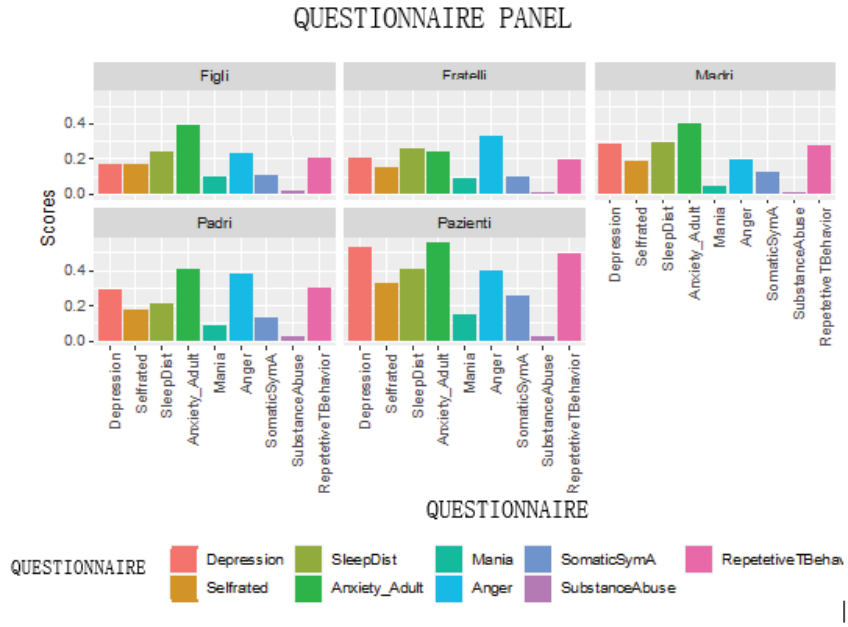
Understood as a cluster of symptoms within the families of psychiatric patients, rather than of specific diagnoses. After the subdivision into groups (patients, fathers, mothers, brothers/sisters, children) and the standardization of the data, which made it possible to obtain the normalization of the results, we proceeded with the identification of the means and their graphic representation on a histogram:



It was therefore possible to note that the patients all had higher average scores than the relatives, as we might have expected given that the group of relatives was composed of mainly healthy individuals or in any case without diagnosis or treatment in place. This was a first important fact that first of all made it possible to verify that the administration of the questionnaires had taken place correctly; if on the contrary we had obtained homogeneous scores between patients and relatives or on average higher in relatives, this would have meant that something in the administration or compilation of the questionnaires had not gone correctly, since otherwise the healthy relatives would have resulted, paradoxically, more symptomatic than patients diagnosed with psychiatric pathology. The values of the survey "Self-administered level 1 transversal symptom assessment scale - Adult" further confirms the above: the values of the relatives are on average lower than those of the patients, testifying an attested reduced symptomatology in all groups of relatives (mothers, fathers,

brothers/sisters, children). A fundamental fact that was able to verify is that, albeit with lower values than the patients, most of the clusters occurred in the different kinship groups. In fact, with the exception of the "substance use" cluster which showed zero or almost zero values in all groups, the other symptom dimensions showed values with a certain degree of significance, some with low values, others with more relevant averages. Mean sleep values are higher in mothers (M = 0.29) than in other family groups and, although patient values are fundamentally higher (M = 0.41), greater heritability among mothers could be assumed. and patients in the onset of sleep disturbances. As for anxiety, in the relatives of fathers, mothers and children there is a high and homogeneous recurrence with almost coincident values (M = 0.4), while for siblings the values are slightly lower but always relevant (M = 0.26); all degrees of kinship therefore have lower averages than the values of the patients (M = 0.56) but, overall, there is still a homogeneous presence of the cluster in the different groups, with values greater than the other symptom dimensions. Therefore, it is possible to suppose an important family aggregation within the family unit and to hypothesize a possible heritability of the symptomatological cluster. The mania survey showed broadly homogeneous scores between patient and relative groups, with mothers, however, being lower. Going to review the original database it was noted that many elements of different households had scores equivalent to zero (compared to the non-normalized scale). Obtaining low but homogeneous scores between patients and family members therefore means that the cluster recurs and that the not particularly high scores are due to totally asymptomatic subjects in the analysis cluster; in other words, when the mania is there it also occurs in family members. The anger has in a peculiar way values coinciding between fathers (M = 0.383) and patients (M = 0.398) and markedly higher than the mothers, from which we can assume a greater paternal influence on the

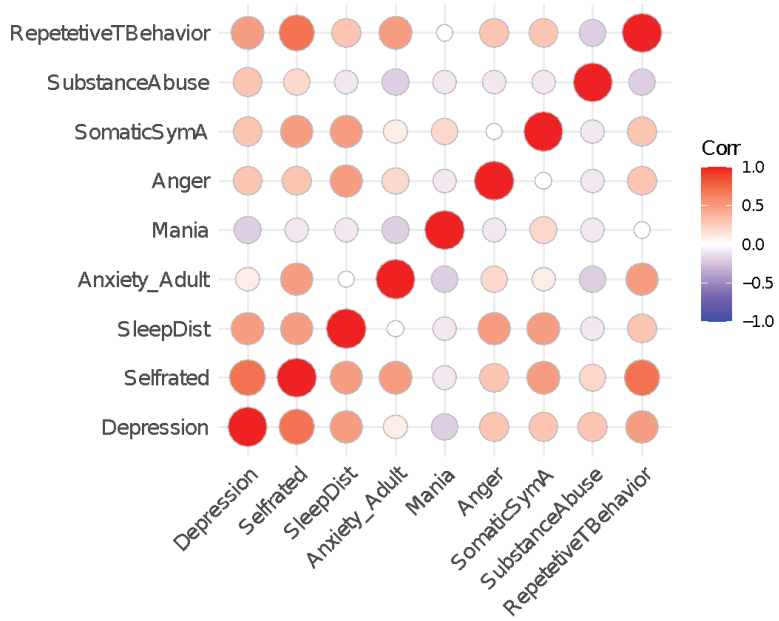
heritability of anger than the maternal origin. The somatic symptoms showed significantly lower average values between the groups of relatives and the group of patients, with rather homogeneous values between them. The use of substances showed average values close to zero in all groups. Repetitive thoughts and behaviors and depression showed the greatest gap between patients and relatives; in both clusters it is interesting to note how fathers and mothers have similar and higher values than the group of brothers/sisters and children; moreover, these last two groups equally show comparable values for both clusters.



A sub-threshold symptomatology or peculiar recurring profiles are however relevant clinical data that could be traces of an increased risk of developing certain psychiatric disorders or a predisposition to the development of more relevant symptoms. When it comes to psychopathological emotions and symptoms, in fact, various shades of the same can fall within physiological or pathological pictures; the recurrence of certain symptoms and the aggregation in family units, even if below the threshold, can

signal the presence of a genetic basis and therefore of a family heritability. For the identification of the symptomatological profiles we proceeded with the representation of the Corrplot correlation matrix graphs, which are read by color and size: the greater the size of the circle and the intensity of the red color, the greater the positive correlation between the symptomatic clusters; if the color tends to white and the circle is of minimal size, there is no correlation between the clusters; if the color turns purple, the correlation between symptom dimensions is negative.

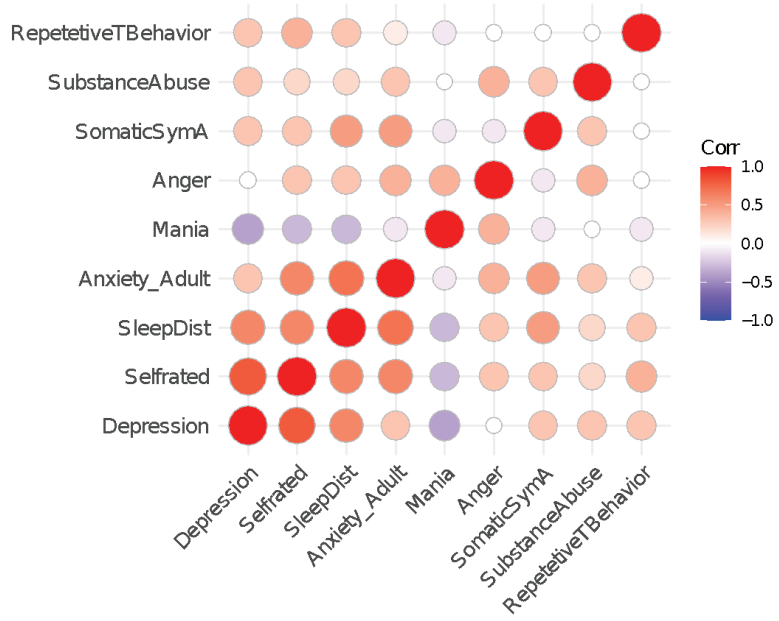
Mothers:



The main correlations found in the group of mothers are: depression and sleep problems, depression and obsessive-compulsive symptoms, anxiety and obsessive-compulsive symptoms. Sleep also appears positively correlated with anger and somatic symptoms. It is interesting to note that sleep is the cluster that correlates most with the other dimensions

(depression, anger, somatic symptoms). The most interesting correlation highlighted is precisely that between depression and sleep problems, given the known association between these two clusters in major depressive disorder in which the same definition of major depressive episode contains among the possible symptoms "insomnia or hypersomnia" (APA, DSM - 5). Furthermore, several studies have shown that the relationship between sleep disturbances and psychiatric disorders (specifically major depressive episode or manic episode) is actually bi-directional (Krystal, 2012; Breslau et al., 1996; Ford et al., 1989; Chang et al.; 1997). The importance of this cluster had already been highlighted by the initial histogram, with mean maternal values higher than in all other family groups, and could suggest that sleep problems have a greater heritable component of maternal origin. Mania in mothers appears instead as an independent symptomatological cluster, not correlating with other dimensions; its recurrence in family nuclei therefore seems disconnected from the other clusters and this could mean that the heritability of mania is also independent of the other symptom dimensions. Of particular interest is the null or negative correlation that characterizes depression and mania, in line with what is stated in the scientific literature: an independent family transmission of manic episodes and major depressive episodes (Merikangas et al., 2014).

The fathers:



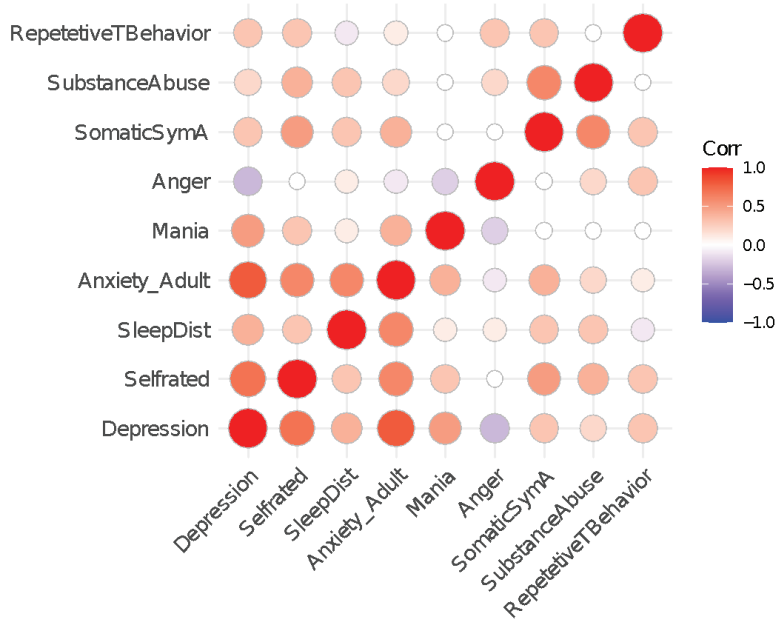
In fathers, the very strong correlation between depression and sleep returns, confirming that the recurrence of these two symptoms even in healthy subjects is in line with what has been demonstrated by the scientific literature (Krystal, 2012; Breslau et al., 1996; Ford et al., 1989; Chang et al.; 1997). Equally relevant is the correlation between sleep and anxiety, also confirmed in this case by the bi-directional relationship demonstrated by the literature. While generalized anxiety disorder is characterized by sleep changes, such as difficulty falling asleep and/or staying asleep, as outlined in the diagnostic criteria of the DSM-5 (DSM-5, 2013), on the other hand the literature has shown how sleep problems significantly increase the risk of developing anxiety disorders, with a doubled risk in subjects suffering from insomnia compared to healthy controls (Krystal et al., 2012; Breslau et al., 1996). Always positive correlations, however less strong but always relevant, found are: anxiety and somatic symptoms, sleep and somatic symptoms. The fathers in analysis as a whole are more symptomatic than the group of mothers and it is interesting to note how the sleep,

anxiety and anger clusters are transversal, with numerous positive correlations with the other symptomatic dimensions. In particular:

- Anger with sleep, anxiety, mania and substance use
- Anxiety with sleep, somatic symptoms and anger
- Sleep with depression, anxiety and somatic symptoms

Anxiety is the symptomatic dimension that correlates most with the others, in a very similar way to what was found in the corrplot of patients (corrplot 5). The transversal correlation of anger with the other clusters is in line with the initial histogram in which the important paternal prevalence compared to other family members was noted, with scores similar to those detected in patients. The dimension of mania is confirmed with negative or zero correlation with all the other clusters, such as depression and sleep (with the exception of anger), confirming what was stated in the mothers.

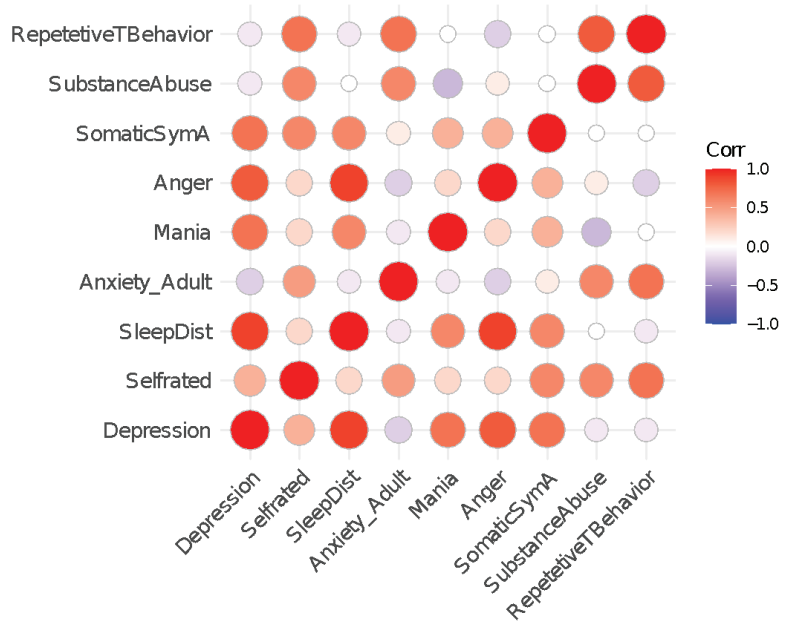
The brothers/sisters:



The correlation between depression and anxiety appears very interesting, as it is very strong and equivalent to the same found in patients. This recurrence of symptomatic clusters had been highlighted by other studies on siblings, which also highlighted how the family setting influenced their onset (Garber, 2006; Rapee et al., 2009; Wood et al., 2003). Anxiety is the cluster that in this group of relatives appears more transversal than the others, with numerous positive correlations. This anxiety behavior is similar to what was found in patients and fathers, as well as being in line with what was seen in the initial histogram. It therefore appears as the most recurrent dimension within families, with important correlations and aggregations between symptoms in different groups of individuals. On the other hand, the mothers did not show such recurrent anxiety in the symptomatological profiles as in the fathers, patients and brothers/sisters; however their mean values were not lower than those of the other kinship groups. This means that anxiety is still present in mothers, but without associating itself in a particular way with other symptomatic dimensions; so "anxious mothers"

will eventually appear only as such. Anger, on the other hand, does not correlate with the other clusters and has an inverse relationship with depression.

Children:



Corrplot of the patients' children appears very interesting, in which the symptoms and correlations between the different dimensions are strong and numerous. In fact, there is a widespread number of large and intense red circles. The correlations that appear most important are:

• Depression and sleep, but also mania, anger and somatic symptoms

• Anger and sleep

• Mania and sleep

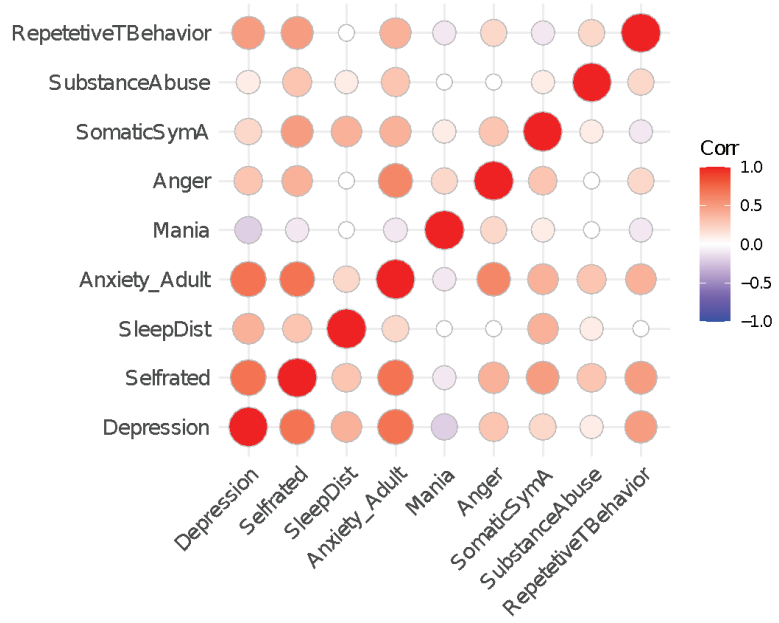
• Anxiety and obsessive-compulsive symptoms, as well as substance abuse

• Obsessive-Compulsive Symptoms and Substance Abuse

If on the one hand there are no symptom profiles that recur in a precise manner between children and patients (with the exception of anxiety and obsessive-compulsive symptoms), as in the cases of fathers and mothers with patients, on the other it emerges that there are many more strong correlations with respect to all other degrees of kinship.

It would therefore seem that, when the offspring is between a child and a sick parent, the symptomatology of the children is more important, as if the heritability of the clusters had a penetrance in the offspring proportional to the symptoms of the parents. On the other hand, the result does not differ from the expected one, given that the offspring of sick parents is characterized by a very high risk of developing numerous psychiatric pathologies (Ayano et al., 2020; Sanchez- Giastau et al., 201 5; Kung et al., 2019) and, consequently, the presence of a number of positive associations between symptom dimensions appears consistent. Overall, they are therefore more at risk of developing various forms of pathological symptoms than patients of healthy or "apparently healthy" parents.

The patients:



As previously explained, comorbidities are classic in psychiatric patients and the very definitions of the various pathologies use diagnostic criteria that range across the various symptomatic clusters, so the positive and strong correlations that unite the symptoms are in line with the expected. The positive correlations that patients share with groups of relatives are interesting:

• Depression and anxiety as in their brothers/sisters

• As in mothers: depression and obsessive-compulsive symptoms (SOC)

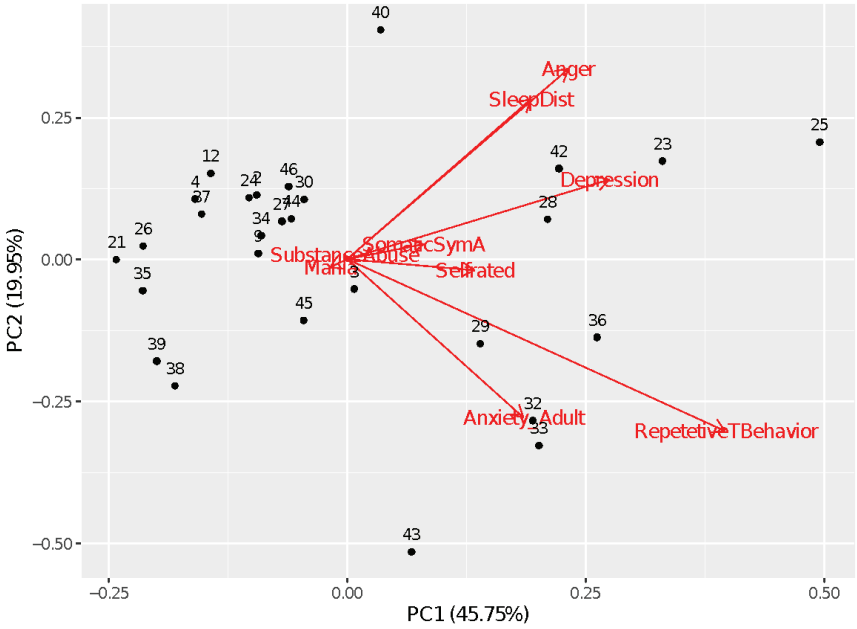
• As in fathers: anxiety and anger or somatic symptoms, sleep and somatic symptoms

• As in children and mothers: anxiety and obsessive-compulsive symptoms

Anxiety in patients is a cross-sectional and recurrent cluster that correlates positively with others, such as in fathers,

brothers/sisters and children. Mania, as in mothers, is an independent cluster with respect to the others, with predominantly zero correlations and negative correlations with depression, in accordance with the literature (Merikangas et al., 2014). Subsequently, the analysis of the main components was carried out and subsequently the respective bplot.

Mothers:



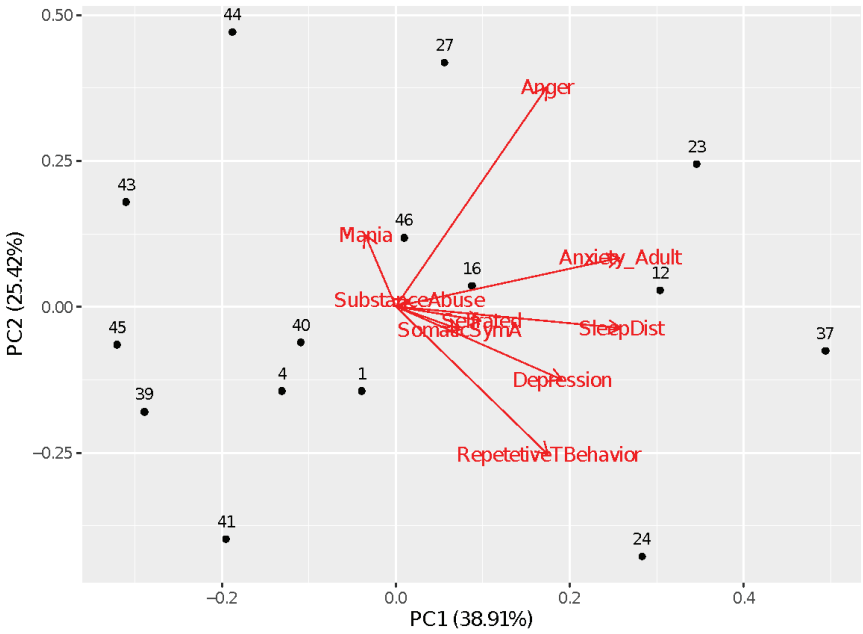
The first two principal components comprise approximately 65% of the variance of the sample.

Here, too, it can be noted as depression, anxiety and obsessive-compulsive symptoms have a greater weight in determining the symptom profile of mothers, thus confirming the correlation between depression and obsessive-compulsive symptoms, anxiety and obsessive-compulsive symptoms, depression and anxiety underlined by previous corrplots (and common with patients). However, it should be noted that the data obtained

require further confirmation through the enlargement of the sample, although it has been possible to obtain similar symptomatological profiles using different statistical techniques.

The small sample, in fact, causes a greater loss of data, as can be seen from the dots located to the right of the diagram. The next step will therefore be to expand the analysis sample to confirm the symptomatological profiles also highlighted by this mathematical-statistical model.

Fathers:



Fathers have two main components which together account for 75% of the sample variance.

It is possible to identify a positive influence on main component 1 (PC1) between sleep, obsessive-compulsive symptoms, depression and anxiety, even if the strength of the correlations

is also influenced by the dispersion of the sample, due to the narrowness of the sample.

There are also similarities with patients: a main symptom profile characterized by 3 dimensions joined by a positive correlation (sleep, anxiety and depression) and the absence of correlation between obsessive-compulsive symptoms and anger (90 ° angle), these data they were also highlighted in the corrplots.

The length of the anger vector confirms the importance of this cluster in the fathers group, its acute angle with anxiety and depression instead its positive correlation with the other 2 clusters, similarly to the patients. The data shown so far confirm what was found in the corrplot of the fathers; however there is also a difference due to the somatic symptoms, which previously appeared important, while in the bplot, being the vector close to zero, it appears as a symptomatological dimension of lesser importance.

All this could be related to the narrowness of the sample which leads to a greater loss of variance in the principal component analysis and, consequently, to a nullification of the importance of this cluster.

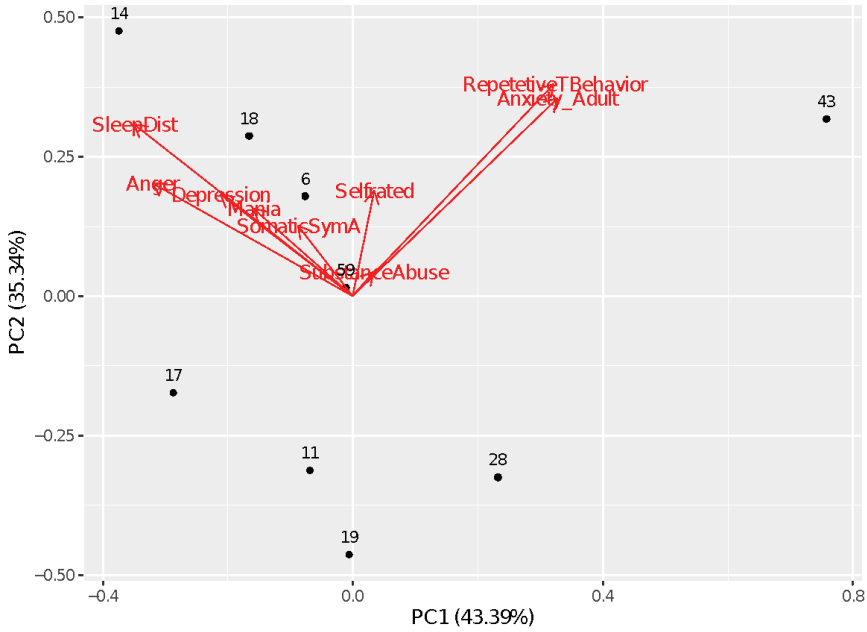
The brothers/sisters:

In brothers/sisters, the two main components comprise about 67% of the sample. It is interesting to see how in fact most of the subjects (PC1) are characterized by a strongly positive correlation between anxiety, depression and sleep.

On the other hand, PC2 is more influenced by obsessive-compulsive symptoms and anger clusters, a symptom profile that was not particularly representative in the first corrplot. Furthermore, the negative correlation of anger with most of the

other clusters is confirmed, as evidenced by the obtuse angle between the vectors.

Children:

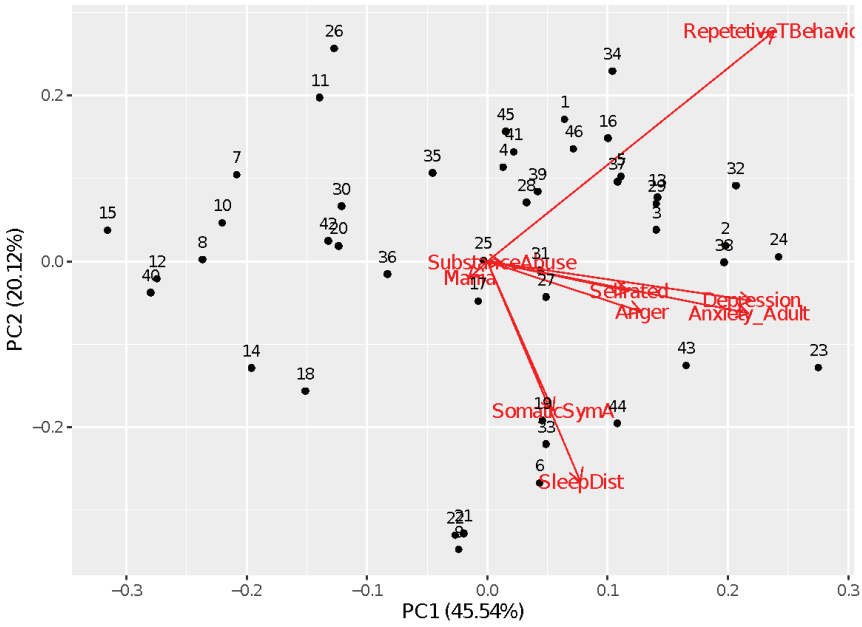


In the children, 78% of the sample is represented by the main components 1 and 2, so the dispersion of the variance is smaller than in the previous groups of individuals.

In the children we had seen how the correlations between clusters were more important than in all the other groups of relatives, thus hypothesizing that the descendants of sick patients could inherit a greater symptomatology from their parents than the other groups of relatives; from the bplot it is possible to outline two main symptomatological profiles: the first represented by subjects who tend to have problems with sleep, anger and depression, the second by subjects with the

presence of anxiety and obsessive-compulsive symptoms. The latter is also found in the bplot of patients.

The patients:



In patients the main component 1 represents 45.54% of the variability of the data obtained, while the main component 2 represents 20.12% (total> 65%) In the bplot of the patients we can see how PC1 is more influenced, through a positive correlation, by the variables: anxiety, depression, anger. These in turn are positively correlated with each other, confirming exactly what was verified with the initial patient corrplot : an association between depression, anxiety. and anger.

It can also be noted that another group of patients who fall into PC2 is instead more influenced by the sleep clusters and somatic symptoms; furthermore, there is an absence of correlation of the same with obsessive-compulsive symptoms (90 ° angle).

Once we analyzed the recurring clusters in family units and the symptomatological profiles that can be delineated in the different kinship groups, we proceeded with the Wilcoxon test to identify the symptomatic dimensions that deserve further study due to non-discardable heritability. The Wilcoxon test is a non-parametric test that is used for the verification of research hypotheses, allowing to identify those clusters worthy of further study.

Our diagnostic hypothesis was that there were symptomatic clusters which, in addition to recurring and aggregating in family nuclei, had a hereditary component of specific origin mainly from paternal or maternal or from both parents. The heritability of multiple clusters was also confirmed when the origin is from sick parents and the aggregation between siblings of some specific symptomatological dimensions. Our hypothesis was that symptom clusters were similar between parents and patients and between patients and offspring; for this reason, using a non-parametric test that recognized the answers between groups as indistinguishable, it would have been possible to identify those clusters worthy of further study due to non-discardable heritability, given the important overlap of the data obtained. In particular, we obtained the p values shown in the Table. The p value of the Wilcoxon test greater than 0.05 means that the test recognized the results of the comparison between patients and group of relatives as indistinguishable.

Specifically, sleep seems to have a prevalent heritable component of maternal origin, in line with what was found in the previous histogram (M = 0.29 vs M = 0.41 and Wilcoxon test p> 0.059); furthermore, anger similarly present between fathers (M = 0.383) and patients (M = 0.398) in the initial histogram demonstrated possible paternal heritability (Wilcoxon test p> 0.83). Anxiety also seems to be of paternal origin, in line with some of the literature already available which has highlighted how the children of anxious fathers develop

anxiety problems and marked emotional dysregulation in adolescence (Shenaar- Golan et al., 2021). Sibling agreement was also confirmed for clusters mania (M = 0.095 vs M = 0.14 and Wilcoxon test, p> 0.069) and anger (M = 0.33 vs M = 0.39 and Wilcoxon Test in p > 0.3).

Finally, a large heritability of clusters and symptomatology relevant to the group of patients' children was highlighted: sleep (p> 0.08), anxiety (p> 0.78), mania (p> 0.12) and anger (p> 0.06). This data is in line with numerous studies that have shown that the children of parents suffering from psychiatric disease have an increased risk of developing numerous psychiatric disorders, not only those inherited from the sick parent, but also a wide range of other disorders characterized from the same genetic susceptibility, as the children of parents with bipolarity who also have a risk of developing mood disorders, anxiety disorders, axis I disorders (Birmaher et al., 2009).

Questionnaires	Patients - Mothers	Patients - Fathers	Patients - Brothers	Patients - Children
Depression	0.00017321	0.0033306	1.56E-05	0.00034811
Sleep	**0.059989486**	0.01018772	0.031365	**0.08120427**
Anxiety	0.00998051	**0.10354131**	4.18E-05	**0.07898574**
Mania	4.49993E-05	0.01231092	**0.069633**	**0.12217806**

Anger	0.00072657 1	**0.8402388 8**	**0.30683 6**	**0.0697391 4**
Somatic symptoms	0.00029752 4	0.0271664 9	0.00091 9	0.0163097 7
Substance abuse	*0.03729192 7*	*0.8300393 5*	*0.12803 6*	*0.7813089 8*
SOC	0.00759010 6	**0.0611478 6**	0.00080 6	0.0175156 9

Note: the values in bold are not significant; they therefore indicate the indistinguishability of tests between kinship couples; the values in italics are recognized by the test as indistinguishable as they are always generated by values equal to or close to zero.

The *p value* of the Wilcoxon test greater than 0.05 means that the test recognized the results of the comparison between patients and group of relatives as indistinguishable.

Specifically, sleep seems to have a prevalent heritable component of maternal origin, in line with what was found in the previous histogram (M = 0.29 vs M = 0.41 and Wilcoxon test p> 0.059); furthermore, anger similarly present between fathers (M = 0.383) and patients (M = 0.398) in the initial histogram demonstrated possible paternal heritability (Wilcoxon test p> 0.83).

Anxiety also seems to be of paternal origin, in line with some of the literature already available which has highlighted how the children of anxious fathers develop anxiety problems and

marked emotional dysregulation in adolescence (Shenaar-Golan *et al.,* 2021). Sibling agreement was also confirmed for clusters mania (M = 0.095 vs M = 0.14 and Wilcoxon test, p> 0.069) and anger (M = 0.33 vs M = 0.39 and Wilcoxon Test in p > 0.3).

Finally, a large heritability of clusters and symptomatology relevant to the group of patients' children was highlighted: sleep (p> 0.08), anxiety (p> 0.78), mania (p> 0.12) and anger (p> 0.06). This data is in line with numerous studies that have shown that the children of parents suffering from psychiatric disease have an increased risk of developing numerous psychiatric disorders, not only those inherited from the sick parent, but also a wide range of other disorders characterized from the same genetic susceptibility, as the children of parents with bipolarity who also have a risk of developing mood disorders, anxiety disorders, axis I disorders (Birmaher *et al.,* 2009).

8. Discussion of the genetics results

BD is a very heterogeneous condition, due to tens of genetic causes still not explained at all (Nishioa M, Azuno AA et al, 2021) Over the years, GWAS analyses allowed to identify up to fifty genome-wide significant loci [Nowrouzi B, McIntyre RS, et al. 2016; Goes FS, Pirooznia M, et al. Exome sequencing of familial bipolar disorder. JAMA Psychiatry. 2016 ;Toma C, Shaw AD, et al. 2020; Ferreira MA, O' Donovan MC, et al. 2008), and WES analyses performed on families allowed to

characterize up to hundred candidate genes for BD, including de novo or segregating variants through generations (Moreno C,Laje G et al., 2007; Rao AR, Yourshaw M et al., 2017; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011). In the present study, we describe WES results identified in a new BD affected family, performed on three affected members, four healthy individuals and one member with some traits of psychiatric disorder (obsessive-compulsive disorder). The analysis allowed us to highlight missense heterozygous variants in ten relevant genes: PDE1C, HERC2, CHD7, MRC2, SPON1, ZNF92, TTC19, RBM12, CLN6, WDR62. In order to identify the variants involved in the phenotypic manifestation, we focused firstly on those shared by the affected subjects, and secondly on those in common also with the symptomatic individual with moderate anxiety and traits of obsessive-compulsive personality. Based on this, ZNF92 and CLN6 were selected as the candidate genes for the bipolar phenotype with a Digenic Inheritance (DI) pattern. DI is the simplest form of oligogenic inheritance, with patients manifesting the disease only when two non- allelic mutations on separated genes are co- inherited (Forstner AJ, Fischer SB, et al. 2020). Even if DI is not so much described in association with BD, a case of digenic neurodegenerative disease has been previously reported in subjects presenting also schizophrenia (Engelbrecht HR, Dalvie S, et al. 2020). Considering this scenario, it is possible to suppose that the milder phenotype shown by Patient V;4 may be due to the presence of the CLN6 variant only, as well as to the incomplete penetrance and variable expressivity of the disease, age-at-onset, environmental factors and so on. Only when combined with other variants, CLN6 may be involved in the complete BD phenotype.

Ceroid Lipofuscinosis Neuronal, 6 (CLN6, #606725) is a gene involved in a group of lysosomal storage disorders with a predominantly autosomal recessive inheritance (Dell'Osso L,

Armani A, et al. ,2002) Neuronal Ceroid Lipofuscinosis (NCL) caused by biallelic variants in the CLN6 gene usually presents in early to late childhood to juvenile, between 1.5 and 8 years of age, with slow motor degeneration, ataxia, loss of vision, epilepsy, and mental disabilities (Fagiolini A, Dell'Osso L, et al,1999). Early death by 12–15 years of age is reported [Fagiolini A, Dell'Osso L, et al,1999). Variants in CLN6 may also lead to a rare adult-onset form of NCL in which the symptoms present in adulthood, typically between 24 and 38 years old (Fagiolini A, Dell'Osso L, et al,1999; Cassano GB, Benvenuti A, et al.2009). The symptomatology includes ataxia, tremor and cognitive impairment around the age of 41 (Cassano GB, Benvenuti A, et al. 2009). Homozygous variants in CLN6 have been reported also in association with depression and anxiety, and some evidence supports the link with psychiatric problems and obsessive-compulsive behavior. (Fagiolini A, Dell'Osso L, et al,1999; Cassano GB, Benvenuti A, et al.2009). Although, to date, heterozygous CLN6 carriers do not seem to show any distinct clinical phenotype, nor most of times psychiatric conditions (Cassano GB, Mula M, et al. 2009; Busner J, Targum S.D.2007). We describe a subject with different phenotypic features and propose to pay particular attention to the possible implications that these variants may have in the development of "borderline" phenotypes, in the context of a multifactorial disease worsened by other concomitant variants in other genes such as that identified in ZNF92.

Zinc Finger protein 92 (ZNF92, #603974) belongs to the ZnF transcription factors family, which contains the Kruppel-associated box, or KRAB domain (Guy W. ECDEU Assessment Manual for Psychopharmacology (028 Clinical Global Impressions [CGI]). The ZNF family presents motifs in DNA- and RNA-binding proteins and appears to be involved in many cellular functions, such as DNA recognition, RNA packaging,

transcriptional activation, apoptosis regulation, protein folding and integration (Guy W. ECDEU Assessment Manual for Psychopharmacology (028 Clinical Global Impressions [CGI]). Increasing evidence suggests that genetic variants on ZNFs influence the susceptibility to psychiatric disease (Montgomery SA, Asberg M. 1979). Multiple evidence documented the involvement of ZNF804A, ZNF298, ZNF506, ZNF101 and ZNF592 in BD (Ferreira MA, O' Donovan MC, et al. 2008;Montgomery SA, Asberg M. 1979; Davidson J, Turnbull CD, et al.1986) changes in ZDHHC8 expression level have been suggested to play a significant role in modulating the development of schizophrenia, as well as many ZNF94 gene polymorphisms can increase the susceptibility to the same disease Davidson J, Turnbull CD, et al.1986). Here, we propose ZNF92 as a new gene candidate for the bipolar phenotype. This study exploited massive parallel sequencing with the aim to identify new disease genes in BD. Despite the small number of the evaluated subjects (8), it provides new molecular information regarding the genetics of BD, suggesting a DI pattern of inheritance. We cannot completely define the role that the observed variants may have in the onset of the psychiatric condition, since they're not already reported in the ClinVar database, nor in other specific BD databases, such as BipEx Consortium. This last browser in particular shows no evidence of association with BD for these two identified variants, in a dataset of 14210 cases and 14422 controls (https://bipex.broadinstitute.org/). Furthermore, there is no strong basis for the assumption that any of the shared variants would be fully penetrant, and those found in common with the healthy subjects may play a role in the family's illness.

For instance, it is well known that mutations in HERC2 produce clinical syndromes in which key neurodevelopmental events are altered, resulting in intellectual disability and other neurological disorders (Young RC, Biggs JT, et al. 1978). Genetic variation

in PDE1C associates with multiple measures of human cognitive function (Martino DJ, Samamé C, et al 2017). CHD7 is a critical player in the epigenetic regulation of neuronal differentiation, disruptions of which are believed to be associated with schizophrenia and autism (Li H, Durbin R.,2010). TTC19 mutations have been identified in a family with severe psychiatric manifestation (Poplin R, Ruano-Rubio V et al., 2017); finally, truncating mutations in RBM12 are associated with psychosis (Gordovez FJA, McMahon FJ, 2020). Based on specific prediction scores reported in DECIPHER database (Table 3).

Some of these genes (Ament SA, Szelinger S, et al.,2015; Goes FS, Pirooznia M, et al.2016,), such as CHD7 and HERC2, seem to be intolerant to loss-of-function variations. It is thus possible that variants in these genes may contribute to the full manifestation of the psychiatric disease, even if they are present also in unaffected individuals. We cannot even be sure that the subjects so far identified as "healthy" cannot manifest psychiatric disorders during their lifetime.

It could be interesting to calculate a polygenic risk score for each genotyped individual; unfortunately, our group of individuals is too small to define significant data that should be compared with an extended set of controls. There is no reported polygenic risk score for BD in the Polygenic Score Catalog (https://www.pgscatalog.org/), but even if it was present, it was not exportable to a different population.

Finally, none of the twelve identified SVs showed clinical correlation with BD; some of these have been classified as "Likely Pathogenic", but the absence of clear associations with psychiatric disorders does not help in their interpretation. This further study reinforces the role of the two genes, ZNF92 and CLN6, found by WES in the manifestation of the disease.

In conclusion, this study suggests new perspectives regarding the BD inheritance patterns, so far described as a multifactorial disease. Further analysis on larger cohorts should be performed to clarify the roles of the genes and the impact of the selected variants in the manifestation of the bipolar phenotype. If additional variants in ZNF92 and CLN6 are detected in psychiatric disorders, this work could support their correlation to BD, help in identifying subjects at risk for the development of the psychosis and improve their clinical management.

Figure 1. Family pedigree. WES analysis was performed on patients highlighted with*. The black arrow indicates the index subject, Patient 1(V;5). A square represents a male individual; a circle, a female individual; a rhombus: undetermined sex. Black symbols: subjects affected by BD symptomatology. Striped symbols: subjects affected by various disorders. In gray: subject with traits of obsessive- compulsive behavior. Genetic counseling was carried out on the family with internal code #17949.

Figure 2. Genes in common between affected and borderline family members. A square represents a male individual; a circle, a female individual. + and – refer to the presence or the absence, respectively, of the heterozygous variant in the indicated gene. The missense variant in ZNF92 is exclusively shared by BD affected subjects; the missense variant in CLN6, also by the «borderline» subject. Supplementary, Figure 1. Segregation of the genes excluded from the analysis in family members. A square represents a male individual; a circle, a female individual. + and – refer to the presence or the absence, respectively, of the heterozygous variant in the indicated gene. Because of the presence of these variants in healthy family members, PDE1C, CHD7, HERC2, MRC2 and TTC19 were excluded from our study. Supplementary, Figure 2. Comparison of the PlotResults from EXCAVATOR2 between Patient's V;5 WES data (2.A) and Patient's V;4 ones (2.B). In these plots,

signals observed around the “zero” are indicative of a normal condition; those moving toward “+1” indicate a duplication; the others moving toward “-1”, a deletion. The figure shows a comparison between data deriving from Patient V;5 (Figure 2.A) and data from Patient V;4 (Figure 2.B). WES data from Patient V;5 were excluded from the analysis of the shared SVs in order to prevent this sample leading to biased results: the sample has indeed passed the quality control required by the EXCAVATOR2 pipeline, but it showed a distortion in the results that could lead to wrong interpretations. The example shown in Figure 2 is related to chromosome 2, but it has to be extended to all the chromosomes.

Table 1. Variants in common among BD subjects from WES analysis.

Gene symbol (#OMIM)	Transcript	HGVS genomic	HGVS_coding	HGVS_protein	Variant Effect	Classification (ClinVar / InterVar)	dbSNP	gnomAD	Splicing effect	CADD phred
PDE1C (#602987)	Chr 7, NM_001191057.4	g.31904581 A>G	c.725A>G	p.Tyr242Cys	Missense	Unreported/ Uncertain	n.a.	n.a.	n.a.	26.5
HERC2 (#605837)	Chr15, NM_004667.6	g.28386774 G>A	c.11819G>A	p.Arg3940Gln	Missense	Unreported/ Uncertain	rs37028963	0.0012%	n.a.	27.6
CHD7 (#608892)	Chr 8, NM_017780.4	g.61735198G>C	c.3094G>C	p.Glu1032Gln	Missense	Unreported/ Uncertain	rs1355615827	0.00040%	n.a.	23
MRC2 (#612264)	Chr 17, NM_006039.5	g.60769687 C>T	c.4315C>T	p.Arg1439Cys	Missense	Unreported/ Uncertain	rs776632141	0.0013%	n.a.	29.2

SPON1 (#604989)	Chr 11, NM_006108.4	g.13963049 C>A	c.145C>A	p.Arg49Ser	Missense	n.a.	n.a.	n.a.	n.a.	1.98	
ZNF92 (#603974)	Chr 7, NM_152626.4	g.64864204 G>A	c.1177G>A	p.Gly393Arg	Missense	Unreported/ Uncertain	rs76403 4193	0.002 4%	n.a.	15.95	
		g.64864203G >T	c.1176G>T	p.Thr392Thr	Synonymous	Unreported/ Likely Benign	rs56601 2007	0.000 37%	n.a.	/	
TTC19 (#613814)	Chr 17, NM_017775.4	g.15928441G >T	c.787G>T	p.Ala263Ser	Missense	Uncertain	rs14189 2030	0.029 %	It creates an alternative splicing site at 3' in exon 8 for two predictive in silico tools (SpliceSiteFinder-like; MaxEntScan)		27

RBM12 (#607179)	Chr 20, NM_006047.6	g.34242641 A>G	c.604A>G	p.Met202Val	Missense	Unreported/ Likely Benign	rs146171962	0.004 0%	It creates an alternative splicing site at 3' in exon 3 for two predictive in silico tools (SpliceSiteFinder-like; MaxEntScan)	13.23

CLN6 (#606725)	Chr 15, NM_017882.3	g.68504183 C>T	c.316C>T	p.Arg106Cys	Missense	Uncertain	rs202226970	0.006 7%	n.a.	24.3
WDR62 (#606725)	Chr 19, NM_001083961.2	g.36595687 G>A	c.4329G>A	p.Gln1443Gln	Missense	Uncertain/ Likely Benign	rs771131709	0.002 8%	n.a.	0.10

In "Classification", a unique output correlates to both ClinVar and InterVar interpretations. CADDphred was calculated for missense variants only (Wellcome Trust Case Control Consortium, 2007). HGVS: Human Genome Variation Society (https://www.hgvs.org). Chr: chromosome; n.a.= not available.

Table 2. Analysis of SVs from Excavator 2 and AnnotSV.

AnnotSV ID/ SV type	SV Chr	SV Start	SV End	SV size (Kb)	Gene Name	Transcript	GnomAD_ID	AnnotSV ranking	Association to Bipolar Phenotypes
1_2181795_2185513_DEL	1	2181795	2185513	3.71 Kb	***SKI***	NM_003036	gnomAD_v2_DEL_1_331	4	No
2_168811559_168855462_DEL	2	168811559	168855462	43.9 Kb	***STK39***	NM_013233	n.a.		No
3_6786589_6863041_DEL	3	6786589	6863041	76.4 Kb	***GRM7-AS3***	NR_110123	gnomAD_v2_DEL_3_34441	2	No
4_56436502_56446416_DEL	4	56436502	56446416	9.9 Kb	***PDCL2***	NM_152401	n.a.	2	No
7_5121870_5182726_DEL	7	5121870	5182726	60.8 kb	***ZNF890P***	NR_034163	gnomAD_v2_DEL_7_90969;gnomAD_v2_DEL_7_90970;gnomAD_v2_DEL_7_90981	2	No

8_144111099_144116293_DEL	8	144111099	144116293	5.19 Kb	**No gene**	-	gnomAD_v2_DEL_8_114898	2	No
10_133456235_133465931_DEL	10	133456235	133465931	9.96 Kb	**No gene**	-	n.a.	2	No
11_117833717_117838299_DEL	11	117833717	117838299	4.58 Kb	**No gene**	-	gnomAD_v2_DEL_11_144738	2	No
12_113206065_113246063_DEL	12	113206065	113246063	39.9 Kb	***RPH3A***	NM_001347952	n.a.	2	No
17_81444368_81463817_DEL	17	81444368	81463817	19 Kb	**No gene**	-	-	2	No
19_4719845_4770779_DEL	19	4719845	4770779	50.9 Kb	***DPP9***	NM_139159	n.a.	2	No
19_4719845_4770779_DEL	19	4719845	4770779	50.9 Kb	***MIR7-3***	NR_029607	n.a.	2	No
19_4719845_4770779_DEL	19	4719845	4770779	50.9 Kb	***MIR7-3HG***		n.a.	2	No
19_15238361_15244668_DEL	19	15238361	15244668	6.3 Kb	**No gene**	-	gnomAD_v2_DEL_19_200694	2	No

AnnotSv ranking scores span from 1-5 according to the AMCG technical standards for the interpretation of constitutional copy number. Pathogenic SVs: class 5; Likely Pathogenic: class 4; Variant of Uncertain Significance: class 3; Likely Benign: class 2; Benign: class 1. The analysis was carried out among Patients IV;10, IV;11, V;4 (Figure 1). Probably due to the low coverage regarding WES data from Patient V,5 analysis could not be ruled out on it. N.a: not available.

Table 3. Predictive Scores for each gene identified from WES analysis according to the DECIPHER database.

Genes	pLI	%HI
PDE1C	0.00	22.08
HERC2	1.00	50.09
CHD7	1.00	2.39
MRC2	0.67	43.17
SPON1	-	-
ZNF92	0.00	92.19
TTC19	0.00	51.13
RBM12	0.00	14.22
CLN6	0.00	41.58
WDR62	0.00	51.07

PLI: PROBABILITY OF LOSS-OF-FUNCTION INTOLERANCE. THE PLI SCORE IS THE PROBABILITY THAT A GIVEN GENE FALLS INTO THE HAPLOINSUFFICIENT CATEGORY, AND THEREFORE IS EXTREMELY INTOLERANT OF LOSS-OF-

function variation. Genes with high pLI scores (pLI ≥ 0.9) are extremely LOF intolerant, whereby genes with low pLI scores (pLI ≤ 0.1) are LOF tolerant (Poplin R, Ruano-Rubio V et al., 2017). %HI: Probability of being a haploinsufficient gene. A haploinsufficient gene is one which requires two functional copies to produce the standard phenotype. The probability that a gene is haploinsufficient based on a set of functional, evolutionary, and network properties of the gene. High ranks (e.g. 0-10%) indicate a gene is more likely to exhibit haploinsufficiency, low ranks (e.g. 90-100%) indicate a gene is more likely to not exhibit haploinsufficiency (Gordovez FJA, McMahon FJ, 2020).

Based on these scores, among these genes *HERC2* and *CHD7* seem to be intolerant to loss-of-function variations; the rest of the genes, including *CLN6* and *ZNF92,* seem to be more tolerant to variations. It is possible that *HERC2* and *CHD7*, although present in healthy individuals, could contribute to a lesser extent to the observed phenotype.

8.1 Discussion of the transmissibility of symptomatic

8.2 Dimensions

Among the primary objectives of the study was the identification of the most suitable collection methodology to expand this pilot study and carry out new ones; for this reason, the choice was made to start two collection arms: the direct method and the indirect method. In the first arm the patients and relatives answered the questionnaires directly, in the second the interviews with the relatives were obtained indirectly through the patients themselves. The direct method has shown numerous limitations, partly due to the frequent reluctance of relatives in answering the questionnaires, partly due to the organizational difficulties secondary to the current epidemiological context. In the second arm of the study, the interviews with the relatives were collected indirectly, by interviewing the patients about their symptoms. This collection method made it possible to overcome the limitations linked to reticence in answering the questionnaires, effectively increasing the number of relatives with known symptoms.

Both methods have been widely used in numerous studies on families based on different needs (Low et al., 2007), so much so that the indirect collection method is known to have a tendency to underestimate the symptoms of relatives and, of consequently, to have a lower sensitivity (Smoller et al., 2003).

Despite this last limitation, however, the current epidemiological context, in association with the other difficulties due to the "stigma" and the false perceptions or beliefs that still hover around psychiatric disorders, make the second method of collection easier, especially for conducting single-center studies which therefore do not have the possibility of accessing thousands of patients and losing large percentages of relatives and/or patients to obtain relevant databases. Furthermore, the psychometric interviews used, in association with the information extrapolated from the medical records, make the conduct of indirect interviews quite easy, managing with the collaboration of the patient to fill in the psychometric investigations with sufficient precision.

Statistical surveys of a non-parametric type have made it possible to find a series of new and important data, however, resulting in a complex interpretation. From the surveys carried out, it was possible to detect the family aggregation of the anxiety cluster which proved to be widespread in all degrees of kinship, with normalized score averages for fathers, mothers and children of 0.4 and 0.26 for siblings, against an average of 0.56 for patients.

Proceeding with the Wilcoxon test, specific heritability was noted between fathers and patients ($p > 0.1$) and between patients and offspring ($p > 0.078$). Anxiety disorders are among the most common disorders in the adult and childhood population, so much so that about 10% of children in childhood manifest the diagnostic criteria for an anxiety spectrum disorder (Copeland et al., 2014; Kerns et al., 2017). Scientific research has highlighted how there is a specificity in the heritability of anxiety disorders between parents and children (Lieb et al., 2000; Merikangas et al., 2003; Biederman et al., 2006); moreover, even in the absence of a specific diagnosis or a symptomatology such as to fall within a spectrum pathology, the presence of parents characterized by anxious symptoms

increases the probability of observing the same symptomatology in the offspring (Adolph et al., 2021), with the anxious temperament that notoriously has a significant family specificity (Iorfino et al., 2021).

The sleep cluster showed significant mean values in all kinship groups, higher in the mothers and lower than the values in the patients. In the mothers' corrplot, the sleep cluster showed the greatest correlations with other symptomatic dimensions: depression and anger in particular, also confirmed with the subsequent bplot.

Furthermore, with the Wilcoxon test, sleep would appear to be a character with maternal inheritance. The risks of psychiatric disorders in individuals with sleep disorders are less investigated in the literature than the risks of sleep disorders in individuals with psychiatric disorders; however, as early as the 1980s, it had been shown that the correlation between these two factors was in fact bidirectional (Krystal, 2012). Individuals suffering from insomnia or hypersomnia have an approximately 10 times greater risk of developing depressive disorder than healthy controls without sleep disturbances (Breslau et al., 1996; Ford et al., 1989). Longitudinal studies have highlighted how chronic insomnia over time leads to a concrete increase in the risk of developing major depressive disorder (Ford et al., 1989; Chang et al. 1997).

Anger, on the other hand, showed coincidental values between fathers and patients and a widespread association with other clusters in the related corrplot. The literature has shown that anger is a prevalent temperament in the male sex and in some of the psychiatric pathologies analyzed, such as bipolar disorder (Okuda et al.; 2015). Anger seems to have a more solid hypothesis of non-discardable paternal inheritability, as does anxiety. The paternal heritability of anger, however, would seem to be in contrast with some of the available literature: if,

as mentioned, anger is a typically male trait that confirms the average values of anger of fathers similar to those of patients (subjects with a known diagnosis), from another point of view some studies show how its onset in children is influenced more by maternal anger than by paternal anger (Plickert & Pals et al. 2019). This confirms the need to enlarge the sample on a large scale to confirm or disprove the heritability of the clusters found in this study.

Among the most important symptomatologic profiles we found the aggregation of depression and anxiety in mothers and patients. It should be noted that the recurrence of anxiety and depression in offspring is greatly influenced by family dynamics, such as conflicts between parents, less loving relationships with their children, excessive involvement or aversion, fewer concessions and greater monitoring of children (Yap et al., 2014).

Consequently, the recurrence of these two clusters, present in both mothers and patients, could have as an explanation the social setting of households in the Italian population, in which mothers tend to be the most present parental figure, who spends more hours with the offspring, thus determining a greater environmental influence on the growth of children.

The concordance shown between siblings in the symptomatologic dimension of mania has a solid scientific basis that supports the data found through studies on siblings, families and GWAS (Bertelsen et al., 1977; Kendler et al., 1993; Cardo et al., 1999).

Finally, the last most relevant data among those found in the study is the major symptomatology and aggregation between clusters in the children of the patients. In particular, without making a distinction in the sex of the parent (choice also due to the narrowness of the sample of children), we found an inheritance of sleep, anxiety, mania and anger. A recent review

(Lejdesdorff et al., 2017) estimated that about 15-23% of the infant population lives with a parent suffering from psychiatric or substance abuse disorder and that this has a decisive impact on the onset of psychiatric pathologies and relevant symptoms in the offspring.

Numerous studies have shown, in fact, that the children of parents affected by psychiatric disorders have a significant risk of developing a wide range of psychiatric pathologies, which do not necessarily agree with those of their parents (Birmaher et al., 2009). The main limitations of the study are due to the narrowness of the sample; the estimates obtained on the specific heritability of the clusters would in fact require large-scale samples to be confirmed. Furthermore, in this kind of studies, source unfortunately, the selection of a large, homogeneous and representative sample of the population is very complex. Lastly, both methods of collection are subject to alterations, more or less significant, in the compilation: on the one hand the responses to direct interviews can undergo voluntary or involuntary alterations by the subject under analysis, on the other indirect interviews they inevitably undergo a distortion or filter of reality due to the subjectivity of the informant. However, the study managed to bring out relevant information, paving the way for more in-depth and targeted investigations; the symptomatic clusters, their co-presence in individuals, their family aggregation and the genetic heritability that constitute the etiological basis are not only important in the pathological field when the subjects under analysis have a diagnosis of psychiatric pathology, but demonstrate their own peculiarities even in healthy subjects who present under-threshold or over-threshold nuances, even without falling within a diagnosis of certainty. In light of all this, the basis exists to affirm that future investigations can move towards the demonstration of a genetic heritability also of symptomatologic clusters.

9. CONCLUSIONS

Finally, the last most relevant data among those found in the study is the major symptomatology and aggregation between clusters in the children of the patients. In particular, without making a distinction in the sex of the parent (choice also due to the narrowness of the sample of children), we found an inheritance of sleep, anxiety, mania and anger. Among the most important symptomatologic profiles we found the aggregation of depression and anxiety in mothers and patients. It should be noted that the recurrence of anxiety and depression in offspring is greatly influenced by family dynamics, such as conflicts between parents, less loving relationships with their children, excessive involvement or aversion, fewer concessions and greater monitoring of children (Yap et al., 2014).The basis exists to affirm that future investigations can move towards the demonstration of a genetic heritability also of symptomatologic clusters. In conclusion, this study suggests new perspectives regarding the BD inheritance patterns, so far described as a multifactorial disease. Further analysis on larger cohorts should be performed to clarify the roles of the genes and the impact of the selected variants in the manifestation of the bipolar phenotype. If additional variants in ZNF92 and CLN6 are detected in psychiatric disorders, this work could support their correlation to BD, help in identifying subjects at risk for the development of the psychosis and improve their clinical management.

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Ethical Statement: The patients gave their informed consent for diagnostics testing and research studies. Studies were performed and samples were obtained in accordance with the Helsinki Declaration of 1964, as revised in October 2013 in Fortaleza, Brazil.

Data Availability Statement: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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