University of Insubria

Department of Medicine and Surgery

Doctoral Research in Clinical and Experimental Medicine and *Medical Humanities*

XXXII Cycle

Coordinator: Professor Marco Cosentino

Doctoral Thesis

TOWARDS PERSONALIZED MEDICINE: THE ROLE OF PHARMACOGENETICS IN THE TREATMENT OF BIPOLAR DISORDER

Scientific areas: 05 – Biological Sciences, 06 – Medical Sciences, 11– Historical, Philosophical, Educational and Psychological Sciences 14 – Political and Social Sciences

Academic fields and disciplines list: BIO/09, BIO/11, BIO/13, BIO/14, M-FIL/02, M-FIL/03, MED/02, MED/04, MED/09, MED/10, MED/11, MED/15, MED/17, MED/25, MED/26, MED/39, MED/41, MED/43, MED/44, SPS/01

PhD student:
Marta Ielmini, MD

Supervisor:
Professor Camilla Callegari

Academic year 2016-2020

To
Greta & Sveva

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Abstract

Background

Bipolar disorder (BD) is a severe psychiatric disease characterized by mood swings between mania and depression, with a life-time prevalence of approximately 2.4%. This is a chronic disease with affective episodes that may produce significant personal distress, social dysfunction and devastating effects on sufferers' psychological, professional, and social welfare. Although several effective treatments have been already proposed, patients suffering from BD frequently present several problems due to drug-resistance and drug-drug interactions, rapid-cycling, and cognitive decline.

Pharmacogenetic tests (PGTs) have been proposed as a method to determine the most efficacious treatment with the lowest side effects, recognizing individual variability in genetics as a key component of drug response. Nevertheless, PGTs have been occasionally used in clinical practice up to now and their clinical utility is an empirical question that has remained largely untested.

This research project aims to evaluate the utility of PGT in routine clinical practice for the treatment of BD in terms of efficacy and cost saving; evaluate the attitude of psychiatrists towards the PGT use in clinical practice; review the literature dealing with lithium, the most common mood stabilizer used, to find any correlation among genes and tolerability/efficacy.

Materials and methods

The first phase of study evaluated 30 patients affected by BD type I or II (according to Diagnostic and Statistical Manual of Mental Disorders, version 5) who underwent the PGT Neurofarmagen® (AB-BIOTICS SA, Barcelona, Spain) between March 2016 and March 2017.

Second phase compares 12 months before the execution of the PGT versus 12 months after, in terms of number and days of hospitalization and accesses to emergency services, in a sample of 30 patients affected by bipolar disorder. Secondarily, it gives an economic value to the data based on the diagnosis-related group (DRG). The third part of the study evaluates psychiatrists' attitude towards the use of PGx into clinical practice employing a five items survey to assess the opinions of

psychiatrists on the benefit of pharmacogenetic data; the last phase of study reviews the literature about lithium salts. Computerized searches of PubMed and Embase databases, for studies published between 1998 and January 2018, was performed: 1162 studies were identified but only 37 relevant papers were selected for detailed review.

Results

In phase I At T0, only 4 patients (13%) had an optimal therapy in line with the PGT suggestions. At 3-month follow-up, 13 patients (40%) had received a change of therapy consistent to the test, showing a significant statistical improvement in the Clinical Global Impression item Severity (CGI-S) score over time compared to those not having changes consistent with the test. Regarding AEs, at baseline 9 out of 10 (90%) of the patients who received a therapy modification according to the test presented AEs, and a significant within-group reduction was observed after 3 months ($p = 0.031$). At 12 months follow-up (T3) 93% of patients (n=28) received a therapy concordant to the test; the others (7%, n=2) had a therapy discordant to the test

Phase II showed statistically significant differences in all the comparisons ($p < 0.0001$). Important cost saving emerged after the use of PGT (€148,920 the first year versus €39,048 the following year).

Phase III showed a positive attitude of the 45 psychiatrists interviewed towards the use of PGX. All respondents 100% (N = 45) believe that pharmacogenetics can help specialists and patients in making decisions about psychopharmacological treatment. All respondents 100% (N = 45) believe that pharmacogenetics can help in setting up therapy, particularly regarding drug interactions. 82% (N = 37) of respondents believe that pharmacogenetic test could become a routine tool in clinical practice. There were no statistically significant differences between those who already had experience with PGTs in clinical practice and those who did not.

Phase IV showed that despite some interesting preliminary findings, the pharmacogenetics of Lithium and the development of a specific pharmacogenetics test in bipolar disorder appears to be a field still

in its infancy, even though the advent of genome-wide association studies holds particular promise for future studies, which should include larger samples.

Conclusion

Despite the small sample size and lack of a control group this study shows promising data about the usefulness of PGT to support clinicians in reaching a more effective and tolerated treatment in the routine approach and the potential role of PGT in cost saving for the treatment of bipolar disorder. Also the attitude of clinicians seemed to be positive towards the use of PGT as a helpful tool into clinical practice. To confirm this result, larger and clinical trials are needed.

Chapter 1: Introduction

It is well established that interpersonal variability in drug response depends on different factors such as diagnostic accuracy, drug–drug interactions, renal and hepatic function, medical and psychiatric comorbidity. Additionally, the drug response can be influenced by genetic determined pharmacokinetic and pharmacodynamic variability, (Mzarek, 2014) and it is known that genetic factors count for 20–40% of differences in individual drug metabolism and response (Saldivar et al, 2016). Pharmacogenetics is defined as *"the study of how genes affect a person's response to drugs*. *It combines pharmacology (the science of drugs) and genomics (the study of genes and their functions) to develop effective, safe medications and doses that will be tailored to a person's genetic makeup*"

Pharmacogenetic tests (PGTs) have been proposed as a method to expedite the process of determining the most efficacious treatment with the lowest side effects, recognizing individual variability in genetics as a key component of drug response (Knisely et al, 2014). Although the Human Genome Project predicted pharmacogenomics would have become the approach for predicting drug responsiveness in the standard practice for many disorders and drugs, (Collins & McKusick, 2001) PGTs have been occasionally used in clinical practice until now. The clinical utility of PGT is an empirical question that has remained largely untested. Pharmacogenetics testing is currently available to guide and support better treatment decisions for some patients. Relevant examples of the impact of pharmacogenomics include the use of HLA-B*1502 genotyping before the initiation of carbamazepine therapy, to prevent Steven Johnson syndrome and toxic epidermal necrolysis; (Dunnenberger et al, 2015) the use of HLA-B*5701 genotyping, prior to initiation of abacavir therapy to avoid serious hypersensitivity syndrome; (John et al, 2017) the use of CYP2C19 genotyping before the initiation of clopidogrel therapy to reduce risk of cardiovascular events; (Alrashid et al, 2016) use of VKORC1/CYP2C9 genotyping prior to initiation of warfarin therapy to determine the appropriate initial dose of warfarin and prevent hemorrhagic incidents. The impact and clinical utility of PGTs is exemplified and further strengthened by the requirements of the United States' Food and Drug Administration, to include human genomic information for over 100 medications labels recommending patient-specific dosing strategies (Anon, 2018). Despite the availability of PGTs, their uptake into clinical patient care appears slow. Previous studies have identified major barriers to healthcare providers' acceptance of pharmacogenetics testing into their practice. These barriers included lack of knowledge, awareness and confidence among healthcare professionals in implementing pharmacogenetic information within patient care (Peterson et al, 2016). Evidences for the clinical benefits of pharmacogenetics testing are needed.

This study aims to evaluate the utility of PGTs in the treatment of bipolar disorder in routine clinical practice. Before getting to the heart of the research I would like to offer a brief excursus on pharmacogenetics and on bipolar disorder.

1.1. History of psychopharmacology

"The desire for an immediate paradise is as old as man himself. Throughout the ages, men have sought artificial means to improve their condition and drugs have played a prominent role in this research"

(J. P. Smith, *The false illusion*, in "U. S. Food and Drugs Administration papers", 1967).

In Greece and throughout the Mediterranean the temples of health spread even before Hippocrates: in Epidaurus, Smyrna, Ephesus, Pergamum and also in the Tiber island of Rome, psychiatric patients were treated with baths, purges, music, suggestive psychotherapy. The patients were guided by a priest who established the cure interpreting the patient's desires. Dealing with ethnopharmacology, in the western culture many examples are alcoholic beverages, in the Middle East many preparations are derived from Cannabis sativa, opium in the Far East, peyote in northern Mexico. These substances are often used to keep in touch with perception.

Men have always been fascinated by the role of substances on the psyche, particularly for mystical-religious or for therapeutic purposes. Opium, for example, was used in antiquity for its analgesic properties. Rauwolfia serpentine seems to have been known for its therapeutic properties in some mental illnesses in Indian folk medicine and perhaps also to Dioscorides, a military surgeon of Nero's period, who treated mania with a snake-shaped root.

The year 1950 could be used to date the birth of the modern psychopharmacology. The first therapeutic successes with reserpine and clorpromazine could be dated back to this period.

The synthesis of chlorpromazine in 1950 marks the beginning of modern psychopharmacology. Chlorpromazine also inaugurated the most remarkable decade in the history of psychopharmacology. During this decade, the pharmaceutical industry synthesized and marketed compounds defined the

future classes of psychotropic drugs. The list includes the antipsychotic drugs (chlorpromazine), anxiolytic drugs (meprobamate in 1950, chlordiazepoxide in 1955), monoamine oxidase inhibitor (MAOI) antidepressants (iproniazid in 1951), and the tricyclic antidepressants (imipramine in 1951) (Marder & Braslow, 2019), as shown in Table 1.1

Table 1.1 Psychopharmacology from 1949 to 1960

1949	John Cade	Antimaniacal effects of lithium salts
1950	Paul Charpentier	Chlorpromazine synthesis
1951	Sigwald & Bouttier	First treatment with chlorpromazine
1952	Hamon et al.	First publication on the effectiveness of chlorpromazine
1952	Jean Delay & Pierre Deniker	First systematic evaluation of chlorpromazine in the clinical practice
1952	Selikoff	Antidepressant properties of Isoniazide
1954	Nathan Kline	Reserpine
1954	Frank Berger	Meprobamate
1955	Roland Kuhn	Imipramine
1955	Jean Delay	Introduction of the term neuroleptic
1956	Frank Ayd	Identification of chlorpromazine dystonia
1957	Nathan Kline	IMAO
1957	Lowell Randall	Behavioral effects of benzodiazepines
1958	P.V. Petersen	Tioxanteni
1958	Paul Janssen	Haloperidol
1959	Sigwald et al.	Tardive dyskinesia
1959		Clozapine
1960	Cohen & Tobin	Anxiolytic effects of chlordiazepoxide

In just ten years, the foundations of modern psychopharmacological knowledge had been laid; the following years have refined the wealth of knowledge and expanded the range of available molecules, but have so far not equaled a paradigmatic leap in the scope of that described in table 1.1.

1.2. The history and definition of pharmacogenetics and pharmacogenomics

Pharmacogenetics and Pharmacogenomics explores the role of genetic variations in the effects of drugs and xenobiotic substances on the body and the body's reactions to drugs

Vogel, in 1959, introduced the term "pharmacogenetics" to define the study of small genetic variations roles relevant to a drugs' disposition or effect. At the moment, the history of pharmacogenetics dates back to 510 B.C. when Pythagoras noted that an ingestion of fava beans gave a potential fatal reaction in some individuals (Nebert, 1999;). The history of Pharmacogenetic is shown in Table 1.2.

Table 1.2 History of pharmacogenetics and pharmacogenomics

510 bc	*Pythagoras*	*Recognition of the dangers of ingesting fava beans, later characterized due to deficiency of G6PD*
1866	Mendel	Establishment of the rules of heredity
1932	Snyder	Characterization of the 'phenylthiourea nontaster' as an autosomal recessive trait
1956	Carson *et al.*	Discovery of glucose-6-phosphate dehydrogenase deficiency
1957	Motulsky	Further refined the concept that inherited defects of metabolism may explain individual differences in drug response
1957	Kalow & Genest	Characterization of serum cholinesterase deficiency
1957	Vogel	Coined the term pharmacogenetics
1960	Price Evans	Characterization of acetylator polymorphism
1962	Kalow	Publication of '*Pharmacogenetics – Heredity and the Response to Drugs*'
1988	Gonzalez *et al.*	Characterization of the genetic defect in debrisoquine hydroxylase, later termed *CYP2D6*
1988–2000	Various	Identification of specific polymorphisms in various phase I and phase II drug metabolizing enzymes, and latterly in drug transporters
2000	Public-private partnership	Completion of the first draft of the human genome
2000	The International SNP Map Working Group	Completion of map of human genome sequence variation containing 1.42 million SNPs

Pharmacogenomics involves the study of a larger collection of genomic factors that contribute to the individual variability of drug responses. This may include genes that regulate phase I oxidative drug metabolism phase II drug conjugation enzymes, drug transporter proteins and drug targets enzymes or receptors. In the past, the variations observed for most drugs in terms of utilization and adverse events were limited to only a few SNPs within one gene (monogenic traits) and “pharmacogenetics” may be the preferred term. However, for warfarin and clopidogrel, multiple SNPs within several genes (polygenic traits) have been determined to influence the drug’s therapeutic effects, and therefore the term “pharmacogenomics” may be preferred. Despite these definitions, these terms are often used interchangeably and the acronym “PGx” is commonly used (Wu & Kiang Teck, 2011).

1.3. The pharmacogenetic approach in evaluating pharmacological response

Pharmacogenetics is the science that deals with hereditary genetic factors responsible of different drug responses between people. For many years it has been possible to ascertain that, in addition to environmental and physiological factors, genetic factors also play a fundamental role in the response to pharmacological treatments, in some cases responsible of 95% of the variability (Vega et al, 2012). The "Genome Project" has defined that the DNA is identical in all individuals for 99.9%, and that the remaining 0.1% is responsible for the differences among individuals. Possible differences in the DNA sequences between two random individuals in the population can be estimated at 2-3 million. The minimum variation that can generate a polymorphism is the change of a DNA base. Polymorphisms for single nucleotides, the so-called SNPs, are a systematic finding extended to the whole genome, with the accumulation of sequencing data (Klug, 2007). More than two milions of these SNPs are already known and there are very detailed SNPs maps of how SNPs are positioned in our DNA.
When the change in a sequence occurs within the coding region of the gene, it can lead to the synthesis of abnormal proteins. While when it occurs in a regulation sequence, there will be a variation in the

amount of protein produced with consequent imbalances in the function, very often defective. In this way, alterations in the DNA sequences can cause the loss or modification of the normal activity of a gene that directs the synthesis of a protein directly or indirectly involved in the pathological process sensitive to the drug, in the mechanism of action, in the metabolism or in the transport of the same drug.

The difference in the pharmacological response can be exercised quantitatively or qualitatively, affecting different phases: a) absorption of the drug; b) metabolism, transport and elimination; c) characteristics of the target; d) adverse reactions.

Pharmacokinetics is the science that quantitatively studies drug absorption, distribution, metabolism and elimination (ADME).

ADME genes are grouped into four categories:

- phase I and phase II metabolism enzymes, responsible respectively for the introduction of functional groups on the molecule and conjugation of the metabolite with a second endogenous molecule in order to make the drug more hydrophilic and ensure its elimination;
- transports proteins, responsible for the absorption and excretion of drugs outside and inside the cell (including the ABC family);
- serological binding proteins responsible for distribution;
- transcription factors that can alter the expression of the other ADME genes or influence their biochemistry.

The first category above mentioned, which includes the superfamily of cytochrome P450 enzymes, is the most studied (Grossman, 2009). Mutations in these genes have been repeatedly associated with the in vitro and in vivo pharmacokinetic properties of various drugs and their correlation with clinical outcomes is still a matter of great interest. The opportunity of a possible individualization drug therapy through the use of genetic information could lead to a shorter time between diagnosis and effective treatment in addition to reducing costs (Gardner et al, 2014).

A genetic test that can identify which drug and in what dose is correct for each patient, represents a desirable goal. The indication of the polymorphic variants of the genes encoding the enzymes, receptors and other proteins involved in the pharmacological response and the understanding of the phenotypic consequences associated with them, facilitates the choice of the most appropriate drug for each patient and consents to avoid toxicity and therapeutic failure.

The goal of pharmacogenetics is to provide routine tests in normal clinical practice aiming to being able to precede "a priori" how a patient will respond to a certain therapy.

Pharmacogenetics into clinical practice will witness a revolution in the way of prescribing drugs with a series of advantages, including:

• a better efficacy of the therapeutic intervention, with a more accurate choice of the dose and a reduction of the adverse reactions. It has been estimated that due to adverse events following properly prescribed medications, hospitalization lengthens on average approximately 1-4 days, increasing health care costs from $ 2300-5600. Some authors calculate that the costs associated with the side effects of the drugs amount to approximately $ 77 billion (Vaismoradi et al, 2019). Other researches carried out an estimate of 2 million hospitalizations and 100,000 deaths in a year recorded at the Institution of side effects; adverse drug reactions emerge from a set of data, are the 6^{th} reason of death in the United States. The a priori knowledge of which drugs to administer, to which patient and in what dosage, the control of the solution is a health and socio-economic problem.

• safer use of drugs by clinicians, which use specific therapies according to the genetic heritage of the individual patient;

• research aimed to using new drugs to be administered to individuals who do not respond;

• a more rational use of resources.

1.4. Pharmacogenetics in the treatment of mood disorders

Mood disorders in psychiatry include bipolar disorder (BD), characterized by recurrent alternating episodes of elevated mood and depression, and major depressive disorders (MDD), which are defined by symptoms associated with pervasively low mood (Amare et al, 2017). Both MDD and BD are among the most disabling mental health worldwide disorders (Ferrari et al, 2013) with a lifetime prevalence of ~ 12% (Merikangas et al, 2011; Andrade et al, 2003) and 1%, respectively.

The causes of mood disorders involve genetic predisposition and non-genetic biological, psychological and social factors. Both MDD and BD are highly heritable, and genetic factors contribute 31–42% to the disease risk in MDD and 59–85% in BD (Lichtenstein et al, 2009). It is estimated that about 47% of the genetic risk factors are shared between MDD and BD. Environmental traumatic risk factors such as childhood abuse are also frequently associated with both disorders.

Pharmacogenetics brought the promise of matching individuals with treatments that would be efficacious, minimizing adverse events. This has been long needed in psychiatry, where treatment options are empirical and treatment choices have been made largely based on clinical judgment and by "trials and errors".

In the context of psychiatry, the main aim of pharmacogenomics is the identification of genes associated with treatment response to psycho-pharmacotherapeutic agents, with the ultimate goal of implementing this information to improve treatment outcomes.

The current psychiatric assessment, clinical decision-making and treatment choice is primarily dependent on the clinical experience and professional judgment of psychiatrists. There are not biological markers available to perform diagnostic or prognostic test. The treatment response of patients with mood disorders treated with the current approaches of psycho-pharmacotherapy varies widely between individual patients and is unsatisfactory in many cases (Papiol et al, 2018; Pisanu et al, 2018).

Genetic association studies have been reported for pharmacokinetic genes such as the CYP450 isoenzymes or MDR1, and pharmacodynamic genes such as the serotonin transporter (SLC6A4) or the serotonin 2A receptor (HTR2A). However, despite the large number of reports, clinically useful predictors are still scarce for antidepressant monotherapy. Efficient pharmacogenetic predictors for mood stabilizers such as lithium and anticonvulsants, have not had a dissimilar fate and clinically meaningful markers are yet to emerge. The lack of consistent results may be in part due to small samples of heterogeneous populations and a lack of consensus on phenotype definitions. Current pharmacogenetic recommendations include testing for HLA-B*1502 when using carbamazepine in Asian ancestry populations to prevent Stevens–Johnson syndrome, CYP2D6 genotypes when using pimozide, and CYP2D6 in polypharmacy to minimize drug interactions.

Today, data on the usefulness of pharmacogenetics in the treatment of mood disorders are still scarce. In the literature some authors have investigated the role of pharmacogenetic tests in the treatment of MDD. Most of the present studies have shown positive results in this regard:

- Perez et al, (Pérez et al, 2017) showed in a 12-week, double-blind, parallel, multi-center randomized controlled trial a higher responder rate in the group treated in accordance with PGT compared to patients treated as usual for 12 weeks (47.8% vs 36.1%, p = 0.0476; OR = 1.62 [95%CI 1.00-2.61]). They also showed a better tolerability of therapies concordant to the PGx (Frequency, Intensity and Burden of Side Effects Rating Burden sub-score ≤2) were higher in the PGx-guided group than in controls at 6 weeks and maintained at 12 weeks (68.5% vs 51.4%, p = 0.0260; OR = 2.06 [95%CI 1.09-3.89]).
- Hall-Flavin and coauthors (Hall-Flavin et al, 2013) showed in an open-label study evaluating the potential benefit of an integrated, five-gene pharmacogenomic test and interpretative report (GeneSight) for the management of psychotropic medications used to treat major depression, greater percent improvement in depression scores from baseline on all three depression instruments (HAMD-17, P < 0.0001; QIDS-C16, P < 0.0001; PHQ-9, P < 0.0001) in the PGx guided group (n = 113) compared with the unguided group (n = 114). Eight-week

QIDS-C16 remission rates were higher in the guided group (P = 0.03). Participants in the unguided group who at baseline were prescribed a medication that was most discordant with their genotype experienced the least improvement compared with other unguided participants (HAMD-17, P = 0.007). Participants in the guided group and on a baseline medication most discordant with their genotype showed the greatest improvement compared with the unguided cohort participants (HAMD-17, P = 0.01).

- Bradley et al. (2018) in a RCT enrolled 685 patients with depression and/or anxiety. Patrients were assesses through HAM-D17 and HAM-A collected at 4 weeks, 8 weeks, and 12 weeks after baseline. In patients diagnosed with depression, response rates (p = 0.001; OR: 4.72 [1.93–11.52]) and remission rates (p = 0.02; OR: 3.54 [1.27–9.88]) were significantly higher in the pharmacogenetics-guided group (NeuroIDgenetix® test) as compared to the control group at 12 weeks. In addition, patients in the experimental group diagnosed with anxiety showed a meaningful improvement in HAM-A scores at both 8 and 12 weeks (p = 0.02 and 0.02, respectively), along with higher response rates (p = 0.04; OR: 1.76 [1.03–2.99]).

1.5. Bipolar Disorder

The following paragraphs summarize the principal characteristics of BD.

1.5.1 History

Aretaeus of Cappadocia began the process of detailing symptoms in the medical field in the 1st century in Greece. His notes on the link between mania and depression went unnoticed for many centuries. The ancient Greeks and Romans were responsible for the terms “mania” and “melancholia,” which are no longer in use and have been substituted by “manic” and “depressive.” They even discovered that lithium salts in baths calmed manic people and relieved depressed people. Aristotle not only acknowledged melancholy as a disturb, but also cited it as the inspiration for great artists of his time.

It was common during this time for people to be executed for having bipolar disorders and other mental conditions. As the study of medicine advanced, strict religious dogma stated that these people were possessed by demons and should therefore be put to death.

In the 17th century, Robert Burton wrote the book “*The Anatomy of Melancholy*,” which addressed the issue of treating melancholy (nonspecific depression) using music and dance.

Later, Theophilus Bonet published a great work titled “*Sepuchretum*”, a text that was possible from his experience performing 3,000 autopsies. He linked mania and melancholy in a condition called “manico-melancholicus.” This was a substantial step in diagnosing bipolar disorders because previously mania and depression were considered separate disorders.

The modern concept of bipolar disorder was born in France with of Farlet’s efforts in “Folie Circulaire” (1851, 1854) and Baillarger (1854) in “Folie A’ Double Forme” (Zaccagni et al, 2008). The two French "alienists" formulated the concept of mania and depression as entities of the same disorder almost simultaneously. In 1951 Farlet described in a congress the concept of "circular madness" as the periodic alternation of phases of excitement, depression and phases of euthymia. Farlet also noted the genetic connection in bipolar disorders.

In the same years, Ballainger spoke of "madness with a double form" characterized by the alternation of excitement and depression phases.

The history of BD changed with Emil Kraepelin, a German psychiatrist that defined the concept of manic-depressive psychosis as a syndrome which includes simple mania, most cases of melancholy and periodic madness. Kraepelin is also responsible for the first classification of mixed states, aiming to indicate the continuity of manic and depressive symptoms within the same disorder. He observed how one or more of the main psychopathological aspects of mania (mood, cognition, motor skills) could be replaced by one or more of the main aspects of depression and vice versa (Vampini & Nifosi, 2014).

Kraepelin's "*Manic Depressive Insanity and Paranoia"* in 1921 detailed the difference between manic-depressive and praecox, which is now known as schizophrenia. His classification of mental disorders put the basis of modern nosography.

The term "bipolar" means "two poles," signifying the opposite poles of mania and depression, first appeared in the American Psychiatric Association's (APA) *"Diagnostic and Statistical Manual of Mental Disorders (DSM)"* in its third revision in 1980. It was that revision that abolished the term mania to avoid calling patients "maniacs." Now in its fifth version (DSM-5), the DSM is considered the leading manual for mental health professionals.

Leonhard in 1957 definitively overcame the unitary Kraepelinian concept of manic-depressive illness. He was responsible for the distinction between depressive disorders and manic-depressive disorders. In fact, he noted how some patients never presented excitement episodes, but only of depression. He defined them unipolar, differentiating them from the patients presenting alternation between the depressive phases and the manic phases, condition that he defined bipolar disorder.

Godwin is responsible for the subsequent distinction between bipolar disorder type I and type II, deriving from the observation of patients who experienced the alternation between depressive episodes and frankly manic episodes, grouped into type I, and patients who experienced depressive episodes alternating with hypomanic phases, belonging to type II.

Over time, the distinction between bipolar and unipolar disorders has evolved towards a real bipolar spectrum which resumes the original unitary setting of Kraepelin. According to this hypothesis, the nuclear characteristics of the mood pathology are distributed along a continuum (manic-depressive spectrum) which includes at one extreme the affective temperaments, cyclothymia and dysthymia, milder clinical pictures and major depression, at the other the most severe forms, bipolar disorder, type I and II (Cassano et al, 2002).

The bipolar area has thus extended to include a spectrum of clinical conditions, no longer just Bipolar I and II, but also the so-called "soft bipolar disorder", also including the territory of substance abuse and some frameworks of axis II. The dilation of the bipolar area expands from the traditional bipolar I up to a hypothetical bipolar disorder in the N dimension.

Surely the concept of spectrum of mood disorders allows clinicians to grasp not only full-expression pathologies, but also atypical and attenuated manifestations (Callegari et al, 2013). This possibility disappears when we consider the nosographic approach forced to a more rigid framework, often unable to describe the complexity and pleomorphism of the existing clinical forms.

1.5.2 Definition of nosography and bipolar spectrum disorders

DSM 5 inserts bipolar spectrum disorders into a separate chapter between schizophrenia and depressive disorders, to indicate the position between the two diagnoses in terms of familiarity, symptoms, history and genetics.

This chapter includes:

-The Bipolar Disorder type I: represents the modern conception of manic-depressive psychosis described in the nineteenth century;

-Bipolar Disorder type II: diagnosed by at least one episode of major depression and at least one episode of hypomanic symptoms in the course of life;

- Cyclothymic Disorder: characterized by at least two years of hypomania and depressive symptoms without ever meeting the criteria for a manic, hypomanic or depressive episode;
- Bipolar disorder or related disorders due to the use of substances or a general medical condition: a large number of abused substances, drug therapies and organic pathologies can be associated with the onset of manic symptoms;
- Bipolar spectrum disorder, unspecified.

In this latest version of the diagnostic manual, the distinction between unipolar and bipolar disorders is reiterated. This differentiation is supported by numerous scientific evidences: first of all by genetic factors that support higher frequency of bipolar disorders in first degree relatives of affected patients (Giusti et al., 2014); the greater role played by genetic factors within bipolar disorders rather than unipolar depression (Jaworska-Andryszewska P, 2019); by the lower number of relapses and by the better prognosis shown in unipolar disorders rather than in bipolar disorders (Fiume et al., 2014).
Han et al (Han et al, 2019) also pointed out that the remission phases of patients with bipolar disorders are characterized by greater extroversion and less neuroticism than the remission phases of patients with unipolar disorder. The depressive phases of bipolar patients are more frequently characterized by psychomotor slowing and psychotic symptoms, while those of unipolar patients by anxiety, hypochondria and insomnia. Finally, DSM 5 includes, within bipolar disorder, rapid-cycle bipolar disorder characterized by poor response to pharmacotherapy.

1.5.3 Epidemiology

It is difficult to frame the prevalence of BD, both for the methodological difficulties encountered in these studies and for the complexity of the diagnostic sub-categories of the bipolar spectrum which are not always taken into consideration. Merikangas et al (2011) of the National Institute of Mental Health, of the Genetic Epidemiology Research Branch in Bethesda, Maryland, conducted an interesting cross-study of 61,392 adults in the United States, Mexico, Brazil, Colombia, Bulgaria,

Romania, China, India, Japan, Lebanon and New Zealand to describe the prevalence, severity of symptoms and co-morbid patterns. In a sample of 61,392 adults from 11 countries, the lifetime prevalence was 0.6% for DB I and 0.4% for DB II, and 1.4% for sub-syndromic symptoms, with an overall estimated prevalence of bipolar spectrum disorders of 2.4% (Merikangas et al., 2011); as regards to the gender distribution, it seems that DB I and sub-syndromic disorders are more frequent in the male gender, while DB II is predominant in the females. These findings have been amply confirmed in literature by studies on smaller populations. According to Kessler and coauthors, (Kessler et al, 2005) it involves 2 to 5% of the population. The incidence of bipolar disorder has been estimated at 9-15 cases per 100,000 inhabitants in males and 7-30 cases in females.

There is a wide debate about the average age of onset; Goodwin and Janet (Goodwin & Janet, 2007) indicated it towards 28.1 years, specifying however that this age is reduced to 15.5 years if we evaluate the first episode of manic-depressive symptoms and it is around 22 years old and 25.8 years old if we take into consideration the first treatment received and respectively the first hospitalization. It should also be noted that the number of late onsets of bipolar disorder appears to be increasing. According to Yassa et al, (1997), late onset after the age of 50, is identified with a frequency of 10% of patients with bipolar disorder. In a subsequent study Depp & Jeste (Depp & Jeste, 2004) have specified that of this 10%, half appear after the age of 60.

1.5.4 Etiopathogenesis

The etiopathogenesis of bipolar disorder is still not fully known, as for many other psychiatric disorders. There is no doubt that it results from the interaction between genetic, biological and environmental factors.

Genetic factors

The complex etiopathogenesis of BD is reflected in its genetic inheritance. Among the different etiopathogenetic factors, familiarity is frequent for bipolar disorder. In literature the presence of psychotic or mood disorders is defined common in families of probands with bipolar disorder (Tomasetti et al, 2015).

Genetic-epidemiological, family, twin and adoption studies have shown a heritability for bipolar disorder of 70-80% with a relative risk of 5-10 times for first degree relatives and 45-75 for monozygotic twins. The genes involved appear to be the 2p, 3p, 4p, 6q, 8q, 9p21, 10p 14-21, 11p, 12q, 13q, 14q, 16p, 16q, 18p, 18q, 21q, 22q and Xq regions (Cassano & Tundo, 2008). In addition, numerous polymorphisms have been associated with susceptibility for bipolar disorders including genes encoded for proteins of the extracellular matrix of brain tissue (NCAN), or for neural membrane proteins (ODZ4), have high concordance in GWAS studies, thus such as genes encoding catechol-O-methyltransferase (COMT), CLOCK genes (which control circadian rhythms), dopamine and serotonin transporters (DAT, SERT), some dopamine receptors (D2, D4) and serotonin (5HT2a), brain-derived neurotrophic factor (BDNF) and glycogen-synatase kinase (GSK3), often also associated with the efficacy of drug treatment with the most common mood stabilizers (Barnett & Smaller, 2009).

It is essential to keep in mind that the genetic account represents only a vulnerability: the clinical phenotype represents the interaction between genes and environment. Recent studies show that high epigenetic reprogramming is associated with the pathophysiology of bipolar disorders, and that dysfunctional epigenetic control activity by pivotal proteins, such as histone deacetylase (HDAC), can be directly related to the disorder.

Organic factors

It is obvious that the involvement of additional genes corresponds to a large number of brain structures. Current researches aim to validate the role of 3 prefrontal-limbic circuits in the

pathophysiology of bipolar disorders: the Salience Network consisting of the Ventromedial Prefrontal Cortex, the Anterior Cingulate Cortex, the Nucleus Accubens, the Pale Globe and the Thalamus and constitutes an iterative circuit that modulates the amygdaloid responses to endogenously generated emotional states. The External Emotional Regulation Network involving the ventrolateral prefrontal cortex, the dorsal girdle, the ventromedial striatum, the pale globe and the thalamus and controls and modulates the emotional states induced by external stimuli. The default network mode, whose function is inherent to the regulation of self-consciousness, social cognition, design / planning, as well as reminiscence, includes medial and dorsomedial fronto-limbic structures and the precuneus (Calhoun et al, 2012). A misconnection between the prefrontal and limbic structures seems to be present in these patients; in addition, there seems to be a hyperactivation of the amygdala in the manic phases and a hyperactivity of the limbic areas with a concomitant reduction in the overall functionality of the prefrontal cortex (Strakowski et al, 2012). There are numerous evidences based on neuroimaging which support a smaller volume of the amygdala and of the prefrontal cortex in individuals with bipolar disorders (Basia & Jovinelli, 2012) and about the increasing size of the third ventricle in adults with BD. There are also numerous supporters of the monoaminergic theory stating that in bipolar disorders there might be an increase of monoamine.

According to the dopaminergic hypothesis, an important dysregulation of glutamate transmission with implications of the NMDA receptors of glutamate at the thalamic and hippocampal level seem to be present in BD. Furthermore, also GDNF (glial-derived neurotrophic factor) seems to be involved, guaranteeing the trophism and correct functioning of the main components of the glia and guaranteeing, together with BDNF, the monoaminergic regulation and neuroimmune functions of microglia (Mandolini et al, 2020). GDNF appears to be regulated by inflammatory cytokines. Patients with BD present a reduced neurotrophic activity and therefore a deficient modulation of monoamines. Among the various intracellular signaling affected by these deficient interactions there would be GSK3, a kinase involved in cellular metabolism capable of modulating neuronal synaptic plasticity and the apoptosis transduction systems (Tomasetti et al, 2015).

Neuroendocrine factors

Endocrine factors play a role in the etiopathogenesis of BD. Dysfunction of the hypothalamic-pituitary-adrenal axis seems to be altered both in the acute phase and during symptomatic remission phase (Watson et al, 2004). The dysregulation of cortisol blood levels in patients with bipolar disorder is intricately linked to the alterations in circadian rhythms. Patients with BD seem to have a dysregulation of melatonin; they nocturnal melatonin levels seem to be lower than in control cases (Gonzalez et al, 2014). This alteration is attributable to a defect in the Suprachiasmatic Nucleus and to the CLOCK genes involved in the serotonin-mediated mechanism of melatonin production. At last, dopamine and norepinephrine alterations seem to lead to a failure of the neuroendocrine efficacy of sleep induction and adaptation to daylight (Abreu & Bragança, 2015).

1.5.5 Clinical presentation

Bipolar disorder is a mood disorder characterized by episodes of major depression and mania or hypomania. Most patients experience chronic symptoms of bipolar disorder approximately half of the time, most commonly subsyndromal depressive symptoms or a full depressive episode with concurrent manic symptoms. Consequently, patients with bipolar depression are often misdiagnosed with major depressive disorders. Health risk behaviors including tobacco use, sedentary activity level and weight gain are highly prevalent in patients with bipolar disorder, as the comorbid chronic diseases such as diabetes mellitus and cardiovascular disease. Patients with bipolar illness have an eight-fold higher risk of suicide and a two-fold increased risk of death from chronic medical illnesses. Recognition of bipolar depression and its associated health risk behaviors and chronic medical problems can lead to the use of appropriate interventions for patients with bipolar disorders, which differ in important ways from treatments used for major depressive disorders.

The following table (Table 1.3) describes the clinical presentation of the different phases of bipolar disorders.

Table 1.3: Symptom Episodes of Bipolar Disorder

Phase of Bipolar Illness*	Episode Symptoms †‡
Manic Episode	Persistently elevated or irritable mood, often with uncontrollable excitement lasting throughout the day. Generally accompanied by increased energy, overactivity, pressure of speech, and decreased need to sleep. Loss of social inhibitions may result reckless and out of character behavior, or an engagement in high-risk pleasurable activities. It may also be accompanied by delusions, or extreme flight of ideas, making the patient incomprehensible.
Depressive Episode	Depressed mood, reduced or decreased energy, diminished capacity of enjoyment, interest and concentration, and guilt or worthlessness are often present. Fatigue often occurs, along with disturbed sleep. Appetite can be diminished or increased. The lowered mood and associated symptoms vary slightly from day to day.
Mixed Affective Episode	Mixture or a rapid alteration of manic and depressive symptoms.
Hypomanic Episode	Persistent mild elevation of mood, increased energy and activity, and usually marked feelings of wellbeing and physical and mental efficiency. Increased sociability, talkativeness, over-familiarity, increased sexual energy, and a decreased need to sleep are often present, but not to the extent that lead to severe disruption of work or result in social rejection. Irritability may also occur.

* The presence of at least one manic episode is required for the diagnosis of BD type I; the presence of at least one hypomanic episode and at least one major depressive episode is required for a diagnosis of BD type II.

‡ Symptoms are not due to general medical problems or to the physiologic effects of substances.

1.5.6. Prognosis

The natural course of bipolar disorder depends on different characteristics. It seems that without treatment, manic and depressive episodes tend to occur more frequently as people get older, causing increasing problems in relationships or at work. Finding the most helpful drug combination with fewer side effects it is not easy. Many people with bipolar disorder are able to function completely normally and have highly successful lives. It is not yet clear which factors determine the different possible prognoses. The following characteristics seem to be associated to an unfavorable disease course in BD type I:

- early onset;
- depressive onset, which remains with a depression / mania ratio of 3: 1 for relapses in women and 3: 2 for men. A later onset, a lower incidence of psychotic symptoms, greater ideas of self-damaging gestures, a higher number of relapses and a more frequent course in rapid or chronic cycles, a longer duration of illness, correlate with the depressive polarity of the first episode;
- a higher number of episodes in life.

Negative prognostic factors related to Bipolar Disorder type II appear to be:

- comorbidity with other psychiatric disorders, especially anxiety, personality disorders and drug abuse;
- rapid cyclicality (ECNP, 2008).

1.5.7 Diagnosis

Bipolar disorder is certainly a diagnosis to be done evaluating the longitudinal course of the disease. Consider information about familiarity, that is present in about 50% of bipolar form cases is also important in the diagnostic process, completing the patient's medical history with family members help, to investigate any previous phase of hypomania or depression.

The differential diagnosis is overly complex and depends on the phase of the disease: a manic phase with psychotic symptoms in an unknown patient has to be differentiated from psychotic disorders and from Schizophrenia itself. In the latter, there are Schneiderian symptoms of rank I such as echo of thought, dialoguing and commenting voices, experiences of bodily influence, arrest and influence of thought (Callegari et al, 2013)

Recognizing a depressive phase of a bipolar disorder from a unipolar depression can be exceedingly difficult. Some clinical characteristics, more often present in bipolarism are slowdown energy, weight loss, hypersomnia and psychotic symptoms (Gordon & Fletcher, 2009). A possible early onset, high number of relapses and resistance to antidepressants could induce the clinician to suspect forms of bipolarity. Diagnoses of personality disorders, particularly of the histrionic type, are frequently attributed to patients suffering from Bipolar Disorder, particular of type II. This is probably because of the difficulty of symptomatic remission frequently due to incorrect diagnostic classification and therefore incorrect therapeutic approach that leads patient to persist complaints about clinical condition.

1.5.8 Therapy

Before treatment, the psychiatrist should perform a diagnostic evaluation and assess the patient's safety and level of functioning to arrive to the decision about the optimum therapy. Subsequently, specific goals of psychiatric management include establishing and maintaining a therapeutic alliance, monitoring the patient's psychiatric status, providing education regarding bipolar disorder, enhancing treatment compliance, promoting regular patterns of activity and of sleep, anticipating stressors, identifying new episodes early, and minimizing functional impairments (Anon, 2018).

The following section describes the psychopharmacological treatments recommended by the guidelines and by the current evidences in literature.

Treatment of bipolar depression

The treatment of bipolar depression is very complex for different reasons, such as the severity of symptoms, the risk of switch, the high disability, the risk of suicide that is 34 times greater than in manic phases (Tundo, 2006). The current "NICE" international guidelines (2014, 2016) recommend treating a depressive episode in BD with the combination olanzapine + fluoxetine or alternatively with quetiapine alone. In case of no response they recommend lamotrigine.

If the patient is already in treatment with lithium or valproate therapy, they recommend evaluating the plasma level, before possible addiction of the combination olanzapine + fluoxetine or quetiapine. If there is no clinical improvement response, they invite to consider lamotrigine addiction. Once clinical remission has been achieved, they recommend maintenance of therapy for 3-6 months.

Furthermore, NICE guidelines recommend psychotherapeutic intervention (psychoeducational, cognitive-behavioral, interpersonal or family) in association with pharmacological treatment.

The debate on the use of antidepressants in the depressive phases of bipolar disorder is still open. In literature there are no univocal opinions. Some authors agree that antidepressants are effective in addition to mood stabilizer therapy only when patients cannot tolerate high doses of lithium (0.8 mEq/l) (Nemeroff et al, 2001), while others consider antidepressant effective (Vieta, 2002).

There have also been numerous efforts to identify the classes of antidepressants less predisposed to switch to the opposite polarity, with heterogeneous results, which seem to agree for a greater risk of switch associated with tricyclics, followed by venlafaxine, MAOI and lastly bupropion and SSRIs.

Treatment of the manic episode

A protective environment is the first step to treat a manic episode, according to the NICE guidelines (2014, 2016). From a pharmacological point of view, if the patient is drug naïve or has stopped antidepressant therapy, the administration of neuroleptics including olanzapine, quetiapine, risperidone or haloperidol must be considered. If an antipsychotic is not enough, a combination of

two antipsychotics could be useful. If there is still no symptomatic remission, the guidelines recommend the addition of lithium or valproate.

If the manic phase appears during a therapy with stabilizer + antidepressant, it is useful to interrupt the antidepressant therapy and possibly add an antipsychotic, after checking the blood levels of the stabilizer and optimizing the therapy accordingly. In resistant forms clozapine is indicated. As a last resort to non-responsive forms, NICE guidelines recommend TEC (electroconvulsive therapy).

Once the acute phase has been resolved, it is advisable to maintain the therapy with a stabilizer or antipsychotic in order to reduce the risk of relapse. Maintenance therapy must be monitored through frequent outpatient assessments which, from the patient's clinical examination, will indicate how to modify the current pharmacotherapy.

Treatment of mixed manic states

The literature on the treatment of mixed states is sparse and controversial. According to Muneer (2017) two essential lines can be derived from the main studies present in the literary panorama:

1. Mixed manias respond more to combined treatments than to monotherapies;
2. Combined therapies, however, present at least two problems compared to monotherapies: possible drug interferences and increased risk of adverse events.

If patient is drug naïve it is advisable to start treatment with a drug in monotherapy. The choice must consider the efficacy of the drug in monotherapy in mixed states, and its suitability and efficacy in combined therapies. In the context of classical stabilizers, valproate is the first choice, because lithium is less active on the mixed component of mania (Frye et al, 2000), while carbamazepine could pose problems of pharmacological interference in the case of the addition of other drugs. Among the new generation antipsychotics, quetiapine is the most recommended for efficacy and for the good risk / benefit ratio in case of subsequent combination with other drugs. Olanzapine, which has proven its powerful therapeutic action in mixed manias (Cuomo et al, 2017), presents metabolic adverse events increased in case of association with other drugs. Ziprasidone and Asenapine, effective in mixed

manias in monotherapy, are second choice options for two reasons: efficacy in combined therapies requires further experimental tests and they cannot be prescribed for long-term treatment.

In case of a patient already in treatment, it is necessary to consider the starting drug. In case of lithium salts, the first operation is to evaluate the plasma level bringing it to the upper limits and, if this is not enough, add a second drug. Among the classic stabilizers, both the addition of valproate and carbamazepine are recommended. Among the new antipsychotics, the two drugs with proven efficacy appear to be quetiapine and olanzapine. It should also be remembered that the addition of aripiprazole is not considered advisable due to a poor risk / benefit ratio. In case of a treatment already in progress with valproic acid, it is possible to add a new generation antipsychotic. The addition of a second stabilizer is not a very rational intervention in this case: lithium is not highly effective and requires slow titration and carbamazepine presents problems of pharmacological interference.

The psychotherapeutic and psychoeducational treatment aimed at strengthening the therapeutic alliance is of extreme importance, as for the other forms described, increasing adherence to treatment and educating the patient and family.

Treatment of mixed depressions

Evidence in literature regarding the treatment of depressive episodes with mixed features is extremely limited. The treatment of the patient with a major depressive episode must consider the aggravating circumstance constituted by the presence of mixed elements. The coexistence of symptoms of opposite polarity increases the suicidal risk and the risk of alcohol and psychoactive substance abuse. With regard to pharmacological intervention, current guidelines generally recommend the use of mood stabilizers, atypical antipsychotics and antidepressants (Fountoulakis et al, 2012).

Quetiapine currently appears to be the most suitable drug for the treatment of bipolar depression with a mixed component due to its proven action on both polarities of bipolar disorder.

Olanzapine is more effective than placebo in the treatment of mixed bipolar depressions, but also indicate that the combined olanzapine-fluoxetine treatment is even more effective without an

increased risk of onset of manic symptoms (Benazzi et al, 2009). Antidepressants are recommended only in combination with a stabilizer as indicated by the very recent CANMAT guidelines (Canadian Network for Mood and Anxiety Treatments, 2013): the use of antidepressants alone in bipolar depressions is not recommended, particularly in mixed states or in case of patients with rapid cyclicality. The possibility of associating antidepressants with mood stabilizers is considered only for bipolar depressions without mixed elements: in these cases, however, tricyclic antidepressants and venlafaxine should be avoided because they are associated with a greater risk of inducing switches. The olanzapine-fluoxetine association appears to be useful and free from the risk of switching, which is more effective than olanzapine alone. Trazodone Contramid is a new antidepressant formulation, approved by the US Food and Drug Administration, used in bipolar depression, with a relatively low risk of inducing manic-switching when prescribed with mood stabilizers (Wichniak et al, 2015).

Chapter 2: Research Project

2.1 Background

This research project evaluates the utility of PGT in the "clinical routine practice" of bipolar disorder. As already mentioned, while data on the usefulness of pharmacogenetics during the treatment of depression are present, the literature regarding BD and pharmacogenomics is still scarce. Therefore, the idea of evaluating the usefulness of pharmacogenetic tests in the treatment of this complex and validating disorder through an observational study. The research project has been developed into IV phases over 3 years; aims, materials and methods and results of each phase of study have been divided to make the reading flowing and clearer.

Before describing the different phases of study, we have to declare that for the genetic analysis a PGT named Neurofarmagen® (AB-BIOTICS SA, Barcelona, Spain) has been used. The decision of its use has been influenced by the number of genes evaluated, the assessment of pharmacokinetic and pharmacodynamic genes and that they are used in the clinical routine practice. The details about its genetic analysis has been reported in the materials section and phase 1 methods.

2.2 Aims

2.2.1 Phase I

During Phase I the following aims have been assessed:

Primary aim: to identify at T0 (corresponding to the test report communication) if the treatment prescribed by the psychiatrists in patients with a Clinical Global Impression item Severity (CGI-S) ≥ 3 was consistent with the treatment suggested by the PGT Neurofarmagen® (AB-BIOTICS SA, Barcelona, Spain).

Secondary aims: to identify if clinicians changed the treatment (in case of discordance) according to the results of the PGT Neurofarmagen at T1, corresponding to 3-month follow-up visit;

To evaluate the psychopathological trend of the groups of patients 3-12 months (T2-T3) after administering the PGT

-describe the polymorphism of the sample.

2.2.2 Phase II

During phase II, the following aims have been studied:

- the utility of PGT in terms of reduction of number of days of hospitalization and number of admissions. Furthermore, this study aims to compare through a mirror analysis 12 months before the execution of the PGT versus 12 months after the execution of the PGT. We analyzed in terms of number and days of hospitalizations and accesses to the emergency services, in a population of psychiatric patients affected by bipolar disorder that received a therapy concordant to the PGT.
- to have an economic value of the data, this being based on the diagnosis-related group (DRG).

2.2.3 Phase III

This phase of study evaluates, through a semi-structured interview (Thomson et al, 2015), the attitude of psychiatrists towards the help of pharmacogenetic information into clinical practice.

2.2.4 Phase IV

The last phase of study focused on the following aim:

- due to the large use of lithium salts for the treatment of BD, literature dealing with this argument has been reviewed in order to see the correlation between the genes and the response to this drug in terms of efficacy and tolerability.

2.3 Materials and Methods

2.3.1 Phase I

Study design

This phase of study is a descriptive, observational and multicentric study, involving the Psychiatric Unit of ASST (Azienda Socio-Sanitaria Territoriale) Settelaghi of Varese and the Psychiatric Unit of ASST Santi Paolo e Carlo Borromeo of Milan.

Sample

In this phase of study, 30 patients corresponding to the following inclusion and exclusion criteria were recruited:
- to be a patient of ASST Settelaghi of Varese or ASST Santi Paolo e Carlo of Milan;
- to have a diagnosis of BD type I or II according to DSM 5;
- to be ≥18 years old;
- to sign an informed consensus both for the execution of the PGT and for the use of data for the study protocol;
- to have a Clinical Global Impression Severity score ≥ 3.

Scales

The psychopathological evaluation has been carried out through a battery of scales administered on several occasions (0-3-12 months) and reported in the appendix section. The following questionnaires have been administered:

- Clinical Global Impression (CGI), used for the global evaluation of the patient and to monitor the clinical improvement. It takes into account three areas: 1 the disease severity; 2 an overall improvement of symptoms; 3 an effect of drugs compared to their side effects (Lam et al, 2005). This scale has been administered to all scheduled appointments and the minimum item severity score ≥ 3 represented a patient inclusion criterion.

- Hamilton Depression Rating Scale (HDRS): it is a tool considered as a gold standard for the evaluation of anxiety-depressive symptoms. In the most common version (Williams & Terman, 2003)

it is composed of 21 voices. The items are variously evaluated: some (10) on a 5-point scale (0-4), others (2) on a 4-point scale (0-3) and the remaining on a 3-point scale (0-2) (Giusti, 2014). It has been administered at different times -T0, T1, T2-.

- Young Mania Rating Scale (YMRS): it is an 11-item scale to assess the severity of maniac symptoms. The information for the compilation is obtained based on subjective symptoms reported by the patient and based on the patient's clinical observation during the interview. The scale is appropriate for both the evaluation of manic symptoms and for the evaluation of response to treatment in patients with a diagnosis of Bipolar Disorder type I or II. Co-administration of a scale such as HDRS for depressive symptoms is indicated. Four of the items are rated on a scale of 0 to 8 points, the remaining 5 items on a scale of 0 to 4 points. The score obtained must support the clinician in assessing the severity of symptoms. A score less than or equal to 12 indicates symptom remission (Diagnostic and symptom interviews for adults Daniel N. Allen, 2019). It will also be administered at all times -T0, T1, T2-.

- The dosages record treatment emergent symptoms scale (DOTES): to assess the occurrence of side effects in relation to the drug therapy. The fundamental characteristic of the scale is to investigate, not only the presence and severity of the symptoms that appeared (or worsened) during the treatment, but also to consider the probability of the correlation between symptoms and treatment. A real score is foreseen only for the severity of the symptoms, to judge the relation with the treatment and for the overall judgment. The scale used always has five points, but the meaning of the score changes:

- in case of severity 0 corresponds to "not assessed", 1 to "absent", 2 to "mild", 3 to "moderate" and 4 to "serious";

- to judge the relation between symptoms and treatment, 0 corresponds to "no relation", 1 to "remote, <10%", 2 to "possible, 10-50%", 3 to probable, 50-90% "and 4 to "safe,> 90%";

- by overall judgment, 0 corresponds to "not at all", 1 to "minimum", 2 to "moderate", 3 to "serious" and 4 to "not evaluated" (Cassano, 2000).

Genetic analysis

The PGT used for pharmacogenetic analysis is Neurofarmagen® (AB-BIOTICS SA, Barcelona, Spain), a PGT for the specific analysis of genetic polymorphisms related to the pharmacokinetics and pharmacodynamics of principles commonly used in neuropsychiatry.

The test evaluates more than 25 different genes (Appendix shows the list of polymorphisms analyzed), comparing them to 59 active substances. The test report contains a table where all active substances matched to a color coding: 1) green: expectation of higher likelihood of good response to treatment or a good tolerability; 2) white: index of a standard response, not different from the general population; 3) yellow: requiring more careful dose monitoring; and 4) red: for high risk of adverse effects or not expected efficacy. The test then allows the clinician to locate the most appropriate dosage for each patient by consulting information in advance, on possible side effects of the drug. The genetic polymorphisms analyzed with this genetic test can be grouped into three different categories, depending on the effect they have been associated with:

- Drug response: the proteins encoded by these genes are direct or indirect targets of drugs (receptors, signaling pathways, etc.). These genes are crucial for evaluating drug efficacy in the patient.
- Risk of unwanted effects: genes that have been associated with adverse effects in subjects receiving the specific psychiatric drugs, and that encode non-metabolic proteins.
- Dose (metabolism): genes involved in drug activation, in penetration, and in its elimination rate. Ultimately, the genes controlling the blood levels of the drug.

The administration of the genetic test is carried out on a patient's saliva sample, collected through a kit; for 30 minutes before the sample collection, the patient should not consume any food, drink, or chewing gum, should not smoke and should have removed all cosmetics from the lips.

After the collection, the saliva sample (about 1 ml) remains stable at room temperature for a maximum of 15 days, the time to be sent to the laboratory. The results are available within 10 working days

from the sample's arrival date at the laboratory of AB-BIOTICS S.A., which has the required authorization to operate as a health laboratory (code E17867643) and to import biological samples.

DNA was extracted from the patients' saliva samples with the Genomic DNA Isolation Kit (Norgen Biotek Corp. Thorold, ON, Canada). DNA quality was evaluated by 2000 nanodrop microvolume spectrometry. Genotyping of single nucleotide polymorphisms was performed by OpenArray® Technology on the QuantStudioTM 12K Flex Real-Time PCR System (Thermo Fisher Scienti c Inc., Waltham, MA, USA) using a custom designed array. CYP2D6 copy number analysis was performed in an Applied Biosystems® 7500 Real-Time PCR System using Hs04083572_cn and Hs04502391_cn TaqMan copy number assays targeting CYP2D6 intron 2 and intron 6, respectively, and RNase P copy number assay as a reference (Thermo Fisher Scienti c Inc.).

Conservation of biological material

Saliva samples were tagged with a code associated to the patient and sent to the laboratory of AB-BIOTICS S.A. to extract genomic DNA. The genetic data and the identification code were stored in a key archive in the participating hospitals (Circle Hospital of Varese, Italy and San Carlo Borromeo Hospital in Milan, Italy). This archive containing these correspondences is necessary to associate each patient's clinical data with the Neurofarmagen report. At the end of the experiment, the above archive will be destroyed. DNA samples will not be conserved.

All information collected in this study was treated in accordance with the Italian Personal Data Protection Act (D.Lgs. 196/2003). Personal data care was processed electronically with all the criteria to make it confidential and used exclusively for the study.

Genetic data are rendered anonymously in electronic treatment, and after collection it was kept separate from the master data. An encryption system allowed only the person in charge to connect the genetic data to the patients. The collected data was used only for scientific research purposes in aggregate form, thus, anonymously.

Statistical analysis

Statistical analysis was performed by Graph-Pad and SAS version 19. Descriptive analysis has been reported as absolute number and percentage (%). The Random Effect Model was used to evaluate the effects of treatment across time in the two subgroups of patients followed up for 3 months. This test allows the estimation of the relationships between two or more variables. It is applicable to a wide range of data types, estimating various types of effects. It is applicable to small comparison groups. The Student's t-test and the Fisher test were used to assess differences between groups at the baseline for continuous and dichotomous variables, respectively, and the McNemar's test to assess within-group differences across time for dichotomous variables. Significance for all tests was set at $p = 0.05$, two-tailed.

2.3.2 Phase II

Sample

Fifty-six patients of the psychiatric ward of the ASST (Azienda Socio-Sanitaria Territoriale) Settelaghi of Varese, Italy, underwent the PGT Neurofarmagen, as in normal clinical routine practice, between March and June 2017. From this sample, 30 patients responded to the following inclusion criteria:

-suffer from BD type I and II (according to criteria of Diagnostic and Statistical Manual of Mental Disorders, version 5);

-aged ≥18 years old;

-signe an informed consent, both for the execution of the test and for the participation in scientific studies;

-non-clinical stability with a score of Clinical Global Impression Item Severity (CGIs) ≥3 before the execution of the PGT;

- have a discordant therapy compared to the test in the 12 months preceding the execution of the PGT;
- have a therapy modified after the execution of the PGT in a consistent manner with it and maintained for the further 12 months of observation.

Sociodemographic data were collected from psychiatrists or residents; data dealing with the number of hospitalization days and the number of emergency room visits were collected from Portale, a software management used within the ASST Settelaghi of Varese (Italy). It is active since 2008 and accessible only by clinicians or medical residents through a personal username and password. The system allows clinicians to view demographic and clinical data of hospitalized patients and to request laboratory, instrumental, or specialist consultations. Data of the admission, of the accesses to emergency service, or of the outpatient visits are stored and can be found even at the end of the procedure through different search methods (search for personal data of the individual patient, search by time period, search by department and so on).

Genetic Analysis

Pharmacogenetic analysis was conducted using Neurofarmagen, described in materials and methods of phase I.

Conservation of Biological Material

Codes associated with the patient tagged saliva samples were sent to the laboratory. An archive in the hospitals where the study was conducted (ASST Settelaghi, Varese, Italy) stored the genetic data and the identification codes. Clinical data were associated with the Neurofarmagen report in this archive, which was destroyed after the examination of samples. All information was confidential and employed exclusively for scientific research purposes, as explained in the previous phase of study, it was processed electronically according to the Italian Personal Data Protection Act (D.Lgs. 196/2003).

Ethics Approval

The Ethics Committee of Insubria approved the research protocol (n. 159; Varese, 1 March 2016). This study observed regulatory and legal requirements (DL n.211, 24 June 2003, and DM 17 December 2004), according to the Declaration of Helsinki's ethical principles. While signing written informed consent for the execution of the PGT, all patients were specifically informed about the opportunity to participate to this study and signed another written consent.

Statistical Analysis

Graph-Pad version 7 and SAS version 19 were used to perform the statistical analysis. Descriptive analysis has been stated as an absolute number and percentage (%). Approximating the normal distribution of the data, we have seen fit to use parametric t-tests to compare the mirror analysis of the number of times emergency services were accessed, the number of hospitalizations, and the number of days of hospitalization. The mirror analysis allowed the comparison of the same population, with the same sociodemographic and clinical characteristics, in two different periods. Statistical significance has been established at $p = 0.05$, two-tailed.

Economic Enhancement

Dealing with the number of days of hospitalization, an economic enhancement has been attributed to compare the expenditure before and after the change of therapy in agreement with the PGT. We have added the PGT cost to the health-care costs of the year's observation following the execution of the PGT. In order to give an economic enhancement, we have used a standard value of a medium-length hospitalization for bipolar disorder diagnosis, using the diagnosis-related groups (DRG). DRG is a system of classification used to quantify the absorption of resources and therefore to remunerate each episode of hospitalization. To assign each episode of admission to a specific DRG, the following

information was required: the main discharge diagnosis, all secondary diagnoses, all surgical interventions, the main diagnostic and therapeutic procedures, age, sex, and discharge modality. The attribution was carried out using an algorithm that analyzed the aforementioned information and determined the group.

2.3.4 Phase III

This phase of study used five questions to assess attitudes towards integration of pharmacogenetic into psychiatric patient care. The eligibility criteria to be part of the survey were to be a psychiatrist, psychiatry resident or professor currently working at the hospital of ASST Settelaghi in Varese and Cittiglio, or Asst Santi Paolo & Carlo of Milan. The survey was administered from January until May 2018. Data analysis was mainly descriptive. Chi-square test were used to compare psychiatrists with experience with PGT with those without experience.

2.3.3 Phase IV

For this last phase a literature search was conducted from PubMed, EmBase, Psychinfo and Google scholar databases from 1998 to January 2018. The string of search was elaborated by a researcher of the Mayo Clinic (U.SA.) using the combination of terms Lithium OR Mood Stabilizer AND Personalized Medicine OR Precision Medicine OR Pharmacogenetics. Inclusion criteria were the following: • Articles published in English • Randomized controlled trials, observational studies, and case-control studies. Genome Wide Association Studies were also included. • Studies evaluating patients between the ages of 18 and 65 years old were included.

Figure 2.1 shows the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analysis) flow-chart of the research. A total of 1162 records were returned after the exclusion of duplicates. Articles were examined independently by two researchers and a third researcher was involved whenever there was a dissenting opinion among the main investigators. 258 articles were identified

as potentially eligible for this study based on title and abstract; of these only 55 studies were retrieved for full-text review. All relevant references were checked for additional records and a total of 37 articles were considered eligible according to the aim of the review (Fig. 2.1).

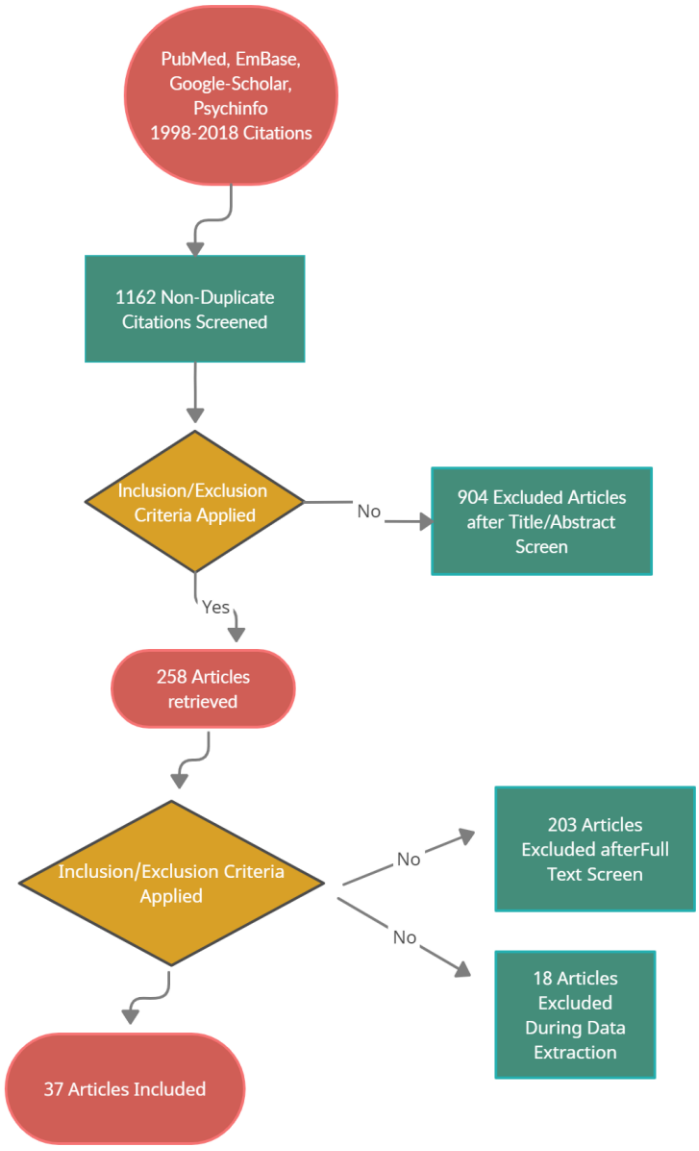

Figure 2.1: Prisma flow chart

2.4 Results

2.4.1 Phase I

Sociodemographic and clinical data

The demographic and clinical characteristics of the participants of phase I are shown in Table 2.1. The average age was 54.8 years old. (SD 15.22), with 52% of males and 48% females. All patients were Caucasian. A total of 52% of patients had a diagnosis of BD type I and 48% of BD type II. During the recruitment, 56% of patients suffered from depression, 24% from mania and 20% were in a mix state. The most prescribed mood stabilizers were lithium (28%) and valproate (24%), followed by lamotrigine (8%). Among antidepressants, paroxetine (20%) and bupropion (12%) were the most prescribed drugs; and antipsychotics, quetiapine, aripiprazole, and olanzapine were the most prescribed (each one in 16% of the patients). At T0, psychopathological evaluation of the 30 subjects recruited yielded an average CGI-S score of 4.8 (SD 3.7), an average YMRS score of 14.7 (SD 5.6), and an average HDRS score of 18.3 (SD 9).

Table 2.1 Demographic characteristics of the patients

Sociodemographic characteristic	**Data**	**N**	**%**
Gender	Male	14	48
	Female	16	52
Nationality	Italian	29	97
	Other	1	3
Average age		55 y.o. (SD 15.22)	
Caregiver	1	12	40
	2	13	44
	3 or more	5	16
Occupation	Employed	12	40
	Unemployed	5	16
	Retired	11	36
	Invalid	2	8
Years on treatment		9.5 y (SD 7.2)	
Number of previous treatments		3.5 (SD 1.3)	

Legend: SD=standard deviation; y.o.=years old; y=years.

Primary result

At T0 according to the Neurofarmagen test, 4 patients (13%) received an optimal therapy in line with the test suggestions (i.e. “green color”). Regarding the remaining patients, the Neurofarmagen test identified: 8 patients (27%) with a standard therapy (i.e. “white color”), 8 patients (27%) with an idiosyncratic negative therapy (i.e.“red color”), 7 patients (23%) with an idiosyncratic positive/negative therapy (e.g. a patient can have one genetic variation associated to good response and a second variation in another gene associated to a specific adverse effect), and 3 patients (10%) with a therapy potentially subject to an altered metabolism rate (i.e. “yellow color”), as shown in Figure 2.2.

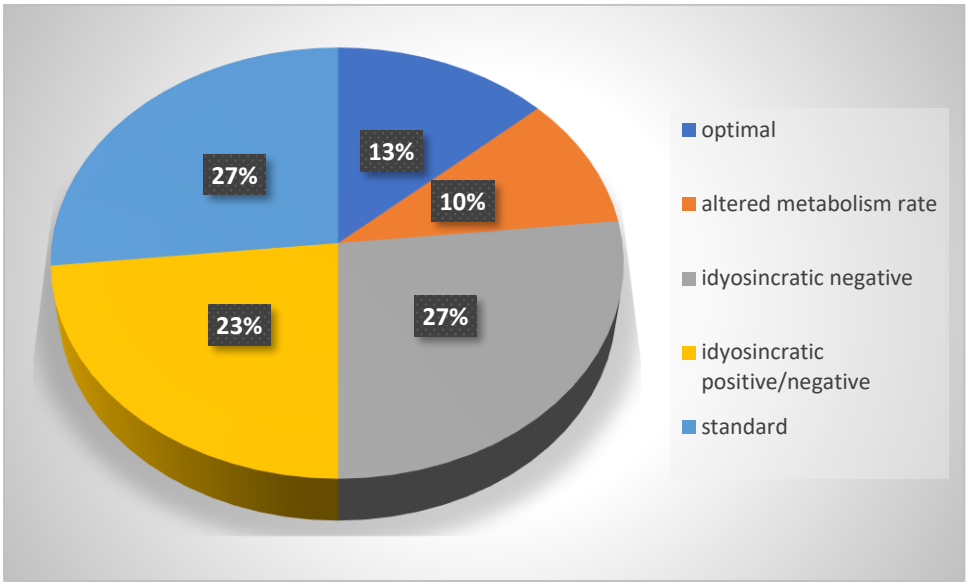

Figure 2.2. PGT suggestions about the therapies received by the sample

At the 3-month follow-up evaluation, 13 patients (40%) had received a change of therapy concerning the Neurofarmagen test; this definition means that the ongoing therapy was appropriate to the test report for efficacy and/or tolerability, without alteration in metabolic rate or high collateral risk. Overall, 10 patients (32%) maintained a therapy discordant to the test. The other 7 patients (28%)

included 4 patients with missing follow-up data, 3 patients who received simultaneously a modification agreeing and a modification not agreeing with the test result at the same time.

Secondary results

A sub-analysis of the sample distribution was performed making a comparison between two small subgroups in terms of psychopathology and tolerability. Comparing the subgroup receiving a therapy consistent to the test after the test result (n = 13 patients, 40%) with the subgroup receiving a therapy not consistent with the test after the test result (n = 10 patients, 32%) in term of psychopathology, a significant statistical difference of treatment over time (i.e. treatment × time interaction) in the CGI-S ($p < 0.001$) emerged: a greater improvement in patients receiving a therapy consistent with the test was observed (Figure 2.3).

Importantly, this effect was still observed when including the baseline HDRS score and baseline AEs as covariates. At the same time, a significant statistical difference over time emerged for HDRS ($p=0.001$), with a greater improvement in the subpopulation which received a therapy consistent to the PGT (Figure 2.4), which was due to the group of patients showing a trend for higher (i.e. worse) HDRS score at baseline ($p < 0.1$). No significant statistical differences between the two subgroups across time emerged for YMRS ($p = 0.9$), as shown in Figure 2.5.

Regarding the adverse events (AEs) recorded through the DOTES scale, an interesting result emerged: at baseline, only 2 out of 10 patients (20%) who did not receive a change in therapy had AEs, while 9 out of 10 (90%) who later received a therapy modification according to the test, presented collateral effects ($p = 0.013$ for the difference at baseline). After 3 months, the incidence of adverse effects in the rest subpopulation did not improve, while the second subgroup presented a significant reduction of AEs as shown in Figure 2.6, with only 3 out of 10 (30%) of patients showing AEs ($p = 0.031$ for within-group change from baseline).

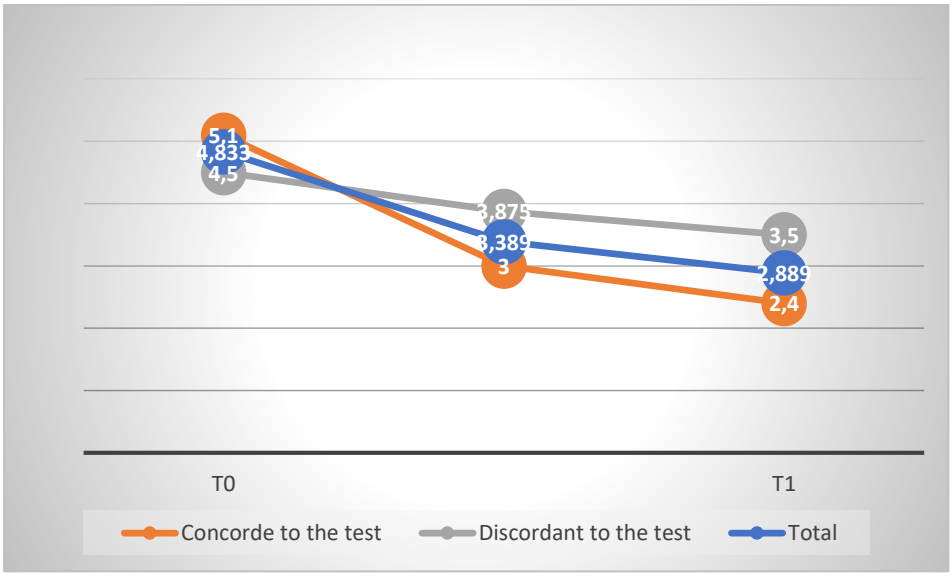

Figure 2.3 Clinical Global Impression Severity (CGI-S) average scores at baseline (T0) and 3-month follow-up (T1).

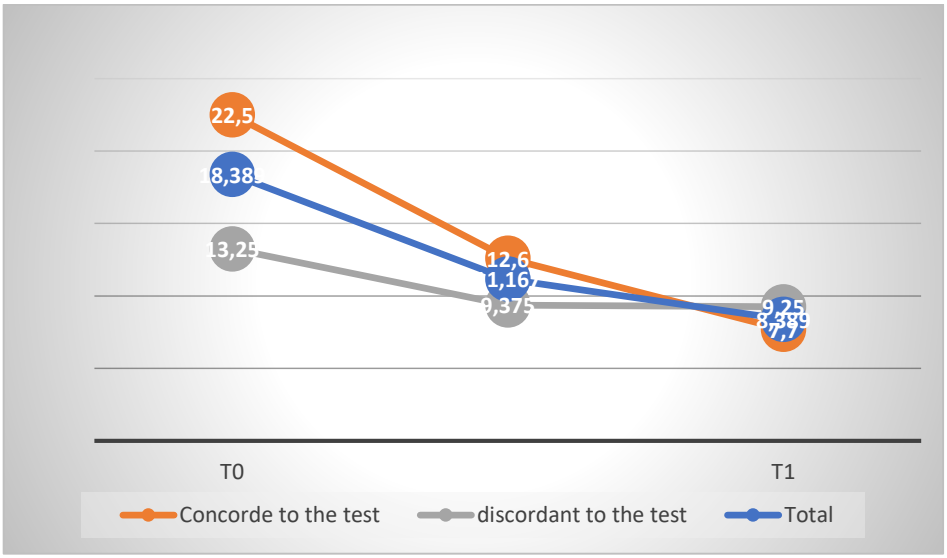

Figure 2.4 The Hamilton Depression Rating Scale (HDRS) average scores at baseline (T0) and 3-month follow-up (T1).

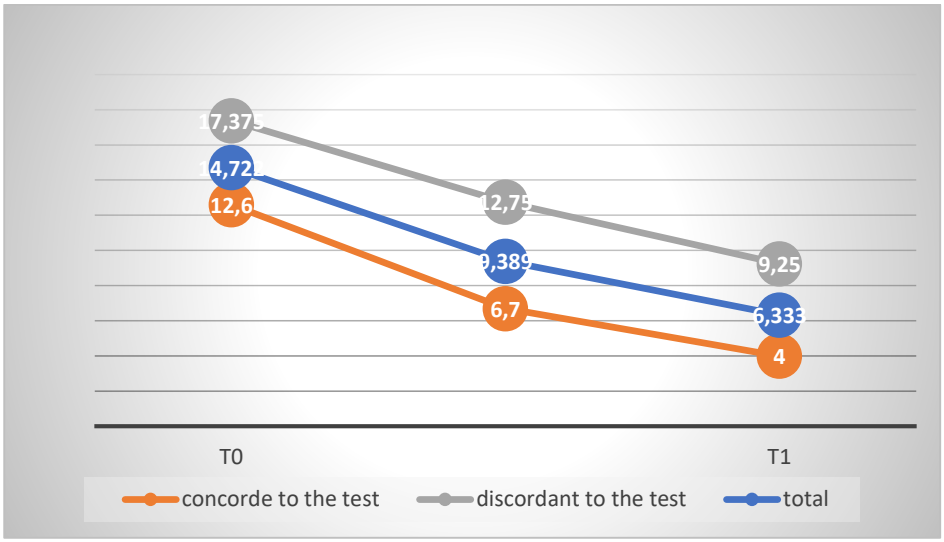

Figure 2.5 Young Mania Rating Scale (YMRS) average scores at baseline (T0) and 3-month follow-up (T1).

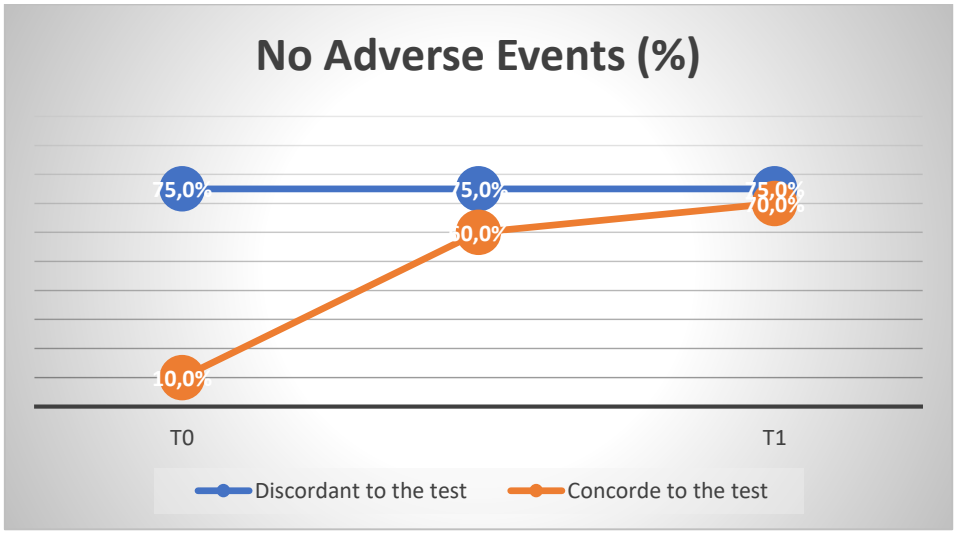

Figure 2.6: Distribution of the patients according to the percentage of no adverse events (AEs) at baseline (T0) and 3 months follow up (T1).

Follow up

Psychopathological trend

At T2 (12 months later) 93% of patients (n=28) received a therapy concordant to the test; the others (7%, n=2) had a therapy discordant to the test (as shown in figure 2.7).

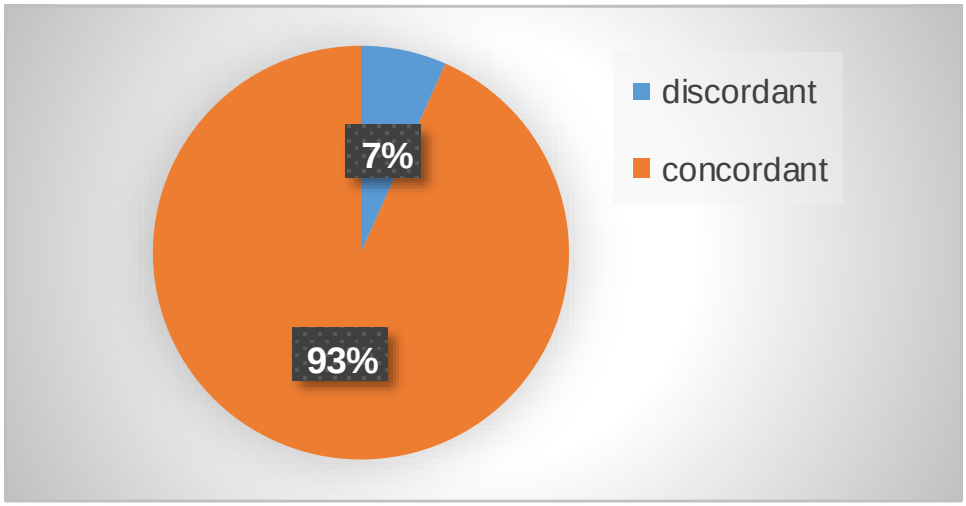

Figure 2.7: concordance of therapy with PGT at 12 months follow up

The global average scores of the scales indicated a clinical improvement as shown in Figure 2.8.

T0: CGI 4.82, HDRS 18.36, YMRS 14.72.

T1: CGI 3.39, HDRS 8.39, YMRS 6.33.

T2: CGI 2.89, HDRS 7.15, YMRS 5.43

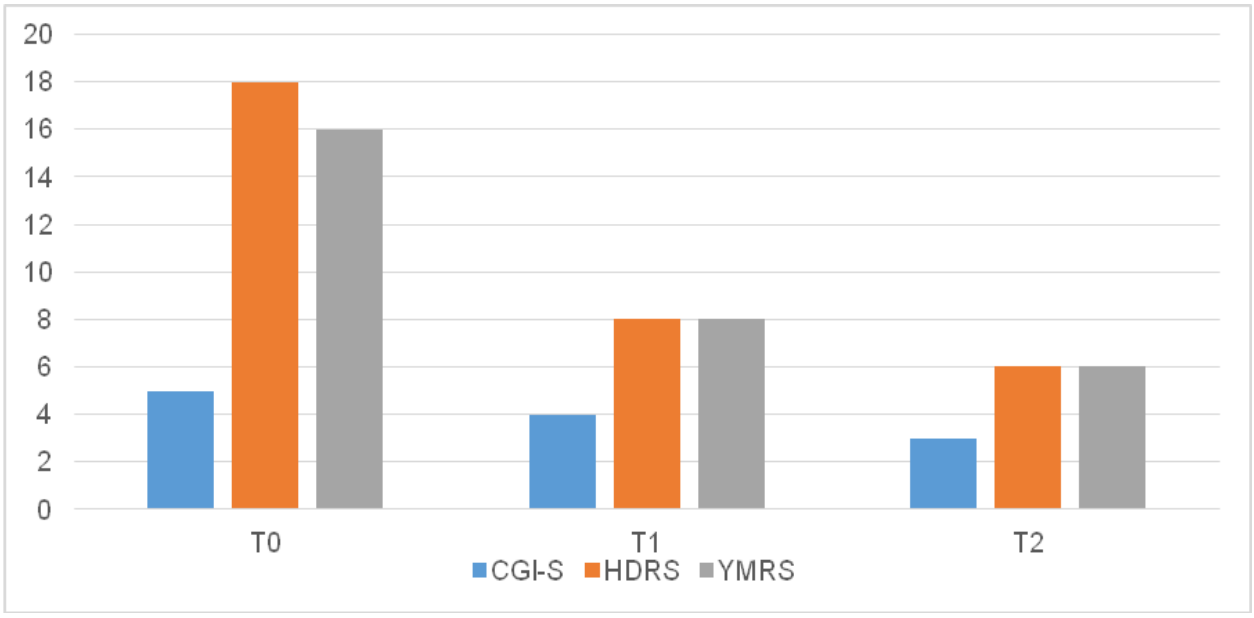

Figure 2.8: psychopathological trend of the sample during time

Polymorphism of the sample

Here are the most frequent and clinically relevant pharmacokinetic mutations within the population (Figure 2.9). The most common is CYP1A2 in 48% of the population that was proven ultrarapid metabolizer (UM). In our population three of the patients carrying this mutation and took olanzapine, that is metabolized at this level; 36% of the population followed by frequency was IM (intermediate metabolizer) for CYP2C9, 24% and 20% for CYP2D6, respectively. Furthermore, a great clinical interest regards the mutation of CYP2D6, cytochrome that metabolizes about 25% of the totality of the drugs in use, in particular antidepressants, antipsychotics, but also antiarrhythmics and B-blockers. To this regard the 4% of PM (poor metabolizer) patients should be evaluated very carefully. In fact, it is documented in the literature how this mutation associated with the use of antidepressants is highly predisposing for the risk of switches in bipolar patients. Luckily, only 1 patient was PM. CYP2C19 has also to be considered for the role in the metabolism of citalopram, escitalopram, amitriptyline, and clomipramine. Among the most frequent and clinically relevant pharmacokinetic mutations within the population CYP1A2 was mutated as UM in 48% of the population (olanzapine and agomelatine are metabolized at this level). In our population 3 of the patients carrying this

mutation took olanzapine); 36% of the population was IM for CYP2C9, 24% and 20% for CYP2D6, respectively.

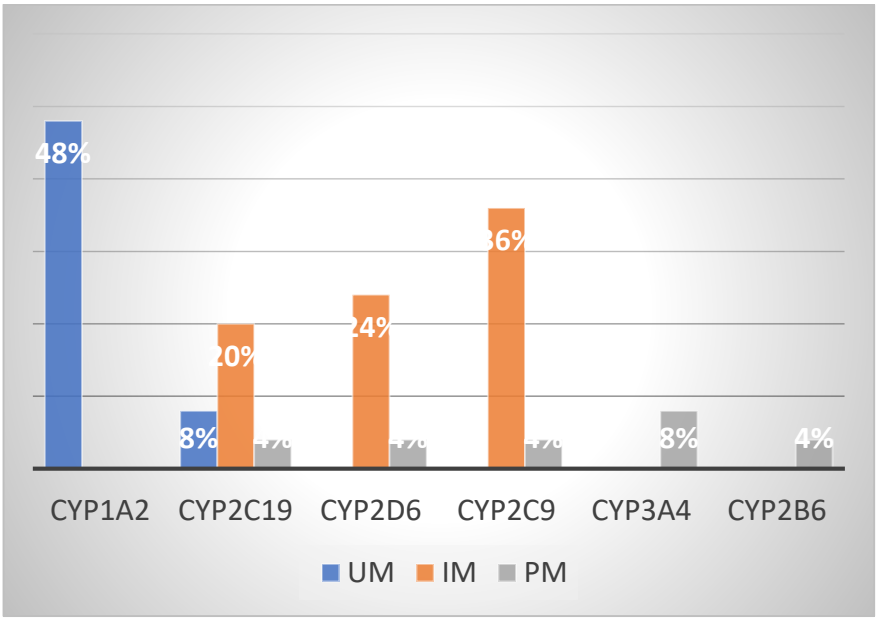

Figure 2.9: Distribution of the sample according to the most relevant pharmacokinetic mutations.

Dealing with the most relevant pharmacodynamic mutations in relation to the use of antipsychotic drugs, as shown in Figure 2.10, the most frequent is in the AKT1 genes, DD1T4-RPTOR-FCHSD1: in 39% of patients indicated a low-risk phenotype of EPS (extrapyramidal symptoms), while in the remaining 40% of cases a high risk of EPS for patients receiving haloperidol, risperidone, zuclopentizole and palieridone. Specifically, within our population, this association was found in 4% of cases in which patients took haloperidol. Current and interesting from a clinical point of view is the HTR2C mutation showed by 24% of patients. It indicates a greater predisposition to the development of metabolic syndrome. Other interesting mutations were found in the NEF3, AKT1-RGS4 genes favorable for the response to antipsychotic.

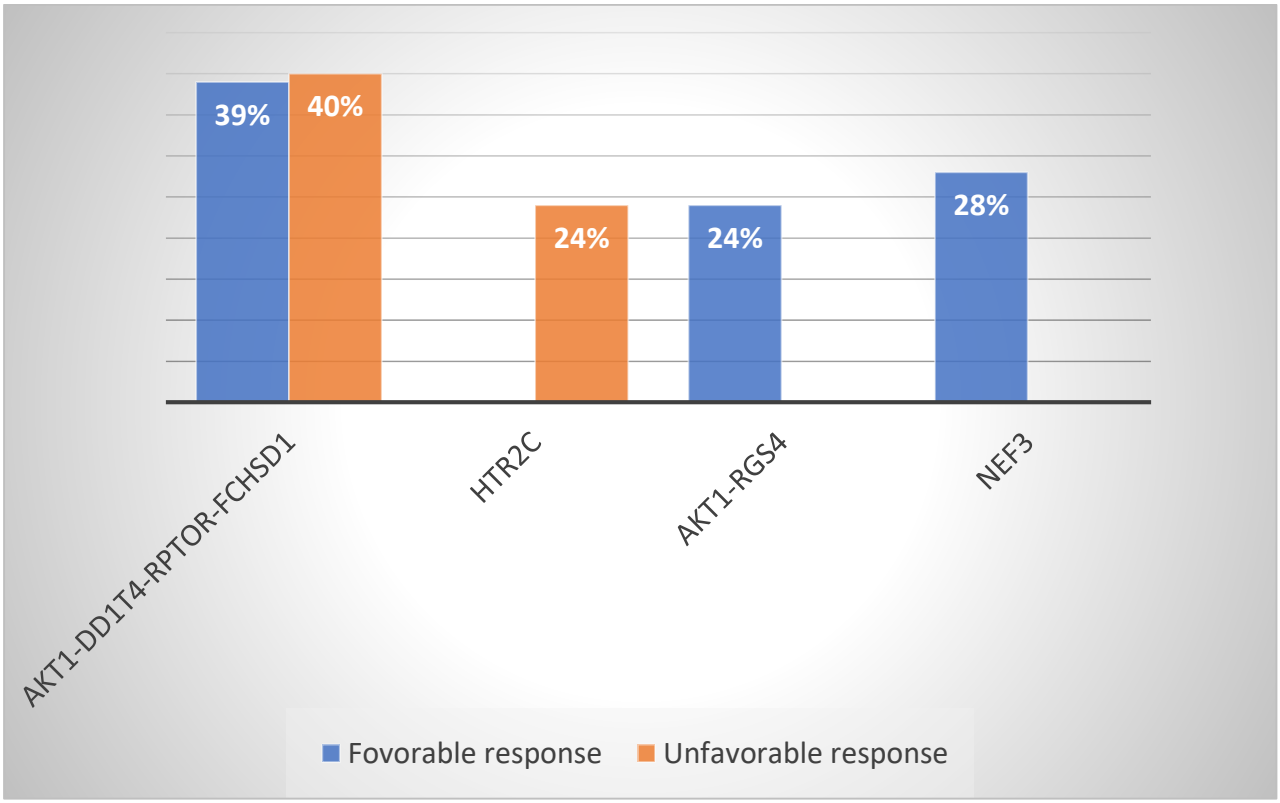

Figure 2.10: Distribution of the sample according to the most relevant pharmacodynamic mutations in relation to the use of antipsychotic.

Dealing with the use of mood stabilizing drugs, the most relevant mutation in our population is in the CACNG2 gene: 48% of patients presented a mutation associated with a good response to lithium. Conversely, the HLA mutation in 16% of patients indicates greater sensitivity and therefore greater probability of adverse effects with carbamazepine. A more complex issue concerns the mutation of the ABCB1 gene, better known as MDR (multi drugs resistant protein), which encodes a protein that regulates the transport of xenobiotics across the blood-brain, blood-testicular and blood-placental barriers. A resistance-related mutation to all AEDs evaluated was found in 12% of our population (Figure 2.11).

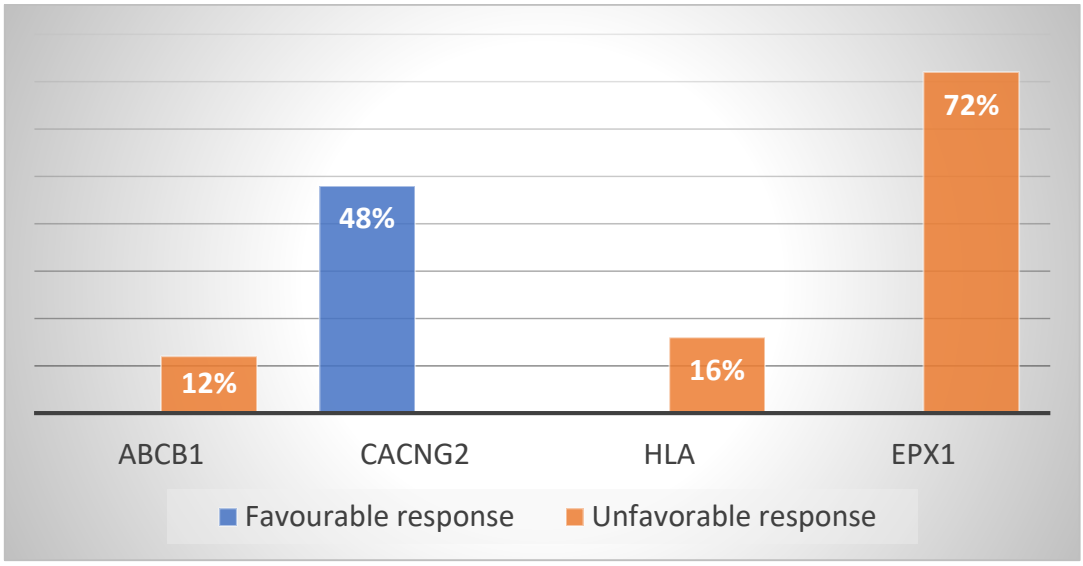

Figure 2.11: Distribution of the sample according to the most relevant pharmacodynamic and pharmacokinetic mutations in relation to the use of antipsychotic.

This same gene was favorably mutated in relation to SSRI treatment in 40% of cases. In relation to the response to antidepressants, favorable mutations in GRICK4 for citalopram, in HTR1A for fluvoxamine and paroxetine response and in BDNF for SSRI were found, as shown in Figure 2.12.

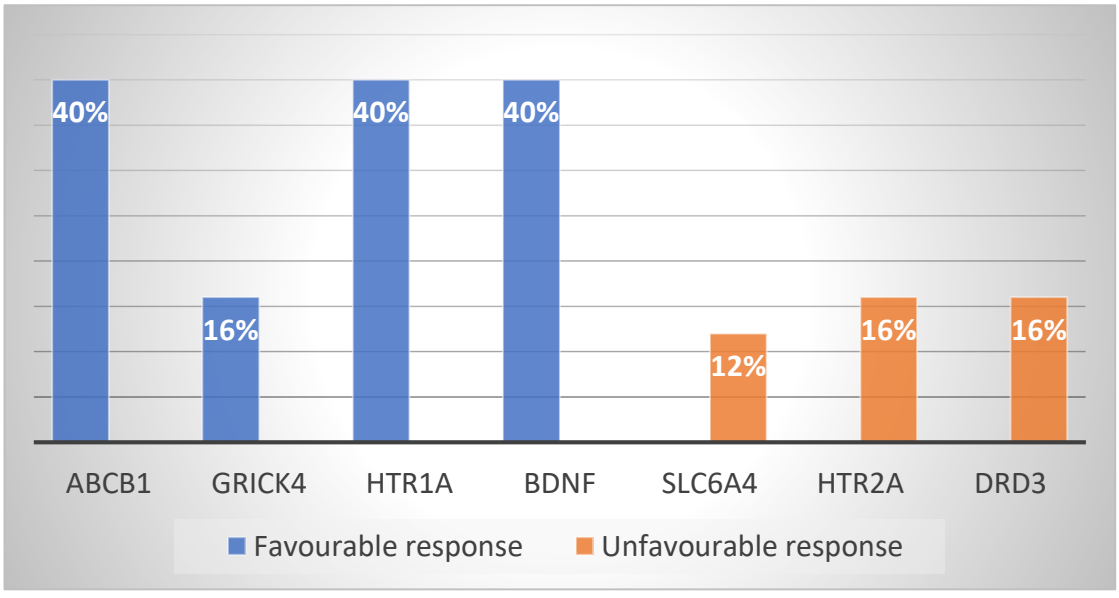

Figure 2.12: Distribution of the sample according to the most relevant pharmacodynamic and pharmacokinetic mutations in relation to the use of antidepressant.

2.4.2 Phase II

Sociodemographic and clinical data

Sociodemographic and clinical data of the sample are shown in Table 2.3. Thirty patients affected by bipolar disorder were recruited. The average age was 48.8 years-old, with 43% of the patients being male and 47% female. All patients were Caucasian. The CGI-S mean score was 4.6 (SD3.8) at the time of recruitment (execution of the test).

Table 2.3 Sociodemographic and clinical characteristics

	Data	%
Mean Age (SD)	48.8 (15.07)	100
Men	13	43
Women	17	47
Nationality		
-italian	28	94
-others	2	6
Job		
-employed	13	43
-unemployed	5	17
-retired	8	27
-invalid	4	13
CGI-s score (SD)	4.6 (3.8)	100
HDRS score (SD)	17.8 (8.2)	100
YMRS score (SD)	14.2 (6.3)	100
Mean age of illness Years (SD)	14.5 (7.08)	100
Diagnosis, N (%)		
-Bipolar Disorder I	13	43
-Bipolar Disorder II	17	47

Legend: SD= standard deviation; CGI-s= Clinical Global Impression- Severity; HDRS=Hamilton Depression Rating Scale.

Number of Hospitalizations

The first mirror analysis compares the number of hospitalizations before and after the modification of the therapy concordant to the test showing a significant statistical difference between the pre-PGT year of observation and the post-PGT year of observation, resulting in fewer hospitalizations after the

assignment of a psychopharmacological treatment concordant to the PGT (p = 0.0001), by paired t-test (Table 2.4, Figure 2.13).

Table 2.4 Number of hospitalizations: mirror analysis.

Group	1-Year Pre PGT	1-Year Post PGT	p
Mean	1.37	0.23	
SD	1.52	0.57	0.00
SEM	0.28	0.10	
N	30	30	

Legend: SD= standard deviation; SEM= Standard Error of Mean; N=Number; PGT=Pharmacogenetic Test.

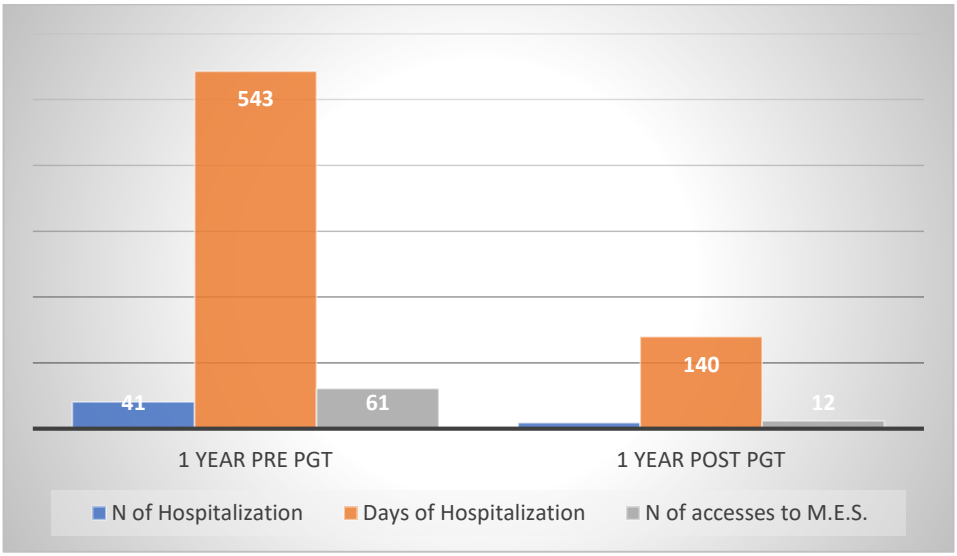

Figure 2.13: Mirror analysis of the number of hospitalizations, days of hospitalizations, and number of times medical emergency services were accessed.

Number of Days of Hospitalization

Also dealing with the number of days of hospitalization, the difference between the pre-PGT year of observation and the post-PGT year of observation is statistically significant (p = 0.0001), (as shown in Table 2.5 and Figure 2.13).

Table 2.5 Number of days of hospitalizations: mirror analysis.

Group	1-Year Pre PGT	1-Year Post PGT	p
Mean	18.10	4.67	
SD	19.19	10.26	0.00
SEM	3.50	1.87	
N	30	30	

Legend: SD= standard deviation; SEM= Standard Error of Mean; N=Number; PGT=Pharmacogenetic Test.

Number of Times Emergency Services were Accessed

It is interesting to highlight that a significant statistical difference emerges between the pre-PGT year of observation and the post-PGT year of observation, resulting in fewer incidents where emergency services were accessed during the year following the setting of a therapy concordant to the PGT, using the paired t-test (Table 2.5, Figure 2.13).

Table 2.5 Number of times medical emergency services were assessed: mirror analysis

Group	1-Year Pre PGT	1-Year Post PGT	p
Mean	2.07	0.40	
SD	1.55	0.56	0.00
SEM	0.28	0.10	
N	30	30	

Legend: SD= standard deviation; SEM= Standard Error of Mean; N=Number; PGT=Pharmacogenetic Test.

Economic Enhancement

Dealing with the economic enhancement, we have used a standard value of a medium-length hospitalization for bipolar disorder diagnosis, using the diagnosis-related groups (DRG). By calculating a daily cost of € 310.25, it is possible to quantify the difference in expenditure for total days of admission in the year before versus the total of days of admission in the year after the agreed therapy check, as shown in Table 2.6. The PGT cost of € 950 has been added to the "1-year post PGT" category, as shown in Table 2.6.

Table 2.6: Economic Enhancement of days of hospitalization (pre- and post-change of therapy).

	Total Number of Days of Hospitalization	Economic Enhancement (€)	Economic Enhancement Adding the PGT cost (€)
1-Year Pre PGT	430	148,920	---
1-Year Post PGT	34	10,548	39.048

Legend: PGT=pharmacogenetic test.

2.4.3 Phase III

Psychiatrist attitudes

Phase III evaluates the attitude of psychiatrists towards pharmacogenetics within daily practice. The specialists interviewed were recruited in the Psychiatry Units of the ASST Settelaghi of Varese and Verbano and ASST Santi Paolo e Carlo Borromeo of Milan (Italy). The questionnaire was administered to medical doctors and residents.

To investigate the opinion of the specialists, we used a questionnaire formulated by Thompson et al (2015) and administered in a study carried out at the University of California.

The questionnaire (Table 2.7) includes 5 questions, to which the specialists answered with "yes" or "no", as well as a preliminary interview on personal data and on any previous experience with PGT in the clinical setting:

Table 2.7: questionnaire about the psychiatrists' attitude towards the use of pharmacogenetics into clinical practice

Question:	Yes %, N	No %, N
Would it be beneficial to have a genetic counselor discuss genetic test results with you and your patient?	72%, 32	28%, 13
Would you refer a patient to a direct to consumer (DTC) testing company to order genetic information that may help in treatment?	100%, 45	0
Do you believe that having genetic data may help you and your patients make better decisions about his or her medications?	91%, 41	9%, 4
Do you believe that you would act on specific data from your patient that may indicate potential drug–drug interactions?	100%, 45	0
Do you believe that genetic testing will eventually become standard practice in patient treatment?	82%, 37	18%, 8

The data analysis is mainly descriptive; furthermore, the data were compared by dividing the psychiatrists into two groups: those who already had experience with PGTs and those who did not, comparing the answers by Chi-square test.

Among the psychiatrists interviewed, 23 are women and 22 men; the average age is 39.3; the average age of job seniority is instead 11.6 years; 20 specialists interviewed have already had experience in the field of pharmacogenetics. Of the interviewees 23 are involved in the outpatient service, 12 in Psychiatric ward, 8 in residential structures and 2 are university professors.

All respondents 100% (N = 45) believe that pharmacogenetics can help specialists and patients in making decisions about psychopharmacological treatment. All respondents 100% (N = 45) believe that pharmacogenetics can help in setting up therapy, particularly regarding drug interactions.

82% (N = 37) of respondents believe that pharmacogenetic test could become a routine tool in clinical practice.

There were no statistically significant differences in the responses between those who already had experience with PGTs in clinical practice and those who did not, in any of the questions asked.

2.4.4 Phase IV

Systematic review

The lithium salts' literature review gave information about several genes investigated in the last twenty years. The most important have been summarized in the following table (Table 2.8).

Table 2.8. Lithium pharmacogenetic over the past two decades: characteristic of the included studies

Study	Year of publication	Sample	Study design	Definition of response	Gene	Marker	Results
Serretti et al. [2]	1998	55	Prospective	Difference between a pre-treatment index and an ongoing treatment index	DRD-3	DRD-3 variants	No association
Steen et al. [3]	1998	Sample a) 43 Sample b) 104	Retrospective study	(a) Demonstrated ''complete lithium response (b) Demonstrated ''long and complete remission'' on lithium alone	INNP1	A682G, G153T, G348A, C973A	C973A better response in sample a); did not replicate in sample b).
Turecki et al. [4]	1998	136 excellent responders and 163 controls	Case-control association study	No affective episodes	PLCG-1	Dinucleotide repeat	More common in responders
Serretti et al. [5]	1999	25	Prospective study	Difference between a pre-treatment index and an ongoing treatment index	DRD-2, DRD-4 and GABA- 1	Genes variants	No association
Serretti et al. [6]	2000	124	Prospective study	Number of affective episodes before and after treatment	5HT2A and 5HT2C	Genes variants	No association
Ftouhi et al. [7]	2001	133 excellent responders and 99 controls	Case-control association study	No affective episodes	PLCG-1	Gene variants	Uncertain association
Lovlie et al. [8]	2001	61	Retrospective study	Number of affective episodes before and after treatment	PLCG-1	Gene variants	Uncertain association

Serretti et al. [9]	2001	201	Prospective study	Difference between a pre-treatment recurrence index and an on-treatment recurrence index	5-HTTLPR	Alleles S and L	s/s genotype showed a worse response than s/l and l/l
Serretti et al. [10]	2002	201	Prospective study	Difference between a pre-treatment recurrence index and an on-treatment recurrence index	COMT, GSK3, MAO-A	Genes variants	No association
Washizuka et al. [11]	2003	54	Retrospective study	No affective episodes	mtDNA	SNPs 5178 and 10398	Significant association between 10398A polymorphism and lithium response
Serretti et al. [12]	2004	83	Retrospective study	Number of affective episodes before and after treatment	5-HTTLPR	Alleles s and l	Genotype l/s associated with a better response
Benedetti et al. [13]	2005	88	Prospective study	Number of affective episodes before and after treatment	GSK3	rs334558 (-50T/C)	C allele associated with a better response
Kakiuchi et al. [14]	2005	56	Retrospective study	Clinical improvement greater than 50%	XBP1	-116C/G	G allele associated with a worse response
Rybakowski et al. [15]	2005	67	Retrospective study	No affective episodes	5-HTTLPR	Alleles s and l	Genotype s/s and s allele more frequent in poor responder
Rybakowski et al. [16]	2005b	88	Retrospective study	No affective episodes	BDNF	Val66Met and 270C/T	Val/Met genotype of BDNF occurred more frequently in excellent responders
Masui et al. [17]	2006	161	Retrospective study	Less frequent and/or severe relapse, including no relapse, compared with the period before the initiation of lithium treatment	BDNF	Val66Met	No association
Masui et al. [18]	2006b	66	Retrospective study	Less frequent and/or severe relapse, including no relapse, compared with the period before the initiation of lithium treatment	XBP1	-116C/G	Lithium more effective in -116C allele carriers than in -116G homozygotes
Michelon et al. [19]	2006	134	Retrospective study	No recurrence of impairing symptoms, or recurrence of mild symptoms, promptly controlled by adjusting the lithium dose	INNP-1, BDNF, 5HTTLPR, GSK-3β	C973A, rs6265, rs3755557	No association
Bremer et al. [20]	2007	184	Retrospective study	Clinical improvement greater than 50%	NTRK2 INPP1	rs1387923 rs2067421	NTRK2: genotype T/T associated with a better response. INNP1: nominal association in patients with comorbid post-traumatic stress disorder
Rybakowski et al. [21]	2007	111	Retrospective studies	No affective episodes	5HTTLPR and BDNF	5HTTLPR and Val66Met interaction	Worse response in genotype S/S + Val/Val
Dmitrzak et al. [22]	2008	108	Retrospective studies	No affective episodes	BDNF NTRK2	rs2030324, rs988748, Val66Met, rs2203877 rs1187326, rs2289656 e rs1187327	rs988748 and Val66Met associated with a better response
Rybakowski et al. [23]	2008	92	Retrospective studies	No affective episodes	DRD1	-48A/G	G allele poor response
Perlis et al. [24]	2009	1177	Genome wide association study	Pre- and post-treatment psychometric tests	-	SNPs in a region on chromosome 4q32	Only 458 were under treatment with Lithium
Campos-de-Sousa et al. [25]	2010	170	Prospective study	Alda Scale	Rev-Erb-α	7 SNPs	rs2314339 poor response
Mc Carthy et al. [26]	2011	282	Retrospective study	Alda Scale	16 SNPs on seven clock genes	Gene variants	Rev-Erb-α: NR1D1 and CRY1 better response

Rybakowski et al. [27]	2012	101	Retrospective study	No affective episodes	Multiple SNPs on 14 different genes	Genes variants	Possible role in Lithium response of 5HTT, DRD1, COMT, BDFN and FYN
Rybakowski et al. [28]	2013	78	Retrospective study	No affective episodes	GSK3	-50T/C	Genotype -50C/C better response in terms of tolerability
Chen et al. [29]	2014	188	Genome wide association study	Alda Scale	-	rs1702668 and rs 17026651 on GADL1 high sensibility	Patients of Han Chinese descent
Iwazashi et al. [30]	2014	42	Retrospective study	Clinical improvement greater than 50%	GSK3	-50T/C and 1727A/T	Genotype -50T/T e -1727A/A associated with a better response to Lithium
Rybakowski et al. [31]	2014	115	Retrospective study	No affective episodes in patients in monotherapy	Several clock genes	Genes variants	6 SNPs of ARNTL and three haplotypes of TIMELESS associated with a better response
Mitjans et al. [32]	2015	131	Retrospective study	Clinical improvement greater than 50%	Multiple SNPs on 16 genes	Genes variants	INPP1: rs3791809, rs4853694 e rs909270 associated with Lithium response GSK3: rs1732170, rs11921360 e rs334558 associated with Lithium response
Cruceanu et al. [33]	2015	41 Caucasian families with high bipolar disorder incidence	Case-control association study	Alda Scale	GADL1	rs1702668 e rs 17026651	No association
Kotambail et al. [34]	2015	151	Retrospective study	No affective episodes	GADL1	rs1702668 e rs 17026651	No association
Geoffroy et al. [35]	2016	151	Retrospective study	Alda Scale	22 Clock genes	Several SNPs	PGC-1α and RORA involved in Lithium response
Hou et al. [36]	2016	2563	Genome wide association study	Alda Scale	-	rs79663003, rs78015114, rs74795342, rs75222709 on chromosome 21 associated with Lithium response	Patients collected by 22 participating sites from the International Consortium on Lithium Genetics
Song et al. [37]	2016	3874	Genome wide association study	Pre- and post-treatment psychometric tests	-	rs116323614 on SESTD1	No significant association within bipolar patients, but strong association comparing lithium responders with healthy controls
Moreira et al. [38]	2017	36 patients and 20 controls	Case-control association study	Alda Scale	GADL-1	-	No association

Table 2.8 - DRD-3: Dopamine Receptor D3; DRD-2: Dopamine Receptor D2; DRD-4: Dopamine Receptor D4; GABA- 1: gamma-aminobutyric acid; INNP1: inositol polyphosphatase-1-phosphatase; PLCG-1: plyphospholipase C-gamma 1; 5HT2A: Serotonin 2A receptor; 5HT2C: Serotonin 2C receptor; 5-HTTLPR: serotonin transporter linked polymorphic region; COMT: catechol-O-methyltransferase; GSK3: glycogen synthase kinase 3; MAO-A: monoamine oxidase A; XBP1: X-box binding protein 1; BDNF: brain derived neutrophic factor; NTRK2: neurotrophic receptor tyrosine kinase 2; Dopamine Receptor D1; GADL1: glutamate decarboxylase like 1; SESTD1: SEC14 and spectrin domain containing 1

As shown in table 2.8, several genes have been investigated: candidate genes participating in monoaminergic neurotransmitter systems, the circadian system, neurotrophic mechanisms, or the inositol signalling pathway have been the most studied in the literature (Papiol et al, 2018) .

Polymorphisms in dopaminergic receptors genes were the first to be investigated, since the dopaminergic neurotransmitter system seems to play a pivotal role in the pathophysiology of Bipolar Disorder, as highlighted over the last decades (Berk et al, 2007; Cousins et al, 2009; Wittenborn, 1974). Nonetheless, at the end of 90s', Serretti et al, in two retrospective studies involving respectively 55 and 125 patients, did not find any association between polymorphisms in dopaminergic receptor genes (DRD2, DRD3 and DRD4) and patients' response to Lithium (Serretti et al, 1998, 1999). However, approximately ten years later, Rybakowsy et al, in a retrospective study on 92 patients diagnosed with Bipolar Disorder, have highlighted the existence of strong association between polymorphism -48A/G in gene coding for dopaminergic receptor-1 and the response to lithium: specifically, the genotype G/G seems to be associated with an excellent response (Rybakowski et al, 2009). These results had been confirmed subsequently in another retrospective study on 101 patients affected by BD, in which 14 genes, previously reported as potentially involved in patients' response to Lithium, were investigated (Rybakowski et al, 2012).

Serotonin is one of the most important neurotransmitters and with lots evidence that supports the association between the serotonergic system with Mood Disorders, including Bipolar Disorder (Lin et al, 2014). All things above considered, the interest shown by the scientific community in finding polymorphisms on genes involved in serotonin's metabolism could explain the variability in response to lithium, is pretty much explained. Serretti and co-authors first and, subsequently Rybakowski and his colleagues, failed to identify any polymorphisms on genes coding for serotoninergic receptors 5HT2A and 5HT2C that could account for the differences, commonly observed in the population, in Lithium response (Rybakowski et al, 2012; Serretti et al, 2000). In the recent years, the role of the functional polymorphism in the regulatory region of the serotonin transporter gene in BD has been a matter of intensive research (Cho et al, 2005). The 44-base pair insertion/deletion within the promoter

region of the serotonin transporter gene (5-HTTLPR) can exist in two allelic forms: the long variant (L) and the short variant (S) (Mynett-Johnson et al, 2000); the presence of the latter, both in homozygosis and heterozygosis, has been associated with a lower transcriptional activity and a consequent reduction in serotoninergic transmission (Collier et al, 1996; Lesch et al, 1996). Thus, the presence of the allele S has been investigated as a predictive factor for clinical response to Lithium. Furthermore, variants of this gene have been associated with individuals' variation in harm avoidance; this personality traits seems to mediate the effects of functional polymorphisms in the regulatory region of the serotonin transporter gene in response to treatment in bipolar patients (Mandelli et al., 2009). As shown in Table 2.8, to date the association between clinical Lithium's efficacy and polymorphism in the regulatory region of the serotonin transporter gene remains not clear and unconvincing. In fact, some studies had pointed out as the presence of the allele S is associated with a worse response to therapy (Rybakowski et al, 2005; Serretti et al, 2001, 2004), whereas others did not confirm these results (Michelon et al, 2006). Moreover, in a retrospective study involving 111 patients diagnosed with BD, Rybakowski et al has shown that excellent Lithium responders presented the simultaneous presence of allele S and polymorphism Val66Met on gene coding for Brain Derived Neurotrophic Factor (BDFN) (Rybakowski et al, 2007).

As mentioned above, inositol signalling pathway has been a matter of intensive study in the last two decades, in view of the hypothesis according to which lithium-blockable enzyme inositol polyphosphate 1-phosphatase is a putative target for the mood-stabilizing effects of lithium. An association with such response was obtained with polymorphism C973A of the inositol polyphosphate 1-phosphatase (*INPP1*) gene (Steen et al, 1998). Moreover, Bremer et al, in a study on 184 patients recruited from family for linkage studies, found a significant interaction between lithium response and single-nucleotide polymorphism (SNP) rs2064721 in INNP1 gene, particularly in patient with an associated post-traumatic stress disorder (Bremer et al, 2007). More recently, Mitjans and colleagues (Mitjans et al, 2015), investigating the potential association of genetic variability at genes related to INNP1, glycogen synthetase kinase-3 (GSK3), hypothalamic-pituitary-

adrenal and glutamatergic pathways with lithium response, in a sample of 131 patients diagnosed with BD, identified a significant association for the SNPs rs3791809, rs4853694 and rs909270 in INNP1 gene with a better response to lithium therapy.

Bipolar Disorder was first linked to glycogen synthetase kinase-3 hyper-activation in 1996 (Klein and Melton, 1996), following the observation that Lithium is a direct inhibitor of this enzyme. From this perspective, it is easy to understand the interest shown by researchers for the gene coding for GSK3 as a potential genetic marker to predict patients' response to Lithium. Regardless of these assumptions, most studies failed to find any association between Lithium response and SNPs on gene coding for GSK3 (Michelon et al, 2006; Serretti et al, 2002). However, there are several evidences which highlight the possible role of this gene in modulating response to Lithium: Benedetti et al, in 2005, showed that carriers of the allele C had a better improvement on Lithium therapy (Benedetti et al, 2005), as later confirmed in a more recent study, carried out by Rybakowski and other, in which authors highlighted that the presence of this allele is associated with a greater tolerability, particularly for what concerns renal functioning (Rybakowski et al, 2013). Contrary to Benedetti's claim and of his group, the study of Iwahashi and co-workers, in 2014, pointed out that carriers of GSK3 haplotype T-A presented a higher Lithium response; this might suggest that patients with the T allele, which gives greater transcriptional activity, are more affected by Lithium, which inhibits GSK3 activity, comparing to those with the C allele (Iwahashi et al, 2014).

Concerning neurotrophic factors, one of the most investigated is Brain-Derived Neurotrophic Factors (BDFN), whose serum levels could represent a potential biomarker of disease activity in Bipolar Disorder (Fernandes et al, 2015). Furthermore, the presence of the SNP Val66Met on gene coding for BDFN has been associated with a greater susceptibility for the development of BP (Green et al, 2006; Sklar et al, 2002). Rybakowski and colleagues, in a retrospective study involving 88 patients with BD, were the first to highlight the existence of a strong association between this SNP and an excellent response to Lithium (Rybakowski et al, 2005). Such evidence was later confirmed by Dmirtzak and his group, in 2008 (Dmitrzak et al, 2008), and by Rybakowski himself, in 2012

(Rybakowski et al, 2012). Furthermore, it seems to be a significant interaction between BDFN gene and 5-HTTLPR polymorphism in determining patients' response to Lithium: the combination of Val66Met polymorphism and allele S seems to be more common in poor responders (Rybakowski et al, 2007).

Starting from the evidence that BDFN could be involved in BD pathogenesis, several researchers have wondered whether Neurotrophic Tyrosine Kinase Receptor 2 (NTRK2), a specific BDFN receptor that regulates neuronal differentiation (Luberg et al, 2010), might have a role in modulating response to therapy with Lithium. Despite these premises, only Bremer and co-workers, in a study with 184 patients with Bipolar Disorder, successfully demonstrated a strong association between SNP rs1387923 and Lithium response, but only in a subset of patients with euphoric rather than dysphoric mania and without suicidal intention (Bremer et al, 2007).

In recent years, interest of scientific community shifted on "clock" genes and their possible role as predictive factors to identify patients that could benefit the most from therapy with Lithium. It is well known, from many years now, that abnormalities in circadian rhythms often are the first sign of illness acute exacerbation (Kripke et al, 1978; Shi et al, 2008). Lithium salts acts by correcting these abnormalities through the modulation of "clock" genes' expression (Kripke et al, 2009; McClung, 2007). Campos De Sousa and his colleagues were the first to investigate the role of seven SNPs on Rev-Erb-α in determining response to therapy, in a sample of 170 patients under long-term treatment with Lithium, demonstrating how the presence of SPN rs2314339 was associated with a poor response to treatment (Campos de Sousa et al, 2010). One year later, McCarthy and collaborators identified two SNPs of on Rev-Erb-α (rs2071427, rs8192440) which were nominally associated with a better response to Lithium (McCarthy et al, 2011), confirming the involvement of Rev-Erb-α in patients' Lithium response. Over the following years, the interest in the possible role of clock genes in determining response to Lithium has spread through researchers, and other genes were identified as possibly involved. In 2014, Rybakowski and colleagues suggested that the six SNPs and three haplotypes of ARNTL gene and two SNPs and one haplotype of TIMELESS gene might be associated

with the lithium prophylactic response in bipolar patients (Rybakowski et al, 2014). Two years later, Geoffrey et al, by testing the association between 22 core clock genes with Lithium response in BD in two independent samples, found an association between PPARGC1A (PGC-1α) and RORA genes and Lithium response (Geoffrey et al, 2016).

A separate mention should be made with respect to Genome Wide Association Studies (GWAS) (Table 2.9). As shown in table 2.9, as concerns Lithium pharmacogenetic, at present only four GWAS were made, the first of which dates back to 2009. Perlis et al examined the hazard for mood episode recurrences among 1,177 patients with bipolar disorder, including 458 individuals treated with Lithium. SNPs showing the greatest evidence of association were then examined for association with positive lithium response among patients treated with Lithium. Five regions showing suggestive evidence of association with lithium response, were further associated with positive lithium response (Perlis et al, 2009), including SNPs in a region on chromosome 4q32 spanning a gene coding for the glutamate/alpha-amino-3-hydroxy-5-methyl-4-isoxazolpropionate (AMPA) receptor *GRIA2*, whose expression has been shown to be regulated by lithium treatment (Seelan et al, 2008). In 2014, Chen and colleagues, performed a genome wide association study on samples from one subgroup of 294 patients of Han Chinese descent, with BD treated with Lithium. They identified two SNPS, rs17026688 and rs17026651, located in the introns of GADL1, that showed a sensitivity of 93% to predict a response to Lithium (Chen et al, 2014). However, this surprising result was not confirmed by subsequent studies involving different ethnicities (Kotambail et al, 2015; Moreira et al, 2017). Two years later, Song and his group performed a GWAS on 2,698 patients with subjectively defined lithium response and 1,176 patients with objectively defined lithium response; no significant association were found within bipolar patients. However, in a second phase, Song's working group conducted GWAS comparing lithium responders with health controls, finding out a strong association with SPN rs116323614 on chromosome 2q31.2 in the gene SEC14 and spectrin domains 1 (SESTD1), which encodes a protein involved in the regulation of phospholipids (Song et al, 2016). In the same year, Hou and colleagues, in a GWAS involving 2,563 patients collected by 22 participating sites from the International Consortium on Lithium Genetics, identified four SNPs on

chromosome 21, meeting the genome-wide significance criteria for association with lithium response (rs79663003, rs78015114, rs74795342, rs75222709) (Hou et al, 2016).

Table 2.9: Genome wide association study.

Study	Sample	Definition of response	Gene	Results	Notes
Perlis et al. (2009)	1177	Pre- and post-treatment psychometric tests	GWAS	SNPs in a region on chromosome 4q32	Only 458 were under treatment with Lithium
Chen et al. (2014)	188	Alda Scale	GWAS	rs1702668 and rs 17,026,651 on GADL1 high sensibility	Patients of Han Chinese descent
Song et al. (2016)	3874	Pre- and post-treatment psychometric tests	GWAS	rs116323614 on SESTD1	No significant association within bipolar patients, but strong association comparing Lithium responders with healthy controls
Hou et al. (2016)	2563	Alda Scale	GWAS	Rs799663003, rs780015114, rs74795342, rs75222709 on chromosome 21 associate to lithium respone	Patients collecting from 22 partecipating centres from the International consortium on lithium genetic

Legend: Single Nucleotide Polymorphisms (SNPs) associated with Lithium response. GADL: glutamate decarboxylase like-1; SESTD1: SEC14 and spectrine domain containing 1.

Chapter 3: Discussion

In psychiatry, as in medicine, the discrepancy in response to the same therapies among patients cannot be explained only by physiological, pathological, and environmental factors, but frequently it arises from multiple components, not fully understood. In routine clinical practice, the trial-and-error-based approach is the main method used to decide patients' therapies. This is often not efficacious for treating complex disorders, such as mood disorders and particularly bipolar disorders (Casetta et al, 2019). Many studies have demonstrated a clinical validity of treatments guided by pharmacogenomics in major depressive disorder (Hall Flavin et al, 2013; Sanchez-iglesias et al, 2018), while fewer studies have been applied to bipolar disorder. Therefore, our decision to address the issue of pharmacogenetics in the approach to this complex and pleomorphic disease.

Patients suffering from BD are intrinsically complex, due to the several phases of the disturb that often lead the clinician to misdiagnosis, and also for the difficulty in identifying an effective treatment for different symptoms and to be safe even if used for a prolonged time. (Singh & Rapjut, 2006).

Moreover there is no greater psychotropic pharmacopoeia in psychiatry than that of BD. Treatment selection with lithium, mood stabilizing anticonvulsants, mood stabilizing atypical antipsychotics, typical antipsychotics, unimodal antidepressants, and benzodiazepines, most commonly as a multimodal therapy, must be based on a number of factors that include: clinical base evidence, phase of illness and symptom severity, BD-I vs BD-II subtype, level of cyclicity, and additional mental health and medical diagnoses that may impact on the efficacy and/or side effect burden. Molecular drug mechanisms of action, biomarkers of treatment response or adverse events, are not part of any clinical algorithm decision in BD. Due to the patient's genomic profile, it is possible to recognize such risks and at the same time characterize specific genetic assets associated with bipolar spectrum disorder, as well as with the individual response to the various therapeutic options. This provides the basis for the definition of pharmacogenomic profiles, thus guiding therapeutic choices and allowing a safer and more effective use of psychotropic drugs.

As already mentioned, the physiopathology of bipolar disorders has not been completely clarified yet. No objective biological marker is available to determine with precision the condition of the disease. Many authors are evaluating depressed patients' pharmacogenetic profile, but fewer data are available about BD.

For these reasons, we focused our attention on bipolar patients and on the utility of PGT in the clinical practice routine of this disturb.

In the first phase of study, we focused on the impact of PGTs exclusively on patients with BD type I and II, with an average disease history duration of 9.5 years (SD 7.2).

Our results showed that 4 patients (13%) received an optimal treatment. Despite this data, at the 3-month follow-up visit, 13 patients (40%) changed therapy according to the Neurofarmagen test, while 10 patients (32%) maintained a therapy discordant to the test.

In this phase of study, some psychiatrists decided to keep the therapy unvaried even if the test suggested a more effective or more tolerated alternative. When occurring, the most frequent reason to change the initial therapy and follow the test results was related to AEs rather than the lack of effectiveness. Adverse effects represent a dramatic issue in clinical routine practice. Clinicians frequently attribute a lack of compliance and a consequent high risk of therapeutic failure to AEs. Moreover it is well established that adverse effects represent a huge cost in public health, accounting for approximately USD 300 billion on drug prescriptions and USD 136 billion for adverse drug reactions in the US Health Care System in 2014 (Schwendimann et al, 2018). This result reflects the mistrust that psychiatrists still have in following the indications of PGTs.

From the sub-analysis comparison between the two subpopulations, it was possible to see how the guided test treatment determined a better outcome in terms of efficacy, particularly on the overall severity of the patient; this result is concordant with other studies (Winner et al, 2013; Hall-Flavin et all, 2013) showing that when PGTs are used to guide the pharmacological treatment of depression, the likelihood of treatment response and remission doubled. Maniac symptoms decreased in both

subgroups without differences; this fact may depend on the role of other factors, such as hospitalization and the passing of time.

Dealing with tolerability, the PGT seemed to be useful in guiding the clinicians choosing a more tolerable treatment in those patients that complained collateral effects.

At T2 (12 months later), 93% of patients (n=28) received a therapy concordant to the test (as shown in figure 2.7). This datum shows that clinicians, after an initial phase of distrust, tend to follow the PGT suggestions. During this period, the sample showed an overall psychopathological improvement.

The initial phase of study investigated the efficacy and tolerability of the therapies set according to the PGT. The topic of this second phase is the potential cost savings associated with the use of genetic reporting. Cost saving has been evaluated in terms of the number of hospitalizations, days of hospitalizations, and the number of times emergency services were accessed; mirror analysis showed statistically significant differences in all the evaluations ($p < 0.0001$). The major efficacy and tolerability of the therapy set according to the PGT could be a hypothesis of the origin of lower number of hospitalizations. Another factor could be related to a major confidence of clinicians in changing therapies, and if necessary, to their outpatients, with less resorting to hospitalization.

Our study showed a significant difference also in the economic enhancement, considering the evaluation of the PGT cost. This evaluation is discussed in literature. It is difficult make a comparison because the cost and the kind of genetic analysis are different among the available studies. Verbelan et al. in a review (Verbelen et al, 2017) found thirty-three economic evaluations (75%) supporting PGx-guided treatment, with 11 studies (25%) finding it cost-effective and 22 studies (50%) considering it dominant and cost-saving; five studies (11%) concluded that PGx testing was not cost-effective, while 3 studies (7%) were inconclusive.

Dealing with the data regarding mental-health in the US, as shown by Benitez et al. (2015), the Center for Medicare Services released a specific coverage decision for the combinatorial GeneSight Psychotropic test, thus increasing the access to combinatorial testing for patients. Additionally,

multiple private insurance companies and the U.S. Department of Veterans Affairs decided to cover the GeneSight combinatorial test. Our economic exploitation, even if approximate, shows considerable cost savings per patient; however, being a rough datum, it deserves further study to be confirmed. Cost studies should therefore be of great interest for public health if they involve treatment changes that could improve the wellbeing for severely ill patients. The disabling characteristics of mental disorders should make such improvements, in particular in areas of public health. (Herbild et al, 2011). An association between local recommendations of pharmacogenetic testing and a significantly lower consumption of primary care services have been observed. The current literature seems to suggest that PGx testing will become a core clinical service, part of health-care infrastructure and as electronic health records, but pharmacoeconomic studies are needed.

To complete the evaluation of PGx into clinical practice, the third phase of study tested psychiatrists' attitude towards the use of PGT into daily clinical routine. All respondents believed that pharmacogenetics could help specialists and patients in better decisions about psychopharmacological treatment, in setting up therapy, and particularly regarding drugs interactions. Drug-drug interactions represent a serious problem in BD. Combined therapy appears to be almost the norm for bipolar patients during the main three phases (mania, maintenance, and depression); nevertheless literature doesn't provide easy resources for clinicians to find descriptions of the pharmacodynamic and pharmacokinetic drug-drug interactions in bipolar patients (De Leon & Spina, 2018). Patients having drug-drug interactions due to polypharmacy with three to four drugs, will never be studied in RCTs. In front of patients that need polypharmacotherapy, clinicians are alone without help. The need of an instrument to guide combined therapy is very felt among psychiatrists. 82% of respondents believe that pharmacogenetic tests could become a routine tool in clinical practice, but are skeptical for the obstacles encountered in their use. PGT cost is still a problem, hospital pharmacies refuse the PGT purchase, even when requested on the basis of justified clinical reasons; the reporting time is still very long and finally there is no education on the argument.

No statistically significant differences emerged in the responses between those who already had experience with PGTs and those who did not. This last result could be because the psychiatrists interviewed, worked in the hospitals where our research project was done; this fact may have influenced their knowledge on the argument.

After observing the impact of PGTs from a clinical and pharmacoeconomic point of view, aware of the limitations of our evaluation useful for observing clinical routine practice, but limited from a scientific point of view due to the large number of polymorphisms evaluated, in the last phase of study we decided to carry out a review on lithium salts. This mood stabilizer is the emblem of the complexity for the treatment in BD. This therapy is frequently associated to side effects if not carefully monitored, but at the same time it is highly effective. Its use is historical, but in recent times it is even more widespread thanks to a new slow-release formulation which, avoiding concentration peaks, it seems to be associated with fewer adverse events. Literature results seem to be contrasting, but after an accurate analysis, it has been possible to focus on genes implicated into lithium response. Among these, BDNF with its SNP Val 66Met (Dmitzak et al,2008), SEST1 (GWAS 2016) and different SNPs located on chromosome 21 (Hou et al, 2016) seem to be related to a better lithium response in terms of efficacy. Dealing with tolerability, different authors (Benedetti et al 2005, Iwazashi et al 2014, and Rybakowsky et al, 2013) found a correlation between a SNP of GSK3 and good renal tolerability to lithium salts.

There are currently several positive findings in Lithium pharmacogenetics' studies but none of them have been replicated in a satisfactory manner. According to available data, patients' response to Lithium appears to be polygenic; furthermore, a single gene, which at best accounts for a small portion of the observed variability, could have multiple polymorphic alleles.

Moreover, at present, there are number of limitations within the available studies on Lithium's pharmacogenetics response. One of these is the selection of candidate genes which was based on Lithium's supposed action mechanism; however, the reality is that our understanding is far from being clearly complete. From this perspective, it appears clear how GWAS could by-pass this

permitting large numbers of single nucleotide polymorphisms (SNPs) to be examined across whole regions of interest within the genome.

Another important limitation, according to authors' opinion, is the sample's selection. In particular the considerable clinical heterogeneity in BD: future works should as far as possible recruit large and representative samples of patients, including those with significant comorbidities, in order to offer a closer picture to reality. Moreover, as shown in table 2.8, there is a great quantity of variability about what constitutes a good response to lithium: authors suggest that future studies should specify precise a priori definitions of lithium response, which could be categorical or dimensional.

Despite the enormous number of patients analyzed so far, with a high number of SNPs possibly implicated in Lithium response, it is important not to overestimate the evidence available up to now, even though it is becoming increasingly precise and reproducible. Dealing with literature reviews on lithium response, we can say that difficulties related to this argument were different. Above all, the extreme etiopathogenetic and diagnostic pleomorphism represented by bipolar spectrum. One of the challenges in psychiatry is surely of defining in a more precise way the diagnostic subgroup within this broad disorder, to be able to find more homogeneous populations. Another limit is represented by the selection of candidate genes, which is based on Lithium's supposed mechanism of action; however, in reality our understanding is far from being clearly complete. In conclusion although bipolar disorder is one of the major psychiatric disorders, knowledge is still poor regarding etiopathogenetic and physio pathogenetic mechanisms. Genetic studies are needed to deepen the knowledge regarding both the pathophysiology and the response to pharmacological treatments.

Chapter 4: Conclusions

At the beginning of my career I have been amazed by BD because it represents a continuous challenge for clinicians. Moreover, it is one of the few psychiatric disorders that, even if during the phases of relapse it is extremely disabling and compromises the psychosocial functioning of the patient, when well treated it may give a complete "restitutio ad integrum".

The difficulties encountered by clinicians during the treatment of this disorder are many: the complexity of the different phases of the disease; the high rates of non-response and poor compliance. Adherence to therapies has always been a problem in mental health; in the treatment of BD, as in other major psychiatric diseases, it is certainly influenced by an insight not always present, but it is also due to the drugs' adverse reactions, that too often occur, particularly in polytherapy regimes.

The tolerability of psychotherapies seems to be the cornerstone related to the spread of pharmacogenetics into the clinical practice (Saldivar et al, 2016). Personally, I believe that in mental health the evolution towards the personalization of drug treatments is a precious resource for the benefit of patients, often worried by the idea to take psychiatric drugs for not define periods. I also believe that the spread out of the personalization of psychiatric treatments may change the prejudice associated to our discipline, marked by a history of treatments, pharmacological and not, which too often have caused important side effects and functional limitations to patients.

The high inter-individual variability in the therapeutic response depends in part on factors such as age, sex, weight, the presence of any liver or kidney disease, the concomitance of other therapies, heterogeneity of diseases, nutritional status or unhealthy lifestyles such as smoking. In addition to these, we must consider variability of drug metabolizing enzymes, transport proteins, receptors and molecules that activate transduction cascades. Pharmacogenetic attempts to define the influence of all these genetic factors on the efficacy and tolerability of therapy, focusing on the study of genetic mutations' effects. The personalization of drug treatment still represents a very distant vision from the current clinical practice; recognition of genetic factors that contribute to the variability in drug

response has an important impact in the clinical setting, especially for drugs with a low therapeutic index and in multi-therapy regimen treatments.

The expectation that by 2020 the use of pharmacogenetic tests could become a standard practice (Collins & McKusick, 2001) has not been completely satisfied and the diffusion of pharmacogenetic tests in the clinical routine is occurring with moderate slowness (Lee Fan, 2013). This situation seems to be dictated by several factors including the PGT costs, in particular genotyping procedures costs. In relation to the economic issue, it is useful to keep in mind that this genetic evaluation is carried out one time in the patient's life, but it offers useful indications for the therapeutic regimen of the entire duration of the person's clinical history (Sanchez-Iglesias et al., 2016). It is also necessary to reflect on the huge public cost related to psychiatric diseases in terms of years of disability (Espadaler et al, 2016), adverse effects and hospitalizations. The direct and indirect costs associated with bipolar disorder in the US are estimated to be around $ 15 billion annually (Begleyet al, 2001). Further studies are needed. However, many difficulties have already encountered in addressing the clinical utility of pharmacogenetic tests, since there are few clinical trials on the subject. In particular for bipolar disorder, there are still few specific studies for diagnosis and evaluating the clinical course of a specific phase of the affective disorder (Salloum et al, 2014).

This observational study has showed promising data on the use of PGT in setting more effective and tolerable psychopharmacological therapies in patients affected by BD.

Furthermore, the mirror analysis with a pharmacoeconomic approach showed a significative reduction of the number of days of hospitalization, hospitalizations and accesses to emergency services after setting the therapy concordant with the PGT used. Surely these phases of study have important limitations, such as the small sample size, dictated primarily by the difficulty in procuring many PGTs and the observational nature of the study. On this last point, however, I believe that for this kind of analysis, the observational nature allowed to evaluate clinical practice in the real world, decreasing the risk of patient selection bias.

Then, the semi-structured interview confirmed that even among psychiatrists there is a need to go beyond the “trial and error" approach and that there is hopefulness about the introduction of PGx into routine clinical practice.

I strongly believe that therapeutic relationship and empathic listening are the basis of our discipline. In front of patients’ desperation suffering from such a disabling disease, which often requires the use of therapies with also frequent adverse events though, it is necessary to find how to adapt as much as possible the treatment to the single person.

The tool used in our research is one of the various PGT currently available in the psychiatric field. Its advantages are it is easy to use, the high number of drugs evaluated and a dedicated section for the drug-drug interactions. This last part was very appreciated by clinicians. Obviously, however, this tool has some limitations such as that it does not take epigenetics into account. Furthermore, like all PGTs available, it needs continuous reviews, following the genetic studies.

Thanks to this first part of research it has been possible to appreciate the complexity of the PGx field and, at the same time, the clinical impact that PGx data could have on the most used and most problematic active drugs. So, the last phase of research focused on lithium, the mood stabilizer historically associated with BD. The lithium review was useful in order to identify the genes most involved in pharmacological response in terms of efficacy and tolerability.

In spite of the limitations encountered, such as the difficulty in finding PGT to broaden the sample, such as to study pleomorphic and complex disorder or review the literature on a complicated and very broad topic, this PhD confirmed my desire to do more for our patients, continuing to do research in the pharmacogenetics’ field applied to the pharmacological response and deepening the pharmacoeconomic aspects.

This period in our profession is complicated, due to the state of emergency linked to the pandemic and few health resources, it is necessary to reaffirm and carry on the desire to improve our discipline. I thank my PhD course because it strongly reiterated the desire for open-mindedness and that

interdisciplinary discussion is the right way to proceed, looking at a better future for our patients in the light of past experiences.

Appendix

List of genes and SNPs analysed

Gene symbol	Gene Name	Polymorphisms
ABCB1	ATP binding cassette subfamily B member 1	rs2235048, rs11983225
AKT1	V-akt murine thymoma viral oncogene homolog 1	rs1130214
BDNF	Brain-derived neurotrophic factor	rs6265
CACNG2	Calcium channel, voltage-dependent, gamma subunit 2	rs2284017
CES1	Carboxylesterase 1	rs71647871
COMT	Catechol-O-methyltransferase	rs4680
CRHR1	Corticotropin releasing hormone receptor 1	rs4792888
CYP1A2	Cytochrome P450 family 1 subfamily A member 2	*1, *1F
CYP2B6	Cytochrome P450 family 2 subfamily B member 6	*1, *6
CYP2C19	Cytochrome P450 family 2 subfamily C member 19	*1, *2, *3, *5, *7, *8, *17, *27
CYP2C9	Cytochrome P450 family 2 subfamily C member 9	*1, *2, *3, *6, *8, *27
CYP2D6	Cytochrome P450 family 2 subfamily D member 6	*1, *2, *2A, *3, *4, *5, *6, *7, *8, *9, *10, *11, *12, *14, *15, *17, *19, *20, *29, *35, *30, *40, *41, *69, *1xN, *2xN, *35×2
CYP3A4	Cytochrome P450 family 3 subfamily A member 4	*1, *22
DDIT4	DNA damage inducible transcript 4	rs1053639
DRD3	Dopamine receptor D3	rs963468
EPHX1	Epoxide hydrolase 1, microsomal (xenobiotic)	rs1051740
FCHSD1	FCH and double SH3 domains 1	rs456998
GRIK2	Glutamate receptor, ionotropic, kainate 2	rs2518224
GRIK4	Glutamate receptor, ionotropic, kainate 4	rs1954787
HLA-A	Major histocompatibility complex, class I, A	rs1061235
HTR1A	5-HTT (serotonin) receptor 1A, G protein-coupled	rs10042486
HTR2A	5-HTT (serotonin) receptor 2A, G protein-coupled	rs6311, rs6314, rs9316233
HTR2C	5-HTT (serotonin) receptor 2C, G protein-coupled	rs1414334
LPHN3	Latrophilin 3	rs6551665
NEFM	Neurofilament, medium polypeptide	rs1379357, rs1457266
OPRM1	Opioid receptor, mu 1	rs1799971
RGS4	Regulator of G-protein signaling 4	rs2661319

Gene symbol	Gene Name	Polymorphisms
RPTOR	Regulatory associated protein of MTOR, complex 1	rs7211818
SLC6A4	Solute carrier family 6 (neurotransmitter transporter), member 4	5-HTTLPR
UGT2B15	UDP glucuronosyltransferase 2 family, polypeptide B15	rs1902023

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PORTFOLIO

Doctoral student: Marta Ielmini

Doctoral period: October 2016-November 2020

Supervisor: Professor Camilla Callegari

Training activity in PhD program (University of Insubria)

- Seminar "Trachea transplant scandal: a tragic consequence of greed and etilism in academic research. Varese, 27 th September 2017

- Seminar "On inflammation and depression, of what…? The effects of inflammation on behavior", Varese, 23rd March 2018

- University of Verona. "Corso di perfezionamento in revisioni sistematiche con metanalisi finalizzato alla stesura di linee guida", 21-26 of april 2018

- Seminar "Genomica e Genetica", Varese 11 th April 2018

- Seminar "La nascita dell'identità", Varese, 31th May 2018

- Seminario "La Legge 180 del 1978: incontro tra etica, diritto e medicina", Varese, 20th june 2018

Attended conferences and seminars

- Convegno "Gli interventi sanitari, giudiziari e di ordine pubblico relativi ai pazienti psichiatrici autori di reato", Varese, 22 marzo 2018

- Convegno "Esiti in psichiatria: qualità e quantità di vita", Bormio, 5-8 aprile 2018

- Convegno "Schizofrenia oggi", Milano, 22-23 maggio 2018

- "Verso una psichiatria di precisione. Poliedricità e personalizzazione dei trattamenti terapeutici". Sala Kursall Palace Grand Hotel Varese, 22 marzo 2019

Organization of training courses

- "Senza oltrepassare una linea sottile: la relazione con il paziente agitato/aggressivo" Supervisione in equipè: 5th march 2018. ASST Sette Laghi, Varese.

Publications

Ielmini M, Poloni N, Caselli I, Espadaler J, Tuson M, Grecchi A, Callegari C. The utility of pharmacogenetic testing to support the treatment of bipolar disorder. *Pharmacogenomics and Personalized Medicine*. 2018; 11:35-42.

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Scientific posters

Marta Ielmini, Alessandro Grecchi, Daniele Zizolfi, Ivano Caselli, Camilla Callegari. "The utility of pharmacogenomic testing to support the treatment of bipolar disorder"
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C. Callegari, I. Caselli, M. Ielmini, M. Lucano, S. Vender. "Influence of the recovery style from psychosis on the distress in psychiatric professionals:

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Callegari C, Caselli I, Poloni N, Isella C, Ielmini M. Test farmacogenetici e riduzione di accessi ai servizi di emergenza e giorni di ricovero. Analisi "mirror" della durata di 2 anni in pazienti affetti da disturbo bipolare, Congresso di Psichiatria Biologica, Napoli, 2-4 ottobre 2019

Teaching activities and invited speeches

- World Cultural Psychiatry Association, "Achieving global mental health equity: making cultural psychiatry count", 5th World Congress of the World Cultural Psychiatry Association, New York City (NYC), 11-13 October 2018

- Simposio parallelo "Dal concetto di somatizzazione al disturbo da sintomi somatici"; relazione dal titolo "Analisi del rapporto costo-efficacia associato agli interventi per MUPS", SOPSI 2019, 20-23 febbraio 2019, Rome (Italy)

- Simposio parallelo "Modelli e prospettive di intervento per disagi emotivi transculturali geograficamente diversificati - Il disagio psicopatologico nei migranti del territorio varesino: uno studio epidemiologico", XXIV Congresso Nazionale SOPSI 2020, febbraio 2020, Rome (Italy)
- Varese, 20 febbraio 2017. Convegno "PSICOFARMACOLOGIA: LIMITI E PROSPETTIVE NELLA RIABILITAZIONE PSICHIATRICA"

- Como, 22 maggio 2017. Partecipazione come correlatore al convegno "Il Contributo della Nutraceutica e della Genetica nelle Patologie di interesse geriatrico, neurologico e psichiatrico".

- Torino, 13-17 ottobre 2018. 48° congresso SIP. Salute mentale nel 3 millennium. Titolo della relazione: "The use of pharmacogenetic testing in routine clinical practice: towards the personalization of pharmacological treatments. Focus on mood disorders"

- Monza, 7 febbraio 2018. Conferenza "Nutrire il benessere: risorse endogene ed esogene". Relazione: "L'uso dei test farmacogenetici nella clinica psichiatrica di routine: verso la personalizzazione dei trattamenti. Focus sui disturbi dell'umore" Varese, 5 marzo 2018.

Participation to other projects

- Joining to the VESPA protocol ("Assessing tolerability and efficacy of Vortioxetine versus SSRIs in elderly patients with depression: a pragmatic, multicenter, open-label, parallel-group, superiority, randomized trial") promoted by the School of Specialization in Psychiatry of the University of Verona. The study involves 14 Italian psychiatric centers engaged in the recruitment of elderly patients with major depression aiming to compare the tolerability, safety and efficacy of vortioxetine *versus* other SSRIs antidepressants in terms of the occurrence of adverse effects, mortality, suicidal events, quality of life and comorbidities. The subjects involved will be evaluated after 1, 3 and 6 months through the administration of the following rating scales: *Montgomery – Åsberg Depression Rating Scale* (MADRS), *Antidepressant Side-Effect Checklist* (ASEC), *EuroQual 5 Dimensions* (EQ-5D), *Charlson Age-Comorbidity Index* (CACI).

- Collaboration with the STAR Network Group in research on the use of second generation long-acting antipsychotics in clinical practice in Italy. The project involves the realization of several multicenter longitudinal observational studies aiming the investigation of the characteristics of the pharmacological prescription and the differences over the use of first generation long-acting antipsychotic drugs.

- Design the study protocol, perform the data analysis and draft the manuscript “Clinical implications of subjectivity in patients with schizophrenia spectrum disorders: an observational study” by Ielmini M., Caselli I., Gasparini A., Amorosi S., Poloni N., Callegari C. [Abstract. The paper assumes that nowadays, mental illness can no longer be considered as a mere list of symptoms corresponding to localized brain dysfunctions but rather as a disturbance of the patient’s subjectivity. Thus, a solid, qualitative study of patients’ subjectivity could represent a useful tool in the complex evaluation of efficacy of pharmacotherapy in schizophrenic persons. In this perspective, authors performed a phenomenological oriented investigation on 49 patients, diagnosed with schizophrenia spectrum disorder, who were receiving long-acting injectable (LAI) antipsychotic therapy. From data analysis, authors found a positive correlation between general psychopathology and the use of long-acting injectable antipsychotic therapies, highlighting the necessity of a

careful investigation of patients' subjectivity in a phenomenological way as an irreducible part of both psychopathological and psychopharmacological matters.