

Endophenotypes and biomarkers: an approach to molecular genetic studies of mental disorders

Adriana Díaz-Anzaldúa,¹ Junior Velázquez-Pérez,¹ Andrés Nani Vázquez,¹ Carlos Berlanga²

Artículo original

SUMMARY

Many precise aspects of the etiology and pathophysiology of mental disorders are still unknown. Susceptibility to these disorders depends in part on variability in the genome sequence among individuals. The genotype, a given environment, a specific epigenetic profile and stochastic factors affect the phenotype, which includes body structures, physiological processes, and behavior. Since the access to the Central Nervous System is generally difficult and in most cases there are still no biological tests that necessarily contribute to diagnosis, psychiatric phenotypes are usually limited to clinical symptoms and functioning. Therefore, researchers are currently seeking alternatives to facilitate the identification of genetic risk factors. One strategy is to identify measurable biological, cognitive, and behavioral markers, intermediate phenotypes, or endophenotypes, which in the best case may be simpler than general psychiatric diagnoses, ideally with a precise biological meaning and a more direct relationship with the action of specific genes. Endophenotypes have been very useful in other fields of medicine. Currently, there are several proposed criteria and specifications for endophenotypes. Examples of possible types of endophenotypes or biomarkers, as well as treatment response phenotypes in some psychiatric disorders will be discussed in this review.

Key words: Endophenotypes, mental disorders, biological markers, pharmacological biomarkers.

RESUMEN

Aún se desconocen diversos aspectos de la etiología y fisiopatología de los trastornos mentales. La susceptibilidad a éstos depende en parte de la variabilidad en la secuencia genómica en las personas. El genotipo, un ambiente dado, un perfil epigenético específico y factores estocásticos afectan el fenotipo, el cual incluye estructuras corporales, procesos fisiológicos y conducta. Los fenotipos psiquiátricos generalmente se limitan al funcionamiento y a los síntomas clínicos, debido a que el acceso al Sistema Nervioso Central es complicado y en la mayoría de los casos no hay pruebas biológicas que necesariamente contribuyan al diagnóstico. Por esta razón, en la actualidad se buscan alternativas para facilitar la identificación de factores de riesgo de tipo genético. La identificación de marcadores biológicos, cognitivos o conductuales medibles, fenotipos intermedios o endofenotipos podría ser una de estas nuevas opciones. Estos marcadores podrían ser más simples que el diagnóstico psiquiátrico general, y de manera ideal tendrían un significado biológico preciso y una acción más directa en los genes. El empleo de endofenotipos ha sido útil en otras ramas de la medicina. Hasta ahora se han propuesto diversos criterios y especificaciones para los endofenotipos. En esta revisión se describirán posibles tipos de endofenotipos o biomarcadores, así como fenotipos relacionados con la respuesta farmacológica en algunos trastornos psiquiátricos.

Palabras clave: Endofenotipos, trastornos mentales, marcadores biológicos, biomarcadores farmacológicos.

INTRODUCTION

Mental disorders severely affect the quality of life of patients, relatives and caregivers. They impact millions of people worldwide and are situated among the leading causes of years of life lost to premature death, as well as years lived with disability. Despite their relevance, so much remains unknown about their etiology and pathophysiology. Research over the past several decades has shown that susceptibility to these disorders is affected in part by variability in the genome se-

quence among individuals. In fact, it has been estimated that approximately 0.1% of a person's genetic recipe differs from the recipe of a non-related person, so between them there are more than three million differences which include some that affect vulnerability to any mental disorder.

The genomic sequence is contained in trillions of nucleated cells in the human body. Instructions are encoded in the language of deoxyribonucleic acid (DNA), in which nitrogenous bases Guanine, Adenine, Thymine, and Cytosine are combined instead of letters or numbers such as ze-

¹ Departamento de Genética Psiquiátrica, Subdirección de Investigaciones Clínicas, INPRFM.

² Subdirección de Investigaciones Clínicas. INPRFM.

Correspondence: Dra. Adriana Díaz-Anzaldúa. Departamento de Genética Psiquiátrica. Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz. Calz. México-Xochimilco 101, San Lorenzo Huipulco, Tlalpan, 14370, México, DF. Tel. 4160-5114. E-mail: adianza2@imp.edu.mx

ros and ones. DNA allows specific ribonucleic acid (RNA) chains to be formed, which in turn serve as a mold to produce polypeptide chains for proteins that are necessary for the body's functioning and structure; additionally, RNA participates in regulating protein production. The genome could be considered a large masterpiece in which the chromosomes represent different volumes. Each of these volumes is formed by a long strand of DNA that is accompanied by some proteins. There are hundreds to thousands of genes in each chromosome.

We can detect differences even within any of our nucleated cells. Although the two versions of our nuclear DNA, the maternal and the paternal genomic copy, could seem almost identical, they do have differences generated mainly by polymorphisms, but also as a result of mutations.

Genetic variations within our species range in size from very short to very long. Currently, there are more than 54 million known short variants, which include single nucleotide polymorphisms (SNPs), insertions/deletions (indels) or somatic mutations. As for structural changes, which affect a sequence at least 1000 nucleotides long, more than 9 million have been described.¹ Structural changes can be deletions, duplications, copy-number variants, insertions, inversions or translocations. Mutations are de novo or spontaneous genetic changes which create new alleles. They are caused mainly by errors during DNA replication, transcription to RNA or exposure to mutagens such as certain chemicals or radiation. Mutations are not always transmitted to the next generations because if they are exclusively somatic, they do not affect germ cells. However, when they do, effects on the phenotype are not always present. When there are at least two alleles and the rare one is carried by in 1% or more of the population, then the locus is considered a polymorphism (different forms) instead of a mutation.

The term genotype refers to all the genetic makeup of an individual, represented by all the genes and the rest of the DNA sequence derived from both parents. In a concrete way, genotype can be used in reference to paternal and maternal genetic information at a particular locus of the genome. In general, nucleated cells of an individual have the same genotype, whether they are neurons, liver cells or leukocytes, or if the person is a neonate or has reached an old age. However, according to a recent finding, since the time when fertilization occurs there is subtle genomic variation between different healthy differentiated tissues.²

In recent years human genetics, particularly in the field of psychiatry, has turned to investigate the relationship between genes and environment. Gene-environment interactions occur when the effect of a particular environment depends on a person's genotype or, in an opposite way, when the effect of a person's genotype depends on the environment. The interest in gene-environment research originates from the knowledge that genotypes do not work alone since their expression must depend on environment and epigenetic

contribution. This explains, for example, why in response to a similar severe life stressor condition, one person becomes depressed while another does not. Recent studies on this field of genetics have used a candidate gene-environment interaction approach. This has not been a simple task since there are several ways in which genotypes and phenotypes interact in most psychiatric disorders. Because of this complex relation, many genome-wide association or linkage studies have failed to replicate findings from the candidate gene literature.³

A frequently cited study by Caspi et al. conducted several years ago,⁴ demonstrated that environmental factors could modulate the effect of a single genetic variant on the onset of depression. In each person, the maternally and paternally inherited promoter region of the serotonin transporter gene (SLC6A4) may contain partially different DNA sequences, which includes a polymorphic region called 5-HTTLPR, characterized by long or short variants. The short allele is associated with a lower messenger RNA production for the serotonin transporter. In this study it was found that stressful events increased the onset of major depression only in individuals who had at least one short variant of 5-HTTLPR, but did not produce the same effect in subjects with two copies of the long allele. This finding was an example of the effect of gene-environment interactions on the phenotype, and thus increased the scientific interest in studying this phenomenon.

The phenotype includes the traits and characteristics that identify an individual. It involves body structures, physiological processes, and conducts that result from the expression of the genotype in a given environment, a specific epigenetic profile and the effect of stochastic factors. In this way, genetic variability contributes to make us unique in our phenotype. As mentioned before, this includes a higher or lower vulnerability to any mental disorder.

From a biomedical point of view, mental disorders tend to be classified as discrete diagnostic entities. The specific psychiatric phenotype is usually limited to clinical symptoms and functioning. The access to the Central Nervous System is generally difficult, and in most cases there are still no biological tests that necessarily contribute to the diagnosis. Usually, the phenotype is considered as a categorical variable (affected *vs.* non-affected subjects). Categorical definitions are used in the *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)* and the *International Classification of Diseases (ICD-10)*. However, these phenotypes may be somehow arbitrary and may encompass a heterogeneous group of patients.⁵

At present, despite the advances in molecular genetics, the complete spectrum of the genetic component of mental disorders is still largely unresolved. This partial lack of success can be explained by the complexity of the Central Nervous System, the interaction among different genetic, epigenetic, and environmental components, the unique-

ness of the set of risk factors in each patient, pleiotropy (a single genetic locus could affect two or more distinct traits or phenotypes), and incomplete penetrance (lack of clinical symptoms in some patients who carry risk alleles). Furthermore, an ambiguous and inconsistent description of the studied phenotype has also contributed to the difficulty in replicating findings. As for the description of the phenotype, it is proposed that the current classifications such as ICD-10 or DSM-IV could too broadly describe mental disorders.⁶

In response to this problem, researchers are currently seeking alternatives to facilitate the identification of the genetic component in psychiatric disorders, with possibly more homogeneous phenotypes. One strategy is to identify intermediate phenotypes, endophenotypes, or biomarkers, which in the best case may be simpler than general psychiatric diagnoses, ideally with a precise biological meaning and a more direct relationship with the action of specific genes. Thus, it has been proposed that the perfect endophenotype could reduce the heterogeneity at the level of phenotype and thus facilitate genetic association and linkage studies.

Endophenotypes have been very useful in other fields of medicine. Several genes that cause long QT syndrome were identified by means of an endophenotype-based method. This cardiac syndrome is characterized by syncope, ventricle arrhythmias, and sudden death, especially in young otherwise healthy individuals.⁷ Not all family members who carry the disease alleles show symptoms, but a much greater percentage show QT elongation. This allowed the identification of QT elongation genes through linkage analysis. Moreover, genetically modified mice are being used to develop novel medication.⁸ Endophenotype strategies have also been successful in the study of disorders such as idiopathic hemochromatosis, juvenile myoclonic epilepsy, and familial adenomatous intestinal polyposis. Furthermore, it is well known that research in diabetes, hypercholesterolemia, obesity, hypertension or osteoporosis is related with physiological measures and challenges, as well as biochemical assays to obtain a primary evidence of pathology. Glucose or cholesterol levels, as well as blood pressure measurement, body mass index or bone mineral density are objective and quantifiable ways to obtain a diagnosis; they constitute endophenotypes which allow performing successful linkage or association tests.⁶

The term endophenotype in Psychiatry was initially used to describe a possible intermediate character or trait, a potentially more basic phenomenon, or internal attribute that in some cases could seem hidden to the naked eye; it could fill the space between genes and disorders in the causal chain of the disease.⁹ Fewer susceptibility genes with a stronger effect were expected to be associated with the endophenotype, and thus, the latter could help understand the basic molecular mechanisms of risk and disease manifestation. The rationale for the study of endophenotypes is that the number of genes involved in a psychiatric condition may

be directly related to both the complexity of the phenotype and the difficulty to perform genetic analysis, i.e., a larger number of genes involved as the complexity of the phenotype increases (for example, in schizophrenia, depression, or addiction) and vice versa.⁶ In addition, since disease-free relatives of a patient may share part of the vulnerability genes with him or her, they may show some abnormalities not present in relatives of non-affected individuals.

Recent definitions of endophenotypes include some of the following criteria or specifications:^{6,10-12}

1. They are associated with the disorder in the population.
2. They are heritable (at least partially).
3. They are not influenced by the state of the patient, as they are stable and present even if symptoms are not manifested.
4. They co-segregate with the disorder.
5. They are found more frequently in unaffected relatives of patients than in the general population.
6. They should be associated with alleles that help distinguish patients and their unaffected relatives from healthy controls on quantitative measures.
7. They should be associated with vulnerability to the disorder rather than with the effects of it.
8. Different endophenotypes could be associated with a given mental disorder.
9. They should vary in a continuous way in the general population.
10. In order to be confirmed, they should be measured accurately across different levels of analysis.
11. If they affect multiple disorders, they should be identified for genetically related disorders.

The family designed studies are the first method used to search for endophenotypes. Patients, first-degree relatives, and demographically similar control subjects are compared under anatomical, physiological and/or functional indicators. However, genetic and environmental influences are confounded in this method. An alternative with a more feasible separation between genetic and environmental variation analyzes monozygotic and dizygotic twins discordant for a disorder and its corresponding control pairs. A third option is a longitudinal study designed to compare the measurement of the endophenotype before and after the disorder manifests or before and after an episode of the illness.¹²

Currently, some researchers propose that a group of genes increases the risk for the categorical mental disorder but also for continuous endophenotypes. In this way, pleiotropy would produce variation in the endophenotype but also in the risk for the disorder. Nevertheless, the successful treatment of the endophenotype would not necessarily prevent disease onset. This can be shown in one study that suggested neuroticism reflected the genetic risk to depression, but the connection from genes to major depression would not necessarily pass through neuroticism.

Others still argue that the pathway from genetic variation to psychiatric conditions necessarily passes through the endophenotype and if the endophenotype is treated, there could be a decline in risk for the mental disorder.¹³

In both cases, unaffected individuals with high scores on the endophenotype measurement would be at elevated risk for the development of the disorder.

The proposed methodology to identify endophenotypes is varied. Measurements are obtained through physiological, cognitive, neurophysiological, endocrinological, neuroanatomical, neuropharmacological, and biochemical studies, among others.

Besides endophenotypes and the general mental disorder, there are traits and characteristics that are also relevant. For example, phenotypes related with drug response, which are studied in the pharmacogenetic field.

DNA variation has been associated with individual differences in pharmacokinetics (absorption, distribution, metabolism, and excretion of drugs) and pharmacodynamics (effect of the drug related with membrane receptors, ion channels, and transporters). It has been shown that the response of patients who are given the same dose of the same drug can vary considerably, which is related in part with genetics. Pharmacogenetic studies may help improve our understanding of the effect of certain genetic variants, but they could also allow researchers to propose specific phenotypes instead of general outcomes such as response or remission.

This paper describes examples of possible types of endophenotypes or biomarkers, as well as treatment response phenotypes for some psychiatric disorders.

SEROTONERGIC SYSTEM

It has been documented that the transport of serotonin is involved in the pathophysiology of depressive disorders. The functional 44-bp indel polymorphism located at the promoter region of the SLC6A4 gene has been associated with unipolar and bipolar disorders.¹⁴⁻¹⁷ As mentioned before, this polymorphism is commonly identified with the alternative name of 5HTTLPR. The L allele was associated with better response to certain antidepressants, such as paroxetine, fluvoxamine, and clozapine.¹⁸⁻²⁰ Another study identified an association between 5HTTLPR and subclinical depressive symptoms in a mentally healthy population.²¹ Therefore, alterations in serotonin transport may underlie certain clinical features or endophenotypes of vulnerability to mood disorders.

It has been estimated that 30% to 40% of patients with depression do not respond at all to the first antidepressant drug and 60% to 70% do not achieve remission.²² Plasma concentration recordings of antidepressants correlate poorly with clinical efficacy, and pharmacogenetic analyses could help find biomarkers that predict individual drug response. Besides the 5HTTLPR polymorphism, genes such as

COMT and GSK3 β have also been consistently associated with antidepressant efficacy and side effects.

In addition, it has been proposed that unaffected relatives of bipolar disorder (BD) patients may be more sensitive to acute tryptophan depletion, and in this case, they may have increased impulsivity and impaired planning and memory, when compared with control subjects.^{23,24} Another study showed that intravenous injection of L-tryptophan produced affective symptoms and neuroendocrine responses in unaffected relatives of BD patients.²⁵ Thus, the sensitivity to acute tryptophan depletion may be a heritable trait or endophenotype of vulnerability for BD.

ENDOCRINOLOGY

Sleep and circadian rhythm disruptions have been extensively reported in neuropsychiatric disorders, sometimes even before the symptoms of the disorder are shown. It has been postulated that alterations in the secretion of melatonin caused by oscillations in circadian rhythms may be associated with various psychiatric conditions such as seasonal affective disorder, BD, unipolar depression, bulimia, anorexia, schizophrenia, panic disorder, and obsessive compulsive disorder.²⁶ Other studies suggest that suppression of melatonin with light may represent a genetic vulnerability marker in BD patients. Heritability of both melatonin secretion and sensitivity to bright nocturnal light is high.²⁷ Therefore, certain clinical conditions with abnormal production of melatonin may constitute vulnerability endophenotypes for BD. Besides, a possible influence of the clock gene on the circadian fluctuations of affective states and on the long-term recurrence of bipolar depression was proposed; a polymorphism on this gene could be involved in the dysregulation of sleep in patients with major depressive disorder and BD.²⁸ In addition, it has been suggested that the therapeutic effect of lithium could be related in part with its effect on circadian instability.

Major depressive disorder has been associated with hypothalamic-pituitary-adrenal-axis hyperactivity. A comparison of salivary morning and evening cortisol concentrations was made between a group of 187 remitted and highly recurrent patients and a group of 72 control subjects. Patients had higher cortisol concentrations than controls ($p < 0.001$), and this was not modified by depressive episodes during follow-up. It was proposed that patients had persistently increased cortisol concentrations, irrespective of stress, so hypercortisolemia may fulfill the endophenotype criterion of state-independence.²⁹ In contrast, low cortisol levels could be a risk factor for post-traumatic stress disorder. Research suggests there may be a transgenerational impact on cortisol reactivity, which should be further explored.³⁰ Cortisol social stress response has also been proposed as a candidate endophenotype in suicidal behavior, as well as in

aggression, impulsivity, early-onset major depression, and neurocognitive function.³¹ In addition, impairment in stress processing was described in medication-naïve patients with a first episode of psychosis, ruling out that this observation was caused by illness progression or antipsychotic medication, according to a recent publication.³² The corticotropin-releasing hormone (CRH) mediates endocrine, autonomic, behavioral, and immune responses to stress. Among the CRH receptor subtypes, CRHR1 has been studied in alcoholic individuals. The P3 amplitude of the event-related potential and alcohol dependence were found to be associated with polymorphisms in the CRHR1 gene.³³

In Autism Spectrum Disorders (ASD), decreased circulating oxytocin levels in affected individuals have been reported; the administration of this hormone to patients may be associated with a reduction in repetitive behaviors, a better retention of social cognition, and improvement in emotional recognition. In addition, the oxytocin receptor gene (OXTR) has been consistently associated with ASD. Regarding social and emotional processes, the oxytocin system interacts with the serotonergic system. In mice, it was described that the anxiolytic effects of oxytocin may be mediated via oxytocin receptors expressed on serotonergic neurons.³⁴

Other phenotypes such as insulin resistance, obesity, and hyperglycemia are very common among BD patients, suggesting some degree of pathophysiological overlap affected by a common genetic background.³⁵

NEUROANATOMY

Basal ganglia are involved in cognition, development, and integration of psychomotor performance, as well as in memory and learning mechanisms. In schizophrenic patients, structural magnetic resonance studies have shown a significant volumetric reduction of the amygdalo-hippocampal system and the thalamus, when compared with healthy controls. Some individuals at high risk of developing schizophrenia showed similar structural characteristics than those reported in schizophrenics, which suggests that certain structural abnormalities represent vulnerability markers for schizophrenia.³⁶ In addition, striatal dopamine alterations have been reported in schizophrenic patients, but also in at risk individuals. It has been proposed that subtle sub-clinical abnormalities in the striatum of subjects at risk could be a biomarker that detects vulnerability to psychosis. On the other hand, an investigation showed grey matter volume reductions in schizophrenic patients, but not in unaffected relatives, which could suggest an absence of heritability of this trait. However, a prefrontal executive functioning deficiency was shared by patients and unaffected relatives.³⁷

In patients with BD, structural and functional alterations in the anterior limbic network have been reported. This network includes prefrontal regions and subcortical

structures (striatum, amygdala, thalamus, and midline cerebellum). Magnetic resonance imaging studies have shown brain volume changes in the anterior and ventral surfaces of the striatum of some BD patients. In one case, 54 healthy subjects were genotyped for the Leu657Phe polymorphism of the Disrupted in Schizophrenia-1 gene (DISC1).

The Phe carriers had larger striatal volume bilaterally (right striatum $p=0.016$ and left striatum $p=0.017$); the left hemisphere was also different between carriers and non-carriers ($p=0.0074$).³⁸ Studies of diffusion tensor imaging in patients with BD have shown a loss of fractional anisotropy of white matter tracts in the prefrontal cortex. This may be related to axonal pathology (e.g. demyelination), which involves the loss of coherence of axonal bundles.³⁹ Moreover, the presence of alterations of the right hemisphere in patients with BD as shown with functional magnetic resonance is consistent with the hypothesis that the right brain is critical in affect regulation.⁴⁰

Recently, size reductions of 11% in hippocampus and 13% in amygdala were described in patients with borderline personality compared to control subjects. When comorbidity was taken into consideration, depression, post-traumatic stress disorder, and substance use disorders were unrelated to volumetric findings. Nevertheless, relatives were not studied and further research is needed.⁴¹

COGNITIVE FUNCTION

Cognitive deficits or deficits in any mental process that involves memory, learning, executive functions, language skills, attention and judgment have been reported frequently in euthymic BD patients and healthy first-degree relatives.^{24,25,42,43} Moreover, it has been observed that patients in a state of mania show alterations in tests of memory and planning, while depressed patients have alterations in the ability to change the focus of attention.⁴⁴ In addition, euthymic bipolar patients have shown alterations in visual-spatial recognition, verbal and non-verbal learning in information retrieval of semantic memory, and also an increase in reaction time in performing task planning.⁴⁵ Studies in first-degree non-affected relatives of BD patients show significant alterations in retrograde digital capability in spatial ability and verbal memory visuospatial tasks.⁴⁶ The deficit in executive control and verbal memory has also been reported in euthymic BD patients, suggesting that they may represent endophenotypes of genetic vulnerability to BD.⁴⁷ In other studies in healthy first-degree relatives of patients with BD, a cognitive deficit enhanced both by tryptophan depletion and its intravenous injection was found. As mentioned before, this may also reflect serotonergic vulnerability in the frontal lobes and suggests that cognitive deficits in these patients provide evidence for a biological marker as a possible endophenotype for BD.^{25,42}

In previous research, schizophrenic patients, their offspring and parents have shown cognitive deficits. Patients in some cases had a recent onset of schizophrenia, and the deficits were found in psychotic episodes as well as in clinically remitted periods.⁴⁸ Working memory, verbal learning and memory, as well as executive function have been studied in schizophrenia. First-degree relatives (≤ 30 years of age) of patients with schizophrenia have shown moderate deficits in vocabulary and single word reading tests, declarative memory, sustained attention, and working memory when compared with controls.⁴⁹ More than 1500 polymorphisms in 94 candidate genes for schizophrenia were genotyped and evaluated against possible quantitative endophenotypes. Schizophrenic patients who were 18 to 65 years old and had no drug abuse or dependence within the past six months or neurological insults were compared with a control group. Patients had significant deficits on ten neurophysiological or neurocognitive endophenotypic measures, mainly on working memory and executive function. Some genes have been shown to interact on a molecular level, revealing associations with three or more endophenotypic measures. The V-ERB-B2 avian erythroblastic leukemia viral oncogene homolog 4 (ERB4) and the neuregulin 1 (NRG1) were among the associated genes.⁵⁰

Significant differences have been found between individuals with obsessive-compulsive disorder and healthy controls in tests that involve verbal memory, attention and psychomotor speed, as well as visuospatial and executive functions. These patients may have difficulty in using an effective learning strategy due in part to insufficient mental flexibility and poor planning ability. Furthermore, there are studies in which drug-naïve patients and their first degree relatives showed similar impairment on neurocognitive domains of delayed verbal recall, set shifting, response inhibition, and visuoconstructive ability when compared with controls.⁵¹

Cognitive function has also been assessed in other kind of psychiatric patients. For example, when stimulant-dependent individuals were compared to their siblings without a history of drug dependence, and with healthy controls, deficits in executive function and response control were shown to be impaired in stimulant-dependent subjects and in their non-dependent siblings, when compared with controls. Patients and relatives tended to have an elevated anxious-impulsive personality.⁵²

NEUROPHYSIOLOGY

Inhibitory deficits have been described in schizophrenia, which could involve sensory and sensorimotor gating, as well as oculomotor control. These are evaluated through measures of P50 suppression, prepulse inhibition of the startle response, and the antisaccade task. P50 suppression

is one of the proposed endophenotypes for schizophrenia. When paired auditory stimuli are presented, an 80% decrease in the second P50 wave is normally observed with respect to the first one, which could mean there is activation of inhibitory neural circuits by the first auditory stimulus; the hippocampus is thought to be involved in this process. It has been shown that P50 suppression abnormalities resolve in patients and relatives after nicotine administration. P50 suppression has been linked with an allele in the promoter of the nicotinic receptor α -7 subunit gene. Prepulse inhibition occurs when a weak sensory event normally inhibits the startle reflex to an intense and abrupt stimulus; deficits in prepulse inhibition have been documented in patients and non-schizophrenic relatives. Finally, differences in oculomotor measures have also been reported between controls and schizophrenic patients, for example, regarding deficits in the control of fast eye movements (saccades).⁵³

DRUG RESPONSE

Cytochrome P450 (CYP) enzymes are responsible for oxidative reactions of many drugs and endogenous substances. CYP2D6 accounts for less than 5% of the total CYP content in the liver, but it is involved in the metabolism of several antidepressants such as fluoxetine, paroxetine, fluvoxamine, citalopram, escitalopram, venlafaxine, duloxetine, and mirtazapine, as well as antipsychotics such as chlorpromazine, thioridazine, haloperidol, risperidone, and aripiprazole. Moreover, codeine is bioactivated by CYP2D6 into morphine. Due to genetic polymorphisms, there is high individual variability regarding CYP2D6 catalytic activity. Among CYP2D6 alleles, there are at least 15 that encode non-functional proteins that result from gene deletion, aberrant splicing or premature translation termination. Alleles *3 to *8 are non-functional, *9, *10, and *41 have reduced functionality, while *1, *2, *35, *4, and *41 can be duplicated, resulting in an increased expression of a functional or non-functional CYP2D6 protein. The allelic frequency varies substantially depending on ethnicity. The presence of two, one or no functional CYP2D6 gene copies result in rapid, intermediate, or poor metabolizer phenotypes.⁵⁴ In humans, CYP2D6 expression may be specific to neurons in cerebral cortex, hippocampus, and cerebellum, which may be impaired in schizophrenia. CYP2D6 may be involved in the transformation of tyramine to dopamine, and in regeneration of serotonin from 5-methoxytryptamine. It has been suggested that CYP2D6 as well as serotonergic and dopaminergic genes could affect variability in antipsychotic drug response, and in psycho-physiological processes and symptoms in schizophrenic patients. A low frequency of poor CYP2D6 metabolizers has been reported in schizophrenia samples. In addition, poor CYP2D6 activity in healthy volunteers is associated with personality traits of social cognitive anxie-

ty, which may resemble mild psychotic-like symptoms.⁵⁵ Other CYP genes that have been studied include CYP3A, relevant for the metabolism of some antipsychotics and antidepressant drugs, carbamazepine, and some benzodiazepines; CYP2C19, involved in the metabolism of antidepressants and diazepam; CYP1A2, for clozapine and olanzapine. Other CYP genes are important for lamotrigine, valproate, some opioids, and secondary pathways for antipsychotics (UDP-Glucuronosyltransferases or UGTs).

It has been shown that individuals who show low levels of response to alcohol are at increased risk for a lifetime diagnosis of alcohol dependence, which depends in part on genetic factors. Some variants of genes that encode for alcohol dehydrogenase (ADH), aldehyde dehydrogenase 2, and CYP2E1, respectively, have been shown to be involved.

DISCUSSION

A broader knowledge of specific phenotypes related with mental disorders will improve our understanding of the pathophysiology of psychiatric entities, and may help accelerate the identification of relevant genetic, epigenetic and/or environmental factors that are associated with the etiology of the disorder. It may also help identify gene-environment interactions that may affect only concrete aspects related with the disorder, propose prevention strategies, establish more personalized treatments, and most importantly, improve the quality of life of patients, relatives, and caregivers, with considerably positive consequences for society. Nevertheless, a clear distinction must be made between potential biomarkers that may associate directly with the disorder (some of which may fulfill the criteria of endophenotypes) and phenotypes that are related with the effects of it.

Ideally, quantitative and objective measurements in large and well-characterized samples may help find suitable phenotypes. Strategies such as the inclusion of affected and non-affected first-degree relatives, twin pairs and/or drug naïve patients in their first episode are required to determine if phenotypes meet some of the criteria for endophenotypes. In addition, longitudinal assessments over longer periods of time are needed to establish if there is state independence.

In order to identify the best initial therapeutic options for each psychiatric patient, genotypes and phenotypes related with treatment response should be further analyzed in samples of all ethnic groups. It is also essential to consider gene-environment interactions that affect phenotypes, and could also affect endophenotypes; the study of epigenetic components, including DNA methylation, histone modifications, chromatin remodeling, and microRNAs represent another important branch of research that will ultimately help to improve our knowledge of mental disorders. Some endophenotypes may be complex, but are still useful to explain aspects of the etiology of mental disorders.

ACKNOWLEDGEMENTS

The authors thank Adriana Estrella González Ramirez for her participation in the initial search of scientific literature.

REFERENCES

1. Flicek P, Ahmed I, Amode MR et al. Ensembl 2013. *Nucleic Acids Res* 2012.
2. O'Huallachain M, Karczewski KJ, Weissman SM et al. Extensive genetic variation in somatic human tissues. *Proc Natl Acad Sci U S A* 2012;109:18018-18023.
3. Duncan LE, Keller MC. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. *Am J Psychiatry* 2011;168:1041-1049.
4. Caspi A, Sugden K, Moffitt TE et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003;301:386-389.
5. Kas MJ, Fernandes C, Schalkwyk LC, Collier DA. Genetics of behavioural domains across the neuropsychiatric spectrum; of mice and men. *Mol Psychiatry* 2007;12:324-330.
6. Gottesman, II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 2003;160:636-645.
7. Keating MT, Sanguinetti MC. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell* 2001;104:569-580.
8. Fabritz L, Damke D, Emmerich M et al. Autonomic modulation and antiarrhythmic therapy in a model of long QT syndrome type 3. *Cardiovasc Res* 2010;87:60-72.
9. Gottesman II, Shields J. Genetic theorizing and schizophrenia. *Br J Psychiatry* 1973;122:15-30.
10. Hasler G. Evaluating endophenotypes for psychiatric disorders. *Rev Bras Psiquiatr* 2006;28:91-92.
11. Preston GA, Weinberger DR. Intermediate phenotypes in schizophrenia: a selective review. *Dialogues Clin Neurosci* 2005;7:165-179.
12. Cannon TD, Keller MC. Endophenotypes in the genetic analyses of mental disorders. *Annu Rev Clin Psychol* 2006;2:267-290.
13. Kendler KS, Neale MC. Endophenotype: a conceptual analysis. *Mol Psychiatry* 2010;15:789-797.
14. Bellivier F, Henry C, Szoke A et al. Serotonin transporter gene polymorphisms in patients with unipolar or bipolar depression. *Neurosci Lett* 1998;255:143-146.
15. Hauser J, Leszczynska A, Samochowiec J et al. Association analysis of the insertion/deletion polymorphism in serotonin transporter gene in patients with affective disorder. *Eur Psychiatry* 2003;18:129-132.
16. Lotrich FE, Pollock BG. Meta-analysis of serotonin transporter polymorphisms and affective disorders. *Psychiatr Genet* 2004;14:121-129.
17. Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT. Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. *Am J Med Genet B Neuropsychiatr Genet* 2005;133B:110-115.
18. Smeraldi E, Zanardi R, Benedetti F et al. Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. *Mol Psychiatry* 1998;3:508-511.
19. Arranz MJ, Bolonna AA, Munro J et al. The serotonin transporter and clozapine response. *Mol Psychiatry* 2000;5:124-125.
20. Zanardi R, Benedetti F, Di Bella D et al. Efficacy of paroxetine in depression is influenced by a functional polymorphism within the promoter of the serotonin transporter gene. *J Clin Psychopharmacol* 2000;20:105-107.
21. Gonda X, Juhasz G, Laszik A et al. Subthreshold depression is linked to the functional polymorphism of the 5HT transporter gene. *J Affect Disord* 2005;87:291-297.
22. Moncrieff J, Kirsch I. Efficacy of antidepressants in adults. *BMJ* 2005;331:155-157.

23. Quintin P, Benkelfat C, Launay JM et al. Clinical and neurochemical effect of acute tryptophan depletion in unaffected relatives of patients with bipolar affective disorder. *Biol Psychiatry* 2001;50:184-190.
24. Hasler G, Drevets WC, Gould TD et al. Toward constructing an endophenotype strategy for bipolar disorders. *Biol Psychiatry* 2006;60:93-105.
25. Sobczak S, Honig A, Schmitt JA, Riedel WJ. Pronounced cognitive deficits following an intravenous L-tryptophan challenge in first-degree relatives of bipolar patients compared to healthy controls. *Neuropsychopharmacology* 2003;28:711-719.
26. Pacchierotti C, Iapichino S, Bossini L et al. Melatonin in psychiatric disorders: a review on the melatonin involvement in psychiatry. *Front Neuroendocrinol* 2001;22:18-32.
27. Hallam KT, Olver JS, Chambers V et al. The heritability of melatonin secretion and sensitivity to bright nocturnal light in twins. *Psychoneuroendocrinology* 2006;31:867-875.
28. Serretti A, Benedetti F, Mandelli L et al. Genetic dissection of psychopathological symptoms: insomnia in mood disorders and CLOCK gene polymorphism. *Am J Med Genet B Neuropsychiatr Genet* 2003;121B:35-38.
29. Lok A, Mocking RJ, Ruhe HG et al. Longitudinal hypothalamic-pituitary-adrenal axis trait and state effects in recurrent depression. *Psychoneuroendocrinology* 2012;37:892-902.
30. Radley JJ, Kabbaj M, Jacobson L et al. Stress risk factors and stress-related pathology: neuroplasticity, epigenetics and endophenotypes. *Stress* 2011;14:481-497.
31. Mann JJ, Arango VA, Avenevoli S et al. Candidate endophenotypes for genetic studies of suicidal behavior. *Biol Psychiatry* 2009;65:556-563.
32. van Venrooij JA, Fluitman SB, Lijmer JG et al. Impaired neuroendocrine and immune response to acute stress in medication-naïve patients with a first episode of psychosis. *Schizophr Bull* 2012;38:272-279.
33. Chen AC, Manz N, Tang Y et al. Single-nucleotide polymorphisms in corticotropin releasing hormone receptor 1 gene (CRHR1) are associated with quantitative trait of event-related potential and alcohol dependence. *Alcohol Clin Exp Res* 2010;34:988-996.
34. Page DT. A candidate circuit approach to investigating autism. *Anat Rec (Hoboken)* 2011;294:1671-1684.
35. Jiang B, Kenna HA, Rasgon NL. Genetic overlap between polycystic ovary syndrome and bipolar disorder: the endophenotype hypothesis. *Med Hypotheses* 2009;73:996-1004.
36. Lawrie SM, Whalley HC, Abukmeil SS et al. Brain structure, genetic liability, and psychotic symptoms in subjects at high risk of developing schizophrenia. *Biol Psychiatry* 2001;49:811-823.
37. Owens SF, Picchioni MM, Ettinger U et al. Prefrontal deviations in function but not volume are putative endophenotypes for schizophrenia. *Brain* 2012;135:2231-2244.
38. Chakravarty MM, Felsky D, Tampakeras M et al. DISC1 and Striatal Volume: A Potential Risk Phenotype For mental Illness. *Front Psychiatry* 2012;3:57.
39. Adler CM, Holland SK, Schmithorst V et al. Abnormal frontal white matter tracts in bipolar disorder: a diffusion tensor imaging study. *Bipolar Disord* 2004;6:197-203.
40. Caligiuri MP, Brown GG, Meloy MJ et al. A functional magnetic resonance imaging study of cortical asymmetry in bipolar disorder. *Bipolar Disord* 2004;6:183-196.
41. Ruocco AC, Amirthavasagam S, Zakzanis KK. Amygdala and hippocampal volume reductions as candidate endophenotypes for borderline personality disorder: a meta-analysis of magnetic resonance imaging studies. *Psychiatry Res* 2012;201:245-252.
42. Sobczak S, Riedel WJ, Booij I et al. Cognition following acute tryptophan depletion: difference between first-degree relatives of bipolar disorder patients and matched healthy control volunteers. *Psychol Med* 2002;32:503-515.
43. Robinson LJ, Thompson JM, Gallagher P et al. A meta-analysis of cognitive deficits in euthymic patients with bipolar disorder. *J Affect Disord* 2006;93:105-115.
44. Murphy FC, Sahakian BJ, Rubinsztein JS et al. Emotional bias and inhibitory control processes in mania and depression. *Psychol Med* 1999;29:1307-1321.
45. Smith DJ, Muir WJ, Blackwood DH. Neurocognitive impairment in euthymic young adults with bipolar spectrum disorder and recurrent major depressive disorder. *Bipolar Disord* 2006;8:40-46.
46. Ferrier IN, Chowdhury R, Thompson JM et al. Neurocognitive function in unaffected first-degree relatives of patients with bipolar disorder: a preliminary report. *Bipolar Disord* 2004;6:319-322.
47. Olley AL, Malhi GS, Bachelor J et al. Executive functioning and theory of mind in euthymic bipolar disorder. *Bipolar Disord* 2005;7(Suppl 5):43-52.
48. Nuechterlein KH, Subotnik KL, Ventura J et al. The puzzle of schizophrenia: tracking the core role of cognitive deficits. *Dev Psychopathol* 2012;24:529-536.
49. Agnew-Blais J, Seidman LJ. Neurocognition in youth and young adults under age 30 at familial risk for schizophrenia: A quantitative and qualitative review. *Cogn Neuropsychiatry* 2012:1-39. En prensa. DOI:10.1080/13546805.2012.676309.
50. Greenwood TA, Light GA, Swerdlow NR et al. Association analysis of 94 candidate genes and schizophrenia-related endophenotypes. *PLoS One* 2012;7:e29630.
51. Rajender G, Bhatia MS, Kanwal K et al. Study of neurocognitive endophenotypes in drug-naïve obsessive-compulsive disorder patients, their first-degree relatives and healthy controls. *Acta Psychiatr Scand* 2011;124:152-161.
52. Ersche KD, Turton AJ, Chamberlain SR et al. Cognitive dysfunction and anxious-impulsive personality traits are endophenotypes for drug dependence. *Am J Psychiatry* 2012;169:926-936.
53. Braff DL, Greenwood TA, Swerdlow NR et al. Advances in endophenotyping schizophrenia. *World Psychiatry* 2008;7:11-18.
54. Zanger UM, Fischer J, Raimundo S et al. Comprehensive analysis of the genetic factors determining expression and function of hepatic CYP2D6. *Pharmacogenetics* 2001;11:573-585.
55. Dorado P, Penas-Lledo EM, Llerena A. CYP2D6 polymorphism: implications for antipsychotic drug response, schizophrenia and personality traits. *Pharmacogenomics* 2007;8:1597-1608.

Declaration of conflict interest: None