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The genetics of depression: What information can new methodologic approaches provide?

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Major depressive disorder is a genetically complex disease involving several minor, or susceptibility, genes whose expression may be modulated by many environmental factors. From the classical early linkage studies to the complete genome-wide association (GWA) study methodologies, it is evident that it is difficult to understand the genetic bases of this mental disorder. Many studies have identified one or more gene regions whose variability confers a small risk for developing depressive disorder, explaining a small percentage of the total genetic component of disease with low predictive value. Although the results to date are inconclusive, new approaches based on gene-environment interactions and biological pathway analysis (based on GWA studies) open new and promising perspectives in the investigation of the genetic and biological basis of major depression.

Keywords:

Major depressive disorder, Genetic risk factors, Association, Linkage, Gene-environment interaction, GWAS

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La genética de la depresión: ¿qué información aportan las nuevas aproximaciones metodológicas?

El trastorno depresivo mayor forma parte del grupo de enfermedades denominadas genéticamente complejas en cuya base se encuentran involucrados una serie de genes de efecto menor o susceptibilidad cuya expresión podría estar modulada por un gran número de factores ambientales. Desde los primeros estudios clásicos de ligamiento hasta las nuevas metodologías de estudio de genoma completo se ha puesto de manifiesto la dificultad para comprender las bases genéticas

de la depresión mayor. En muchos estudios se han identificado una o varias regiones génicas cuya variabilidad confiere un riesgo pequeño para desarrollar un trastorno depresivo; es decir, dichas variantes explicarían un porcentaje muy pequeño del componente genético total de la enfermedad en la población y, por tanto, poseerían un valor predictivo bajo. Aunque los resultados obtenidos hasta el momento no son concluyentes, las nuevas aproximaciones basadas en estudios de interacción gen-ambiente así como los análisis de vías biológicas (basados en los estudios GWAS) abren nuevas y prometedoras perspectivas en la investigación de las bases genéticas y biológicas de la depresión mayor.

Palabras clave:

Trastorno depresivo mayor, Factores genéticos de riesgo, Asociación, ligamiento, Interacción gen-ambiente, GWAS

INTRODUCTION

Major depressive disorder (MDD), or unipolar depression, is considered a serious mental illness from a medical standpoint. The diagnosis is often complex due to the difficulty of defining different symptoms and the syndrome of behaviors and feelings in certain life situations and the broad clinical variability present in depressive pictures. Similarly, we should not forget that there are no biological, biochemical or brain morphology markers that allow an unequivocal diagnosis of depression. Due to the absence of external markers of depressive disorders, the diagnosis is necessarily psychopathological and clinical.¹ In this sense, major depressive disorder is diagnosed based, for example, on DSM-IV-TR (Diagnostic and Statistical Manual of Mental Disorders).² This classification is based on criteria developed and revised over the past three decades by the American Psychiatric Association (APA).

According to this categorical diagnosis, major depressive disorder is characterized by the presence of a depressed

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Table 1		Summary of the symptoms associated with major depressive disorder
Symptoms of depression		
Emotional	Depressed mood, sadness Decreased pleasure or lack of interest in most activities	
Somatic	Weight loss or gain Insomnia or hypersomnia Psychomotor agitation or retardation Fatigue or diminished energy	
Cognitive	Feelings of worthlessness or excessive or inappropriate guilt Decreased ability to think or concentrate, indecision Recurrent thoughts of death or suicide	

mood (hypothymia) and/or loss of interest and diminished pleasure in daily life activities (anhedonia). These symptoms, which become established and persist over time, interfere seriously with the daily life of the patient and tend to be accompanied by somatic or psychological changes and abnormalities in different biological functions. These symptoms include diminished appetite and weight loss, reduced activity of the individual, constipation, sleep disorders such as hypersomnia or early awakening, agitation or generalized inhibition of movement, and decreased libido or loss of libido.³ The depressed person also often exhibits changes in cognitive functions that reduce the capacity to think, concentrate or make decisions. Pessimistic thinking is also common, often including feelings of guilt and inferiority, ideas of hopelessness, and recurrent thoughts of death or suicide. Suicidal ideation should be kept in mind and suicide attempts are common in people affected by major depression, with more than 15% of patients ending their lives by suicide.⁴

In order to diagnose a major depressive episode, the patient must present at least 1 emotional symptom and 4 somatic or cognitive symptoms. Furthermore, according to DSM-IV-TR criteria, these manifestations must be present for a period of at least 2 weeks.

According to the World Health Organization (WHO), about 340 million persons currently have depression, but only 25% of them have access to effective treatment. Depression is one of the five leading causes of disability, illness and premature death in people between the ages of 18 and 44 years and it is believed that by 2020 it will be the second most important cause of disability, preceded only by cardiovascular disease.⁵

Depression can occur at any time in life, most frequently between 18 and 44 years of age,⁶ and with a mean age of onset of about 27 years.⁷ The incidence of this disorder in the population is 10 new cases per 1000 individuals annually.⁸

Epidemiological studies show that, with some exceptions, the prevalence, incidence and morbid risk of depressive disorder are twice as high in women as in men.⁹⁻¹² Thus, the prevalence of this disorder in the general population varies within a range of 2.6 to 5.5% in men and 6.0 to 11.8% in women.¹³ Other studies that contemplate a broader range of depression phenotypes have found much higher prevalences, with a range of variation from 10-12% in men and up to 20-25% in women.^{14, 15}

GENETICS OF DEPRESSION

Major depressive disorder, like most diseases that affect humans, is part of the group of diseases known as genetically complex diseases, in which both genetic and environmental factors have a role in the etiology.

The genetic component of these diseases has been identified from studies in families, twins or adopted children. Complex diseases, despite having a genetic basis, do not conform to the classic Mendelian inheritance pattern. In general, the *sensitivity threshold* model is considered one of the most useful for explaining how the disease is transmitted. This model assumes that the "disease susceptibility" variable is distributed continuously in the population, so that only those individuals who surpass a certain threshold manifest the disorder. It is hypothesized that a number of minor effect genes are involved in the origin of this complex heredity, whose expression can be modulated by many environmental factors.

EXISTENCE OF A GENETIC COMPONENT

Family studies

The first and simplest approach to studying the hereditary factors involved in a disorder stems from observations of the family and study of the prevalence of the disorder in family members, which allow the familial morbid risk of the diagnosis of interest to be calculated. Consequently, we would start with the hypothesis that the prevalence of a particular inherited disorder is higher among the relatives of those affected than in the general population and that the larger the percentage of genes shared with the affected person, the greater the risk of developing the disorder.

Such studies have confirmed that among the first-degree relatives of a patient with major depression (parents and siblings have 50% shared genes), there is a significant increase in the prevalence of this disease (15%) compared to the general population (5.4%).¹⁶

Family studies, however, have the drawback that they do not control the environmental factor. We think that many important aspects of our behavior and psychopathology may be related to behaviors acquired in the family setting and are thus equally heritable. In order to complete the study of the genetic risk factors involved, adoption and twin studies are essential in which it is possible to control the environmental factor and differentiate it from the genetic factor.

Adoption studies

Different types of adoption studies differ in their experimental design. The goal is always to determine whether the factors linked to the familial transmission of the disorder are biological or environmental.

In this sense, adoption studies involve comparing the risk of disease in the biological children of parents with the disorder (high-risk children) who are raised in a healthy adoptive family compared to the risk of children of affected parents raised in their biological family.

Some adoption studies of unipolar depression in children at high risk of depressive disorder raised in healthy families show higher rates of depression than expected in the general population,¹⁷ which reveals the existence of genetic factors in the risk of major depression, especially for the most severe forms of the disease.¹⁸

Studies of twins

Studies of twins allow the relative importance of genes, the environment, and the interaction between them to be estimated in relation to certain complex characteristics of human beings.

These studies compare the concordance rates for a given disorder in monozygotic, or identical, twins (who share all their genes) and in dizygotic twins (who share only half their genes), which allows the relative contribution of genes and environment in the origin of these mental disorders to be evaluated. The comparison of the concordance between the two types of twins used to assess the heritability (h^2) of the disorder is a statistical measure of the degree to which genes contribute to the total variability observed in a character or phenotype.

In a review by Tsuang and Faraone, approximately 60% of the phenotypic variability present in major depression could be attributed to genetic factors.¹⁹ However, in studies by other researchers, lower heritability rates situated around 40% are observed.^{20,21} These differences can be attributed in part to differences between studies in the definition of the inclusion phenotype.

Most studies of this topic generally show the importance of genetic factors and indicate that some of these factors might be specific to forms of depression that are particularly severe, recurrent and specific to women, whereas other genetic risk factors are shared by individuals of both sexes.²² From the perspective of twin studies, early-onset recurrent depression (before age 30) is the form that accumulates the greatest genetic risk.²³

Regarding the approach to environmental risk factors in twin studies, these studies have allowed the investigation of how differential studies of certain environmental factors may explain why in a pair of monozygotic, i.e., genetically identical twins, one develops major depressive disorder and the other does not. Studies by Kendler's team in a sample of over 7,000 twins (men and women) have allowed the identification of important environmental risk factors for major depression, such as certain stressful life events, specifically those related to loss (death, separation, etc.) and humiliation (shameful experiences, separations initiated by others).^{24,25}

SEARCH FOR RISK GENES

Linkage studies

In linkage analysis, genealogies in which the disease occurs in different family members and in which there is a Mendelian inheritance pattern are usually used. In these families, the segregation of a particular genetic marker is studied to determine if disease transmission and different alleles of this marker show independence. Where the disease and a given allele are transmitted together, this may suggest the existence of a gene for disease located near the polymorphism used as a marker.

These studies allow the LOD-score to be calculated. This statistical parameter tells us the likelihood of genetic linkage between the genetic marker studied and the disease, i.e., the probability that they are transmitted together (LOD-score > 3).

Such studies have been relatively numerous in the genetic investigation of mental disorders, but the lack of a Mendelian model of inheritance of these disorders, suspected etiological heterogeneity, involvement of environmental factors in their diagnosis and the phenotypical heterogeneity

Table 2		Linkage studies in major depression and subtypes (recurrent and early-onset recurrent) (table adapted from Middeldorp et al. ²⁶)				
Chromosome	Region (cM)	LOD	N subjects/families	Phenotype	Reference	
1	42	1.7	426/90	MDD-RE	27	
2	90.6	1.7	?/278	MDD	26	
	237-248	2.2	224 possible pairs	MDD (+alcohol)	31	
		2.5 ^b	?/81	MDD	32	
	15	3.19	?/81	MDD-RE	91	
8	2.7	1.9	?/278	MDD	26	
10	5-9	1.6	426/90	MDD-RE	27	
	76	3.0	?/81	MDD	32	
11	85-99	2.5	?/81	MDD	32	
12	105-124	1.6	994/497	R-MDD	30	
17	17q11.2	2.1	?/278	MDD	26	
18	73	3.8	96/21	MDD-RE & anxiety	27	

LOD > 3 = Linkage, -2 < LOD < 3 = inconclusive; LOD < -2 = No linkage
MDD: major depressive disease
MDD-RE: major depressive disease, recurrent early-onset (age at onset < 31)
MDD-R: major depressive disease, recurrent

are, among others, reasons that might explain the inconclusive results obtained to date.²⁶

The most important linkage studies conducted in major depressive disorder are summarized in Table 2.

Among the analyses carried out in major depression are linkage studies that have made it possible to identify candidate genes for this disorder.²⁷⁻³² One of the most interesting findings involves chromosome 11. The study, conducted in samples with recurrent major depression, found an LOD-score of more than 3 (4.2) in the 11pter-p15 chromosomal region.³² This result was partially replicated by a second study that obtained an LOD-score of 1.6 in the same chromosomal region.²⁷ Interestingly, this region of chromosome 11 contains genes that have been considered candidates for MDD, such as tyrosine hydroxylase (TH), a key enzyme in dopamine synthesis. In fact, some studies have shown that TH inhibition can cause depressive symptoms in healthy subjects.³³

Another interesting result is found in chromosomal region 17q11.2, in which a study with a sample of patients with MDD found an LOD-score of 2.1.²⁶ This chromosome region harbors the serotonin transporter gene (SLC6A4), which has gained prominence in gene-environment interaction (GxE) studies, which will be discussed later in this chapter.³⁴

A recent study has shown evidence of linkage with the 2q33.34 chromosomal region found in the CREB1 gene in women with early-onset recurrent major depression.³² This gene encodes for the CREB1 transcription factor that regulates the expression of growth factors involved in synaptogenesis and neurogenesis, which makes it a good candidate in view of the hypothesis that alterations in the cellular pathways involved in synaptic plasticity contribute to increasing the risk of developing depression.³⁵

Linkage studies, although not the most powerful methodology for detecting genes involved in complex diseases such as MDD, have been useful in detecting regions containing genes that have been subsequently implicated in the origin of the disease by association studies or GxE interaction studies.

Association studies

Analysis of genetic associations is an alternative to linkage studies and one of the best strategies for identifying the genes responsible for genetically complex diseases in which there is no known inheritance model, in which many genes with minor effects are probably involved.

The classic design of a genetic association analysis is a case-control study in which the frequency of a possible risk allele of a candidate gene in unrelated individuals affected by the same disease (case group) is compared to the frequency observed in healthy individuals of the same ethnic group (control group). If the risk factor analyzed is found more frequently in the case group than in controls, an association exists between the factor and the disease. This means that the exposure, or presence, of this factor increases the risk of, or susceptibility to, the disease.

In genetic association studies, the risk factor analyzed is always a genetic marker or polymorphism, usually located on a candidate gene for the disease. The results are reported as an *odds ratio* (OR), which indicates how much more common the disease is in carriers with genetic variants of risk than in non-carriers.

Since the first case-control study conducted by Beckman et al. linking major depression and genetic variability,³⁶ a large number of studies have been published, but few susceptibility genes for the origin of depression have been recognized and replicated.

These inconsistent results may be due to methodological differences between studies, such as the study design, study population, diagnosis of major depression or even the lack of statistical power due to a small sample size.³⁷

A meta-analysis of genetic association studies in major depression was recently conducted in which 20 polymorphisms in 18 genes were analyzed. Five of these genes showed a statistically significant association with major depression (APOE, GNB3, MTHFR, SLC6A3 and SLC6A4).³⁷ The results of this meta-analysis are summarized in Table 3.

Most researchers have focused on genes encoding proteins that are involved in central nervous system

neurotransmission pathways, especially serotonergic neurotransmission.³⁸

In this sense, the SLC6A4 gene has been one of the genes most studied because it encodes the protein that is a therapeutic target of selective serotonin reuptake inhibition drugs. This gene (chromosome 17q11.1-Q72) encodes the serotonin transporter and has a polymorphism (5-HTTLPR) in the promoter region, with two allelic variants: 528 (L) and 484 (S). *Studies in vivo* have determined that the presence of the short 484 allele was associated with decreased gene expression and, consequently, with fewer serotonin transporters in the neuronal membrane.³⁹⁻⁴¹ Likewise, various association studies have described the relationship between the presence of the short allele of the 5-HTTLPR polymorphism and the presence of depression severity traits (suicide or melancholy)⁴²⁻⁴³ and increased vulnerability to develop major depression when the person has been abused in childhood.^{42, 44, 45}

Similarly, when considering serotonergic neurotransmission, other studies show an association between other genes of the serotonergic neurotransmission system and the presence of depression severity traits such as seasonality^{46, 47} or suicide.^{48, 49} However, the identification of genetic variants of interest, or relatively specific genetic variants related to the most endogenous forms of depression, remains to be established.

Gene-environment interaction (GxE) studies

Analysis of the gene-environment relation opens new perspectives for understanding the etiology of major depression, in which the genetic profile of a person and its continuous interaction with the environment are the focus of study. This interaction could be explained by genetically mediated sensitivity to the environmental factors to which a person is exposed throughout life. This means that certain genotypes (risk genotypes) confer a higher probability of suffering the disorder than others (non-risk genotypes), given the same exposure to an environmental risk factor. According to this model, individuals differ in their sensitivity to adverse environmental factors, so that genetically vulnerable persons are at increased risk of developing the disease when exposed to the same dose of a particular environmental risk factor.⁵⁰

In 2003, a paradigmatic study was published in the search for gene-environment interactions in the origin of depression. Caspi's team showed that individuals carrying the short allele (S) of the 5-HTTLPR polymorphism of the serotonin transporter gene had experienced stressful life events in childhood and youth, and presented more depressive symptoms, depressive episodes and suicidal behavior at age 26.³⁴

Table 3 Meta-analysis of genetic association studies in major depression. (Table adapted from López-León et al..³⁷)

Gene	Region	Polymorphism	Studies	Analysis	Heterozygotes			Homozygotes		
					Comparison	OR (95% CI)	I2	Comparison	OR (95% CI)	I2
ACE (angiotensin I converting enzyme)	17q23.3	Ins/Del -Int16	8	Fixed	IV vs II	0.94(0.78-1.13)	27	DD vs II	1.15 (0.94-1.42)	26
APOE (apolipoprotein E)	19q13.2	ε2/ε3/ε4	5	Fixeds	ε2/ε3 vs ε3/ε3	0.90 (0.71-1.13)	72*	ε2/ε2 vs ε3/ε3	1.11 (0.85-1.45)	NA
				Random		0.41 (0.15-1.07)				NA
BDNF (brain-derived neurotrophic factor)	11p13	Val66Met	5	Fixeds	ε3/ε4 vs ε3/ε3	1.02 (0.78-1.35)	0	ε4/ε4 vs ε3/ε3	1.02 (0.44-2.37)	32
				Random		1.02 (0.78-1.35)			0.91(0.25-3.33)	
COMT (catechol-O-methyltransferase)	22q11.21	Val158Met	8	Fixeds	Val/Met vs Val/Val	0.98 (0.89-1.09)	0	Met/Met vs Val/Val	1.05 (0.84-1.32)	53*
				Random		0.98 (0.89-1.09)			1.09 (0.72-1.65)	
DRD3 (dopamine receptor D3)	3q13.3	Ser9Gly	6	Fixeds	Val/Met vs Val/Val	1.14 (0.86-1.52)	29	Met/Met vs Val/Val	0.95 (0.67-1.33)	12
				Random		1.13 (0.86-1.59)			0.95 (0.67-1.33)	
GABRA3 (gamma-aminobutyric acid A receptor. alpha 3)	Xq28	CA repeat	4	Fixeds	Ser/Gly vs Ser/Ser	0.92 (0.67-1.26)	33	Ser/Ser vs Ser/Ser	1.31 (0.80-2.14)	72*
				Random		0.96 (0.64-1.45)			1.71 (0.57-5.13)	
GNB3 (guanine nucleotide binding protein beta polypeptide 3)	12p13	C825T	6	Fixeds	*1 vs 1/1	0.74 (0.49-1.12)	46	*1* vs 1/1	0.92 (0.40-2.11)	10
				Random		0.63 (0.32-1.25)			0.97 (0.36-2.62)	
HTR1A (5-hydroxytryptamine receptor 1A)	5q11.2-q13	C-1019G	3	Fixeds	CT vs CC	1.25 (0.91-1.72)	50	TT vs CC	2.13 (1.39-3.28)**	67*
				Random		1.30 (0.81-2.09)			2.09 (0.97-4.51)	
HTR1B (5-hydroxytryptamine receptor 1B)	6q13	G861C	4	Fixeds	CG vs CC	0.98 (0.72-1.33)	18	GG vs CC	1.33 (0.95-1.87)	79**
				Random		0.98 (0.69-1.38)			1.63 (0.72-3.70)	
HTR2A	13q14-q21	A-1438G	3	Fixeds	GC vs GG	0.99 (0.74-1.33)	42	CC vs GG	0.81 (0.46-1.41)	25
				Random		0.98 (0.66-1.44)			0.79 (0.41-1.53)	
			4	Fixeds	AG vs AA	1.23 (0.88-1.73)	62*	GG vs AA	1.06 (0.73-1.55)	75**

Table 3	Continued										
	Gene	Region	Polymorphism	Studies	Analysis	Heterozygotes			Homozygotes		
						Comparison	OR (95% CI)	I2	Comparison	OR (95% CI)	I2
(5-hydroxytryptamine receptor 2A)			T102C	8	Random	TC vs TT	1.15 (0.65-2.03)	0	CC vs TT	0.98 (0.45-2.14)	17
MAOA (monoamine oxidase A)	Xp11.3	VNTRprom	4	Fixed	12 vs 22		1.00 (0.79-1.27)	17	11 vs 22	0.92 (0.71-1.21)	0
MTHFR (methylene-tetrahydrofolate reductase)	1p36.3	G677T	6	Random	CT vs CC		1.00 (0.79-1.27)	26	TT vs CC	0.92 (0.68-1.25)	0
SLC6A2 (noradrenalin transporter 2)	16q12.2	T-182C	3	Fixed	TC vs TT		1.34 (0.95-1.89)	52	CC vs TT	0.71 (0.49-1.03)	64
SLC6A3 (DAT1) (dopamine transporter 3)	5p15.3	40bp VNTR	3	Random	9/10 vs 10/10		1.29 (0.87-1.91)	0	9/9 vs 10/10	0.71 (0.49-1.03)	0
SLC6A4 (SERT) (serotonin transporter 4)	17q11.2	44bp Ins/del	22	Fixed	LS vs LL		1.22 (1.03-1.44)*	0	SS vs LL	1.38 (1.08-1.76)*	0
TPH1 (tryptophan hydroxylase 1)	11p15.3-p14	A218C	9	Random	10/12 vs 12/12		1.23 (0.95-1.58)	24	10/10 vs 12/12	1.38 (1.08-1.77)*	0

All the studies used random and/or fixed effects meta-analysis methods. OR, odds ratio CI, 95% confidence interval. The references of these studies can be found in the review by López-León et al.³⁷

Although several authors had postulated that stressful situations involving sense of defeat, loss, humiliation and frustration influence the age at onset of depression,⁵¹⁻⁵⁴ the Caspi study team was the first to demonstrate this GxE interaction empirically.³⁴

However, the debate about the role of this polymorphism in the risk of depression is evident in the recent literature. A meta-analysis by the team of Risch et al. concluded that life events have a strong relation with the increased risk of major depression. However, the addition of genetic variability associated with the serotonin reuptake gene does not appear to increase the predictive power of negative life events *per se* on the risk of major depression.⁵⁵ The results obtained from this meta-analysis show the same tendency as the study by Munafo et al., in which it is concluded that the associations found to date could be consistent with random results.⁵⁶ Despite the results obtained, a new study supports the existence of an interaction between the serotonin transporter gene and major depression initially found by Caspi et al.³⁴ The authors of this meta-analysis⁵⁷ show that the results of two previous meta-analyses did not take into account all the published studies, so their results are not valid.

It should be noted that, despite the data collected in these studies, not all people who experience stressful events develop depression. This may be related to the genetic substrate of vulnerability. In this sense, this hypothesis is supported by the genetics of behavior, since it has been documented that the risk of depression after a stressful event is higher among individuals who are in a group at high familial genetic risk than in those who do not exhibit this increased genetic risk.⁵⁸

In this sense, early traumatic experiences like child abuse have been described as one of the most important environmental risk factors leading to the onset of major depression in adults.⁵⁸⁻⁶⁰ Evidence from neurobiology and epidemiology suggests that disruptive adverse events that occur during an individual's development can cause persistent cerebral dysfunction.^{61, 62} The impact of such events on brain neurobiology would be moderated by individual genetic variability and the literature indicates that both the gene encoding the serotonin transporter^{34, 63} and the gene encoding brain-derived neurotrophic factor (BDNF)^{64, 65} seem to play a key role in modulating the impact of childhood abuse and the risk of the emergence of depressive symptoms in adulthood.⁶⁶ Thus, early experiences can affect the development of the hypothalamic-pituitary-adrenal (HPA) axis and neurobiological responses to stress in adulthood, and predispose the individual to the development of MDD.⁶⁷

Such studies help to understand how early life stressors can leave an indelible mark on the central nervous system,

especially in individuals with high genetic vulnerability, and thus increase the risk of suffering a disorder of the depressive spectrum in adulthood.

Given the complexity of the depressive phenotype, not only the approaches based on genetic association or GxE interaction studies help to explain the origin of this disease. Other approaches based on epistatic or gene-gene interaction (GxG) models should also be considered. In this sense, it should be noted that the action of a gene can be modified by that of one or more other genes (modifiers), i.e., the phenotypic consequences of an allele generally depend on multiple alleles with a complex interaction.

Genome-wide association (GWA) studies in depression. Is this approach valid?

One of the most recent methodologies used in the search for genetic risk factors in complex diseases is based on genome-wide association (GWA) studies. This methodology is based on genotyping arrays or microarrays that allow the variability of the human genome (up to a million genetic markers in a subject in a single text) to be traced in order to assess the hypothesis of common disease-common variant without the need to conduct a hypothesis-guided study of the etiology of the disease.

Currently, GWA studies unguided by hypotheses are transforming our understanding of the genetic and pathophysiological architecture of complex medical conditions. Since 2005, nearly 100 genetic risk variants have been replicated in up to 40 common diseases, such as diabetes or cancer. Many of these variants are found either in genes that were not previously considered candidates for disease, or in genomic regions that do not contain genes.⁶⁸ Similarly, promising results have been found in disorders with a low prevalence and high heritability, such as Crohn's disease.⁶⁹

With regard to GWA studies in MDD, the first study was developed by Muglia et al. in 2008, in two independent samples of recurrent major depression: the first consisting of 1022 patients diagnosed of recurrent major depression and 1000 controls and the second consisting of 492 patients diagnosed of the same disease and 1052 controls. Unfortunately, no significant results were obtained in any of the samples used, meaning that none of the polymorphisms examined showed an association with the phenotype with a p-value below the limit of significance established in GWA studies ($p < 10^{-8}$). In order to increase statistical power, the authors made a meta-analysis of the two studies in which the results confirmed those obtained by the two previous independent analyses. According to the findings, the authors suggest the possibility that there is no genetic marker that

provides a significant OR for major depression *per se*, understood from a categorical diagnosis.⁷⁰

In this regard, in a study made after this GWA study in 1738 patients with early-onset (onset before age 31) recurrent depressive disorder and 1802 controls, no significant association was found in relation to established statistical parameters for GWA studies.⁷¹ However, marginal significance was observed in chromosomal region 18q22.1 (rs17077540, $p=1.83 \times 10^{-7}$). Previous studies have shown the existence of a genetic association between this region and major depressive disorder.³⁸ This region is approximately 75 Kb from the DSEL (dermatan sulfate epimerase-like) gene, a gene expressed in the brain with unknown function, in which two non-synonymous mutations were observed in patients with bipolar affective disorder but not in controls.⁷²

In the same line, a new GWA study (GAIN MDD) achieved significant results, in which the signals of maximum significance were detected over the region of the chromosome 7 gene occupied by the Piccolo (PCLO) gene, whose protein is located in the presynaptic active zone cytomatrix and is important in serotonergic neurotransmission. Two SNPs (single nucleotide polymorphisms) showed maximum significance values: rs2715148 ($p=7.7 \times 10^{-7}$) and rs2522833 ($p=1.2 \times 10^{-6}$),⁷³ but only the second could be replicated in independent samples that were phenotypically similar to the first sample. Recently, this association with the PCLO gene rs2522833 polymorphism has been confirmed in an independent sample of depressed patients of Dutch origin.⁷⁴

Another GWA study (UK study) conducted by the Lewis team of in a sample of 1636 patients with recurrent major depression and 1594 control subjects showed evidence of an association between an SNP polymorphism in the BICC1 gene (bicaudal C homologue 1 gene).⁷⁵ The product of this gene, expressed in all brain regions, is an RNA binding protein that forms complex interactions with RNA and other proteins. The association of BICC1 and depression is a novel finding since no previous evidence existed of the role of this gene in neuropsychiatric diseases. This association was stronger when analyzed in a population of women diagnosed of depression.⁷⁵ Various interesting signs of association were identified but, as in previous genome-wide association studies, it was suggested that the contributions of individual genes to major depression may be only minor.⁷⁵

The recent MDD2000+ study conducted by the team of Wray et al. is the largest GWA study of major depression reported to date, with 2431 cases of MDD and 3673 controls.⁷⁶ This group compared their results to those published on other GWA studies of major depression.^{73, 75} In this study, a meta-analysis was also made of autosomal SNPs with the samples of the MDD200+, GAIN MDD⁷³ and UK studies⁷⁵ (i.e.,

the three largest GWA studies of MDD). No polymorphism reached genome-wide significance in either the study itself or in the meta-analysis, with a total of 5763 cases and 6901 controls. These results imply that either common variants of intermediate effect do not seem to have important effects on the genetic architecture of major depression,⁷⁶ or that the phenotypic heterogeneity of the disease impedes the detection of overall genetic risk factors.

GWA studies have undoubtedly opened a new door in the investigation of the importance of genetic factors in the origin of depression. However, we must be aware that some genes may be associated with the disease (as demonstrated in classic association studies) without attaining the level of significance required in a genome-wide association study. This means that we must take into account that, while the analysis of individual SNPs has been useful in identifying variants of disease-related susceptibility, this mode of analysis can be quite limiting in certain situations because of the difficulty of achieving the levels of significance established in genome-wide association studies. Specifically, and for the purpose of controlling type I errors, the statistical level of each test must be adjusted. Due to the large number of hypotheses considered, the threshold of significance for GWA studies can be extreme and difficult to attain. Remember that for a GWA study analyzing the effect of 500,000 SNPs, each statistical test is performed at a significance level of at least 10^{-8} , which is highly restrictive.⁷⁷ The small p-value of GWA studies thus requires a sample size on the order of thousands of subjects to achieve sufficient statistical power to allow the detection of polymorphisms in genes with a minor effect. The need for inclusion of a sample of this size is related to the greater heterogeneity of the samples due to inclusion criteria variability between evaluators and/or sites or the use of broader inclusion criteria to ensure an adequate sample size. It would be desirable to have large samples with a restrictive or extreme phenotype to reduce sample variability. The working definition of the phenotype is a prerequisite for successful genetic studies.⁷⁸

In summary, the results of GWA studies suggest that we are far from being able to identify the genes responsible for the diseases studied. Many studies have identified one or more gene regions that confer a small risk, which indicates that there is only a small percentage of total genetic component of the disease in the population and that it has a low predictive value.

CONCLUSIONS

Depression undoubtedly has a complex and heterogeneous phenotype in terms of its biology and etiology, in which both genetic and environmental factors

play a fundamental role. In this sense, from the vantage point of quantitative genetics, family and twin studies have confirmed the importance of genetic factors and suggest that depressive disorder, like other common mental illnesses, is a complex condition that reflects the influence of many genes with a minor effect. Likewise, we must not forget that understanding any complex characteristic of human beings is impossible without simultaneously considering the effect of genes and environment, environment being understood in its broadest sense, as a factor in continuous interaction with individual genotype.

Molecular studies have helped to establish the genetic basis of disease more specifically. Among them, the classic linkage studies have made it possible to identify chromosomal regions at risk and, thus, to identify some candidate genes by their genomic position and function. Similarly, association studies have shown that a certain degree of genetic variability, particularly associated with genes of the serotonergic system, seems to contribute to the risk of disease and certain clinical aspects of the disease, such as the clinical response to pharmacological treatment with antidepressants.⁷⁹

Interestingly, GxE interaction studies have shown the importance of environment in the risk of developing MDD. In the literature, evidence had already been found that the presence of stressful life events in the course of life, such as feelings of defeat, loss, humiliation and frustration, or child abuse, increased the risk of MDD.⁵¹⁻⁵⁴ However, these studies revealed that the impact of these adverse events on the neurobiology of the brain is moderated by certain individual genetic variability, as was demonstrated in the study by Caspi et al. mentioned above.³⁴

Finally the results of GWA studies in disorders with a high prevalence and lower heritability, such as major depressive disorder, present a more complicated challenge when analyzing the results. These are expensive studies that have not yet met expectations in the field of mental illness. For instance, although the results of GWA studies have identified candidate genes for depression, as of yet it has not been possible to replicate the association of specific candidate genes previously identified by classical association studies.

Consequently, these early GWA studies have generated a number of important questions regarding the genetic variants identified to date. In first place, the results of GWA studies raise the issue of "missing heritability" as a serious problem in GWA study design. Missing heritability is the difference between the large proportion of the phenotype of major depression explained by genetic factors, as estimated by heritability studies, and the scant risk genes identified by GWA studies. Most of the explanations and

possible solutions proposed are related to both genetic and methodological issues that are poorly controlled in current designs. Among the genetic issues per se are trait penetrance, i.e., the frequency with which a trait or phenotype is expressed when a specific gene combination is present, the existence of epistasis and epigenetic processes, the genetic heterogeneity of the disease, the presence of undetected rare variants with more penetrance (a less common allele with a frequency of at least 1%), or even the existence of an incomplete linkage imbalance between the SNP marker and true causal variants.

On the other hand, among the methodological issues, possible errors in genotyping should be taken into account, including copy number variations (CNV) and the control of GxE interactions.⁸⁰⁻⁸⁷ However, how a complex trait is measured and how phenotypic information is used are just as important as the correct detection of genetic variants.⁸⁸ A stricter redefinition of phenotype could, *a priori*, increase the power to detect more robust effects.

In addition, we must not forget that the search for statistically significant associations between a genetic polymorphism and mental illness is just the first step in understanding the role of genetic variants in the pathogenesis of the disease. The next step involves knowledge of the functional effect of these genetic variants and how they act in the expression of disease phenotype.

We can conclude that GWA studies are still in their infancy and new approaches are being perfected to improve the performance of the vast amounts of data provided by this analysis. One option proposed would be to conduct a meta-analysis in which all the information from multiple GWA studies is pooled, thus increasing the chances of finding true positives among the false positives. A second approach is to search for epistasis in each GWA study to identify more robust results that would appear when gene-gene interactions are taken into account. Finally, one of the most interesting options would be to prioritize certain genes and alleles using information from known biological pathways [89]. In this sense, GWA study pathway analysis (GWASPA) seems to be the next step in understanding the genetic basis of complex diseases.⁹⁰ These new approaches focus on examining a collection of predefined genes based on available biological knowledge of the genes and their possible implication in the disease. It is well known that genes do not function in isolation. They generally form part of complex molecular networks and different cellular pathways that are frequently involved in susceptibility and disease progression.

Although these new approaches configure the near future of the investigation of the molecular basis of complex disease, there are still many unknowns to be clarified, largely

related to the role of genetic variability and CNVs (copy number variants), which are currently not included in GWA analysis. Likewise, the role of rare structural variants (with higher penetrance) and their interaction with the common type SNP variability is under study.

Finally, we must not overlook the role of the environment, understood in its broadest sense. GxE interaction studies have shown that individuals are probably genetically more or less susceptible to a particular environment, and this interaction is what increases the risk for the onset of disease.⁵⁰ In this sense, the inclusion of environmental variables in GWA studies is a pending issue. Similarly, epigenetic modifications can provide highly relevant information on changes at the level of environment-mediated expression.

The results obtained so far with regard to the genetic risk for major depression are not powerful enough to support predictions that attain the levels of sensitivity and specificity required to be clinically useful. However, evidence from research studies on vulnerability to mental disorders seems to have a number of consequences in terms of clinical practice, suggesting a change from the categorical diagnostic model toward dimensional approaches to disorders. Research on genetic risks, aside from its impact on diagnosis, will contribute to advances in the prevention and pharmacological treatment of psychiatric disorders.

In short, although the results obtained to date on the genetic basis of major depression are not entirely conclusive, the new avenues of methodological and biological investigation that are now opening are new routes toward better understanding the etiology of this disease.

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