

Hypothesis of the neuroendocrine cortisol pathway gene role in the comorbidity of depression, type 2 diabetes, and metabolic syndrome

Claudia Gragnoli^{1,2}

¹Center for Biotechnology and Department of Biology, College of Science and Technology, Temple University, Philadelphia, PA, USA;

²Molecular Biology Laboratory, Bios Biotech Multi-Diagnostic Health Center, Rome, Italy

Abstract: Depression, type 2 diabetes (T2D), and metabolic syndrome (MetS) are often comorbid. Depression per se increases the risk for T2D by 60%. This risk is not accounted for by the use of antidepressant therapy. Stress causes hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis, by triggering the hypothalamic corticotropin-releasing hormone (CRH) secretion, which stimulates the anterior pituitary to release the adrenocorticotropin hormone (ACTH), which causes the adrenal secretion of cortisol. Depression is associated with an increased level of cortisol, and CRH and ACTH at inappropriately “normal” levels, that is too high compared to their expected lower levels due to cortisol negative feedback. T2D and MetS are also associated with hypercortisolism. High levels of cortisol can impair mood as well as cause hyperglycemia and insulin resistance and other traits typical of T2D and MetS. We hypothesize that HPA axis hyperactivation may be due to variants in the genes of the CRH receptors (*CRHR1*, *CRHR2*), corticotropin receptors (or melanocortin receptors, *MCR1-MC5R*), glucocorticoid receptor (*NR3C1*), mineralocorticoid receptor (*NR3C2*), and of the FK506 binding protein 51 (*FKBP5*), and that these variants may be partially responsible for the clinical association of depression, T2D and MetS. In this review, we will focus on the correlation of stress, HPA axis hyperactivation, and the possible genetic role of the *CRHR1*, *CRHR2*, *MCR1–5*, *NR3C1*, and *NR3C2* receptors and *FKBP5* in the susceptibility to the comorbidity of depression, T2D, and MetS. New studies are needed to confirm the hypothesized role of these genes in the clinical association of depression, T2D, and MetS.

Keywords: depression, type 2 diabetes, metabolic syndrome, cortisol, CRH, ACTH

Introduction

Type 2 diabetes mellitus (T2D) is associated with an increase in the risk of major depressive disorder.^{1,2} Conversely, depression confers a 60% increased T2D risk.³ This association between depression and T2D cannot be attributed to antidepressant therapy.⁴ T2D is associated with metabolic syndrome (MetS), and T2D, MetS, and depression are associated with high cortisol levels.^{5–9} It is possible that neuroendocrine dysfunction in depression increases the risk for T2D and MetS, and that inherited gene variants involved in stress responses might account, at least in part, for the comorbidity of depression with T2D and MetS.^{6,10,11}

In fact, considerable evidence suggests that genetic vulnerability to depression may be conferred by variation in genes regulating systems of stress response,^{12,13} such as the hypothalamic–pituitary–adrenal (HPA) axis.^{14,15} The HPA axis is a neuroendocrine

Correspondence: Claudia Gragnoli
BioLife Science Bldg, Suite 431, Center
for Biotechnology, College of Science and
Technology, Temple University, 1900 N
12th Street, Philadelphia, PA 19122, USA
Tel +1 508 246 9330
Fax +1 717 482 1123
Email claudia.gragnoli@gmail.com

system modulating the stress response via interactions with serotonergic, noradrenergic, and dopaminergic brain systems. Stress leads to high cortisol levels; under stress, the HPA axis releases corticotrophin-releasing hormone (CRH) to stimulate adrenocorticotropin hormone (ACTH) secretion via the CRH receptor (CRHR) from the anterior pituitary, thereby elevating cortisol. The CRHRs (CRHR1 and CRHR2) and the melanocortin receptors (MC1R–MC5R) mediate the axis responsiveness to integrated signals from diurnal rhythms, cortisol negative feedback via the glucocorticoid receptor, and superimposed stressors. Recently, *MC2R* variants leading to strong responses to ACTH have been described.¹⁶ Further, *MC4R* variants causing functional impairment have been reported.¹⁷ Increased cortisol causes serotonergic dysfunction, which is a substrate for depression.¹² While acute stress with associated cortisol levels is a physiological phenomenon, persistent or chronic stress associated with persistent or chronic hypercortisolism may lead to a pathological condition. Hypercortisolism and altered feedback inhibition are HPA abnormalities of depression, T2D, and MetS (Figure 1).^{7–9,18} Depressed patients, given their hypercortisolism and compared to control subjects, have inappropriately “normal” plasma ACTH and cerebrospinal fluid CRH;¹⁹ that is, their levels should be lower in the presence of high cortisol, which triggers a negative feedback at the level of the hypothalamus and pituitary gland, thereby reducing CRH and ACTH. Thus CRHR, ACTH receptor, and glucocorticoid receptor dysfunctions are possible. Several of these HPA axis receptor genes are associated with metabolic abnormalities; the known physiologic effects of glucocorticoids suggest that inherited predisposition to HPA axis activation may contribute to glucose intolerance,²⁰ visceral obesity, and increased triglycerides and blood pressure.^{21,22}

It is possible that common gene pathways are responsible for the association of depression with T2D and MetS and that

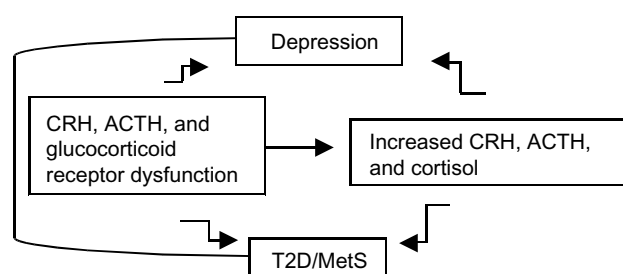


Figure 1 Hypothesized relationship between CRH, ACTH, and glucocorticoid receptors dysfunction, increased CRH, ACTH and cortisol levels, depression, T2D, and MetS.

Abbreviations: ACTH, adrenocorticotropin hormone; CRH, corticotrophin-releasing hormone; MetS, metabolic syndrome; T2D, type 2 diabetes.

genetic variation in the HPA axis receptors may explain the link between depression, T2D, and MetS.

T2D, MetS, and depression and possible genetic stratification for their comorbidity

T2D (with 11% prevalence after age 50),²³ MetS (with 42% prevalence after age 60),²⁴ and depression (with 9% prevalence in adults)²⁵ are heterogeneous polygenic and complex disorders and they are often comorbid. T2D is defined by at least two fasting glucose levels ≥ 126 mg/dL or a glucose level of 200 mg/dL at 2 hours of the oral glucose tolerance test or by a random glucose level of 200 mg/dL associated with symptoms. MetS is defined by the National Cholesterol Education Program (2002) Adult Treatment Panel III as the presence of at least three of the following: abdominal obesity (waist circumference in men > 102 cm, in women > 88 cm), high triglycerides (≥ 150 mg/dL), low high-density lipoprotein (HDL) cholesterol (HDL < 40 mg/dL in men, < 50 mg/dL in women), high blood pressure ($\geq 130/85$ mmHg and/or on antihypertensive medications), and high fasting glucose (≥ 110 mg/dL and/or on antidiabetic medications). Visceral fat deposition, high blood pressure, and hyperglycemia are all aging phenomena.

The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) criteria for major depressive disorder (depression) are: presence of depressed mood or a loss of interest or pleasure in daily activities for more than 2 weeks; mood change from the person's baseline; impaired function at least either social or occupational or educational. Further, at least five of the following nine symptoms must be present nearly every day: depressed or irritable mood most of the day, nearly every day, per either subjective report (eg, the patient feels sad or empty) or observation made by others (eg, appears tearful); decreased interest or pleasure in most activities, most of each day; significant weight change (5%) or change in appetite; change in sleep: insomnia or hypersomnia; change in activity: psychomotor agitation or retardation; fatigue or loss of energy; guilt or worthlessness: feelings of worthlessness or excessive or inappropriate guilt; concentration: diminished ability to think or concentrate, or more indecisiveness; and suicidality: thoughts of death or suicide, or the patient has a suicide plan.

Both the serotonergic and HPA system are implicated in depression²⁶ and both may play a role in T2D and MetS.^{6,27,28} Thus, it is unlikely that a single pathway or a few genes in a specific pathway will explain any one of these three disorders or their comorbidity. We believe that there is a genetic stratification in comorbid-diseases predisposition

correlated to phenotype heterogeneity. As not all depressed patients develop T2D and MetS, and not all patients with T2D and MetS have depression, different gene pathways will be responsible for each disorder independently, but the HPA axis function and underlying genes could be related to the symptoms and signs of depression and potentially contributing to the increased risk for T2D and MetS. However, the genetic risk conferred by the HPA axis pathway for the comorbidity of depression, T2D, and MetS is expected to be stratified in patient groups and to correlate with sub-phenotypes of T2D, MetS, and depression. An investigational study of the correlation of the HPA-axis-related genes with the phenotype(s) of T2D, MetS, and depression, or the lack thereof, may elucidate the genetic basis of the T2D and MetS association with depression. Such an investigational study will provide the foundation upon which other data and pathogenetic hypotheses could subsequently be built.

Novelty of hypothesis and new investigational needs

Our hypothesis for the potential link of depression with T2D and MetS challenges the currently accepted paradigm that T2D and MetS are only metabolic disorders, and that depression is only a mental disorder. In fact, we believe that they are all, at least partially and in the subgroup of patients with T2D, MetS, and depression comorbidity, associated with hyperactivity of the neuroendocrine cortisol pathway. There are no extensive data regarding genetic screening of the HPA axis receptor genes jointly exploring T2D, MetS, and depression traits in humans. It would be a powerful and innovative strategy for gene risk identification to perform linkage and association tests not only in a group of patients with T2D, MetS, and depression, but also with the pre-phenotypes and associated mental traits potentially contributing to these diseases. The hypothesis is that T2D and MetS are not merely disorders affecting glucose levels, lipid levels, and blood pressure, rather they are complex disorders characterized by increased predisposition to stress-related hyperactivity and abnormal psychological traits leading to abnormal behavioral and compensatory mechanisms.

Ideally, the impact of single and/or complex gene variants should be tested in the major traits as well as in the sub-phenotypes of these two apparently distinct disorder groups of T2D-MetS and depression. There is a need to test not just genotypes and alleles of candidate genes, but also haplotypes, diplotypes, and multilocus alleles to prove the risk effects of complex gene variants in common disorders.

A linkage strategy would be helpful in identifying rare variants rather than common variants as major players in the

diseases' causes.²⁹ Also, the interaction among gene variants should be tested.

To test the abovementioned hypothesis, it would be essential to build an interdisciplinary approach of human genetics and clinical phenotyping of T2D and MetS with a focus on depression and related traits and to perform a joint study of the HPA axis receptor genes in both human T2D and MetS with the depression phenotypes.

Implication of the HPA axis and related candidate genes in the pathogenesis of the comorbidity of T2D, MetS, and depression

High cortisol increases glycogenolysis, gluconeogenesis, insulin resistance, free fatty acids, and visceral obesity, and indirectly and directly reduces insulin secretion; all these effects per se and jointly lead to T2D²⁰ and MetS. High cortisol may cause depressive symptoms as well^{30,31} and stress plays a major role in depression: early life stress (eg, separation anxiety at an early life stage)³² associated with HPA axis hyperactivation¹⁴ induces long-lasting changes linked to adult anxiety and depressive behavior.³³ While some subjects thrive and some break down under similar adverse circumstances due to neuroendocrine stress-related psychopathology, differential genetic predisposition to neuroendocrine stress response is expected. The CRH system plays a role in the stress response of depression^{13,15} and *CRH* single nucleotide polymorphisms are depression risk variants.¹⁵ The CRH system resistance leads to hypercortisolism. A subgroup of hypercortisolemic patients with depression, if stimulated with CRH, show a blunted ACTH response, whereas CRH infusion to healthy subjects reproduces the hypercortisolism of depression, suggesting that hypercortisolism in depression represents a defect at the *CRHR* level, resulting in CRH hypersecretion.³⁴ Furthermore, a significant cortisol response to a blunted ACTH response suggests that the adrenal glands hyper-respond to ACTH. Thus, there may be an ACTH receptor abnormality as well.³⁵ Aging is associated with both the HPA axis reduced feedback and hyperactivity,³⁶ which could be due to glucocorticoid receptor resistance, and may lead to T2D, MetS, and depression. However, depression prevalence decreases with age. Consequentially, the stress response pathway may lead to the T2D–depression and MetS–depression comorbidity. The genes possibly conferring predisposition to the clinical association of T2D, MetS, and depression are the *CRHRs* (*CRHR1* and *CRHR2*), the corticotropin receptors or melanocortin receptors (*MC1R–MC5R*), the glucocorticoid receptor (*GCR*), the mineralocorticoid receptor (*MCR*), and the FK506 binding protein 51 (FKBP5) (Table 1).

Table I Candidate genes, their locus and linkage data, and related risk human phenotypes

Gene	Locus; linkage	Risk variants in humans
<i>CRHR1</i>	17q12; T2D, MetS	Depression, antidepressant response, hypertension
<i>CRHR2</i>	7p21-p15; T2D, glycemia, triglyceride, HDL, depression, bipolar disorder	
<i>MC1R</i>	16q24; bipolar disorder, diabetic nephropathy, left ventricular thickness in MetS	Depression, antidepressant response
<i>MC2R</i>	18p11; bipolar disorder, T2D, BMI, obesity, insulin	Hypoglycemia, glucocorticoid deficiency
<i>MC3R</i>	20q13.2; bipolar disorder, T2D, early-onset hypertension in African-Americans	Obesity, T2D, protective against increased weight and blood pressure
<i>MC4R</i>	18q22; depression, bipolar disorder, T2D 18q22 del metabolic defects; glycemia, triglyceride, fat mass	Hyperphagia, increased BMI, T2D Weight regulation, depression
<i>MC5R</i>	18p11; bipolar disorder, T2D, BMI, obesity, insulin	T2D, BMI
<i>NR3C1</i>	5q31; bipolar disorder	Vulnerability, stress response, depression, hyperglycemia, insulin resistance, T2D treatment, hypertension
<i>NR3C2</i>	4q31.1-31.2; T2D, MetS, bipolar disorder, blood pressure	Antidepressant response, depression
<i>FKBP5</i>	6p21.31; T2D, obesity, blood pressure, bipolar disorder	Depression, antidepressant response

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; MetS, metabolic syndrome; T2D, type 2 diabetes; del, deletion; *CRHR1*, corticotropin receptor 1; *CRHR2*, corticotropin receptor 2; *MC1R*, melanocortin receptor 1; *MC2R*, melanocortin receptor 2; *MC3R*, melanocortin receptor 3; *MC4R*, melanocortin receptor 4; *MC5R*, melanocortin receptor 5; *NR3C1*, glucocorticoid receptor 1; *NR3C2*, mineralocorticoid receptor 2; *FKBP5*, FK506 binding protein 5.

The *CRHR1* gene is expressed in the brain. *CRHR1*–/– mice show reduced anxiety behavior.^{37,38} *CRHR1* variants are implicated in human depression^{39–42} and antidepressant response.^{43,44} However, there are no studies in human T2D and MetS. *CRHR1* is expressed on beta cells and stimulates beta cell proliferation and insulin secretion in a glucose-dependent manner;⁴⁵ some *CRHR1* variants are associated with hypertension,⁴⁶ thus *CRHR1* dysfunction may lead to hyperglycemia, T2D, and high blood pressure of MetS. Interestingly, the *CRHR1* genetic locus 17q12 is linked to T2D⁴⁷ and MetS.⁴⁸

The *CRHR2* gene is also expressed in the brain. *CRHR2*–/– mice have reduced stress coping behaviors⁴⁹ and show hypersensitivity to stress and anxiety behavior.^{50,51} *CRHR2* is not yet identified as a risk gene in human depression,⁵² T2D, or

MetS. However, previous studies report the *CRHR2* locus 7p21-p15 in linkage with depression⁵³ and bipolar disorder.^{54,55} Of note, genetic overlap exists between bipolar disorder and depression.⁵⁶ The *CRHR2* genetic locus 7p21-p15 is also linked to T2D,^{57,58} glycemia, triglyceride, and HDL levels.⁵⁹ *CRHR2*–/– mice show high blood pressure and reduced sustained hypophagia;⁴⁹ thus *CRHR2* variants may lead to high blood pressure and obesity of MetS, the latter potentially due to impaired mediation of food intake control.

The corticotropin receptor or melanocortin 1 receptor (*MC1R*) gene is expressed in the brain, liver, and pancreas, and is implicated in depression and antidepressant response.⁶⁰ However, there are no studies in human genetics of T2D or MetS. The *MC1R* genetic locus 16q24 is linked to bipolar disorder,^{61,62} T2D nephropathy,⁶³ and left ventricular thickness in the setting of MetS.⁶⁴

The corticotropin receptor or melanocortin 2 receptor (*MC2R*) gene is expressed in the brain and adrenal gland; *MC2R* mutations cause hypoglycemia and glucocorticoid deficiency.⁶⁵ There is a lack of studies in human depression and T2D. The *MC2R* genetic locus 18p11 is linked to bipolar disorder,^{67–70} T2D, insulin, obesity, and body mass index (BMI).^{71–75}

The corticotropin receptor or melanocortin 3 receptor (*MC3R*) gene is expressed in the hypothalamus and brain; it is involved in obesity^{76,77} and may play a role in human T2D.^{66,78} *MC3R*–/– mice have increased adipose mass;⁷⁹ *MC3R* variants may protect against weight and blood pressure increase.⁸⁰ There are no studies in human MetS and depression. The *MC3R* genetic locus 20q13.2 is linked to bipolar disorder,⁵⁵ T2D,^{81–83} and early-onset hypertension in African Americans.⁸⁴

The corticotropin receptor or melanocortin 4 receptor (*MC4R*) gene is expressed in the brain; it is involved in hyperphagia and increased BMI,⁸⁵ as well as T2D,^{86–89} but data are inconsistent.^{90,91} Some *MC4R* variants are implicated in weight regulation and BMI.^{92,93} *MC4R* is also implicated in depression.⁹⁴ The *MC4R* genetic locus 18q22 is linked to depression,⁵³ bipolar disorder,^{67,69} and T2D.⁷¹ It is known that 18q22 deletion causes metabolic defects.⁹⁵ The 18q22 locus affects glycemia,⁹⁶ is linked to hypertriglyceridemia,⁹⁷ and is associated with fat mass.⁹⁸

The corticotropin receptor or melanocortin 5 receptor (*MC5R*) gene is expressed in the brain and has a role in T2D.⁶⁶ *MC5R* variants are associated with BMI.⁹⁸ There is a lack of studies regarding depression. The *MC5R* genetic locus 18p11 is linked to bipolar disorder,^{67–69} T2D,^{71–73} BMI,⁷⁵ obesity, and insulin.⁷⁴

The glucocorticoid receptor gene or nuclear receptor sub-family 3, group C, member 1 (*GCR* or *NR3C1*), expressed ubiquitously, and the mineralocorticoid receptor gene or nuclear receptor sub-family 3, group C, member 2 (*MCR* or *NR3C2*), expressed in multiple tissues including the central nervous system and the hippocampus, may also predispose to the T2D, MetS, and depression comorbidity. Glucocorticoids mediate the HPA axis negative feedback via the GCR at the hypothalamus and pituitary level. Cortisol has effects on both MCR and GCR. Of note, the *GCR* locus 5q31 is associated to hypertension⁹⁹ and linked to bipolar disorder.¹⁰⁰ The *MCR* locus chromosome 4q31.1-31.2 is linked to T2D and MetS¹⁰¹ and to bipolar disorder.¹⁰²⁻¹⁰⁵ *MCR* and *GCR* variants affect coping style and depression vulnerability, antidepressant response, stress, and depression endocrine response.¹⁰⁶⁻¹¹⁵ *MCR* expression is increased in the hypothalamus of diabetic rats¹¹⁶ and *MCR* is linked to blood pressure.¹¹⁷ *GCR* affects glucose levels¹¹⁸ and is associated with insulin resistance;¹¹⁹ *GCR* antagonism improves insulin sensitivity.¹²⁰ Thus, *GCR* and *MCR* dysfunction may lead to depression, T2D, and MetS, and contribute to the comorbidity of these three disorders.

In addition, the FK506 binding protein 5 (*FKBP5*), a heat shock protein 90 co-chaperone of the steroid receptor complex and component of the chaperone receptor hetero-complex, reduces ligand sensitivity of the GCR¹²¹ and is implicated in cortisol effects. Interestingly, the *FKBP5* gene locus 6p21.31 is associated to T2D¹²² and linked to obesity,¹²³ blood pressure,¹²⁴ and bipolar disorder.¹²⁵ The gene *FKBP5* is therefore a candidate gene for the comorbidity of T2D, MetS, and depression. *FKBP5* has in fact been implicated in depression^{126,127} and antidepressant response,¹²⁸ even if results for antidepressant response are conflicting.¹²⁹ There are not yet data on *FKBP5* and T2D and MetS.

For all the above-mentioned reasons, these genes alone or jointly may confer the genetic susceptibility to the HPA axis hyperactivation under stressful circumstance and cause depression and confer increased risk for the T2D and MetS phenotypes.

Suggested methods to empower gene identification for the comorbidity of depression, T2D, and MetS

Families phenotyping

Ideally, to identify the gene variants in the HPA axis receptor genes responsible for the clinical association of depression,

T2D, and MetS, families with all three disorders, as well with only two and one disorder, should be studied. At best, recruiting families with early onset of disease phenotypes and their comorbidity will increase the genetic load for risk predisposition and the probability to discover the susceptibility gene variants. The families should be characterized not only for depression but also for associated traits such as anxiety, insomnia, symptom duration and severity, substance abuse and dependence, life stress level, and resilience. The cases should be characterized for disease severity, age of onset, associated mental comorbidities, subtype of melancholic depression and atypical depression, to empower gene identification strategy and to link risk gene variants to specific traits. Melancholic depression is characterized by the following: depressed mood; anhedonia as per loss of pleasure in most or all activities, inability to react to pleasurable stimuli; insomnia or early-morning waking; symptom worsening in the morning hours; psychomotor agitation or retardation; excessive or inappropriate guilt, worthlessness; fatigue; decreased appetite and excessive weight loss; and inattention or poor concentration. Atypical depression is characterized by the following: improved mood in response to positive events; irritable-anxious mood; increased appetite as per comfort eating or significant weight gain; sensation of heaviness in the limbs; hypersomnia as per excessive sleep or sleepiness; over-sensitivity to perceived interpersonal rejection causing significant social impairment.¹³⁰

Similarly the families should not only be characterized for T2D but also for all the T2D-associated common phenotypes, including, but not only, behavioral and compensatory mechanisms related to stress, nutrition, and physical exercise. In the characterization of MetS, beyond the classical features of MetS, other potentially associated traits should be included; for example psychological traits, behavioral traits, nutrition and exercise preferences, sleep duration and quality, and quality of life.

Linkage and association tests

By characterizing the families with all three disorders, and with only two or one of them, as well as defining new groups of traits potentially associated with the disease comorbidity, it would then be feasible to create different categories of joint endophenotypes and traits and to test them for linkage and association with the gene variants under study. The creation of categories with T2D and melancholic or atypical depression or both or with specific depression traits (eg, anhedonia, insomnia, psychomotor agitation or retardation, decreased appetite), psychoactive medication intake, earlier age of onset,

extreme phenotype severity, and life stress level, will enrich the genetic load and likelihood for stress-related risk-gene identification, which could be compared with one category with either only T2D or a depression trait. Similarly, it would be helpful to test gene variants for linkage with MetS (as traditionally defined) as well as for just single phenotypes of high blood pressure, visceral obesity, high triglycerides, and low HDL, and for the positivity of only two phenotypes; and for the additional presence of depression, anxiety, insomnia, and therapy with antipsychotics, antidepressants, and anxiolytics. To increase the probability of discovering risk-genes, the linkage tests should also be performed on a group of families with severe MetS by including at least four positive criteria as well as increasing the criteria threshold (eg, BMI >35, blood pressure >160/95 mmHg, and triglycerides >200 mg/dL), with the addition of depression, anxiety, insomnia, psychotropic medications, and high level of stress. By creating a subject category for multiple MetS–depression traits, the probability that those families will carry the gene variants related to chronic stress vulnerability and HPA axis hyperactivation will be greater, as well as the chance to identify those gene variants compared to: either a category with only two MetS traits; or one MetS trait or depression trait; or neither MetS nor a depression trait.

Another helpful method to identify the gene variants responsible for the comorbidity of depression, T2D, and MetS is to perform variance-component trait analyses for each quantitative trait associated with T2D and mental-behavioral traits (eg, stress, sleep duration and quality, diet, physical activity, and medication compliance), using the other available traits as covariates. The metabolic quantitative traits for T2D could be basal glucose levels at T2D onset, average glycated hemoglobin 1c % (HbA1c%) levels, total cholesterol and low-density lipoprotein levels, age at disease onset, BMI at onset, and years of therapy defined by diet only, oral hypoglycemic agents only, insulin only, and insulin combined with oral hypoglycemic agents. For the MetS phenotype, the quantitative traits could be blood pressure, waist circumference, maximum life BMI, triglycerides, HDL, and glucose levels, both independently and by using these traits as covariates with age and sex. Further, phenotypes of depression, anxiety, insomnia and, psychotropic medication, could be used as covariates. Another approach could be to test gene variants for linkage with a newly created quantitative scale with: depression, anxiety, insomnia, psychotropic medication use, pre-diabetes, T2D, hypertension, high triglycerides, low HDL, and visceral obesity. For the depression phenotype, the quantitative traits could be age of onset,

number of DSM-V criteria met, quantification of severity of episodes, associated mental diseases, and treatment types and years of antidepressant therapy.

Both linkage and association tests should be performed using the nonparametric and parametric methods and data should be validated with random simulations. Linkage analysis should also be conditioned on the presence of already identified loci.

The association tests should be performed with alleles, genotypes, haplotypes, and diplotypes; in fact when two or more adjacent single variant polymorphisms are considered, haplotype analysis is crucial since single variant polymorphisms may affect complex traits acting as cis-regulatory elements. In addition, haplotype analysis is more powerful than single variant polymorphisms analysis.¹³¹ Epistatic interaction ought to be studied as well, as a disorder often involves a multifactorial underpinning to which many genes contribute, and evidence shows that the effects of some genes are not additive; rather, the effects are via interactions or epistasis.^{132,133} Because of epistasis, two or more genes may increase or reduce disease risk more than expected from their independent effects.

If large case–control groups are used, to avoid false negative associations, association tests should be performed with case subjects positive for comorbid diseases or a trait category against control subjects negative for those comorbid diseases or trait category. In fact, if a gene variant causes depression, T2D, and MetS, it would be difficult to identify it by association unless the case group is enriched of those phenotypes and the control group is unaffected by all those phenotypes. For disease-associated quantitative traits (eg, HbA1c%), logistic regression analysis should be performed.

If association is not identified, the tests should be repeated with the probands of only the families showing a positive linkage, in order to increase the likelihood of gene identification. In fact, those families with a positive linkage are more likely to carry the disease variant and to highlight a positive association if tested against a control group.¹³⁴

T2D–depression and MetS–depression reciprocity

The T2D and depression association should be, for some genes and gene variants and with different significance levels, comparable to the MetS and depression association. However, some discordance is expected that, if identified, could guide into gene risk stratification for the T2D, MetS, and depression comorbidity. Thus, while some families with T2D–depression and/or MetS–depression will have shared

gene-risk vulnerability within the cortisol pathway, other families will not show genetic susceptibility within the cortisol pathway and will likely present phenotype differences.

However, for the gene variants not contributing to the T2D–depression and MetS–depression association, linkage and association within just T2D–MetS or depression could be present. Ultimately, some genes and gene variants will contribute neither to T2D–MetS comorbidity with depression nor to T2D–MetS or depression. In fact, some variability in the genetic burden of T2D, MetS, and depression is expected, and once identified will help in defining more homogeneous clinical phenotypes for future studies by increasing gene risk detection power.

Other studies and possible outcomes

It would be interesting to set up epigenetic studies to explore in depth the hypothesis that stress may indeed induce functional regulatory changes in the abovementioned candidate genes, which may per se lead to hypercortisolism and to the comorbidity of depression, T2D, and MetS. Furthermore, to fully assess the impact of depression on T2D and MetS and study the comorbidity and interaction of these three disorders phenomenon, it would be ideal to perform longitudinal studies to determine the conferring risk of one disorder onto the other.

The final goal of our proposed studies would be to implement preventive plans targeting subjects identified at risk for T2D, MetS, and depression, who, by knowing their genetic make-up and risk for such disorders, may be inclined to undergo behavioral–cognitive therapy treatments and lifestyle modifying behaviors. Such preventive plans may significantly decrease the burden of T2D, MetS, and depression comorbidity on subjects and on the health care system. In the long-term, it would be possible to therapeutically target the receptors implicated in the HPA axis disruption in subjects at risk for T2D, MetS, and depression, thereby reversing the HPA axis hyperactivity to a physiological state, and preventing the comorbidity of T2D, MetS, and depression. The results of a study targeting the abovementioned comorbidity should prompt new research in the area of associated mental and metabolic disorders, thereby creating a new focus on the neuroendocrine–mental–metabolic dysfunctions, which may characterize pre-disease states. In order to advance the research in the field of T2D, MetS, and depression, there is a need to accept the idea of a joint pathogenesis of such apparently different disorders.

Disclosure

The author reports no conflicts of interest in this work.

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