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Novel implication of the prolactin (*PRL*) gene in the comorbidity of type 2 diabetes and depression

M. AMIN[1,2], R. WU[3,4], T.T. POSTOLACHE[5,6,7], C. GRAGNOLI[3,8,9]

[1]INSERM, US14-Orphanet, Paris, France
[2]Department of Biochemistry and Molecular Biology, Faculty of Medicine, Al-Neelain University, Khartoum, Sudan
[3]Department of Public Health Sciences, Penn State College of Medicine, Hershey, PA, USA
[4]Department of Statistics, Penn State College of Medicine, Hershey, PA, USA
[5]Mood and Anxiety Program, Department of Psychiatry, University of Maryland School of Medicine, Baltimore, MD, USA
[6]Rocky Mountain Mental Illness Research Education and Clinical Center (MIRECC), Veterans Integrated Service Network (VISN) 19, Military and Veteran Microbiome: Consortium for Research and Education (MVM-CoRE), Denver, CO, USA
[7]Mental Illness Research Education and Clinical Center (MIRECC), Veterans Integrated Service Network (VISN) 5, VA Capitol Health Care Network, Baltimore, MD, USA
[8]Division of Endocrinology, Department of Medicine, Creighton University School of Medicine, Omaha, NE, USA
[9]Molecular Biology Laboratory, Bios Biotech Multi-Diagnostic Health Center, Rome, Italy

Abstract. – OBJECTIVE: The prolactin (PRL) system plays important behavioral, social, and metabolic roles, such as mediating social bonding and insulin secretion. Inherited dysfunction of the PRL pathway-related genes is associated with psychopathology and insulin resistance. We have previously suggested that the PRL system might be implicated in the comorbidity of psychiatric (depression) and type 2 diabetes (T2D) owing to the pleiotropy of PRL pathway-related genes. To our knowledge, no *PRL* variants have so far been reported in patients with either major depressive disorder (MDD) and/or T2D.

PATIENTS AND METHODS: In this study, we analyzed 6 variants within the *PRL* gene and tested them for the presence of parametric linkage and/or linkage disequilibrium (LD, i.e., linkage and association) with familial MDD, T2D, and their comorbidity.

RESULTS: We found, for the first time, that the PRL gene and its novel risk variants are linked to and in LD (i.e., linkage and association) with familial MDD, T2D, and MDD-T2D comorbidity.

CONCLUSIONS: *PRL* might play a key role in mental-metabolic comorbidity and can be considered a novel gene in MDD and T2D.

Key Words:
Prolactin, *PRL*, Prolactin receptor, PRLR, Prolactin-releasing peptide, PrRP, Prolactin-releasing hormone receptor, PRLHR, Polypeptide, PRL pathway, Type 2 diabetes, T2D, Depression, Major depressive disorder, MDD, Genes, Single nucleotide polymorphisms, SNPs, microarray, Variant, Risk, Intronic, Allele, Parametric analysis, Linkage, Linkage disequilibrium, Association, LD block, Linked, Model, Inheritance, Dominant, Recessive, Complete penetrance, Incomplete penetrance, Independent, Uncertain paternity, Identical twins, Generations, Genotyping, Mendelian error, Exclusion, PLINK, Pseudomarker, Mental-metabolic, Comorbid, Comorbidity, Anterior pituitary gland, Lactotroph, Milk production, Homeostasis, Stress response, Immune response, Plasma osmolarity, Social bonding, Insulin secretion, Resistance, Psychopathology, Response to antidepressants, Prolonged breastfeeding, Italian, Italy, Peninsular families, Familial history, Tuscany, Population, Childhood-onset mood disorders, Treatment, Response, Schizophrenia, Adipocyte hypertrophy, Multi-Racial, Cohort, Pregnancy, High levels, Gestational diabetes, Chinese, Metabolic-associated fatty liver disease, MAFLD, J-shaped, Subjects, High, Low, Quartile, Variable degrees, Impaired secretion, Rats, Depressive behavior, Mental disorders, Pleiotropy, Novel, First, Ethnic groups, DSM IV diagnostic criteria.

Introduction

The prolactin system exerts very important and diverse physiological roles in mammals[1]. It is comprised of the prolactin-releasing peptide (PrRP) and its receptor (PRLHR) and the prolactin hormone (PRL) and its receptor (PRLR). The actions of the PRL pathway are centered around PRL, a polypeptide of about 200 amino acids. PRL is secreted by the anterior pituitary gland, mainly by the lactotrophs, and acts on target

Corresponding Author: Claudia Gragnoli, MD, Ph.D; e-mail: claudia.gragnoli@gmail.com

tissues to regulate milk production, homeostasis, stress response, immune response, and plasma osmolarity[2]. The PRL system plays important behavioral, social, and metabolic roles, such as mediating social bonding and insulin secretion. Inherited dysfunction of the PRL pathway-related genes is associated with psychopathology[3,4], insulin resistance[5], and response to antidepressants[6]. The gene encoding the *PRL* polypeptide, called the *PRL* gene, has been studied[7] in major depressive disorder (MDD), but no association has so far been found. On the other hand, prolonged breastfeeding protects from type 2 diabetes (T2D)[8,9], an effect that is potentially mediated by PRL[10]. However, to our knowledge, no *PRL* variants have been reported in T2D patients. In this study, we report for the first time the *PRL* gene as a novel risk gene linked to MDD, T2D, and MDD-T2D comorbidity.

Patients and Methods

We accessed the deidentified data of 212 previously recruited Italian peninsular families with T2D, history of familial T2D, and characterized for MDD (DSM IV diagnostic criteria). Subjects were recruited following the Helsinki Declaration Guidelines and provided written informed consent. The study was approved by the Bios Ethical Committee (Prot. PR/Mg/Cg/311708). Subjects with uncertain paternity and identical twins were excluded. The families were at least three generations Italian. Within the families, we amplified 6 microarray-derived single nucleotide polymorphisms (SNPs) in the *PRL* gene and performed genotyping and Mendelian error exclusion using PLINK[11] (available at : https://zzz.bwh.harvard.edu/plink/thresh.shtml).

Statistical Analysis

We used Pseudomarker[12] to analyze the SNPs for 2-point parametric-linkage to and linkage disequilibrium (LD, that is linkage + association) with T2D and MDD using the dominant with complete penetrance (D1) model. Secondarily, we performed the analysis under the models dominant with incomplete penetrance (D2), recessive with complete penetrance (R1), and recessive with incomplete penetrance (R2). We considered $p < 0.05$ statistically significant. We tested the statistically significant SNPs ($p \leq 0.05$) for the presence or absence of LD blocks in the Tuscany Italian population (https://www.internationalgenome.org/data-portal/population/TSI). SNPs were considered "independent" if found not to be within a specific LD block.

Results

We found 1 independent intronic variant (rs849885) significantly linked to and in LD ($p < 0.05$) with T2D across various inheritance models (Figure 1). The rs849885 allele (A) underlies the significant linkage and association. The T2D-risk rs849885 SNP was also significantly linked to MDD (0.05) under D1. The rs849885 allele (G) underlies the linkage to MDD, but does not show significant association, indicating that another variant in LD with rs849885 allele (G) might confer risk for MDD. Thus, the rs849885 SNP appears to be a T2D-MDD comorbid marker; however, while the allele (G) appears to contribute to T2D, the marker contribution to MDD might be mediated by another untested variant in LD with the allele (A). We also found 1 independent intronic variant (rs116358700) significantly linked to/in LD with MDD under R1 (Table I).

Discussion

Both the rs849885 and rs116358700 variants are novel and have not been reported before with MDD or T2D. The comorbid risk variant (rs849885) has been previously reported[13,14] as not associated with either childhood-onset mood disorders[13] or treatment response in schizophrenia[14].

Researchers[15,16] studying abnormalities of the PRL system by measuring serum PRL levels in relation to T2D and T2D-related traits have reported interesting findings. High circulating still normal PRL levels have been correlated with lower risk of T2D in U.S. subjects[15], and low circulating PRL levels have been correlated with insulin resistance and adipocyte hypertrophy in Mexican subjects[16]. In a multi-racial cohort[17], during pregnancy, PRL levels might mediate the need for an increased insulin response in the presence of gestational diabetes. Of interest, researchers[17] found the risk for metabolic-associated fatty liver disease (MAFLD) follows a J-shaped association with the PRL levels in Chinese women with T2D. Within the subjects with high PRL levels, the risk for MAFLD was higher per each quartile with higher PRL levels, potentially indicating the presence of variable degrees of PRLR resistance; and

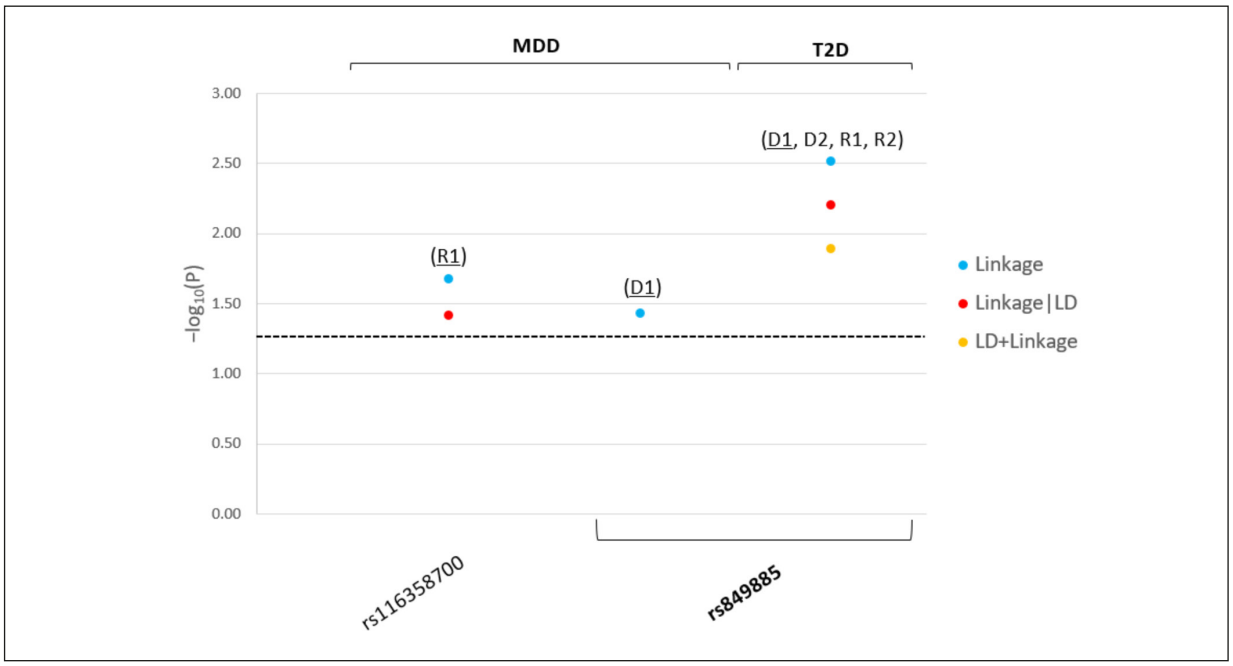

Figure 1. Linkage and linkage disequilibrium analyses results of major depressive disorder and type 2 diabetes risk variants. For the *PRL* gene risk variants rs849885 and rs116358700 in relation to major depressive disorder and/or type 2 diabetes, we present the $-\log_{10}(p)$ as a function of the most significant test statistic [Linkage (logarithm of the odds - LOD - score), Linkage|Linkage Disequilibrium (LD), and LD+Linkage] across the significant inheritance models: D1: dominant, complete penetrance, D2: dominant, incomplete penetrance, R1: recessive, complete penetrance, R2: recessive, incomplete penetrance (the most significant inheritance model is underlined). The bolded SNP is comorbid for major depressive disorder and type 2 diabetes.

within the subjects with normal PRL levels, the risk for MAFLD manifested as higher per each lower PRL level quartile (i.e., the risk was highest with the lowest normal-PRL levels quartile and the highest high-PRL level quartile). Thus, very low PRL levels might increase metabolic risk, due to an impaired PRL secretion, as well as very high PRL levels, potentially due to PRLR resistance[18]. To our knowledge, no previous study has reported variants in the *PRL* gene with either T2D or MDD. Previously, researchers[7,13] studied the *PRL* gene in MDD[7] and childhood-onset mood disorders[13], but no association was established. Of note, in rats, early postnatal administration of PRL resulted in depressive-like behavior in adult period[19]. We previously[20] suggested that the PRL system might be implicated in the comorbidity of mental disorders (e.g., depression, schizophrenia) and T2D owing to the pleiotropy of PRL pathway-related genes.

Conclusions

In this study, we report for the first time the *PRL* gene and its novel risk variants linked to/in LD with MDD, T2D, and MDD-T2D comorbidity, at least in Italian families. However, these results need to be replicated in other ethnic groups.

Table I. *PRL* risk single nucleotide polymorphisms linked/in linkage disequilibrium to/with major depressive disorder and type 2 diabetes.

Disease	SNP	Ref/Alt	Risk allele	Model[1]	Consequence	LD block	Reported in MDD or T2D?
MDD	rs849885	G/A	G	D1	Intronic	Independent	Novel
	rs116358700	A/C	A	R1	Intronic	Independent	Novel
T2D	rs849885	G/A	A	D1, D2, R1, R2	Intronic	Independent	Novel

[1]Models – D1: dominant, complete penetrance, D2: dominant, incomplete penetrance R1: recessive, complete penetrance, R2: recessive, incomplete penetrance.

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Ethics Approval
Families were recruited following the Helsinki Declaration Guidelines. The Bios Ethical Committee approved this study (Prot. PR/Mg/Cg/311708).

Informed Consent
Individuals provided written informed consent prior to participation.

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Authors' Contributions
C.G. conceived and supervised the project, including statistical analysis and manuscript drafting. M.A. helped with the bioinformatic analysis, literature search, and manuscript drafting. R.W. and T.T.P. critically helped in data interpretation and critical revision of the manuscript. All authors have approved the final manuscript.

ORCID ID
C.G.: 0000-0002-3873-6617
M.A.: 0000-0003-2876-0784
R.W.: 0000-0002-2334-6421
T.T.P.: 0000-0001-6056-4244

Availability of Data and Materials
The study data are available on reasonable request, and due to lacking specific patients' consent and privacy restrictions, they are not publicly available.

Conflict of Interests
The authors have declared that have no conflicts of interest.

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