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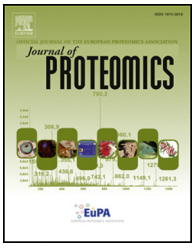

Characterization of salivary proteins of schizophrenic and bipolar disorder patients by top-down proteomics

Federica Iavarone[a], Marianna Melis[b], Giovanna Platania[b], Tiziana Cabras[c], Barbara Manconi[c], Raffaele Petruzzelli[d], Massimo Cordaro[e], Alberto Siracusano[b], Gavino Faa[f], Irene Messana[c], Marco Zanasi[b], Massimo Castagnola[a,*]

[a]Istituto di Biochimica e Biochimica Clinica, Facoltà di Medicina, Università Cattolica e Istituto di Chimica del Riconoscimento Molecolare CNR, Roma, Italy
[b]Dip. di Medicina dei Sistemi, Unità Operativa di Psichiatria, Univ. di Tor Vergata, Roma, Italy
[c]Dip. di Scienze della Vita e dell'Ambiente, Sezione di Biochimica e Biologia Molecolare Università di Cagliari, Italy
[d]Dip. di Scienze Sperimentali e Cliniche, Univ. G. D'Annunzio, Chieti, Italy
[e]Istituto di Clinica Odontostomatologica, Facoltà di Medicina, Università Cattolica, Roma, Italy
[f]Dip. di Scienze Chirurgiche, Sez. Anatomia Patologica, Università di Cagliari, Italy

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ABSTRACT

The analysis of whole saliva of 32 subjects with diagnosis of schizophrenia (SZ), 17 with diagnosis of bipolar disorder (BD), and 31 healthy subjects divided in non-smokers (HN; n = 19) and smokers (HS; n = 12) using an HPLC–ESI-MS top-down platform is reported in this study. Both SZ and BD revealed more than 10 fold mean increase of α-defensins 1–4, S100A12, cystatin A and S-derivatives of cystatin B levels with respect to the HN and HS control groups. No differences of protein levels were observed between SZ and BD groups and between HN and HS groups. Moreover, the correlation coefficients among the different proteins were significantly better in BD group than in SZ group.

Biological significance
This study on whole saliva confirms a schizophrenia-associated dysregulation of immune pathway of peripheral white blood cells and suggests that the dysregulation of BD group could involve the activation of more specific cell type than that of SZ group.

1. Introduction

Schizophrenia is a severe psychotic illness affecting about 0.3–0.7% (prevalence lifetime) of the general population and one of the major causes of disability in young and adult people, as well as a serious social burden worldwide. Because of a great overlapping of the symptomatology with other psychiatric illness, it is not characterized by specific pathological features.

Abbreviations: SZ, schizophrenia; BD, bipolar disorder; HN, healthy non-smokers; HS, healthy smokers; DUP, duration of untreated psychosis; XIC, extracted ion current.

* *Corresponding author at:* Istituto di Biochimica e Biochimica Clinica, Facoltà di Medicina, Università Cattolica, Largo F. Vito, 00168 Roma, Italy. Tel./fax: +39 06 3053598.
E-mail address: massimo.castagnola@icrm.cnr.it (M. Castagnola).

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The disorder is diagnosed on the basis of a clinical interview and using criteria of the diagnosis manuals, such as Diagnostic and Statistical Manual of Mental Disorders IV TR; 2000 (DSM) published by the American Psychiatric Association and International Statistical Classification of Diseases and Related Health Problems (ICD) produced by the World Health Organization (WHO).

Because of the variable symptomatology, clinicians commonly get to diagnose schizophrenia after many months and sometimes more. The duration of untreated psychosis is positively relied to worse disease progression and outcome and lasts one year on average.

Bipolar mood disorder is another severe psychiatric illness, affecting the 1% of the population; the lifetime risk of suffering from a bipolar spectrum disorder is 2% in the general population (DSM IV). Bipolar mood disorder is associated with severe disability in lifetime and increased risk of committing suicide. Despite more specific pathological features of this disorder, the duration of untreated illness is, as in schizophrenia, an important predictive element for the disease progression and outcomes.

From these preliminary remarks follows the importance to characterize analytes variations in bodily fluids of subjects suffering these diseases that could be used in the future for the development of predictive diagnostic tests and could help to understand the molecular events underlying these disorders [1].

Recently, several studies showed the presence of significant alterations in the immune system of bipolar and schizophrenic patients. Hope et al. found that the elevated plasma levels of inflammatory markers, particularly IL-1 receptor antagonist (IL-1Ra) and soluble tumor necrosis factor receptor (sTNF-R1), were associated with general disease severity and psychotic features in schizophrenia and bipolar disorder [2]. This is consistent with the results of Dickerson et al. [3] that showed a relationship between the elevated level of C-reactive protein and schizophrenia and the rate of C-reactive protein and the severity of affective symptoms in patients with bipolar disorder in manic phase [4]. These studies support a central role of immune activation in the core pathological mechanisms of severe mental disorders.

Also some proteomic studies have been carried out as recently reviewed by de-Souza and colleagues [5] with the intent to identify specific markers of the disease.

A proteomic study carried out with SELDI-TOF-MS and ELISA on anti-CD3 stimulated and unstimulated peripheral blood T cell lysates evidenced that α-defensins contributed significantly to the separation of schizophrenic patients and control groups [6]. ELISA analysis of plasma samples in affected and unaffected monozygotic twins confirmed significantly elevated α-defensin levels when compared to healthy unaffected twins. Stimulated by these findings we have carried out a top-down proteomic analysis on the acidic soluble solution of whole saliva of different individuals with psychiatric illness (schizophrenia, bipolar mood disorder) comparing them against a group of healthy controls further divided in smokers and non-smokers. Indeed, saliva is a bodily fluid attractive for the non-invasive specimen collection [7] and it can be sometimes considered a good substitute of plasma and blood, with a particular concern for the detection of biomarkers also in the pediatric age [8]. Top-down proteomic analysis of this bodily fluid has allowed us the characterization in the last years of more than 250 naturally peptides and proteins [9]. Top-down proteomic platform can discriminate between different isoforms and it is able to distinguish their levels by the extracted ion current (XIC) procedure [10]. Attracted by the advantages of top-down platforms other groups are applying them to the study of salivary proteome [11–14]. This study confirmed increased levels of α-defensins 1–4 and evidenced increased levels of S100A12, cystatin A and S-derivatives of cystatin B [15] in whole saliva of individuals with psychiatric illness, confirming some of the results obtained by blood analysis and, once again, demonstrating that whole saliva can be a good alternate for the detection of some relevant plasma clinical analytes.

2. Methods and materials

2.1. Sample collection

Resting whole saliva (from 0.2 to 1.0 mL) was collected with a soft plastic aspirator at the basis of the tongue from 10.00 to 12.00 a.m. Samples were collected at least 30 min after any food or beverage had been consumed and teeth had been cleaned. After collection salivary samples were immediately mixed with an equal volume of 0.2% 2,2,2 trifluoroacetic acid (v/v; TFA) in an ice bath. After stirring, the acidic solution was centrifuged at 9000 *g* for 5 min to remove the precipitate and the acidic clear solution was either immediately analyzed by HPLC–ESI-MS (100 μL, corresponding to 50 μL of saliva) or stored at −80 °C until analysis.

2.2. Participants and ethics statements

The study protocol and written consent forms were approved by the Medical Ethics Committee of the Faculty of Medicine of the Catholic University of Rome. Informed consent forms were filled out and all the rules have been complied according to the instructions of the Declaration of Helsinki. Exclusion criterion was the use of drugs of abuse. Whole saliva was collected according to the protocol described in the previous section in 32 subjects with a diagnosis of schizophrenia (SZ) and 17 subjects with a diagnosis of bipolar disorder (BD) classified according to the guidelines of Diagnostic and Statistical Manual of Mental Disorders IV TR, (2000) American Psychiatric Association, and 31 healthy subjects (HT), further divided into non-smoker (HN; n = 19) and smoker (HS; n = 12) groups.

2.3. Reagents and apparatus

Chemicals and reagents, all of LC–MS grade, were purchased from J.T.Baker (Deventer the Netherlands), Merck (Darmstadt, Germany) and Sigma Aldrich (St. Louis, MI, USA). HPLC–ESI-IT-MS apparatus was a Surveyor HPLC system (ThermoFisher, San Jose, CA, USA) connected by a T splitter to a PDA diode-array detector and to an LCQ Deca XP Plus mass spectrometer. The mass spectrometer was equipped with an ESI source. The chromatographic column was a Zorbax SB300 C8 (Agilent) column, with 5 μm particle diameter (column dimensions 150 × 2.1 mm).

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