Research Article

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Journal of Translational Neurosciences

2016
Vol. 1 No. 1: 5

VDAC Differential Interactome in Chicken Brain: Possible Hints to an Intrinsically Distinct Metabolism

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Citation: Crepaldi CR, Laure HJ, Rosa JC, et al. VDAC Differential Interactome in Chicken Brain: Possible Hints to an Intrinsically Distinct Metabolism. J Transl Neurosci. 2016, 1:1.

Abstract

The kinetic assembly of protein complexes containing the VDAC (voltage dependent anion channel) follows a quite different pattern in bovine brain in comparison with rat neuronal cells. In order to investigate if the differential expression of VDACs between chicken brain when compared with bovine and rat brain mitochondria was linked to a differential VDAC interactome, we utilized BN-PAGE of mitochondria treated with dodecyl maltoside, followed by a second-dimensional SDS-PAGE. Unusually perhaps, several VDAC interactant proteins in chicken brain were not members of VDAC interactome in rat and bovine brain, which possibly suggests that these cells exhibit intrinsically differential responses to events such as neuronal cell death, bioenergetics, oxygen consumption and oxidative insults.

Keywords: Hexokinase; VDAC; Interactome; BN / SDS PAGE; Mitochondria; Chicken; Brain

Received: February 18, 2016; **Accepted:** March 23, 2016; **Published:** March 30, 2016

Introduction

Hexokinase (HK, EC 2.7.1.1) mediates the first step of glucose catabolism, phosphorylating glucose to produce glucose 6-phosphate. The regulation of HK activity plays a major role in governing the rate of cerebral Glc utilization while avoiding production of neurotoxic lactate [1, 2]. The Type I isozyme of hexokinase (HK 1) accounts for more than 90% of the total hexokinase activity in mammalian brain, where it exists predominantly in a mitochondrially bound form [2].

HK I attaches to the voltage dependent anion channel (VDAC) via their N-terminal region facilitating access to intra-mitochondrial ATP. It has been suggested that binding of HK modulates VDAC's role in apoptosis [3]. VDAC proteins constitute the major pathway for metabolic exchange across the mitochondrial outer membrane (MOM). Two isoforms of VDAC, i.e., VDAC1 and VDAC2 are known to be expressed ubiquitously in chicken brain mitochondria [4].

VDAC1 regulates metabolic and energetic functions of mitochondria; its down-expression should affect cell metabolism and normal mitochondrial function [5]. The VDAC isoform 2 has anti-apoptotic roles as a specific inhibitor of BAK-dependent mitochondrial apoptosis [6]. Changes in the levels of VDAC1 and VDAC2 expression were observed under various pathological conditions [7]. It is interesting to note that cells with low levels of VDAC1 showed 4-fold-lower ATP-synthesis capacity and contained low ATP and ADP levels. In these cells there was a strong correlation between ATP levels and cell growth, suggesting limited metabolite exchange between mitochondria and cytosol [8].

It has been studied the possibility that differences in the relative expression of VDAC isoforms could be a factor in determining the differences in species-dependent ratio of hexokinase binding sites on bovine, avian and rat brain mitochondria. In this research, VDAC1 was the most abundantly expressed of the three isoforms. Moreover, chicken brain mitochondria showed the highest VDAC1 expression and the lowest VDAC2 levels between the three species [4]. The same phenomenon, increase of VDAC1 and decrease of VDAC2 has been detected in pharmaco-resistant epilepsy [9].

There are a variety of conventional (liquid chromatography, ultracentrifugation, and sucrose density gradient centrifugation) and nonconventional methods (co-immunoprecipitation, epitope-tagging, tandem affinity purification, and GST-pull-down) available for isolation of multiprotein complexes. However, most of these techniques often separate a population of such assemblies. To isolate individual complexes, further separation is required, which can be achieved by two-dimensional blue native SDS-PAGE [10]. In BN-PAGE, the Coomassie Brilliant Blue G-250 dye is added into the electrophoresis buffer. This anionic dye binds to the surface of the membrane proteins and facilitates their migration in the native polyacrylamide gel [11]. As a result, distinct protein complexes are separated due the sieving effect of the polyacrylamide gel, but protein – protein interactions among the multiprotein complex subunits are still retained. Although initially developed for the separation of mitochondrial and chloroplast membrane proteins, recent contributions of this approach have been made in studies of protein complexes in termophilic (*Clostridium termocellum*), antibiotic productive (*Streptomyces coelicolor*), sulfate-reducing bacteria and in pathogens associated with chronic periodontitis in humans [11-15]. BN / SDS-PAGE have also been used to study protein complexes in a variety of tissues, such as erythrocytes, rat muscle, colorectal cancer and bladder epithelial cells [10, 12, 16, 17].

Recent studies indicate that the kinetic assembly of protein complexes containing the VDAC follows a pattern quite different in bovine and rat brain [18]. In order to investigate if the differential expression of VDACs between chicken brain when compared with bovine and rat brain mitochondria was linked to a differential VDAC interactome, we utilized BN-PAGE of mitochondria treated with dodecyl maltoside. After BN-PAGE, a second-dimensional SDS-PAGE was performed to separate polypeptides as components of VDAC complexes.

Materials and Method

Preparation of brain mitochondria

Chicken brains were obtained from the school slaughterhouse located on campus. Mitochondria were isolated from avian brain as described previously [2]. Mitochondrial protein concentrations were determined by the Bicinchoninic Acid Method, using the assay kit from Thermo Scientific (Rockford, IL, USA) with bovine serum albumin as standard.

Sample preparation

To determine the optimal conditions for the solubilization of VDAC protein complexes, a series of five different concentrations of dodecyl-maltoside (DDM) (0.25, 0.5, 0.75, 1.0 and 1.5%) were evaluated. The solubilization with 1.0% (w/v) was found to be the most effective. Mitochondrial membranes were solubilized as previously reported [18]. The detergent extracts of mitochondria containing approximately 200 µg of protein before solubilization, were loaded per lane.

2D – BN / Tricine SDS PAGE

BN PAGE was performed with linear 6% - 13% gradient gels, overlaid with a 4% stacking gel [19]. GE Healthcare HMW-Native protein markers (GE Healthcare, Buckinghamshire, UK) were used. For a second dimension, BN-PAGE gel lanes containing the proteins of interest were excised and incubated for 30 min. in equilibrating buffer A, containing 12,5 mM Tris (pH 6.8), 4% SDS, 20% Glycerol and 9% β-mercaptoethanol. The lanes were then dipped into equilibrating buffer B supplemented with 12,5 mM Tris HCl pH 6.8, 4% SDS, 20% Glycerol, and 2.5% Iodoacetamide for 15 min. at room temperature.

A 10% Tricine SDS-PAGE was utilized for the separation of proteins [20]. Proteins were subjected to Coomassie Blue R-250 staining or immunoblotting, to detect the complexes in which VDACs 1 and 2 were found.

Each stained gel was digitized and processed using the ImageQuantTL Capture software system (GE Healthcare). This software contains a graph that shows the intensity at each point along the length of the current lane. This is a threshold parameter, which discards peaks under a certain size (30) in relation to the highest peak on the gel. The higher the percentage value entered here the fewer the peaks likely to be detected in the profile. The sizes of the peaks were calculated after background subtraction.

Protein detection by western blotting

Proteins separated as described above were transferred to nitrocellulose membranes using TE62 Transfer Unit (GE Halthcare) and the buffer system of [21]. Membranes were blocked by incubation (overnight, 4°C) with 5% non-fat dried milk in TBS (20 mM Tris - 0.5 M NaCl, pH 7.5). Blots were probed with suitable dilutions of primary antibodies, and then washed extensively with TBS containing 0.1% (v/v) Tween 20, and incubated with 1:10,000-15,000 dilutions of horseradish peroxidase conjugated secondary antibodies; both primary and secondary antibodies were diluted in TBS containing 1% (w/v) gelatin. After further washing with TBS -Tween, chemi-luminescence was developed using the Super Signal West Pico system from Thermo Scientific (Rockford, IL, USA).

Mass spectrometry and protein identification

The second dimension gel bands were selected and proteins were identified by MALDI-TOF-TOF mass spectrometer after in gel trypsin digestion. Briefly, selected gel bands were excised and combined. SDS and CBB were removed by washing the gels three times with 50% ACN in 0.1 M ammonium bicarbonate, pH 7.8, followed by dehydration in neat ACN. Gel bands were dried in a Speed Vac instrument (Savant, New York, NY) and were swollen in 20 mL of 0.5 mg trypsin (Promega, Madison, USA) in 0.1 M ammonium bicarbonate, pH 7.8, followed by the addition of 50 mL of 0.1 M ammonium bicarbonate to cover the entire gel piece. Trypsin hydrolysis was carried out at 37°C for 24 h and the reaction was stopped by the addition of 5 mL of neat formic acid. Peptides were extracted from gel pieces and desalted in

microtips filled with POROS R2 (PerSeptive Biosystems, Foster City, CA) previously equilibrated in 0.2% formic acid. After loading, the sample was desalted with two washes of 150 mL 0.2% formic acid. Peptides were eluted from the microtips with 30 mL of 60% methanol / 5% formic acid. The sample was concentrated, and samples were mixed with matrix solution (5 mg/mL α-cyano-4 hydroxycinnamic acid in 50% acetonitrile / 0.1% trifluoroacetic acid), applied on the MALDI target plate and air dried at room temperature. MALDI-TOF-TOF-MS instrument (Axima Performance, Kratos-Shimadzu, Manchester, UK) was calibrated with a mixture of bradykynin fragment, angiotensin II, renin and ACTH (mass accuracy < 50 ppm). The spectra of CID-MS / MS of each gel band were obtained in data dependent acquisition mode. The peak list was obtained from CID-MS / MS spectra using Launchpad v. 2.8 (Kratos-Shimadzu, Manchester, UK) and submitted to a database search using MASCOT version 2.2.04 against NCBI database version 52.9 selected for taxonomy filter of Gallus gallus. The database search parameters accept one missing trypsin cleavage, carbamidomethylation and methionine oxidation. Mass tolerance was 1.2 Da for precursor ions and 0.8 Da for product ions. Protein was considered to be identified by MASCOT for proteins corresponded to a level of significance of $p < 0.05$ and FDR less than 0.2%.

Results and Discussion

To analyze proteins interacting with VDAC, BN / SDS-PAGE was performed. The VDACs 1 and 2 associated protein complexes were revealed by antibody detection and confirmed by mass spectrometry. In the present paper, we utilized a well-characterized antibody against VDAC1, which was first raised by Poleti et al., which is highly specific for their desired targets [4]. However, the antibody against VDAC2 showed cross-immunoreactivity with VDAC1 from chick brain mitochondria. VDACs were detected in four pre-complexes (II, III, IV and V) and one complex (I) in avian brain mitochondria **(Figure 1).** The apparent masses of complex I and pre-complexes II, III, IV and V were 762, 707, 625, 571 and 464 kDa, respectively.

The general interpretative rule for the analysis of the BN / SDS-PAGE is that all protein spots which are situated vertically in the gel are potential subunits of a same protein complex. A second rule is that if it is combined information about the subunit composition of the protein complexes (vertical) and the molecular mass of the complexes (horizontal) and is assumed that the molecular mass of a protein complex is increasing during assembly of the structural subunits of this complex, the analysis of the protein pattern after BN / SDS-PAGE resolves the direction of the stepwise subunit assembly from the lower towards the higher molecular mass of the protein complexes [22].

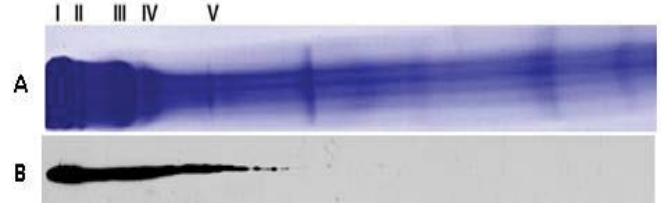

Figure 1 (A) Blue Native gel gradient (6% - 13%) of DDM solubilized chicken brain mitochondria (B) Immunoblot to VDACs 1 and 2, showing the overlap proteins in the first complex (762 Kda), pre-complex II (707 KDa), pre-complex III (625 KDa), pre-complex IV (571 KDa) and pre-complex V (464 KDa) in chicken brain.

BN / SDS-PAGE were performed on five replicates of five different mitochondrial preparations (each preparation extracted from 3 adult chickens). The analysis of the representative gel from **Figure 2** detected 85 spots of VDAC interacting proteins. Among these spots, 54 different proteins were identified with a MASCOT score above the threshold to validate MS data, i.e., 32. The identification of subunits of individual OXPHOs complexes, as VDAC interacting proteins in chicken brain, confirmed the validity of our MS approach. These interactants represent a large variety of functions such as components of TCA cycle, ketone body's metabolism, scavengers of reactive oxygen species, members of mitochondrial permeability transition pore and cell proliferation between many others.

The identified proteins and their corresponding MASCOT score, sequence coverage, and number of matched MS / MS are listed in **Tables 1**-**6**. The four pre-complexes and the major complex from chicken brain mitochondria can be observed in **Figure 2** and **Table 1**.

Twenty eight identified non-OXPHOS proteins were interacting with VDAC in avian brain mitochondria. The identified components of VDAC interacting chicken brain proteins in pre-complex V **(Table 1** and **Figure 2)** are ADP / ATP Translocase 1 (ANT1); Adenine Nucleotide Translocator 3 (ANT3); Mitochondrial 10-formyltetrahydrofolate dehydrogenase (AL1L2); ATP / ADP Antiporter (avANT); Aspartate Aminotransferase (AATM); D-Beta-Hydroxybutyrate Dehydrogenase (BDH1); 60 kDa heat shock protein (CH60); Cytochrome C Oxidase subunit II (COXII); Cytochrome C Oxidase subunit 4 Isoform 1 (COX4I1); Trifunctional enzyme subunit alpha (HADHA); Malate Dehydrogenase Mitochondrial (MDHM); unnamed protein product (BLAST: xin actin-binding repeat-containing protein 1, XIRP1); Voltage-Dependent Anion-selective Channel Protein 2 (VDAC2); Thioredoxin-dependent Peroxide Reductase Prdx3 protein (PRDX3); Phosphate Carrier Protein Mitochondrial (MPCP); Cytochrome b-c1 complex subunit 2, mitochondrial-like isoform X4 (QCR2) and similar to WW, C2 and coiled-coil domain containing 2 (WWC2). The proteins AL1L2, avANT, BDH1, CH60, COXII, COX4I1, HADHA, MDHM, PRDX3, XIRP1 and WWC2 were not VDAC interactants in bovine and rat brain mitochondria [18].

D-Beta-Hydroxybutyrate Dehydrogenase (BDH1) catalyzes the interconversion of acetoacetate and (R)-3-hydroxybutyrate, the two major ketone bodies produced during fatty acid catabolism. The authors showed that ketolytic (BDH1) and glycolytic enzymatic (Hexokinase) profiles of malignant brain tumors were different from the normal non-neoplastic brain tissue. A decrease in the mitochondrial enzymes BDH1 and OXCT1(3-Oxoacid CoA Transferase) was coupled with positive expression of the glycolytic enzymes HK2 and PKM2 (Pyruvate Kinase M2 isoform), supporting

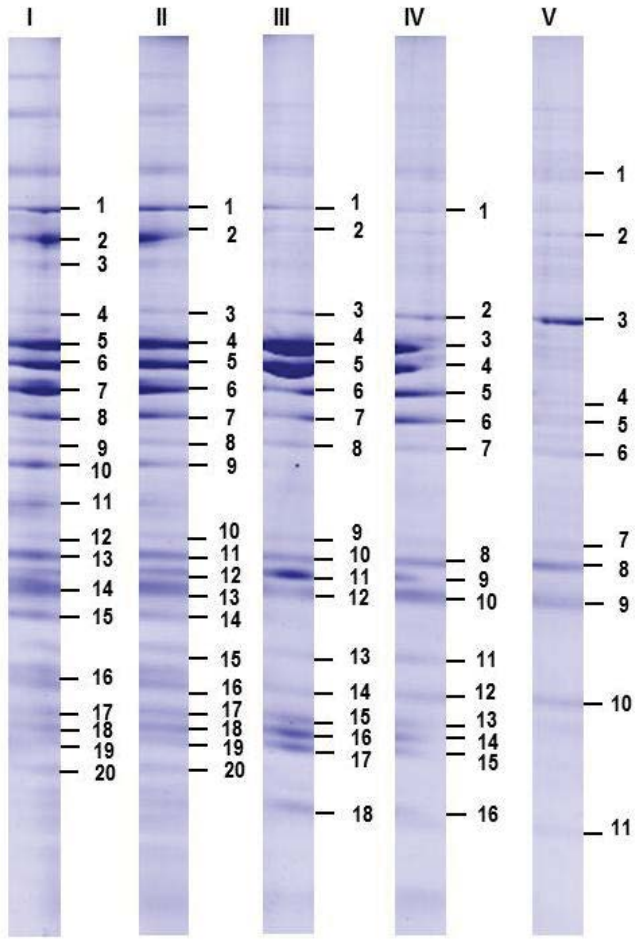

Figure 2 Identification of protein subunits in 2D BN / SDS gels of the pre-complexes II to V and complex I from dodecylmatoside-solubilized chicken brain mitochondria. Corresponding spots 1-20 from complex I, 1-20, 1-18, 1-16, 1-11 from pre-complexes II, III, IV and V, respectively, were excised from the gel and identified by MALDI-TOF / TOF MS. Results are summarized in **Table 1**.

the notion that many high grade brain tumors in humans have aberrant metabolism of ketones, and may preferentially use glucose for their energy needs [23].

PRDX3 is a mitochondrial antioxidant protein and a member of the peroxiredoxin family that can scavenge not only hydrogen peroxide (H_2O_2) in co-operation with thiol, but also peroxynitrite (ONOO–). PRDX3 markedly reduced gliosis, a post- neuronal cell death event and seems to be neuroprotective against oxidative insults [24].

Another VDAC interactant protein just observed in chicken brain mitochondria was xin actin-binding repeat-containing protein 1 (XIRP1). It was found as one of the proteins with an altered level in serum from schizophrenic patients [25]. The phosphate carrier protein (MPCP) was also found as a VDAC interactant in bovine brain mitochondria [18], and is a key component of the mitochondrial permeability transition pore (mPTP) [26], in the same way as VDAC [27]. MPCP undergoes a calcium-induced conformational change to induce pore formation.

In addition to the proteins identified in pre-complex V, fifteen other proteins were identified in pre-complex IV **(Table 1 and Figure 2)**. They are Aconitate hydratase (ACON); similar to ADP / ATP translocase (ANT); ATP synthase subunit alpha (ATPA); ATP synthase subunit

Table 1 Identification results of VDAC-associated protein complex (I) and pre-complexes (II to V) in chicken brain mitochondria.

I	II	III	IV	V
3CWB_D	3CWB_A	3CWB_A	ANT	
ANTI	ANT1	ANT1	ANT1	ANT1
ANT3	ANT3	ANT3	ANT3	ANT3
	AATM	AATM	AATM	AATM
	ACON		ACON	
	ATPG	ATPG	ATPG	
AT5F1	AT5F1	AT5F1	AT5F1	
ATP5H	ATP5H	ATP5H	ATP5H	
ATP5O	ATP5O	ATP5O	ATP5O	
ATPB	ATPA	ATPA	ATPA	
ATPA	ATPB	ATPB	ATPB	AL1I2
ARALAR2		ATPD	ATPD	
avANT	avANT	avANT	avANT	avANT
	BDH1	BDH1		BDH1
CH60	CH60	CH60	CH60	CH60
CHPF2				
		CMBL	CMBL	
COXII	COXII	COXII	COXII	COXII
		COX4I1	COX4I1	COX4I1
	DOCK2	DESM		
	E1C825	HADHA		HADHA
IMMT	IMMT	IMMT	IMMT	
			QN1	
		MRP1	MRP1	
	MDH2	MDH2		MDH2
NDUA9			MYH11	
NDUAA	NDUAA			MPCP
NDUFA8	NDUFA8	NDUFA8		
		NDUFB4		
NDUFA12	NDUFA12			
NDUFB8	NDUFB8			
NDUFB10	NDUFB10	NDUFB10		
NDUFS7	NDUFS7	NDUFS7		
NDUFS8	NDUFS8			
NDUFS1	NDUFS1	NDUFS1		
NDUFS3	NDUFS3			
NDUV2	NDUV2	NDUV2		
PHB	PHB	PRDX3	PRDX3	PRDX3
PHB2	PHB2	RBBP8	RBBP8	
QCRC1		QCRC1		
QCRC2	QCRC2	QCRC2	QCRC2	QCRC2
VDAC2	VDAC2	VDAC2	VDAC2	VDAC2
				XIRP1
				WWC2

beta (ATPB); similar to ATP synthase subunit b (ATP5F1); ATP synthase subunit gamma (ATPG); ATP synthase subunit delta (ATPD); similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d isoform 3 (ATP5H); similar to LOC446923 protein isoform 1 (BLAST: ATP synthase subunit O, mitochondrial isoform 2) (ATP5O); Carboxymethylenebutenolidase homolog isoform 3 (CMBL); DNA endonuclease RBBP8 (RBBP8); Myosin-11 (MYH11); Mitochondrial inner membrane protein (IMMT); similar to Multidrug Resistance Protein 1a (MRP1) and similar to QN1 orf (QN1). CMBL, RBBP8, MYH11, MRP1 and QN1 do not interact with VDACs in bovine and rat brain mitochondria [18]. Schizophrenic patients showed differences in activities of some enzymes from TCA cycle, like Aconitase (ACON) which presented a decreased activity [28]. The proteins ACON, F1-ATPase chains α (ATPA) and β (ATPB) were found differentially decreased in response to an acute hypobaric hypoxic episode and the subsequent re-oxygenation in rat brain cortex [29]. The authors suggest that these results could be due to the loss of proteins coupled with the destabilization of the mitochondria found after a hypobaric hypoxic insult, which would alter both the structure and functionality of ATPase and more specifically its catalytic subunit F1.

CMBL serves as a key enzyme in the activation of olmesartan medoxomil a prodrug type angiotensin II type I receptor antagonist. It is distributed in 20 tissues, including whole brain,

Table 2 Summary of proteins from 2D BN / SDS Gels Identified by MALDI-TOF-TOF in chicken brain mitochondria.

Complex I								
Spot	Accession NCBI	Name of protein	Function	Mascot score	Sequence coverage (%)	Matches MS / MS	Theoretical	
							pI	Mr
1	gi|57530041	Mitochondrial inner membrane protein	Other functions	578	13,2	14	5,72	79249
2	gi|57529753	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	Oxidative phosphorylation	1024	18,7	16	6,5	79576
3	gi|61098440	Calcium-binding mitochondrial carrier protein Aralar2	Other functions	142	4	4	8,93	74102
4	gi|61098372	60 kDa heat shock protein, mitocondrial	Chaperone; Cellular defense	122	5,2	3	5,72	60972
5	gi|118109616	PREDICTED: similar to mitochondrial ATP synthase alpha subunit, partial	Oxidative phosphorylation	77	29,2	2	5,8	5292
6	gi|71897237	ATP synthase subunit beta, mitochondrial precursor	Oxidative phosphorylation	988	27,4	20	5,59	56627
7	gi|50754375	PREDICTED: cytochrome b-c1 complex subunit 1, mitochondrial	Oxidative phosphorylation	145	4,6	4	6,58	52758
8	gi|118098350	PREDICTED: cytochrome b-c1 complex subunit 2, mitochondrial-like isoform X4	Oxidative phosphorylation	458	19	11	9,04	48579
10	gi|71895153	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial	Oxidative phosphorylation	350	14,4	8	6,15	41431
11	gi|57529307	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9, mitochondrial	Oxidative phosphorylation	474	20,1	12	9,4	43079
12	gi|46048903	Voltage-dependent anion-selective channel protein 2	Transport and carrier protein	334	19,1	6	8,61	30197
13	gi|124249322	Prohibitin-2	Regulates cell proliferation. May play a role in regulating mitochondrial respiration activity	302	14	8	9,89	33336
14	gi|295148230	Prohibitin	Regulates cell proliferation. May play a role in regulating mitochondrial respiration activity	207	12,5	6	5,57	29892
	gi|196049778	Chain D, Chicken Citocromo Bc1 complex Inhibited By An Iodinated Analogue Of The Polyketide Crocacin-d	Oxidative phosphorylation	164	10,8	4	6,32	26939
	gi|22775582	ATP/ADP antiporter	Transport and carrier protein	105	6,7	3	9,78	32847
	gi|118089692	PREDICTED: similar to ADP/ATP translocase	Transport and carrier protein	105	7,6	3	9,72	29307
	gi|54020693	ADP/ATP translocase 3	Transport and carrier protein	102	6,7	3	9,73	32748

15	gi|226437575	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	Oxidative phosphorylation	273	17,1	8	6,55	29232
16	gi|118086790	PREDICTED: similar to NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial precursor (NADH-ubiquinone oxidoreductase 24 kDa subunit) (NADH dehydrogenase subunit II) isoform 1	Oxidative phosphorylation	308	22,4	7	7,6	26893
	gi|5834847	Cytochrome c oxidase subunit II (mitochondrion)	Oxidative phosphorylation	127	9,3	3	4,57	25568
	gi|50755667	PREDICTED: NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10	Oxidative phosphorylation	62	6,3	2	5,98	20497
17	gi|118103240	PREDICTED: NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial-like	Oxidative phosphorylation	120	8,6	4	10,02	20473
	gi|118102465	PREDICTED: ATP synthase subunit b, mitochondrial	Oxidative phosphorylation	107	8,5	2	9,34	31751
	lgi|118090950	PREDICTED: NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial	Oxidative phosphorylation	104	11	2	5,84	23824
	gi|118085386	PREDICTED: chondroitin sulfate glucuronyltransferase-like	Tissue development and morphogenesis	45	1,4	1	5,81	83804
18	gi|50745451	PREDICTED: similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d isoform 3 (BLAST: PREDICTED: ATP synthase subunit d, mitochondrial isoform 1)	Oxidative phosphorylation	313	30,4	8	8,73	18343
	gi|118099484	PREDICTED: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	Oxidative phosphorylation	97	11,6	4	8,42	20125
19	gi|118099484	PREDICTED: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	Oxidative phosphorylation	91	11,6	4	8,42	20125
	gi|118083809	PREDICTED: similar to LOC446923 protein isoform 1 (BLAST: ATP sinthase subunidade O, isoforma mitocondrial 2)	Oxidative phosphorylation	66	6,7	2	9,88	22803
20	gi|296090732	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12	Oxidative phosphorylation	165	27,4	6	9,57	16892
	gi|57529832	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8, mitochondrial precursor	Oxidative phosphorylation	151	18,3	6	7,9	21945

but the highest level is found in liver [30]. And the protein RBBP8 or CtIP (C-terminal binding protein Interacting Protein) is a multifunctional protein involved in transcription, DNA replication, DNA repair by homologous recombination and the G1 and the G2 checkpoints. Both its functions and interactions point to a putative oncogenic potential of RBBP8 loss [31].

Our results demonstrated in avian brain several VDAC interactant proteins associated with neurological diseases, like the Multidrug resistance-associated protein 1 (MRP1). Drug resistance is one of the most serious problems in treatment of epilepsy [32]. Accumulating experimental evidence suggests that increased expression of MRP1 has been determined in epileptogenic brain regions of patients with pharmacoresistant epilepsy [33]. Over-expression of such transporters in epileptogenic tissue is thus likely to reduce the amount of drug that reaches the epileptic neurons, which would be a likely explanation for pharmacoresistance [32].

In addition to the proteins identified in pre-complex IV, nine other proteins were identified in pre-complex III **(Table 1** and **Figure 2).** They are Chain A, Chicken Cytochrome Bc1 complex Inhibited By An Iodinated Analogue Of The Polyketide Crocacin-D (3 CWB_A); Cytochrome b-c1 complex subunit 1 (QCRC1); Desmin, partial (DESM); NADH dehydrogenase [ubiquinone] 1 alpha sub complex subunit 8 (NDUFA8); NADH dehydrogenase [ubiquinone] 1 beta sub complex subunit 4 (NDUFB4); NADH dehydrogenase [ubiquinone] 1 beta sub complex subunit 10 (NDUFB10); NADH dehydrogenase [ubiquinone] iron-sulfur protein 7 (NDUFS7);

Table 3 Summary of proteins from 2D BN / SDS Gels Identified by MALDI-TOF-TOF in chicken brain mitochondria (Pre-complex II).

Pre-complex II								
Spot	Accession NCBI	Name of protein	Function	Mascot score	Sequence coverage (%)	Matches MS / MS	Theoretical	
							pI	Mr
1	gi|57530041	Mitochondrial inner membrane protein	Other functions	586	12,7	13	5,72	79249
	gi|45383738	aconitate hydratase, mitochondrial	Metabolic enzyme (TCA); mitochondrial DNA stability	52	1,9	1	8,05	85790
2	gi|57529753	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	Oxidative phosphorylation	766	16,1	16	6,5	79576
3	gi|61098372	60 kDa heat shock protein, mitocondrial precursor	Chaperone; Cellular defense	49	3,1	1	5,72	60972
	gi|45383566	ATP synthase subunit alpha, mitochondrial	Oxidative phosphorylation	40	2,4	2	9,29	60186
4	gi|118084029	PREDICTED: uncharacterized protein LOC418583		34	0,9	1	5,64	146912
	gi|118097244	PREDICTED: dedicator of cytokinesis protein 2	Modulates microglia secretion, phagocytosis and paracrine neurotoxicity	34	0,7	1	8,3	219618
5	gi|71897237	ATP synthase subunit beta, mitochondrial precursor	Oxidative phosphorylation	887	23,5	17	5,59	56627
6	gi|196049775	Chain A, Chicken Cytochrome Bc1 complexo Inhibited By An Iodinated Analogue Of The Polyketide Crocacin-D	Oxidative phosphorylation	406	12,6	10	5,95	49441
7	gi|118098350	PREDICTED: cytochrome b-c1 complex subunit 2, mitochondrial-like isoform X4	Oxidative phosphorylation	577	19	11	9,04	48579
8	gi|45382953	Aspartate aminotransferase, mitochondrial precursor	Amino acid metabolism	633	19,4	12	9,38	47241
9	gi|71895153	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10, mitochondrial	Oxidative phosphorylation	455	18,6	9	6,15	41431
10	gi|50758110	PREDICTED: malate dehydrogenase, mitochondrial	Metabolic enzyme (TCA);	318	12,5	5	8,83	36968
	gi|57529615	D-beta-hydroxybutyrate dehydrogenase, mitochondrial precursor	Ketogenesis pathway	61	2,9	2	8,37	38237
	gi|46048903	voltage-dependent anion-selective channel protein 2	Transport and carrier protein	46	3,9	1	8,61	30197
11	gi|46048903	voltage-dependent anion-selective channel protein 2	Transport and carrier protein	464	31,1	9	8,61	30197
	gi|124249322	Prohibitin-2	Regulates cell proliferation. May play a role in regulating mitochondrial respiration activity	140	13,6	6	9,89	33336
12	gi|118123062	PREDICTED: ATP synthase subunit gamma, mitochondrial isoform 1	Oxidative phosphorylation	269	11,3	6	9,39	32608
	gi|295148230	Prohibitin	Regulates cell proliferation. May play a role in regulating mitochondrial respiration activity	187	12,5	6	5,57	29892
13	gi|22775582	ATP/ADP antiporter	Transport and carrier protein	206	10,7	4	9,78	32847
	gi|54020693	ADP/ATP translocase 3	Transport and carrier protein	204	10,7	4	9,73	32748
	gi|57530120	ADP/ATP translocase 1	Transport and carrier protein	97	8,4	2	9,74	32968
14	gi|226437575	NADH dehydrogenase [ubiquinone] iron-sulfur protein 3, mitochondrial	Oxidative phosphorylation	561	27,2	14	6,55	29232

16	gi|5834847	cytochrome c oxidase subunit II (mitochondrion)	Oxidative phosphorylation	287	25,1	8	4,57	25568
	gi|50755667	PREDICTED: NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10	Oxidative phosphorylation	155	7,4	4	5,98	20497
	gi|118086790	PREDICTED: similar to NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial precursor (NADH-ubiquinone oxidoreductase 24 kDa subunit) (NADH dehydrogenase subunit II) isoform 1	Oxidative phosphorylation	121	9,8	3	7,6	26893
17	gi|118103240	PREDICTED: NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial-like	Oxidative phosphorylation	125	8,6	4	10,02	20473
	gi|118102465	PREDICTED: ATP synthase subunit b, mitochondrial	Oxidative phosphorylation	71	4,2	1	9,34	31751
	gi|118090950	PREDICTED: NADH dehydrogenase [ubiquinone] iron-sulfur protein 8, mitochondrial	Oxidative phosphorylation	56	5,7	2	5,84	23824
18	gi|50745451	PREDICTED: similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d isoform 3 (BLAST: PREDICTED: ATP synthase subunit d, mitochondrial isoform 1)	Oxidative phosphorylation	324	30,4	8	8,73	18343
	gi|118099484	PREDICTED: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	Oxidative phosphorylation	59	7,6	2	8,42	20125
	gi|118083809	PREDICTED: similar to LOC446923 protein isoform 1 (BLAST: ATP sinthase subunidade O, isoforma mitocondrial 2)	Oxidative phosphorylation	46	6,7	2	9,88	22803
19	gi|118083809	PREDICTED: similar to LOC446923 protein isoform 1 (BLAST: ATP sinthase subunit O, isoforma mitocondrial 2)	Oxidative phosphorylation	144	11,9	3	9,88	22803
	gi|118099484	PREDICTED: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	Oxidative phosphorylation	50	7,6	2	8,42	20125
20	gi|296090732	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12	Oxidative phosphorylation	142	15,8	2	9,57	16892
	gi|57529832	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8, mitochondrial precursor	Oxidative phosphorylation	116	11	2	7,9	21945

NADH-ubiquinone oxidoreductase 75 kDa subunit (NDUS1) and similar to NADH dehydrogenase [ubiquinone] flavoprotein 2 (NDUV2). Between these 8 proteins, just DESM was not interacting with VDACs in rat and bovine brain mitochondria [18]. Regarding molecular evidence of abnormal mitochondrial function in psychiatric disorders, in studies of schizophrenic patients, the 24 kDa and 51 kDa subunits of complex I from the electron transport chain were significantly decreased in the pre frontal cortex [34]. Mitochondrial dysfunction and abnormal brain bioenergetics have also been implicated in autism, with reduced expression of eleven genes of electron transport chain complex I [35].

Desmin plays an essential role in maintaining cell cytoarchitecture, positioning and functioning of organelles, and the intercellular signaling pathway [36]. Desmin was shown to regulate mitochondria affinity to ADP and oxygen consumption supposedly through direct binding to VDAC [37].

The identified components of pre-complex II, not found in pre-complex III **(Table 2)** are uncharacterized protein LOC418583 (E1C825); Dedicator of cytokinesis protein 2 (DOCK2); NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10 (NDUAA); NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12 (NDUFA12); NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8 (NDUFB8); NADH dehydrogenase [ubiquinone] iron-sulfur protein 8 (NDUFS8);

Table 4 Summary of proteins from 2D BN / SDS Gels Identified by MALDI-TOF-TOF in chicken brain mitochondria (Pre-complex III).

Pre-complex III								
Spot	Accession NCBI	Name of protein	Function	Mascot score	Sequence coverage (%)	Matches MS / MS	Theoretical	
							pI	Mr
1	gi|57530041	Mitochondrial inner membrane protein	Other functions	344	8,2	10	5,72	79249
2	gi|57529753	NADH-ubiquinone oxidoreductase 75 kDa subunit, mitochondrial	Oxidative phosphorylation	90	4	4	6,5	79576
	gi|45384238	Trifunctional enzyme subunit alpha, mitochondrial	Lipid metabolism; fatty acid beta-oxidation	39	1,4	2	9,19	83186
3	gi|61098372	60 kDa heat shock protein, mitocondrial precursor	Chaperone; Cellular defense	184	5,2	3	5,72	60972
	gi|45383566	ATP synthase subunit alpha, mitochondrial	Oxidative phosphorylation	35	2,4	1	9,29	60186
4	gi|45383566	ATP synthase subunit alpha, mitochondrial	Oxidative phosphorylation	658	17,5	12	9,29	60186
5	gi|71897237	ATP synthase subunit beta, mitochondrial precursor	Oxidative phosphorylation	886	23,5	16	5,59	56627
6	gi|50754375	PREDICTED: cytochrome b-c1 complex subunit 1, mitochondrial	Oxidative phosphorylation	273	9,2	9	6,58	52758
	gi|196049775	Chain A, Chicken Cytochrome Bc1 complexo Inhibited By An Iodinated Analogue Of The Polyketide Crocacin-D	Oxidative phosphorylation	273	9,9	9	5,95	49441
7	gi|118098350	PREDICTED: cytochrome b-c1 complex subunit 2, mitochondrial-like isoform X4	Oxidative phosphorylation	360	15,1	9	9,04	48579
	gi|71897237	ATP synthase subunit beta, mitochondrial precursor	Oxidative phosphorylation	72	2,6	1	5,59	56627
8	gi|45382953	Aspartate aminotransferase, mitochondrial precursor	Aminoacid metabolism	337	15,4	11	9,38	47241
9	gi|50758110	PREDICTED: malate dehydrogenase, mitochondrial	Metabolic enzyme (TCA)	233	12,8	6	8,83	36968
	gi|46048903	voltage-dependent anion-selective channel protein 2	Transport and carrier protein	57	7,1	2	8,61	30197
	gi|57529615	D-beta-hydroxybutyrate dehydrogenase, mitochondrial precursor	Ketogenesis pathway	45	7,1	3	8,37	38237
10	gi|46048903	voltage-dependent anion-selective channel protein 2	Transport and carrier protein	584	24	9	8,61	30197
11	gi|118123062	PREDICTED: ATP synthase subunit gamma, mitochondrial isoform 1	Oxidative phosphorylation	300	11,3	8	9,39	32608
	gi|50734923	PREDICTED: carboxymethylenebutenolidase homolog isoform 3	Prodrug bioactivation	40	4,9	2	6,45	28170
	gi|22775582	ATP/ADP antiporter	Transport and carrier protein	35	3	2	9,78	32847
	gi|54020693	ADP/ATP translocase 3	Transport and carrier protein	35	3	2	9,73	32748
	gi|118089692	PREDICTED: similar to ADP/ATP translocase	Transport and carrier protein	35	3,4	2	9,72	29307
12	gi|54020693	ADP/ATP translocase 3	Transport and carrier protein	309	17,8	7	9,73	32748

14	gi|5834847	cytochrome c oxidase subunit II (mitochondrion)	Oxidative phosphorylation	345	25,1	9	4,57	25568
	gi|50755667	PREDICTED: NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 10	Oxidative phosphorylation	142	7,4	4	5,98	20497
	gi|118086790	PREDICTED: similar to NADH dehydrogenase [ubiquinone] flavoprotein 2, mitochondrial precursor (NADH-ubiquinone oxidoreductase 24 kDa subunit) (NADH dehydrogenase subunit II) isoform 1	Oxidative phosphorylation	106	9,8	3	7,6	26893
	gi|118093103	PREDICTED: thioredoxin-dependent peroxide reductase, mitochondrial isoform X4	Antioxidant protein	72	4,9	2	8,4	30992
15	gi|118102465	PREDICTED: ATP synthase subunit b, mitochondrial	Oxidative phosphorylation	239	11,6	5	9,34	31751
	gi|118103240	PREDICTED: NADH dehydrogenase [ubiquinone] iron-sulfur protein 7, mitochondrial-like	Oxidative phosphorylation	103	8,6	4	10,02	20473
	gi|118085922	PREDICTED: similar to multidrug resistance protein 1a	Transport protein, limit access of drug to the central nervous system	42	1,3	1	8,87	148466
	gi|50745451	PREDICTED: similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d isoform 3 (BLAST: PREDICTED: ATP synthase subunit d, mitochondrial isoform 1)	Oxidative phosphorylation	40	6,2	2	8,73	18343
16	gi|50745451	PREDICTED: similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d isoform 3 (BLAST: PREDICTED: ATP synthase subunit d, mitochondrial isoform 1)	Oxidative phosphorylation	354	30,4	7	8,73	18343
	gi|118083809	PREDICTED: similar to LOC446923 protein isoform 1 (BLAST: ATP sinthase subunidade O, isoforma mitocondrial 2)	Oxidative phosphorylation	58	6,7	2	9,88	22803
	gi|118099484	PREDICTED: NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8	Oxidative phosphorylation	50	7,6	2	8,42	20125
	gi|50737115	PREDICTED: DNA endonuclease RBBP8	Cell cycle progression, DNA repair and transcriptional regulation	42	1,1	1	6,14	103530
17	gi|118083809	PREDICTED: similar to LOC446923 protein isoform 1 (BLAST: ATP sinthase subunidade O, isoforma mitocondrial 2)	Oxidative phosphorylation	154	11,9	4	9,88	22803
18	gi|118124369	PREDICTED: ATP synthase subunit delta, mitochondrial-like, partial	Oxidative phosphorylation	100	35	2	4,75	4159
	gi|71895513	citocromo c oxidase subunidade 4 isoforma 1, mitocondrial	Oxidative phosphorylation	47	7	2	8,91	19631
	gi|118083465	PREDICTED: NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 4 isoform X1	Oxidative phosphorylation	37	6,2	2	9,46	18701
	gi|2959450	Desmin	Cell morphology	36	2,9	1	5,3	51663

Table 5 Summary of proteins from 2D BN / SDS Gels Identified by MALDI-TOF-TOF in chicken brain mitochondria (Pre-complex IV).

Pre-complex IV								
Spot	Accession NCBI	Name of protein	Function	Mascot score	Sequence average (%)	Matches MS / MS	Theoretical	
							pI	Mr
1	gi|57530041	Mitochondrial inner membrane protein	Other functions	314	8,2	9	5,72	79249
	gi|45383738	Aconitate hydratase, mitochondrial	Metabolic enzyme (TCA); mitochondrial DNA stability	106	3,8	3	8,05	85790
	gi|45383566	ATP synthase subunit alpha, mitochondrial	Oxidative phosphorylation	53	2,4	2	9,29	60186
2	gi|61098372	60 kDa heat shock protein, mitocondrial precursor	Chaperone; Cellular defense	468	11,7	8	5,72	60972
	gi|45383566	ATP synthase subunit alpha, mitochondrial	Oxidative phosphorylation	107	2,4	2	9,29	60186
4	gi|71897237	ATP synthase subunit beta, mitochondrial precursor	Oxidative phosphorylation	816	23,5	16	5,59	56627
5	gi|118088850	PREDICTED: similar to QN1 orf	Cell division	53	0,6	2	5,86	170342
6	gi|118098350	PREDICTED: cytochrome b-c1 complex subunit 2, mitochondrial-like isoform X4	Oxidative phosphorylation	541	19	11	9,04	48579
7	gi|45382953	Aspartate aminotransferase, mitochondrial precursor	Aminoacid metabolism	364	15,4	12	9,38	47241
	gi|71897237	ATP synthase subunit beta, mitochondrial precursor	Oxidative phosphorylation	76	2,6	2	5,59	56627
8	gi|46048903	Voltage-dependent anion-selective channel protein 2	Transport and carrier protein	603	30,4	8	8,61	30197
9	gi|118123062	PREDICTED: ATP synthase subunit gamma, mitochondrial isoform 1	Oxidative phosphorylation	296	11,3	8	9,39	32608
	gi|50734923	PREDICTED: carboxymethylenebutenolidase homolog isoform 3	Prodrug bioactivation	40	4,9	2	6,45	28170
10	gi|22775582	ATP/ADP antiporter	Transport and carrier protein	51	3	2	9,78	32847
	gi|54020693	adenine nucleotide translocator 3	Transport and carrier protein	51	3	2	9,73	32748
	gi|118089692	PREDICTED: similar to ADP/ATP translocase	Transport and carrier protein	51	3,4	2	9,72	29307
11	gi|5834847	cytochrome c oxidase subunit II (mitochondrion)	Oxidative phosphorylation	260	16,3	8	4,57	25568
12	gi|118093103	PREDICTED: thioredoxin-dependent peroxide reductase, mitochondrial isoform X4	Antioxidant protein	64	4,9	2	8,4	30992
13	gi|118102465	PREDICTED: similar to ATP synthase subunidade b	Oxidative phosphorylation	428	15,8	12	9,34	31751
	gi|50745451	PREDICTED: similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d isoform 3 (BLAST: PREDICTED: ATP synthase subunit d, mitochondrial isoform 1)	Oxidative phosphorylation	66	6,2	2	8,73	18343
	gi|45384060	Myosin-11	Transport of vesicles	40	0,9	1	5,5	228891
	gi|118085922	PREDICTED: similar to multidrug resistance protein 1a	Transport protein, limit access of drug to the central nervous system	40	1,3	1	8,87	148466
14	gi|50745451	PREDICTED: similar to ATP synthase, H+ transporting, mitochondrial F0 complex, subunit d isoform 3 (BLAST: PREDICTED: ATP synthase subunit d, mitochondrial isoform 1)	Oxidative phosphorylation	345	30,4	8	8,73	18343
	gi|118083809	PREDICTED: similar to LOC446923 protein isoform 1 (BLAST: ATP sinthase subunidade O, isoforma mitocondrial 2)	Oxidative phosphorylation	56	6,7	2	9,88	22803
	gi|50737115	DNA endonuclease RBBP8	Other functions	45	1,1	1	6,14	103530
15	gi|118083809	PREDICTED: similar to LOC446923 protein isoform 1 (BLAST: ATP sinthase subunidade O, isoforma mitocondrial 2)	Oxidative phosphorylation	53	6,7	2	9,88	22803
16	gi|118124369	PREDICTED: ATP synthase subunit delta, mitochondrial-like, partial	Oxidative phosphorylation	101	35	2	4,75	4159
	gi|71895513	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	Oxidative phosphorylation	37	7	2	8,91	19631

Table 6 Summary of proteins from 2D BN / SDS Gels Identified by MALDI-TOF-TOF in chicken brain mitochondria (Pre-complex V).

Pre-complex V								
Spot	Accession NCBI	Name of protein	Function	Mascot score	Sequence coverage (%)	Matches MS / MS	Theoretical	
							pI	Mr
1	gi|118082834	PREDICTED: mitochondrial 10-formyltetrahydrofolate dehydrogenase (Alternative name: Aldehyde dehydrogenase family 1 member L2	Detoxification, cell death	39	1,4	2	6,5	101470
2	gi|45384238	Trifunctional enzyme subunit alpha, mitochondrial	Lipid metabolism; fatty acid beta-oxidation	181	5,5	7	9,19	83186
3	gi|61098372	60 kDa heat shock protein, mitocondrial precursor	Chaperone; Cellular defense	539	16,4	11	5,72	60972
	gi|4521320	unnamed protein product (BLAST: xin actin-binding repeat-containing protein 1)	Protects actin filaments from depolymerization	39	1,6	1	7,3	87986
4	gi|118090114	PREDICTED: similar to WW, C2 and coiled-coil domain containing 2	Celular death	32	0,8	1	5,07	132717
5	gi|118098350	PREDICTED: cytochrome b-c1 complex subunit 2, mitochondrial-like isoform X4	Oxidative phosphorylation	207	10,5	7	9,04	48579
6	gi|45382953	Aspartate aminotransferase, mitochondrial precursor	Aminoacid metabolism	207	13,5	10	9,38	47241
7	gi|50758110	PREDICTED: malate dehydrogenase, mitochondrial	Metabolic enzyme (TCA)	195	17,4	7	8,83	36968
	gi|46048903	voltage-dependent anion-selective channel protein 2	Transport and carrier protein	116	11	3	8,61	30197
	gi|57529615	D-beta-hydroxybutyrate dehydrogenase, mitochondrial precursor	Ketogenesis pathway	48	2,9	2	8,37	38237
8	gi|46048903	voltage-dependent anion-selective channel protein 2	Transport and carrier protein	492	31,1	10	8,61	30197
9	gi|22775582	ATP/ADP antiporter	Transport and carrier protein	251	13,4	7	9,78	32847
	gi|54020693	adenine nucleotide translocator 3	Transport and carrier protein	249	13,4	7	9,73	32748
	gi|57530120	ADP/ATP translocase 1	Transport and carrier protein	185	11,1	5	9,74	32968
	gi|57525378	Phosphate carrier protein, mitochondrial	Transport and carrier protein	58	3,6	2	9,33	37421
10	gi|5834847	Cytochrome c oxidase subunit II (mitochondrion)	Oxidative phosphorylation	248	16,3	8	4,57	25568
	gi|118093103	PREDICTED: thioredoxin-dependent peroxide reductase, mitochondrial isoform X4	Antioxidant protein	148	8,7	4	8,4	30992
11	gi|71895513	Cytochrome c oxidase subunit 4 isoform 1, mitochondrial	Oxidative phosphorylation	469	36,3	14	8,91	19631

NADH dehydrogenase [ubiquinone] iron-sulfur protein 3 (NDUFS3); Prohibitin (PHB) and Prohibitin-2 (PHB2).

DOCK2 and E1C825 interact with VDACs just in chicken brain mitochondria [18]. DOCK2 is a guanyl nucleotide exchange factor expressed exclusively in brain microglia, and is regulated by PGE2 receptor EP2. It has been described that ablation of DOCK2 reduced amyloid burden in a model of Alzheimer´s disease [38].

PHBs have been functionally linked to diverse processes, such as transcriptional regulation, cell proliferation, development, and mitochondrial function [39]. Prohibitins exert a neuroprotective effect, by suppressing Reactive Oxygen Species (ROS) production and have a critical role in the activity of respiratory chain complex I [40]. It has been proposed that prohibitins promote longevity by moderating fat metabolism, mitochondrial proliferation and energy levels [41]. Its interaction with chicken brain VDAC, a gatekeeper in mitochondria mediated apoptosis is noteworthy.

In addition to proteins found in pre-complex II, four other proteins were identified in complex I. They are Calcium-binding mitochondrial carrier protein Aralar2 (Aralar2); Chondroitin sulfate glucuronyltransferase-like (CHPF2); Chain D Chicken Cytochrome Bc1 Complex Inhibited By An Iodinated Analogue Of The Polyketide Crocacin-D (3CWB_D) and NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9 (NDUA9).

Proteins Aralar2, CHPF2 and 3CWB_D interact with VDAC just

in chicken mitochondria [18]. Aralar2 or Citrin is an aspartate-gluatamate carrier [42]. The presence of citrin in this study highlights a differential VDAC interactome in avian neuronal mitochondria in comparison with bovine and rat brains.

The protein CHPF2 is involved in glycosaminoglycan biosynthesis and plays a key role in tissue development and morphogenesis, and also contributes to tumor formation and development [43].

Analysis of the data reported above indicate that the kinetic assembly of protein complexes containing the VDAC follows a pattern quite different between chicken, bovine and rat brain [18]. The presence of ACON, Aralar2, CHPF2, CMBL, DOCK2, MRP1, PRDX3, RBBP8, Xirp1 and other proteins associated with VDAC only in chicken brain mitochondria, is in fact remarkable, and differentiate them from those of mammals, certainly in terms of developmental mechanisms of diseases, cell death and bioenergetics. Further studies are required to investigate if the differences in VDAC interactome reflect in differential metabolic and pathologic mechanisms between these species.

Acknowledgement

The work was supported by a grant from Fundacao de Amparo a Pesquisa do Estado de Sao Paulo (2010/05560-6). Carla Rossini Crepaldi had a fellowship from Fundacao de Amparo a Pesquisa

do Estado de Sao Paulo (2009/06697-2). Biological samples were provided by Globoaves.

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