Neuroanatomical Pattern of Mitochondrial Complex I Pathology Varies between Schizophrenia, Bipolar Disorder and Major Depression

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Abstract

Background: Mitochondrial dysfunction was reported in schizophrenia, bipolar disorderand major depression. The present study investigated whether mitochondrial complex I abnormalities show disease-specific characteristics.

Methodology/Principal Findings: mRNA and protein levels of complex I subunits NDUFV1, NDUFV2 and NADUFS1, were assessed in striatal and lateral cerebellar hemisphere postmortem specimens and analyzed together with our previous data from prefrontal and parieto-occipital cortices specimens of patients with schizophrenia, bipolar disorder, major depression and healthy subjects. A disease-specific anatomical pattern in complex I subunits alterations was found. Schizophrenia-specific reductions were observed in the prefrontal cortex and in the striatum. The depressed group showed consistent reductions in all three subunits in the cerebellum. The bipolar group, however, showed increased expression in the parieto-occipital cortex, similar to those observed in schizophrenia, and reductions in the cerebellum, yet less consistent than the depressed group.

Conclusions/Significance: These results suggest that the neuroanatomical pattern of complex I pathology parallels the diversity and similarities in clinical symptoms of these mental disorders.

Introduction

The past decade has witnessed an abundance of studies focusing on mitochondrial abnormalities in several mental disorders including schizophrenia, bipolar disorder and major depression. The role mitochondria play in mental disorders has been investigated using a wide array of experimental techniques ranging from imaging studies through ultrastructural methods to genetic and molecular means.

Imaging studies using phosphorous magnetic resonance spectroscopy (^{31}P-MRS) and ^{1}H- MRS demonstrated reduced mitochondrial originated high energy phosphates, such as ATP and phosphocreatine (PCr) as well as other cellular factors whose metabolism is strongly suggested to be linked to mitochondrial ATP production, in schizophrenia relevant brain structures of schizophrenic patients [1–8]. In bipolar disorder similar mitochondrial abnormalities have been reported [9–11], while in major depression, the current literature on MRS studies is sparse and inconsistent [12–14].

Genetic studies also implicate mitochondria abnormalities in schizophrenia and in affective disorders. For example, two single nucleotide polymorphisms (SNPs) in a nuclear encoded subunit of complex I, NDUFV2, were found to be associated with schizophrenia and with bipolar disorder [15,16]. Additional genetic variations in mitochondrial DNA encoded ND3 and ND4 subunits of complex I were associated with bipolar disorder and schizophrenia, respectively [17,18]. These studies suggest the genetic variation in complex I as a risk factor in both disorders.

Finally, accumulating molecular, transcriptomic, proteomic and metabolomic approaches as well as biochemical data points to abnormalities in mitochondria in both periphery and brain in schizophrenia [19–28]. Focusing on the mitochondrial oxidative phosphorylation system (OXPHOS) in schizophrenia, revealed alterations in the enzymatic activities of complexes IV, II and I–III and in mRNA and protein levels of complex I subunits, NDUFV1 and NDUFV2, in post-mortem brain specimens [26,29–32]. Similarly, alterations both in complex I activity and its subunit expression were observed in peripheral blood cells of schizophrenic patients [22,27,33,34]. In bipolar disorder a reduction in the expression level of mitochondrial genes, including those of the OXPHOS was observed in hippocampal and prefrontal postmortem specimens [35–37], while an increase in complex I subunits NDUFV1 and NDUFV2 was observed in the parieto-occipital cortex [26]. In major depression, although most studies did not show cortical modifications in mitochondrial related genes, some reports suggest alterations in the expression of nuclear as well as in mitochondrial DNA encoded genes in the prefrontal cortex [26,37]. In addition, it was demonstrated that muscle mitochon-

dria in depressed patients produced less ATP and that the activity of the OXPHOS complexes I+III and II+III was impaired [38].

The studies described hitherto suggest a dysregulation of mitochondrial function in schizophrenia and mood disorders, consequently raising the question as to whether mitochondrial impairment displays disease-specific characteristics or is rather a general non-distinguishing pathology of these disorders. Complex I, the focus of the present study, plays a major role in controlling oxidative phosphorylation, and therefore mitochondrial function [39]. The aim of the present study was to determine whether complex I abnormalities show disease-specific characteristics. mRNA and protein levels of three subunits of complex I, NDUFV1, NDUFV2 and NDUFS1, all forming one functional subunit, were assessed in postmortem brain specimens of striatum and cerebellum of patients with schizophrenia, bipolar disorder or major depression and normal subjects, and analyzed together with our previous data from prefrontal and parieto-occipital cortices of the same cohorts. Tables 1, 2, 3 summarize the main clinical, functional, biochemical and pathogenic characteristics of the different mental disorders, brain areas and complex I subunits investigated in the present study. The abnormality in mitochondrial complex I subunits' parameters demonstrated disease-specific regional distribution discriminating between schizophrenia, bipolar disorders and major depression.

Materials and Methods

Post-mortem tissues

Frozen samples from the striatum including the nucleus accumbens, and the lateral cerebellar hemisphere, were provided by the Stanley Foundation Neuropathology Consortium (Bethesda, MD). Samples were obtained from individuals diagnosed (DSM-IV criteria) with schizophrenia, bipolar disorder or major depression and normal controls 15 subjects in each group. Medication

undertaken by each patient is summarized in Table 4. The four groups are matched by age, sex, race, postmortem interval (PMI), pH, laterality, and mRNA quality. Demographics are presented in Table 5. A more detailed description of the Stanley Brain Collection, and protection of human rights is reported in [40]. All 60 samples were analyzed in parallel, blind to patients' diagnosis.

RT-PCR

RNA was extracted from tissue using RNA STAT-60 kit (TEL-TEST, INC, Frienwood, TX,) and treated by DNase as described previously [27,41]. RNA integrity depicted in the form of three bands corresponding to 28S, 18S and 5S-RNA was assessed by electrophoresis, and its amount and purity was determined spectophotometricaly. The expression of NDUFV1, NDUFV2 and NDUFS1 encoding for 51-kDa, 24-kDa and 75-kDa subunits of complex I, respectively, was studied by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Amplification of RT-cDNA was first performed on a control specimen at different concentrations to define a linear range for all genes. Number of cycles, cDNA amount and primers concentration were established according to a stringent calibration process determining the log-linear phase of amplification for each gene. After establishing the optimal reaction conditions, amplification was performed at least twice for each individual. Sequences of PCR primers for NDUFV1, NDUFV2, NDUFS1, 18S-RNA and β-actin are summarized in Table 6. β-actin and 18S-RNA were used for assessment of RNA quality and yield. β-actin and 18S-RNA were used for assessment of RNA quality and yield. The β-actin was used for normalizing variations in RNA aliquots, as its levels were not affected by disease. A single batch of human platelets RNA, on which PCR was performed at three different concentrations, was assayed in parallel with each set of samples as a positive control, to control for the log-linear phase of amplification for each reaction and for between sample-sets normalization.

Table 1. Clinical characteristics of the three different mental disorders investigated in the present study.

Disease	Population prevalence	Typical age of onset	Gender differences	Symptoms according to the Diagnostic and statistical Manual of Mental Disorders –DSM-IV
Schizophrenia	1.1% [a]	M – 18 yr F – 25 yr	F = M	The essential features of Schizophrenia are a mixture of characteristic signs and symptoms (both positive and negative) that have been presented for a significant portion of time during a 1 month period with some signs persisting for at least 6 months. These signs include cognitive, emotional and behavioral anomalies.
				Positive symptoms: delusions, hallucinations, disorganized speech (eg. frequent derailment or incoherence), grossly disorganized or catatonic behavior, Negative symptoms, i.e. affective flattening, alogia or avolition.
				Two or more of these symptoms each present
Bipolar disorder (BP)	2.6% [a]	20–35 yr	F = M	The essential feature of BP is the occurrence of one or more Manic episodes. Often individuals have on or more MD episodes whose symptoms are summarized for MD.
				Manic episode: inflated self-esteem or grandiosity, decreased need for sleep, flight of ideas, distractibility, increase in goal-directed activity or psychomotor agitation, excessive involvement in pleasure activitiesthat have high potential of painful consequences.
				Three or more of these symptoms lasting for at least 1 week.
Major depression (MD)	5.3% [a]	30–40 yr with wide variations	F>M	The essential feature of MD is a period of at least 2-weeks during which there is either depressed mood or lost of interest or pleasure in nearly all activities.
				Five or more of the following symptoms presented during 2-weeks and represent change from previous functioning: Depressed mood, markedly diminished interest or pleasure, significant weight loss or weight gain, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, felling worthless or excessive inappropriate guilt, diminished ability to think or concentrate, recurrent thoughts of death.

Table 2. Functional characteristics of the four different brain areas investigated in the present study.

Brain area sections	Location	Function	Reference:
Striatum including the nucleus accumbens		Caudate: motor control, learning and memory (especially feedback processing), language comprehension	[91–98]
		Putamen: reinforcement learning	
		Nucleus accumbens: reward, addiction and emotions such as pleasure and laughter, fear, and the placebo effect.	
Cerebellum		Balance, coordination, muscle tension, posture, balance of limbs, fine motion control and eye movement. Recent findings suggest a role in mood and cognition (70)	[99–102]
BA 46/9	Dorsolateral prefrontal cortex	Motor planning, organization, and regulation. It plays an important role in the integration of sensory and mnemonic information and the regulation of intellectual function and action and working memory.	[103–106]
BA 19	Extrastriate cortex in the occipital cortex	A visual association area, with feature-extracting, shape recognition, attentional, and multimodal integrating functions	[107–109]

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Immunoblotting

Protein isolated from frozen specimens was analyzed by immunoblotting three times for each individual [26]. Protein samples were separated on SDS-PAGE. Primary customized recombinant rabbit antihuman antibodies used; anti-51-kDa 1:500, anti-24-kDa 1:1500, anti-75-kDa 1:1000 synthesized by Sigma-Aldrich, Israel. Secondary antibodies used; anti rabbit-IgG 1:15,000 for 24-kDa, and 1:10,000 for 51-k-Da and 75-kDa subunits (Santa Cruz Biotechnology Santa Cruz, CA). β-actin was used for normalizing variations in protein aliquots, as its levels were not affected by disease. In addition, a single batch of rat brain mitochondrial protein in three different concentrations was used as a positive control and for between sample-sets normalization.

Statistical analysis

Normal distribution of data was analyzed by Kolmogorov-Smirnov test. For further analysis parametric tests were used, as most data (90%) presented normal distribution. For data not distributed normally, non-parametric and parametric tests showed similar differences between groups. Data were analyzed by two-way ANOVA followed by Bonferroni post-hoc test. Age, gender, laterality, PMI, brain pH, disease duration, drug and alcohol abuse and medication were added as covariates and persistence of the significant difference in main effect between diagnostic groups was assessed by ANCOVA. Correlations were analyzed by Pearson correlation test. SPSS version 14.0 software was used.

Table 3. Biochemical and pathogenic characteristics of complex I subunits investigated in the present study.

Complex I subunit	Biochemical function	Pathology associated with mutations or polymorhism
NDUFV1	Flavoprotein, contains the NADH-binding site, and binds NADH released electron together with NADUV2.	Leigh-Like syndrome with early onset Ophthalmoplegia (611A→G (Y204C))/ 616T→G (C206G) [112]
	Catalytic sites:	Mitochondrial Complex I Deficiency - 611A→G (Y204C); 616T→G (C206G); 640G→A (E214K);1294G→C (A432P) ; Deletion nt 989–990 [113]
	flavin mono-nucleotide (FMN)	Leukodystrophy and myoclonic epilepsy 175C→T (R59X); 1268C→T (T423M) [114]
	4Fe-4S cluster (N3) [110,111]	
NDUFV2	Flavoprotein that together with NDUFV1 binds the electron and passes it probably to NDUFS1 Catalytic sites:	Schizophrenia – polymorphisms – rs56506640 (−3542A>G); rs 51156044 (−602G→A) [115]
	2Fe-2S cluster (N1a)[110,111]	Bipolar disorder – polymorphisms- rs56506640 (−3542A>G); rs 51156044 (−602G→A) [115,116]
		Early onset hypertrophic cardiomyopathy and encephalopathy- 4-bp deletion- IVS2 +5÷+8 (GTTA) [117]
		Parkinson's disease Parkinson's disease – polymorphism (182C→T) [118]
NDUFS1	Iron sulfur protein. The largest transmembrane subunit of complex I.	Mitochondrial Complex I Deficiency –721C→ T (R241W); 755 A→G (D252G); 2119 A→G (M707V);664–666 3 bp deletion 222 [113]
	Catalytic sites:	
	2Fe-2S cluster (N1b)	
	4Fe-4S cluster (N4)	
	and probably 4Fe-4S cluster (N5) [110,111]	

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Table 4. Summary of medication undertaken by each patient in each diagnostic group.

Psychiatric diagnosis		
Schizophrenia	**Bipolar**	**Major depression**
Subject #		
1 Thiothixene, desipramine	Thiothixene, carbamazepine, lithium, trazadone	Imipramine, amitriptyline, nortiptyline, clonazepam
2 None; untreated for over 20 yrs.	Valproate, sertraline, chlorprothixene, carbamazepine	Lithium
3 None; untreated for several months	Lithium, bupropion, clonazepam, lorazepam	Fluoxetine, imipramine, lorazepam
4 None; had ECT but probably never treated otherwise	Lithium, carbamazepine	Phenytion for a single seizure; no other meds for 5 yrs
5 Thioridazine, amitriptyline	Lithium, clozapine	No medication for 6 yrs
6 Clozapine	Never treated	Diphenhydramine, cloxazepam
7 Clozapine	Haloperidol, diphenhydramine	Fluoxetine, lithium
8 Haloperidol, iphenhydramine	Risperidone, valproate, venlafaxine	Nefazadone, hydroxyzine
9 Risperidone, paroxetine	Untreated for over 20 years	Never treated
10 Haloperidol, carbamazepine, fluoxetine, clonazepam, benzotropine	Halperidol, trazadone, trihexphenidyl	Temazepam but off medications for more than 2 months
11 Clozapine, chlorpromazine, lithium	Valproate, bupriopion	Sertraline
12 Haloperidol, lithium, diphenhydramine, chloral hydrate	None, untreated for several months	Venlafaxine, buspirone, alprazolam
13 Clozapine, chlorpromazine, maprotiline, benzotropine, diphenhydramine	Fluoxetine, valproate	None
14 Haloperidol, clozapine, clonazepam	Valproate, clozapine, flurazepam, benzotropine	Trimipramine
15 Risperidone, thioridazine	Valproate, clomipramine	Fluoxetine, nefazadone

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Results

Protein and mRNA levels of complex I subunits, 51-, 24- and 75-kDa, were analyzed in two brain specimens obtained from the striatum and the lateral cerebellar hemisphere (Fig 1) of patients with schizophrenia, bipolar disorder, major depression and normal subjects. In a previous study we have shown alterations in the same three subunits, in specimens obtained from the prefrontal (BA9/46) and the parieto-occipital (BA19) cortices of the same subject cohorts [26]. Results of the two studies were combined and analyzed by two-

Table 5. Demographic data for post mortem brains.

Variable	Control (n = 15)	Schizophrenia (n = 15)	Bipolar disorder (n = 15)	Major depression (n = 15)
Age (years, means±S.D.)	48.1±10.7	44.53±13.11	42.3±11.7	46.4±9.3
Gender (male, female)	9M, 6F	9M, 6F	9M, 6F	9M, 6F
Postmortem interval (h, means±S.D.)	23.7±9.94	33.94±14.62	32.5±16.1	27.5±10.7
Cause of death				
Cardiac	13	6	4	7
Accident	2	2	1	
Suicide		4	9	7
Other		3	1	1
Age of onset (years, means±S.D.)	N/A	23.20±7.95	33.93±13.29	21.47±8.35
pH (means±S.D.)	6.3±0.2	6.2±0.26	6.2±0.2	6.2±0.2
Brain hemisphere used (right:left)	8:7	9:6	7:8	9:6
Lifetime antipsychotic dose[a] (mg, means±S.D.)	0	52267±62061	20827±24016	0
History of psychosis		15	11 with	
			4 without	
Current alcohol/drug abuse or dependence	0	3	4	3
Past alcohol/drug abuse or dependence	2	3	3	1

[a]Lifetime antipsychotic dose in fluphenazine milligram equivalents. N/A – not applicable.
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Table 6. Primer sequences and PCR conditions.

mRNA		primer sequence	Denaturing temperature and time °C (s)	Annealing temperature and time °C (s)	Elongation temperature and time °C (s)	Number of cycles	Product size (bp)
NDFUV1	S	5'-TACATCCGAGGGGAATTCTACA-3'	94 (60)	60 (60)	72 (60)	35	426
	NS	5'-GTTCTTTCAAGGGCACAGACAT-3'					
NDUFV2	S	5'-GGAGGAGCTTTATTTGTGCAC-3'	94 (60)	55 (60)	72 (60)	35	640
	NS	5'-CCTGCTTGTACACCAAATCC-3'					
NDUFS1	S	5'- TACTCGCTGCATCAGGTTTG-3'	94 (60)	58 (60)	72 (60)	35	299
	NS	5'-CATGCATACGTGGCAAAATC-3'					
β-actin	S	5'-TGAAGTGTGACGTGGACATCCG-3'	94 (60)	60 (60)	72 (60)	25	447
	NS	5'-GCTGTCACCTTCACCGTTCCAG-3'					
18S-RNA	S	5'-AGGAATTGACGGAAGGGCAC-3'	94 (60)	60 (60)	72 (60)	25	324
	NS	5'-GTGCAGCCC CGGACATCTAAG-3'					

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way ANOVA with the four subject cohorts and 4 brain areas as the two independent variables and mRNA and protein levels of 51-, 24- and 75-kDa as the dependent variables. The 75-kDa subunit was assessed in three brain regions, as parieto-occiptal cortex specimen was insufficient. A highly significant interaction between disease cohorts and brain areas for all dependent variables was observed
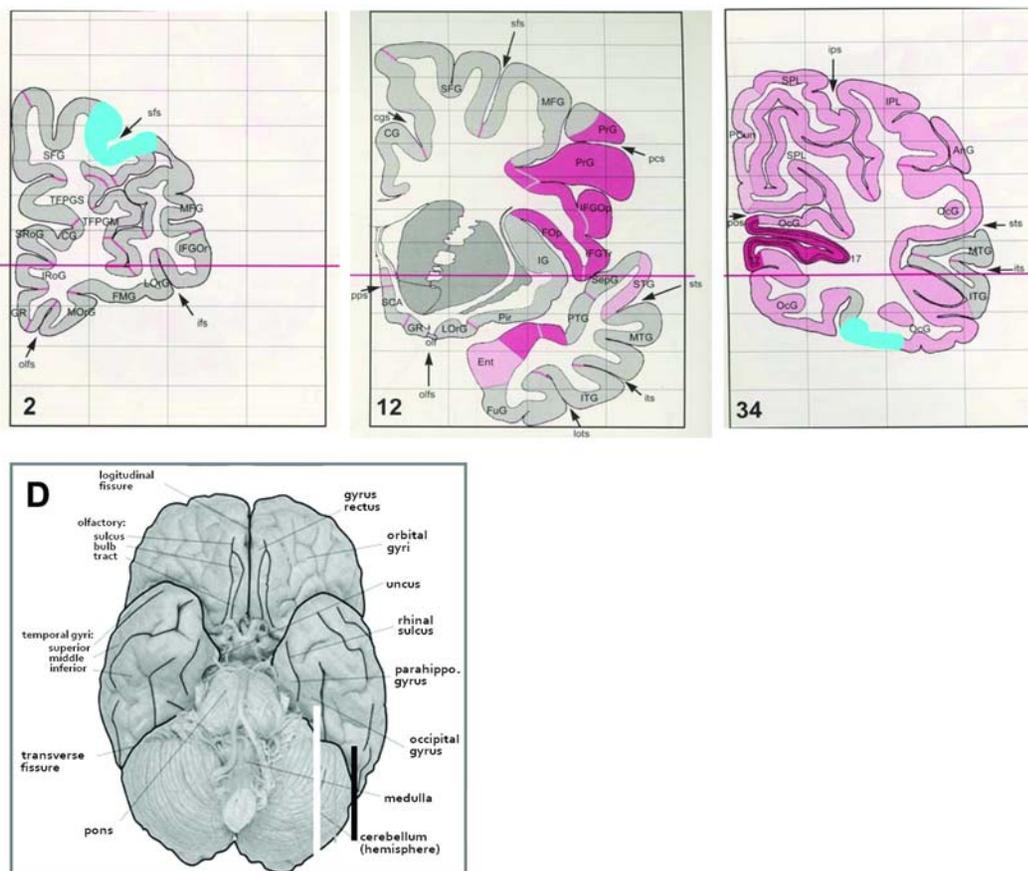
Figure 1. Diagrams of brain section presenting the four different brain areas in which complex I subunits were assessed. A) The prefrontal cortex (BA 9/46) sections are 14 μm frozen coronal sections though the area marked in blue B) The striatum sections are 14 μm frozen coronal sections through the head of the caudate nucleus and putamen at the level of the nucleus accumbens. C) The parieto-occipital cortex (BA 19) sections are 14 μm frozen coronal sections though the area marked in blue. D) The cerebellar sections are 14 um frozen sagittal sections through the lateral cerebellar hemisphere at the level marked by the green line. Diagram are obtained from the Atlas of the Human Brain by Jurgen K. Mai, Joseph Assheuer and George Paxinos, 1997 3rd Ed. pp. 123, 124, 126 Elsevier Ltd.
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Table 7. Two way ANOVA results of disease and brain area dependent alterations in complex I subunits.

	Dependent Variable	df	F	Sig.
Cohort	NDUFV1	3	8.131	0.000
	NDUFV2	3	11.297	0.000
	NDUFS1	3	9.646	0.000
	51-kDa	3	3.513	0.020
	24-kDa	3	5.663	0.001
	75-kDa	3	6.935	0.000
Area	NDUFV1	3	43.306	0.000
	NDUFV2	3	152.980	0.000
	NDUFS1	2	16.815	0.000
	51-kDa	3	368.109	0.000
	24-kDa	3	432.844	0.000
	75-kDa	2	399.590	0.000
Cohort*Area	NDUFV1	9	2.795	0.004
	NDUFV2	9	9.008	0.000
	NDUFS1	6	4.773	0.000
	51-kDa	9	7.148	0.000
	24-kDa	9	7.819	0.000
	75-kDa	6	4.295	0.000

The NDUFV1, NDUFV2 and NDUFS1 and 24-, 51- and 75-kDa stand for mRNA and protein levels of the three subunits of complex I, respectively. All subunits were analyzed in 4 brain areas, the striatum, the lateral hemisphere of the cerebellum, the prefrontal cortex (BA9/46) and the parieto-occipital cortex (BA19) in schizophrenia, bipolar disorder and major depression and normal subjects. The 75-kDa subunit was analyzed in all brain areas except the parieto-occipital cortex (BA19).
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(Table 7), suggesting that the different disease cohorts differentially affect complex I subunits expression in various brain areas.

Alterations in complex I subunits in the prefrontal and the parieto-occipital cortices which were previously discussed [26] and are presented in Table 8. Data of complex I subunits' expression in the striatum and the cerebellum are further analyzed for disease related effects.

Complex I subunits in the striatum

The significant difference in mRNA levels between the four subject cohorts for each subunit of complex I (NDUFV1-$F_{(3,56)} = 5.838$, $p = 0.002$; NDUFV2-$F_{(3,56)} = 3.781$, $p = 0.015$; NDUFS1-$F_{(3,56)} = 10.155$, $p = 0.0001$) was due to a significant decrease in levels of NDUFV1 (41%; $p = 0.003$), NDUFV2 (46%; $p = 0.0001$), and NDUFS1 (60%; $p = 0.0001$) in the schizophrenic group as compared to controls. The bipolar and depressed groups did not differ from the controls. Interestingly, the schizophrenic group differed significantly from both mood disorder groups (NDUFV1, $p = 0.004$ vs. bipolar; NDUFV2, $p = 0.03$ vs. depressed; NDUFS1, $p = 0.009$ and $p = 0.000$ vs. bipolar and depressed, respectively). However, no difference was observed between depressed and bipolar patients (Fig 2).

Protein levels of the 51-kDa (NDUFV1) and 24-kDa (NDUFV2) subunits, but not of 75-kDa (NDUFS1), showed a parallel pattern of change to that of the mRNA, throughout the four cohorts ($F_{(3,56)} = 5.604$, $p = 0.002$, $F_{(3,56)} = 6.704$, $p = 0.001$ and $F_{(3,56)} = 6.067$, $p = 0.001$, respectively) (Fig 3). Thus, a significant decrease of 51-kDa (31%; $p = 0.040$) and 24-kDa (30%; $p = 0.045$) subunits, was observed in the schizophrenic group. While the depressed group did not differ from the controls for all three subunits, the bipolar group showed a slight, yet significant, decrease in the 75-kDa subunit (17%; $p = 0.045$). Similar to the findings in mRNA, the schizophrenic group differed significantly from both mood disorder groups (51-kDa, $p = 0.002$ vs. depressed; 24-kDa, $p = 0.001$ vs. bipolar; 75-kDa,

Table 8. Summary of mRNA and protein levels in four different brain areas.

	Striatum		Cerebellum		Prefrontal cortex (BA 46/9)		Parieto-occipital cortex (BA 19)	
NDUFV1 (51 kDa)								
	mRNA	protein	mRNA	protein	mRNA	protein	mRNA	protein
Normal	1.83±0.51	1.78±0.37	1.25±0.27	1.63±0.32	1.43±0.68	0.80±0.21	0.37±0.14	1.66±0.30
SCH	**1.09±0.26 ***	**1.35±0.24 ***	1.28±0.3	1.25±0.60	**0.83±0.16 ***	**0.66±0.31 ***	**0.59±0.44***	**1.92±0.17 ***
MD	1.52±0.46	1.95±0.34	**1.00±0.31 ***	**0.83±0.24 ***	1.02±0.50	0.77±0.40	**0.28±0.35***	1.73±0.28
BP	1.81±0.84	1.53±0.68	**0.88±0.24 ***	**0.92±0.22 ***	1.49±1.20	0.76±0.32	0.40±0.23	**2.24±0.63 ***
NDUFV2 (24-kDa)								
	mRNA	protein	mRNA	protein	mRNA	protein	mRNA	protein
Normal	1.24±0.32	1.03±0.22	1.59±0.48	1.04±0.17	0.50±0.07	0.89±0.24	0.31±0.17	1.35±0.18
SCH	**0.67±0.18***	**0.73±0.12 ***	1.34±0.64	1.06±0.45	**0.34±0.09***	**0.39±0.19 ***	**0.66±0.37***	**1.92±0.36 ***
MD	1.23±0.81	0.93±0.19	**0.85±0.30 ***	**0.57±0.22 ***	0.43±0.16	**0.49±0.31 ***	0.29±0.28	1.5±0.32
BP	1.12±0.61	1.23±0.49	1.82±0.62	0.78±0.21	0.56±0.19	0.74±0.25	0.39±0.25	**1.81±0.42 ***
NDUFS1 (75-kDa)								
	mRNA	protein	mRNA	protein	mRNA	protein	mRNA	protein
Normal	1.27±0.58	2.63±0.52	0.82±0.19	1.40±0.13	1.17±0.69	1.08±0.19	ND	ND
SCH	**0.55±0.16 ***	2.61±0.33	0.73±0.50	1.04±0.34	1.14±0.32	1.01±0.13	ND	ND
MD	1.45±0.55	2.85±0.50	**0.52±0.25***	**0.95±0.29***	1.30±0.65	0.94±0.15	ND	ND
BP	1.09±0.56	**2.20±0.27 ***	0.62±0.14	**0.97±0.23 ***	0.92±0.54	0.98±0.19	ND	ND

Results are Mean±SD of arbitrary standardized densitometry values. *p<0.05 compared to control. MD-major depression, BP-bipolar, SCH- schizophrenic. (The data of the prefrontal and parieto-occipital cortices are calculated from the data published in Karry et al. 2002).
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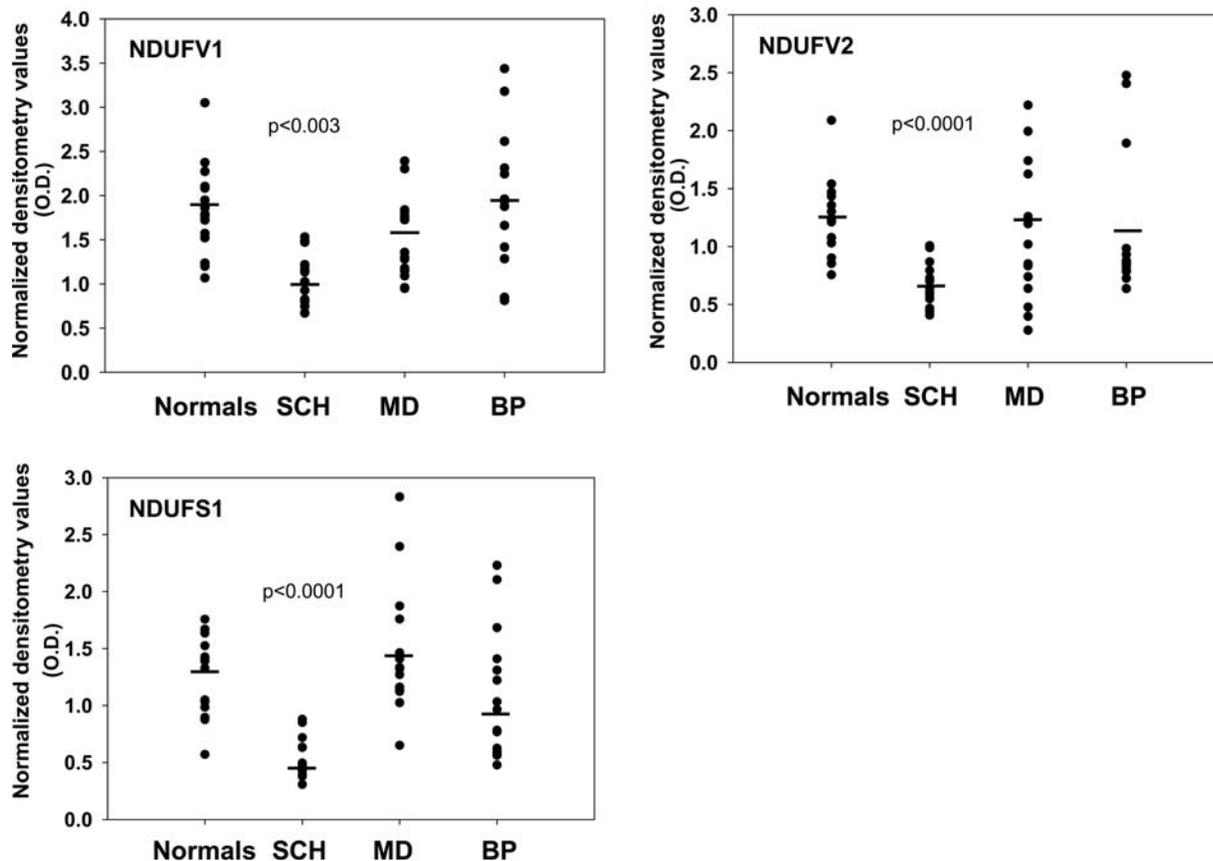
Figure 2. mRNA levels of NDUFV1, NDUFV2 and NDUFS1 subunits of complex I in post mortem striatum including the nucleus accumbens of patients with schizophrenia (SCH, n = 15), major depression (MD, n = 15) and bipolar disorder (BP, n = 15), and of normal controls (n = 15). Statistical significant differences vs. the control group were observed only in the schizophrenic group in all three subunits of complex I.

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p = 0.017 vs. bipolar). The bipolar and the depressed patients did not differ in 51-kDa and 24-kDa subunits but differed significantly in the 75-kDa subunit (p = 0.000).

Complex I subunits in the cerebellum

The lateral cerebellar hemisphere, which was planned to serve as a control area, showed interesting significant inter-group differences in mRNA of complex I subunits (NDUFV1-F(3,56) = 23.780, p = 0.0001, NDUFV2-F(3,56) = 12.234, p = 0.0001 and NDUFS1-F(3,56) = 4.331, p = 0.008). These changes were primarily due to the decrease in NDUFV1 (57%, p = 0.014), NDUFV2 (47%, p = 0.003) and NDUFS1 (36%, p = 0.038) in the depressed group as compared to controls, with less pronounced, but still significant, changes in the bipolar group in NDUFV1 (57%, p = 0.001) and NDUFS1 (30%), which did not reach significance (Fig 4). Unlike the findings in striatum, the schizophrenic group did not show significant changes from control in any of the three subunits. However, a significant difference was observed between the schizophrenic group and both groups with mood disorders. (NDUFV1, p = 0.000 vs. both bipolar and depressed; NDUFV2, p = 0.000 vs. depressed). No significant difference was observed between the depressed and the bipolar groups.

Protein levels showed a parallel pattern of change to that of mRNA in the lateral cerebellar hemisphere (51-kDa-F(3,56) = 13.833, p = 0.0001; 24-kDa-F(3,56) = 8.044, p = 0.0001; 75-kDa-F(3,56) = 4.331, p = 0.008) (Fig 5). Thus, a significant decrease in the levels of the 51-kDa (43%, p = 0.001) and 75-kDa (30% p =

0.024) subunits was observed in the bipolar group and of 51-, 24- and 75-kDa subunits (49%, p = 0.0001, 45%, p = 0.001, 32% p = 0.014, respectively) in the depressed group. The schizophrenic group showed no significant difference from the control group in any of the subunits, similar to its mRNA findings, but was significantly different from the depressed group (51-kDa, p = 0.015; 24-kDa, p = 0.001). No significant difference was observed between the bipolar group and both the depressed or the schizophrenic group.

Demographic parameters, covariance analysis

To control for potential confounds, age, gender, PMI, brain pH, side of brain, duration of disease, age of onset, severity of alcohol and drug abuse and psychotropic medication were added as covariates, and assessed by ANCOVA for all three subunits in the striatum and in the cerebellum. Severity of alcohol and drug abuse was scored from 0-no use to 6-heavy use. Disease related significant differences, observed in mRNA and protein levels of 51-, 24- or 75-kDa subunits, were not altered by any of the parameters used as covariant in all groups, including pH, alcohol and drug abuse and lifetime antipsychotic dose, in both brain areas for all patient groups, except for protein and mRNA levels of the 75-kDa, in which onset and duration of illness obliterated the disease related significance, but showed no significant correlation with 75-kDa mRNA or protein levels in any patient group. Table 8 presents disease-related adjusted means±SD of mRNA and protein after ANCOVA, for all three genes in the four cohorts.
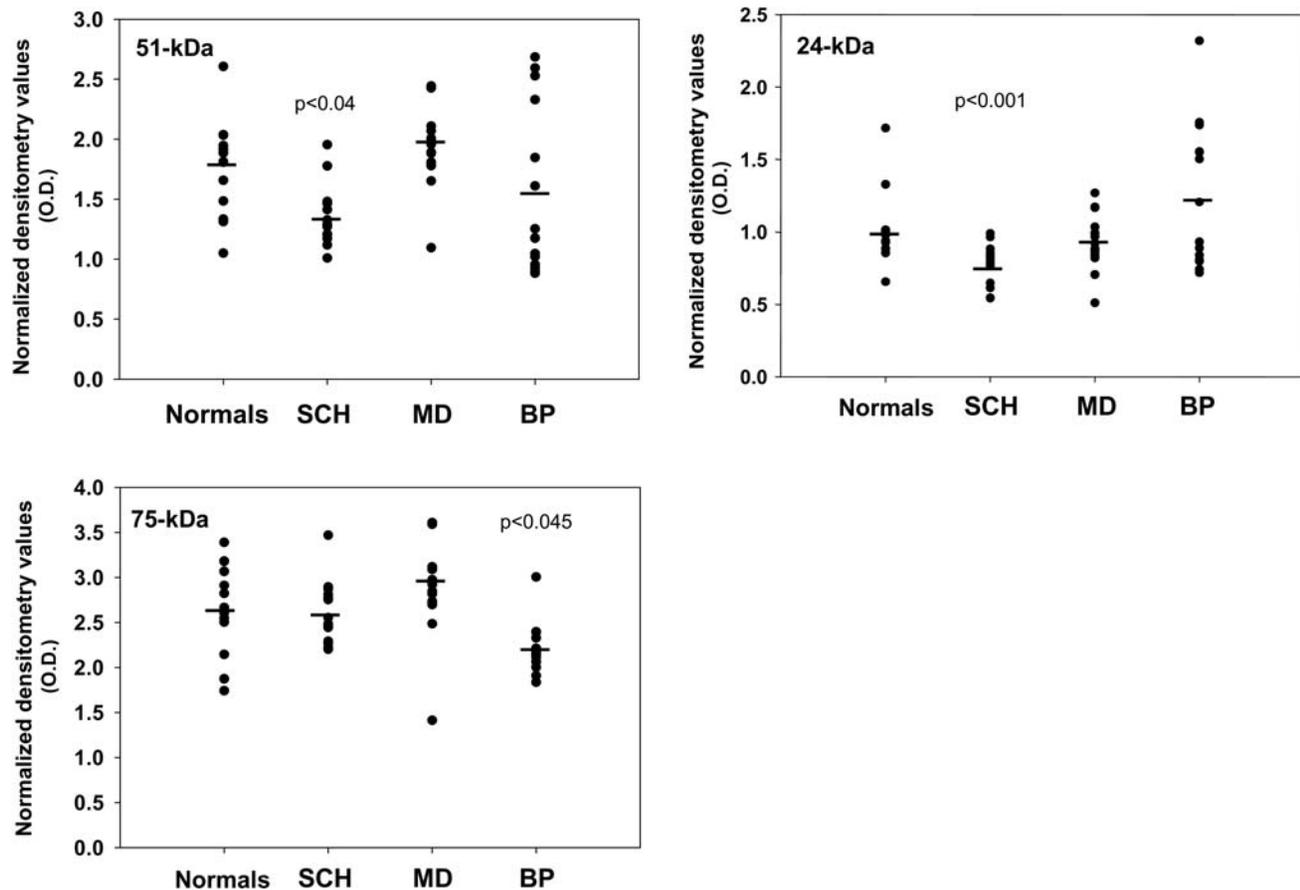
Figure 3. Protein levels of 51-, 24-and 75-kDa subunits of complex I in post mortem striatum including the nucleus accumbens of patients with schizophrenia (SCH, n = 15), major depression (MD, n = 15) and bipolar disorder (BP, n = 15) and of normal controls (n = 15). Statistical significant differences vs. the control group were observed in the 51- and 24-kDa subunits of the schizophrenic group and in the 75-kDa subunit of the bipolar group. No statistically significant difference was observed in the depressed group.
doi:10.1371/journal.pone.0003676.g003

Effect of antipsychotic medication

Given the reported effects of antipsychotic drugs on brain energy metabolism in motor areas, specifically in the basal ganglia [42–44], together with their inhibitory effect on complex I activity [30,33,45], we redefined our group variable to define 2 subgroups; schizophrenic or bipolar patients medicated (12 schizophrenic, 7 bipolar patients) and unmedicated (3 schizophrenic, 8 bipolar patients) with antipsychotic drugs. Three schizophrenic patients and 3 bipolar patients were medication free and the other 5 bipolar patients received other psychotrophic drugs. No significant difference was observed between the two subgroups in mRNA or protein levels of all three subunits in the striatum and the cerebellum. In addition, there was no significant correlation between antipsychotic dose, expressed in fluphenazine milligram equivalents, and mRNA or protein of any of the three subunits in the antipsychotic medicated patients.

Effect of antidepressant medication

To asses possible effects of antidepressant medications on complex I subunits in depressed subjects, we divided the depressed group into 2 subgroups: subjects medicated with antidepressant drugs (n = 9) and unmedicated (n = 6) subjects (two of them treated with other psychotropic drugs). No significant difference between groups was observed in mRNA or protein levels of all three subunits in the two brain areas.

Effect of mood stabilizers

It was recently reported that lithium can affect genes of the mitochondrial OXPHOS [46]. To asses possible effects of mood-stabilizers on complex I subunits, we divided the bipolar group into 2 subgroups: subjects medicated with mood-stabilizers (n = 10) and umedicated (n = 5) subjects, 2 of them treated with other psychotropic drugs. No significant difference was observed between the two subgroups in mRNA or protein of all three subunits in both brain areas. We then redefined our group variable to include 2 subgroups: subjects receiving mood-stabilizers (n = 16; 10 bipolar, 3 depressed, 3 schizophrenics) and subjects not receiving mood-stabilizers (n = 44), again there was no significant difference between groups in both mRNA and protein of all three subunits in the two brain areas.

Discussion

This study compares mRNA and protein levels of three subunits of mitochondrial complex I in different brain areas of schizophrenic, bipolar and depressed patients and normal subjects. The main finding of the present study is that complex I subunits are altered in all three psychiatric disorders, albeit in a disease specific neuroanatomical pattern. In schizophrenia, but not in affective disorders, a selective reduction in the 51- and 24-kDa subunit expression was observed in the striatum. However, in both affective disorders, reductions in complex I subunits were observed
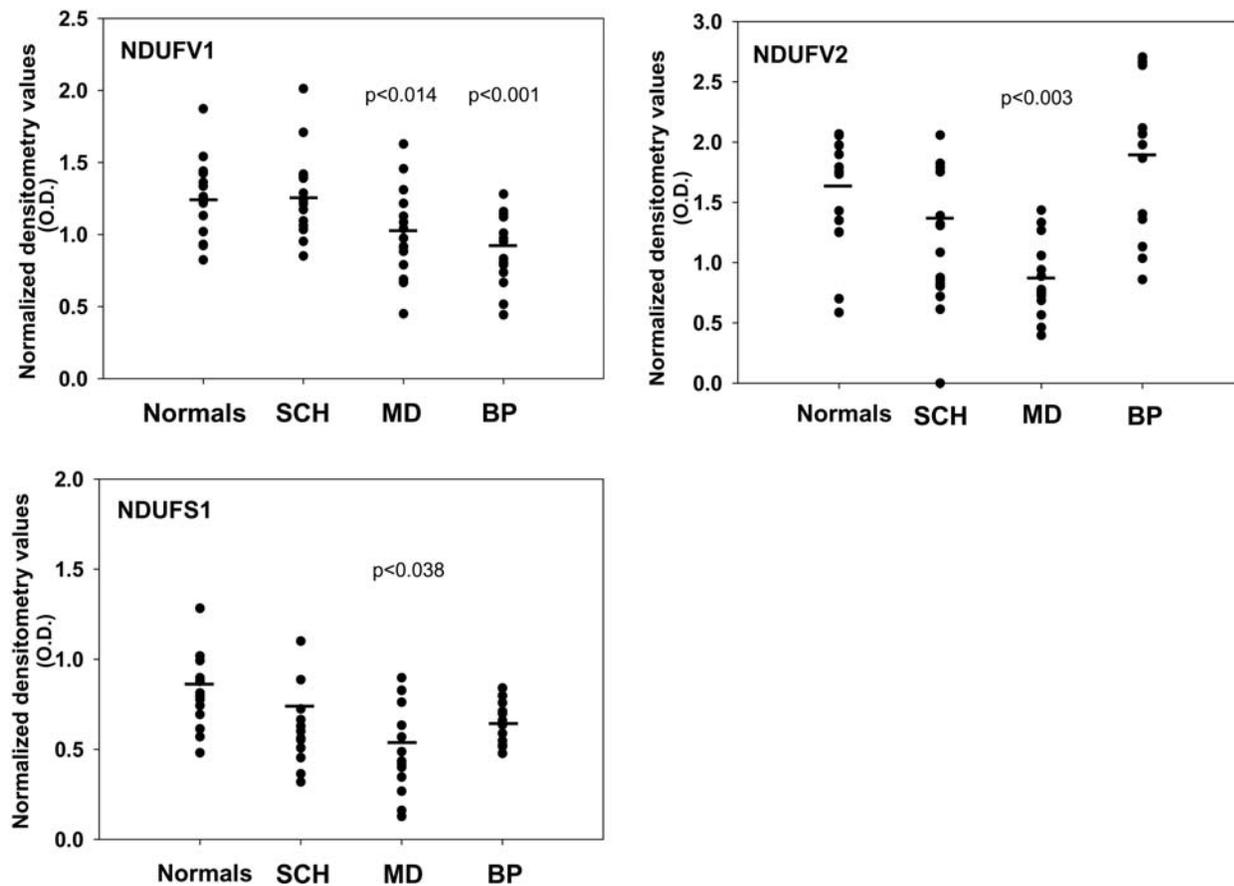
Figure 4. Cerebellar lateral hemisphere mRNA expression of complex I subunits. mRNA levels of NDUFV1, NDUFV2 and NDUFS1 subunits of complex I in post mortem cerebellar lateral hemisphere of patients with schizophrenia (SCH, n = 15), major depression (MD, n = 15) and bipolar disorder (BP, n = 15), and of normal controls (n = 15). Statistical significant differences vs. the control group were observed in all three subunits in the major depression group and in the NDUFV1 in the bipolar group. No statistically significant difference was observed in the schizophrenic group.
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specifically in the cerebellum, with the depressed group demonstrating more consistent alterations. Thus, the depressed patients showed significant reductions in mRNA and protein of all three subunits of complex I, while in the bipolar group mRNA and protein of the 24-kDa subunit and only protein levels of the 75-kDa subunit were reduced, with no significant changes in the 51-kDa subunit. The phenomenon of a disease-specific regional distribution of aberrant expression of complex I subunits was also observed in our previous study on the same subject cohorts [26], demonstrating a schizophrenia-specific reduction in the 51- and 24-kDa expression in the prefrontal cortex (BA 46/9). In the parieto-occipital cortex (BA19), however, an increased expression of these subunits was observed both in schizophrenic and bipolar patients. In the depressed group, no consistent change was observed in both brain areas. Interestingly, similar to schizophrenia, the bipolar group demonstrated some abnormality in the striatum, as the 75-kDa protein levels were decreased in this area. Taken together, the results of both studies indicate that while the schizophrenic and depressed groups display disparate regional distribution of complex I alterations, the bipolar group shares considerable similarities with both the schizophrenic and depressed groups. The latter is in line with the significant overlap among patients with bipolar disorder and patients with schizophrenia or major depression in clinical symptoms, neurocognitive dysfunction, cerebral metabolism abnormalities, as well as brain biochemical and molecular pathophysiological processes [47–50].

Psychotropic medication can conceivably contribute to disease-specific regional alterations in complex I, as well as to the anatomical overlap the bipolar group displays with both the schizophrenic and depressed group. Indeed, it is well established that antipsychotics, typical and atypical, interact with the mitochondrial OXPHOS, specifically with complex I, directly inhibiting its activity, both *in vitro* and *in vivo* in rodents and humans [29,30,33,51]. In addition, mitochondria are a target for mood stabilizers such as lithium and valproate [52] and for antidepressant drugs [53], primarily MAO inhibitors. In the present study, however, we were unable to detect any effect of antipsychotic, antidepressant or mood stabilizing medication on the disorder-related differences in mRNA or protein levels of complex I subunits. Moreover, no differential effect on complex I subunits could be observed upon dividing patients according to the type of medication they were receiving at time of death. Although these results should be taken with caution due to small sample size, this lack of effect of antipsychotic drugs is in line with our previous finding in platelets of schizophrenic patients [27], which suggests that while complex I activity is affected by antipsychotic medication, its subunits' mRNA and protein expression is not, a finding recently corroborated by our recent findings in the neonatal ventral hippocampus lesion rat model of schizophrenia [54] and unpublished data in neuronal cell line.

Another extensively addressed confounding factor in postmortem brain studies is sample pH [36,55,56]. Although the
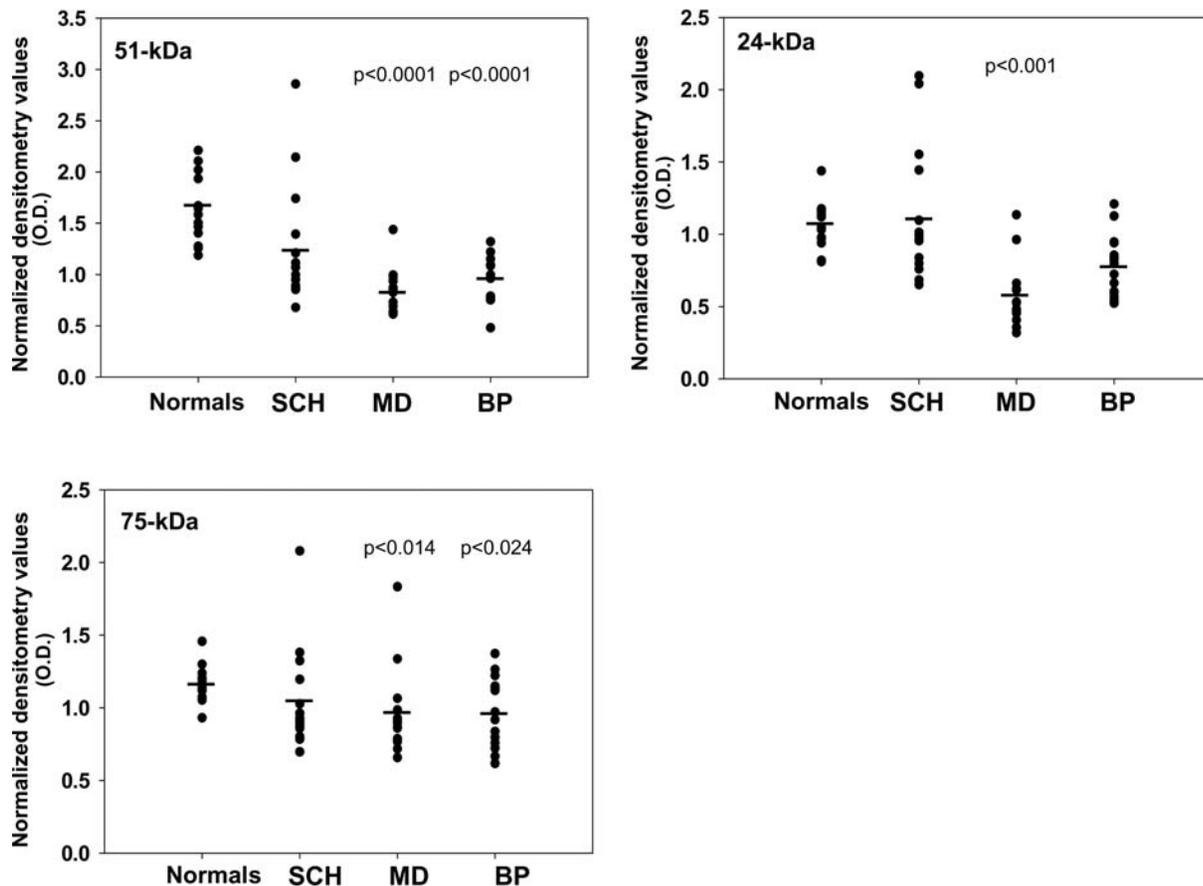
Figure 5. Cerebellar lateral hemisphere protein expression of complex I subunits. Protein levels of the 51-, 24-and 75-kDa subunits of complex I in post mortem Cerebellar lateral hemisphere of patients with schizophrenia (SCH, n = 15), major depression (MD, n = 15) and bipolar disorder (BP, n = 15), and of normal controls (n = 15). Statistical significant differences vs. the control group were observed in all three subunits in the major depression group and in the 51-kDa and the 75-kDa subunits in the bipolar group. No statistically significant difference was observed in the schizophrenic group.
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mechanism by which pH affects the expression of genes is unclear, it has been reported that the expression pattern of some genes differs between low and high pH-brain specimens [36,55]. However, adding pH as a covariate did not change disease-related effects on complex I, which is in line with our previous study [26] and with its lack of effect on other genes such as glucocorticoid receptor, cytosolic protein kinase Cε and kainate receptor 2 [57]. Other covariates such as age, gender, alcohol and drug abuse, laterality and PMI had no effect on the statistical significance of the differences between the diagnostic groups. These results, with the reservation that small sample size may contribute to lack of confounders' effect, minimize the potential effect of possible artifacts due to postmortem analysis, giving further credence to disease-dependent main effects.

The present study examined three nuclear DNA encoded subunits of complex I, the 51- and 24-kDa subunits, two iron-sulfur flavoproteins with catalytic properties including the site for transhydrogenation from NADH to NAD^+, and the 75-kDa, the largest iron-sulfur transmembranous subunit [58]. All three subunits form one functional subunit with a stoichiometry of 1mol of each subunit for 1mol of complex [59]. Therefore, it is plausible that any deviation from this ratio can lead to abnormal complex I activity. Indeed, we have previously shown that abnormal expression of both the 51- and 24-kDa with no change in the 75-kDa subunit, were associated with impaired activity of

complex I in platelets of schizophrenic patients [27]. Since complex I activity measurement in whole tissue has low sensitivity and postmortem delay significantly affects it [60], direct assessment of brain complex I activity is relatively scarce. Taken together, the data suggest that complex I subunits' expression may reflect abnormalities in complex I activity.

In schizophrenic patients we observed a decrease in complex I subunits in prefrontal cortex and striatum, key elements in the cortico–striatal-thalamic circuitry modulating cognitive processes prominent in schizophrenia [61], and critically important in biological processes believed to underlie psychotic symptoms [62,63]. Congruently, most imaging studies report abnormal metabolic activity of the cortico–striatal-thalamic circuitry in schizophrenia [63]. In the parieto-occipital cortex, however, increased levels of complex I subunits have been observed in both schizophrenic and bipolar patients, in line with previous findings of increased brain metabolic rates in both disorders [64]. The parieto-occipital cortex was shown to be involved in cognitive processes of mental visual imagery [65]. Aberrations in the ability to distinguish between perception and mental imagery has been suggested to be associated with psychotic hallucinations and paranoid delusions, characteristic of both disorders [66–68]. Interestingly, in the depressed group, alterations in both prefrontal and parieto-occipital cortices were either sporadic or not expressed at the functional relevant (protein) level.

The results of this study suggest the lateral hemisphere of the cerebellum as a prominent anatomical substrate for depression, as most consistent alterations were observed in the depressed group but also in the bipolar patients, as expected by the overlap of symptoms. The role of the cerebellum has traditionally been limited to coordination of voluntary movement, gait, posture, speech and motor function. However, evidence from studies of patients with overt cerebellar diseases as well as from normal subjects, suggests a possible role for the cerebellum in cognition, mood and behavior [69,70]. For example, cerebellar increase of blood flow is often seen in normal subjects performing cognitive tasks and exposed to sadness evoking challenges. Several studies have shown that patients with various cerebellar pathologies demonstrate flattening of affect or have higher depression scores than control subjects [70,71]. Furthermore, in patients with depression, transient mood challenges produced less activation in the cerebellum, prefrontal and limbic areas, than in healthy subjects [71–73]. Interestingly, it was reported that refractory depression responded to treatment with a chronically implanted cerebellar pacemaker [74]. In line with the suggested role of the cerebellum in mood and behavior is the abundance of serotonergic and noradrenergic inputs to the cerebellum, and their ability to modulate the cerebellar circuitry and affect cerebellar learning and control mechanisms [75].

The four brain areas we have analyzed are part of the neuronal circuitries implicated in all three mental disorders. Structural and functional imaging studies have implicated the cerebellum as part of the cortico-thalamic-cerebellar-cortical circuit, in schizophrenia [70,71]. In this study, however, complex I subunits expression was unaltered in the lateral cerebellar hemisphere of the schizophrenic group. Similarly, the prefrontal cortex has been implicated in depression and in bipolar disorder, but alterations in complex I were either sporadic or absent. This apparent contradiction may be attributed to methodological differences, as imaging studies usually sample a broader brain area and do not exclude circuit-dependent inductions during measurement, whereas molecular studies are more localized.

Finally, in the present and previous studies we have shown a particular molecular pathology, in four discrete brain areas, and detected disease-specific pattern of regional alterations. Given the major role complex I plays in controlling mitochondrial OXPHOS its abnormal activity can result in mitochondrial dysfunction.

Mitochondria, being the main source of high energy intermediates, are of prominent importance in maintaining the cellular energy state, particularly of high-energy consuming cells such as neurons. Hence, we hypothesize that mitochondrial dysfunction and thereby impaired neuronal metabolism can lead to alterations in neuronal function, plasticity and brain circuitry. Mitochondrial dysfunction can be either a causal or a consequential event of abnormal signaling in specific brain circuitries. Previous studies utilizing pharmacological tools to inhibit mitochondria, revealed defects in synaptic potentiation and a failure to maintain neurotransmission under rigorous stimulation [76,77]. Further evidence for the critical role of mitochondria in neuronal activity is the finding that loss of mitochondria from axon terminals result in defective synaptic transmission in Drosophila [78–80] and that dendritic mitochondria are essential in the morphogenesis and plasticity of spines and synapses in hippocampal tissue slices [81,82]. Our findings of mitochondrial impairment in peripheral blood cells in schizophrenia may support the suggestion of mitochondrial dysfunction as a primary event [24,27,83]. However, an opposite interaction between neuronal transmission and mitochondria was also demonstrated by the ability of neurotransmitters, mainly glutamate but also dopamine, to reciprocally affect mitochondrial function and ATP production process in a cellular system as well as in-vivo [84–89]. Given this reciprocal interaction between both, the question of primacy still remains an enigma.

Prefrontal cortex, parieto-occipital cortex, striatum and cerebellum may constitute one, or part of several complex neuronal circuits critical for optimal functioning of normal cognition, emotion and behavior. Considering our data as well as previously reported data such as those on the differential anatomical apolipoprotein D (apoD) abnormalities in schizophrenia and bipolar disorder [90], it is tempting to suggest that the diversity in the clinical spectrum of mental disorders may, at least in part, be attributed to the different anatomical pattern of specific impairments in the neuronal circuits implicated in the disorders.

Author Contributions

Conceived and designed the experiments: DBS RK. Performed the experiments: RK. Analyzed the data: DBS RK. Contributed reagents/materials/analysis tools: DBS. Wrote the paper: DBS.

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