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Mitochondrial dysfunction as an underlying cause of bipolar disorder

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MITOCHONDRIAL DYSFUNCTION AS AN UNDERLYING CAUSE OF BIPOLAR DISORDER

by

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ABSTRACT

Bipolar disorder is a psychiatric disorder with alarming rates of morbidity and mortality. Since the pathophysiology of the disease is not well understood, it is difficult to develop treatments or even explain why the current treatments are successful. An increasingly popular hypothesis is that mitochondrial dysfunction plays a role. This paper examines the relationship between mitochondrial dysfunction and bipolar disorder by examining the following: (i) mitochondrial complex I dysfunction and oxidative damage, (ii) mitochondrial complex I dysfunction, epigenetic modifications, and treatment with lithium, (iii) post-mortem brain studies, (iv) the mtDNA common deletion, (v) calcium, (vi) comorbidity with mitochondrial disorders, (vii) lactate and intracellular pH levels, (viii) phosphocreatine, (ix) apoptosis, and (x) inositol. These studies point to a definitive correlation between the bipolar disorder and mitochondrial dysfunction, but it is too soon to determine causation. Further research is needed.

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LIST OF ABBREVIATIONS

5hmc 5-hydroxymethylcytosine

5mc 5-methylcytosine

ACC ..... Anterior Cingulate Cortex

Bcl-2 ..... B-cell lymphoma protein-2

CK ..... Creatine Kinase

Cr ..... Creatine

CSF ..... Cerebrospinal Fluid

DLPFC ..... Dorsolateral Prefrontal Cortex

DSM ..... Diagnostic and Statistical Manual of Mental Disorders

ETC ..... Electron Transport Chain

HIP ..... Hippocampus

HN ..... Hereditary Sensorimotor Neuropathy

IMPase ..... Inositol monophosphatase

MA ..... Monoamines

MAO-A ..... Monoamine oxidase A

MAO-B ..... Monoamine oxidase B

MDD ..... Major Depressive Disorder

MELAS ..... Mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes

mtDNA ..... Mitochondrial DNA

nDNA ..... Nuclear DNA

NOS ..... Not Otherwise Specified

OXPHOS ..... Oxidative Phosphorylation

PBMC ..... Peripheral blood mononuclear cell

PCr ..... Phosphocreatine

PEO ..... Progressive External Ophthalmoplegia

PI ..... Phosphotidylinositol cycle

PFC ..... Prefrontal Cortex

PTSD ..... Post-Traumatic Stress Disorder

ROS ..... Reactive oxygen species

SCID ..... Structured Clinical Interview for the DSM-IV

UPR ..... Unfolded protein response

VPA ..... Valproic acid

INTRODUCTION

Bipolar Disorder

Bipolar or manic-depressive disorder consists of two states: mania (or hypomania) and depression (Müller-Oerlinghausen, Berghöfer, & Bauer, 2002). Mania and hypomania are defined by the same symptoms (Table 1), but patients are specifically diagnosed with mania when these symptoms drastically affect their work and/or personal life. While the presence of manic episodes distinguishes bipolar disorder from unipolar depression, an individual with bipolar disorder is more likely to be found in a depressive rather than a manic state. The symptoms of the depressive phase of bipolar disorder (Table 2) are the same as those of unipolar depression (Strakowski, 2014).

There is no cure for bipolar disorder, and existing treatments are not as effective as initially thought. The relatively large prevalence, in addition to these treatment difficulties, makes bipolar disorder one of the most common causes of disability (Müller-Oerlinghausen et al., 2002). More significantly, bipolar disorder proves to be deadly as up to 50% of patients attempt suicide and up to 15% of patients commit suicide (Strakowski, 2014). At this point, the pathophysiology is not well-understood. However, evidence is beginning to point to mitochondrial dysfunction as the culprit (Anglin, Mazurek, Tarnopolsky, & Rosebush, 2012). Researchers hope that elucidating the etiology will create better treatment options and improve the prognosis of individuals with bipolar disorder.

Table 1. Symptoms of Bipolar Disorder: Mania and Hypomania. Table adapted from (Strakowski, 2014).

Euphoric, expansive, or irritable mood	Excessive energy
Decreased need for sleep	Racing thoughts/flight of ideas
Rapid speech	Grandiosity
Impulsive pleasure seeking	Distractibility
Mood lability	Hypersexuality
Brief periods of depressed mood	Hallucinations
Delusions	Severe thought disorder
Aggressive impulsivity	Confusion
Hyperreligiosity	Extravagance
Catatonia	

Table 2. Symptoms of Bipolar Disorder: Depression. Table adapted from (Strakowski, 2014).

Depressed mood	Anhedonia
Feelings of worthlessness or excessive guilt	Change in appetite and weight
Psychomotor agitation or retardation	Change in sleep pattern
Fatigue	Impaired concentration
Suicidal thoughts or behavior	

Mitochondrial Function

Mitochondria are double membrane bound organelles (Figure 1). These membranes create two spaces: the intermembrane space (between both membranes) and the matrix (bound by the inner membrane). In addition to its location, the inner membrane can be distinguished from the outer membrane by the presence of cristae. These infoldings of the inner membrane function to increase the surface area, and the number of cristae vary depending on the cell type in which the mitochondria reside with more cristae indicating an increased ATP demand (Alberts et al., 2002).

The outer membrane contains many porins, types of transport proteins that permit the passage of molecules no larger than 5,000 Daltons. This implies that even proteins (small in size) are able to pass through the outer membrane. However, once these molecules enter the intermembrane space, they are unable to pass through the inner membrane. The lipid bilayer of the inner membrane contains a large number of cardiolipin, a phospholipid that contains four phospholipids as opposed to the usual two. Despite this structural difference (that may be responsible for the impermeability of the this membrane to ions), the inner membrane has many transport proteins that allow it to be selectively permeable to products of mitochondrial metabolism as well as molecules needed by the enzymes presence within the matrix (Alberts et al., 2002)

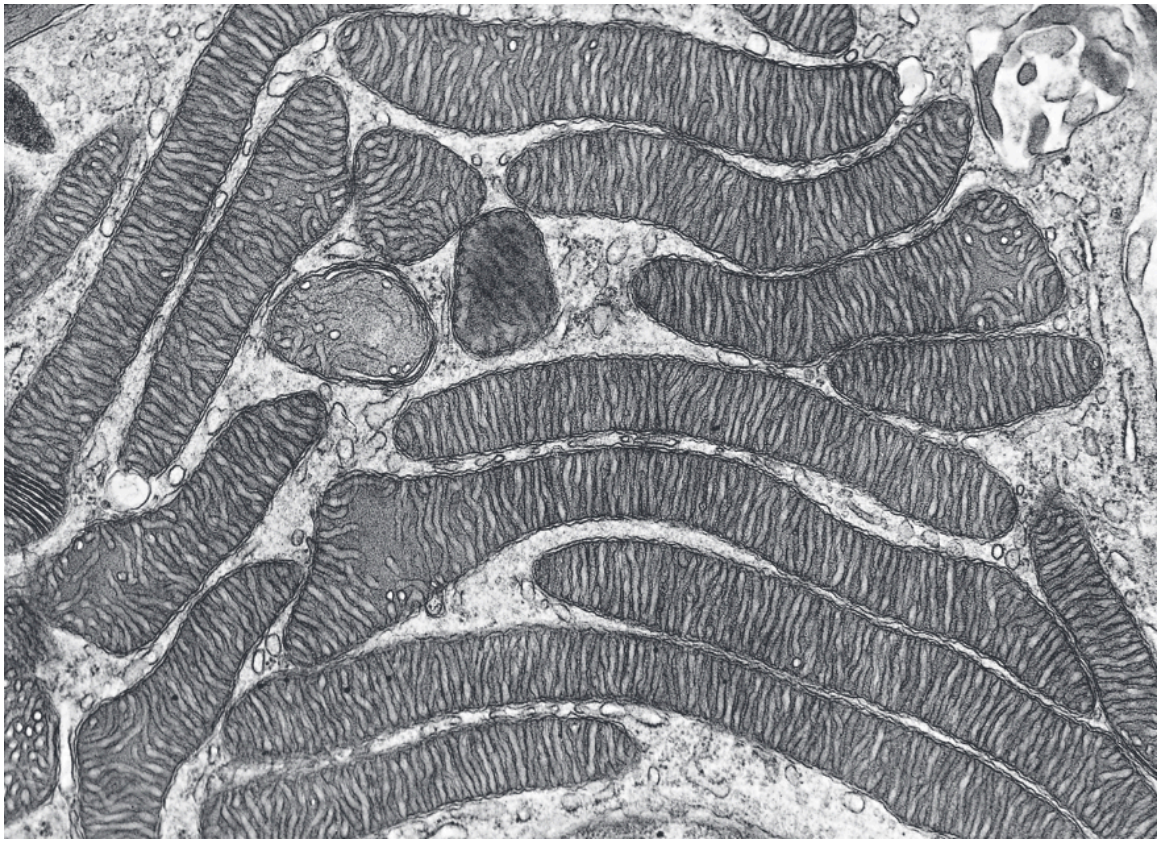

Figure 1: An Electron Micrograph of Mitochondria from the Adrenal Cortex. Figure taken from (Scheffler, 2007).

As mitochondrial proteins are encoded by mitochondrial (mtDNA) and nuclear DNA (nDNA), an alteration in either could lead to mitochondrial dysfunction (P. Chinnery & Schon, 2003). Mitochondria rely on nDNA for proper function of the OXPHOS (oxidative phosphorylation) system, and researchers have determined that there are 97 genes related to this process. mtDNA differs from nDNA in that it is circular, maternally inherited, and contains fewer genes, 37 specifically. The breakdown of mtDNA genes and their products are described in Table 3. mtDNA mutations are relatively common due to errors in mtDNA repair, oxidative surroundings, and the high rate of replication. Thus, mutated versions of mtDNA are interspersed with the wild-type

mtDNA in different proportions. This varied ratio is termed heteroplasmy, and it has important implications in mitochondrial diseases in that that clinical manifestation is directly related to the degree of heteroplasmy (P. F. Chinnery & Hudson, 2013).

Table 3. mtDNA Genes and Their Products. Information taken from (P. F. Chinnery & Hudson, 2013)

Number of Genes	Gene Products	Other Details
13 genes	One polypeptide component of the mitochondrial respiratory chain	
24 genes	Mature RNA product	22 mitochondrial tRNA molecules, a 16s rRNA, and a 12s rRNA

Objective

Although there are many known types of mitochondrial diseases, there are disorders (such as multiple sclerosis, Parkinson's disease, schizophrenia, depression, autism, chronic fatigue syndrome, and bipolar disorder) that are not classified as mitochondrial diseases themselves but have proven to involve mitochondrial dysfunction (Morris & Berk, 2015). The evidence presented in this thesis specifically explores the association between bipolar disorder and mitochondrial dysfunction in an effort to answer the following question: is bipolar disorder a mitochondrial disease?

PUBLISHED STUDIES

Mitochondrial Complex I Dysfunction and Oxidative Damage

Mitochondrial complex I dysfunction has been linked to bipolar disorder (Scola, Kim, Young, Salvador, & Andreazza, 2014). This particular type of mitochondrial dysfunction results in the generation of reactive oxygen species and thus oxidative damage to the following: proteins, lipids, and DNA (Scola et al., 2014). Such oxidative changes have been seen in bipolar disorder (Che, Wang, Shao, & Young, 2010). For example, lipid peroxidation was increased and the activity of antioxidant enzymes was decreased in the plasma of patients with bipolar disorder. In addition, postmortem brain studies showed reduced expression of the antioxidant enzymes. Furthermore, a clinical study on twins, one with and one without bipolar disorder, showed that the twin with bipolar disorder had a higher amount of lipid peroxidation when compared to the healthy twin. Interestingly, this lipid peroxidation decreased to normal levels when the affected twin was treated with mood-stabilizers, although it is not clear why (Che et al., 2010).

Mitochondrial Complex I Dysfunction, Epigenetic Modifications, and Treatment with Lithium

Several studies showed that oxidative stress can impact DNA methylation levels in cancer, but only one study linked this relationship to mitochondrial dysfunction, specifically that of complex I (Scola et al., 2014). The findings of this study suggested that complex I dysfunction may lead to an increase in DNA methylation and hydroxymethylation. Such dysfunction in epigenetic modifications was identified in

neuropsychiatric disorders (Dong, Gavin, Chen, & Davis, 2012), such as bipolar disorder, suggesting yet another possible connection between mitochondrial complex I dysfunction and bipolar disorder.

The study linking complex I dysfunction and alterations in epigenetic modifications, and thus bipolar disorder, was conducted using rotenone, which causes complex I dysfunction by preventing the transfer of electrons to ubiquinone in the electron transport chain (ETC) (Scola et al., 2014). Using immunocytochemistry, the DNA methylation and hydroxymethylation levels were measured. In bipolar disorder, increased levels of 5-methylcytosine (5mc) and 5-hydroxymethylcytosine (5hmc) were identified, so these epigenetic modifications were specifically monitored. 5mc is involved in cell dynamics, while the function of 5hmc is not entirely understood, although it may be involved with the regulation of methylation/demethylation balance. With completion of the experiment, the researchers reached the conclusion that, in rat primary cortical neurons, rotenone-induced complex I dysfunction increased DNA methylation and hydroxymethylation, suggesting a link between mitochondrial dysfunction and bipolar disorder (Scola et al., 2014).

At the same time, the researchers in this study examined the effect of lithium, a drug commonly prescribed for bipolar disorder, on DNA methylation and hydroxymethylation levels. Interestingly, the results indicated that lithium not only decreased the DNA methylation and hydroxymethylation to a level that approximated normal, but it also prevented the effects of rotenone on complex I activity (Scola et al., 2014).

Post-Mortem Brain Studies

Brain tissue heavily relies on the function of mitochondria as it utilizes approximately 20% of the total oxygen in the body and has a high ATP demand. In addition, low levels of catalase compromise its ability to attend to free radicals, which are primarily produced by the mitochondrial ETC (Andreazza, Wang, Salmasi, Shao, & Young, 2013). The following studies use the post-mortem brains of patients with bipolar disorder to illustrate obvious alterations of mitochondrial function present in various regions of these diseased brains.

The prefrontal cortex is a commonly examined region in studies using post-mortem brains. In a study with participants with bipolar disorder, schizophrenia, and controls with no psychiatric diagnosis, results supported the hypothesis that mitochondrial dysfunction is indeed present in the prefrontal cortex (PFC) of patients with bipolar disorder: these results were not seen in the brains of the controls nor in those of the patients with schizophrenia (Andreazza et al., 2013). One part of the experiment showed that there were decreased levels of mitochondrial complex I subunit proteins (NDUFS7 and NDUFS8) in the post-mortem PFC samples of subjects with bipolar disorder. Another part of the experiment looked at myelin, as its lipid composition makes it susceptible to lipid peroxidation. The PFC of the participants with bipolar disorder showed a significant increase in lipid peroxidation marker levels (8-iso and 4-hydroxynonenal) compared to the controls. In addition, the researchers showed that mitochondrial proteins are more susceptible to nitration damage, while synaptosomal

proteins are more disposed to oxidative damage. Interestingly, the study showed no effect of treatment with psychotropic drugs on the examined markers (Andreazza et al., 2013).

The mtDNA Common Deletion

mtDNA is more prone to mutations due to the lack of histones and absence of the repair machinery seen with nuclear DNA (Sequeira et al., 2012). The most frequently seen mtDNA deletion is called the mtDNA common deletion. It is a 4,977 base pair deletion that has been frequently examined in cases of bipolar disorder (Jou, Chiu, & Liu, 2009). However, studies yield conflicting results, and further study is needed to clarify the discrepancy.

One explanation relates to the region of the brain examined. It is likely that the mtDNA deletion ratio varies depending on the part of the brain (Sabunciyan et al., 2007). In one study, researchers examined the dorsolateral prefrontal cortex, and they found that the patients with bipolar disorder showed an increase in the common deletion versus the control group (Sequeira et al., 2012). However, a similar study examined the occipital cortex and found no such correlation, supporting the hypothesis that there are varying levels of the deletion in different areas of the brain (Torrell et al., 2013).

Another explanation relates to the age of subjects. Multiple studies showed that there is a correlation between age and the accumulation of the common deletion (Sequeira et al., 2012; Shao et al., 2008). It is worth mentioning that one study did not find an association with age, but they noted that their conflicting results were most likely due to the narrow age range of their participants (Torrell et al., 2013).

A third explanation was posed by researchers who hypothesized that the pathophysiology of bipolar disorder was due to the combination of the common deletion and other mtDNA deletions (Kakiuchi et al., 2005). They found that the levels of common deletion were small, and thus not likely to play pathophysiologic role. In addition, their search for other deletions was unsuccessful. Despite their rejected hypothesis, they suggested yet another explanation: it is possible that the common deletion is only abundant in specific cell types (Kakiuchi et al., 2005). Two years later, the revised hypothesis resurfaced. Again, it was shown that no difference in the number of common deletions was found when bipolar patients were compared to controls. Despite these results, the authors acknowledged the possibility that the techniques used in their research (global amplification) could not determine cell type specific changes that may yield different results. To alleviate this problem, the researchers suggested using laser micro-dissected cells in future studies (Sabunciyan et al., 2007).

Calcium

Calcium (Ca^{2+}) is a second messenger that has multiple roles, such as regulating neurotransmission, receptor signaling, the generation of action potentials, neuronal periodicity and excitability, gene expression, synaptogenesis, and cell death (Warsh, Andreopoulos, & Li, 2004). Mitochondria have been shown to play several roles in calcium homeostasis (de Sousa, Machado-Vieira, Zarate, & Manji, 2014). These organelles can act as calcium high-capacity sequesters as well as releasers as cytosolic calcium. In addition, without sufficient ATP production by mitochondria, ATP-dependent Ca^{2+} transporters are impaired, leading to a calcium imbalance (de Sousa et al., 2014).

Problems with calcium arise when the levels are elevated (de Sousa et al., 2014). Increases in calcium levels impair post-tetanic potentiation, which results in dysfunction of synaptic signaling. Furthermore, high cytosolic calcium has been shown to cause stress and excitotoxicity, the effects of which can be remedied by mitochondrial buffering. Finally, the resulting cellular injury of increased calcium influx can provoke increased mitochondrial membrane permeability, mediated by the opening of a protein complex called the mitochondrial permeability transition pore. The opening of this complex results in the release of certain mitochondrial proteins, specifically cytochrome C and procaspases, that induce apoptosis (de Sousa et al., 2014). A membrane-associated protein called Bcl-2 also relates to this process (Soeiro-de-Souza et al., 2012). Bcl-2 is found in the outer mitochondrial membrane and the endoplasmic reticulum, and it has been shown to stabilize mitochondrial membrane integrity, preventing the release of these proteins and the resulting apoptosis. Interestingly, the Bcl-2 rs956572 genotype AA was linked to abnormal Bcl-2 expression as well as alterations in calcium homeostasis present in patients with bipolar disorder. These effects were reversed when the patients were treated with lithium (Soeiro-de-Souza et al., 2012).

Bipolar disorder has been associated with disruptions in calcium homeostasis (Warsh et al., 2004). Using lymphoblastoid cells, one group set out to determine the mechanism responsible for the altered intracellular calcium signaling seen in patients with bipolar disorder (Tadafumi Kato et al., 2003). In addition to measuring calcium responses to platelet-activating factor and thapsigargin, they looked at the effect of carbonyl cyanide m-chlorophenylhydrazone (CCCP), which is of interest as it is a

mitochondrial uncoupler that impacts mitochondrial calcium uptake. Their study showed that the calcium response to the CCCP depended on the mtDNA 5178/10398 haplotype, indicating that this (bipolar disorder associated) mtDNA polymorphism, by affecting the mitochondrial calcium regulation, may be a risk factor for bipolar disorder (Tadafumi Kato et al., 2003).

Through the examination of the mtDNA sequence in 35 hybrid cell lines, a different study found two polymorphisms, 8701A and 10398A that resulted in an increase in the basal fluorescence ratio (ratio of 480 nm excitation to 410 nm excitation) of mitochondria-targeted radiometric Pencam (RP), a calcium indicator (Kazuno et al., 2006). While the two polymorphisms are closely linked, 10398A has been previously associated with bipolar disorder. In both mtSNPs (single nucleotide polymorphisms), an alanine is substituted for a threonine. In the 10398A/G mtSNP, the substitution occurs at the C terminus of a subunit of complex I (NADH; ubiquinone oxidoreductase) called ND3. In the 8701A mtSNP, the substitution occurs at the F0 subunit 6 of complex V (ATP synthase), which is called ATPase6. Complex I is responsible for the generation of a proton gradient across the inner mitochondrial membrane, and Complex V takes advantage of this proton gradient to produce ATP. In addition to producing ATP, this proton gradient is considered the driving force of mitochondrial calcium uptake (Kazuno et al., 2006).

Comorbidity with Mitochondrial Diseases

Patients with mitochondrial disease are often characterized by various types of physical impairments. However, more and more research is identifying comorbid

psychiatric symptoms (Clay, Sillivan, & Konradi, 2011). One study examined 19 patients with known primary mitochondrial DNA disorders as well as 10 controls with hereditary sensorimotor neuropathy (HN) (Inczedy-Farkas et al., 2012). Their goal was to assess the presence of psychiatric symptoms in the mitochondrial disease patients. Eight mitochondrial disease patients had a past psychiatric diagnosis (42%), six had a current psychiatric diagnosis (31%), and five had both past and current psychiatric diagnoses (47%). In contrast, only three patients had both a past and current psychiatric diagnosis in the control group with a lifetime prevalence of 30%. The specific results of the Structured Clinical Interviews of DSM-IV axis-I (SCID-I) and axis-II (SCID-II) disorders are outlined in Table 4. The study concluded that there is a high association of psychiatric symptoms with mitochondrial disorders.

Table 4. Gender, Age, and Results of SCID-I and SCID-II Scores of Mitochondrial Disease Patients. Adapted from (Inczedy-Farkas et al., 2012)

ID	Gender	Age	Past Diagnosis SCID-I	Current Diagnosis SCID-II	Personality Disorder SCID-II
1	Female	34	Mixed-anxiety depressive disorder	Major depressive disorder	Avoidant
2	Female	51	Dysthymia	-	-
3	Female	34	-	-	-
4	Female	16	-	-	-
5	Male	32	-	Dysthymia	Personality disorder NOS

6	Female	61	-	-	-
7	Male	34	Adjustment disorder with depressed mood	-	-
8	Male	34	Adjustment disorder with depressed mood	-	Avoidant
9	Female	59	-	-	Obsessive-compulsive
10	Male	23	-	-	-
11	Female	34	-	-	-
12	Female	40	-	-	-
13	Female	38	Bipolar II disorder	Bipolar II, current episode depressive	-
14	Male	20	-	-	-
15	Female	22	-	-	Obsessive-compulsive
16	Female	39	Major depression with psychotic features	Major depressive disorder	Personality disorder NOS
17	Male	20	-	-	Avoidant
18	Female	37	PTSD	Bipolar II, current	Personality disorder NOS

				episode depressive	
19	Female	41	Postpartum depression	Bipolar I, latest episode depressive	-

Another article summarized the findings of 19 case reports, all of which link mitochondrial disease with psychiatric illnesses (Fattal, Budur, Vaughan, & Franco, 2006). The specific diagnoses are listed in Table 5. Researchers are becoming increasingly aware of the comorbidity between psychiatric illnesses and mitochondrial diseases, but the comorbidity with bipolar disorder, specifically, remains to be evaluated.

Table 5. Case Reports of Mitochondrial Disease Patients with Psychiatric Problems. Adapted from (Fattal et al., 2006)

ID	Gender	Mitochondrial Disorder	Psychiatric Symptoms (Age of Onset)
1	Female	MELAS, atypical	Schizophrenia, progressive dementia (23 years)
2	Male	MELAS	Psychosis: auditory hallucinations, persecutory delusions, disorganized behavior, progressive dementia (25 years)
3	Male	MELAS	Psychosis: visual hallucinations, somatic delusions, hyperactivity,

			progressive dementia (32 years)
4	Male	MELAS	Psychosis: auditory and visual hallucinations, delirium, IQ: 87→43 (19 years)
5	Female	MELAS; A3243G mutation of tRNA	Psychosis: auditory hallucinations, paranoid ideation, delusions of reference (22 years)
6	Male	Mitochondrial encephalomyopathy; C3256T mutation of tRNA	Psychosis: “delusion”, “confusion”, progressive dementia (29 years)
7	Male	A3243G mutation of mtDNA	Psychosis: auditory hallucinations, persecutory delusions, frontal lobe syndrome, IQ: 71→59 (31 years)
8	Male	MELAS; A3243 mutation of tRNA	Psychosis (21 years)
9	Female	Kearns-Sayre syndrome	Bipolar disorder, decreased intellectual functioning (23 years)
10	Male	Mitochondrial myopathy and decreased MARPs	MDD, transient auditory and visual hallucinations (22 years)
11	Male	G3274A mutation in tRNA	MDD with psychosis, suicidal

			ideation; deficits in memory, attention, and orientation (27 years)
12	Female	Autosomal dominant PEO, multiple mtDNA deletions	MDD (19 years)
13	Male	A3243G mutation of tRNA	MDD (22 years)
14	Male	A3243G mutation of tRNA	MDD recurrent: IQ 95 (47 years)
15	Female	A3243G mutation of tRNA	Panic disorder, agoraphobia (37 years)
16	Female	A3243G mutation of tRNA	Social phobia; IQ 98 (25 years)
17	Male	A3243G mutation of tRNA	Simple phobia; IQ: 103 (35 years)
18	Male	MELAS; 3243 mutation of tRNA	Personality change: poor impulse control, inactivity, apathy (34 years)

Lactate and Intracellular pH Levels

Metabolic studies of bipolar disorder patients have illustrated a trend in which these patients generally show an increase in lactate levels and a decrease in pH (Stork & Renshaw, 2005). These two levels appear to be inversely linked, and the increased levels of lactate has been shown to result from mitochondrial dysfunction. When the respiratory chain component of cellular metabolism is impaired, the cell defaults to anaerobic

glycolysis for energy production. When this occurs, pyruvate is converted into lactate when it is used as a hydrogen acceptor in order to form NAD from NADH. This relationship between lactate concentration and intracellular pH is reinforced by an animal study that illustrated this inverse relationship when mitochondrial failure was induced with the application of cyanide (Clausen, Zauner, Levasseur, Rice, & Bullock, 2001).

A study that examined cerebrospinal fluid (CSF) lactate concentrations in bipolar disorder patients of varying states (depressed, euthymic, or manic) showed an increase in the lactate levels (Regenold et al., 2009). However, it is important to distinguish between the states as various other studies show that the lactate and pH levels depend on the state of the bipolar disorder patients (Stork & Renshaw, 2005). The studies conducted on bipolar disorder patients in these varying states are discussed below and summarized in Table 6.

Several studies looked at patients with bipolar disorder in the euthymic state. One examined the intracellular pH in the basal ganglia of 13 bipolar disorder patients and found that the pH was significantly reduced. These researchers acknowledged that dysfunctional metabolism may be responsible for this alteration in pH (Hamakawa et al., 2004). This decrease in intracellular pH in euthymic bipolar disorder patients compared to controls was found when researchers examined the frontal cortex as well (T. Kato, Takahashi, Shioiri, & Inubushi, 1993). In addition, the intracellular pH levels in the manic and depressed state were found to be approximately normal (and thus higher than that seen in the euthymic state) (Tadafumi Kato & Kato, 2000). Although the reason for this discrepancy in intracellular pH between states is not known, it was suggested that the

pH in depressed and manic states are altered in response to an over-activation of monoaminergic systems. This is because noradrenaline has been linked to the activation of the Na^+/H^+ exchanger, which increases intracellular pH (Tadafumi Kato & Kato, 2000). It is important to note, however, that a more recent study contradicted these findings, in that no difference was seen in the intracellular pH of euthymic and manic patients (Weber et al., 2013). Weber et al. acknowledged this contribution and attributed the different results to the fact that the age, brain regions examined, and medication status differed between studies (2013).

A study with 32 medication-free bipolar disorder patients looked at the lactate levels while these patients were in depressed or mixed-mood states (Dager et al., 2004). Like the majority of the studies looking at euthymic bipolar disorder participants, they found elevated lactate levels when examining gray matter. Previously, lactate was believed to be a by-product of glycolysis only. However, more recent evidence suggests that lactate has an important role in brain energetics. While a small fraction of energy used during normal neuronal activation is from glycolysis, this amount is increased to one-third of total energy expenditure during rapid neuronal firing, which could explain the elevated gray matter lactate levels seen in bipolar disorder in the study by Dager et al. (2004).

Finally, one study examined the change in lactate levels as the participants switched from a manic state to a euthymic state (Brady et al., 2012). They originally had 15 patients, but only seven returned when in a euthymic state. These researchers found that the bipolar disorder participants had lactate levels similar to the controls when in a

manic state. However, the lactate concentrations were significantly reduced when these patients were in the euthymic state (Brady et al., 2012).

Table 6. Studies of Lactate Levels and/or Intracellular pH in Bipolar Disorder Patients of Varying States

Author	Year	Participants	Focus	Results
Brady et al.	2012	7 bipolar type I patients in a manic state and later in a euthymic state	Anterior cingulate cortex (ACC)	Same lactate levels in mania and decreased lactate levels in euthymia
Dager et al.	2004	32 medication-free bipolar type I and bipolar type II patients in a primarily depressed or mixed-mood state	Gray matter	Increased lactate levels
Hamakawa et al.	2004	13 patients with bipolar disorder in a euthymic state	Basal ganglia	Decreased intracellular pH
Kato et al.	1993	17 patients with bipolar disorder in the euthymic and manic states	Frontal cortex	Increased intracellular pH in the manic state compared to the euthymic state, where manic and depressed state levels are approximately normal; decreased intracellular pH in the euthymic

				state compared to the controls
Regenold et al.	2009	15 patients with bipolar type I disorder in various states	CSF	Increased lactate levels
Weber et al.	2013	14 bipolar disorder patients in the euthymic state and 19 bipolar disorder patients in the manic state	Anterior cingulate cortex (ACC)	Decreased intracellular pH in manic compared to the controls

Phosphocreatine

Phosphocreatine (PCr) is molecule of interest because it is an indicator of the status of cellular energy, and a decreased PCr concentration suggests decreased energy metabolism, which may be caused by mitochondrial dysfunction (Frey et al., 2007). PCr is formed from the reaction of creatine (Cr) and ATP in the mitochondria, and the reaction is catalyzed by creatine kinase (CK) (see Figure 2 below). PCr transports high energy phosphates from mitochondria to the cytosol (Tadafumi Kato & Kato, 2000), and it is necessary for cellular homeostasis (Frey et al., 2007). On the other hand, Cr stabilizes mitochondrial CK, preventing a preliminary aspect of apoptosis in which the mitochondrial transition pore is opened.

$$\mathrm{Cr} + \mathrm{ATP} \underset{\mathrm{CK}_{\mathrm{non-mitochondrial}}}{\overset{\mathrm{CK}_{\mathrm{mitochondrial}}}{\rightleftharpoons}} \mathrm{PCr} + \mathrm{ADP} + \mathrm{H}^{+}$$

Figure 2: PCr is formed from the reaction of Cr and ATP, and the reaction is catalyzed from CK. Figure taken from (Frey et al., 2007).

Decreased PCr has been seen in mitochondrial encephalopathies as well as in bipolar disorder (Tadafumi Kato & Kato, 2000; Young, 2007). This decrease in PCr, along with a decrease in ATP, suggest that mitochondrial dysfunction is the culprit (Tadafumi Kato & Kato, 2000). However, research also shows that decreased PCr is not universal throughout the brain. An *in vivo* phosphorous-31 magnetic resonance spectroscopy study using 12 non-medicated, euthymic bipolar disorder subjects and 16 controls revealed that this decrease in PCr was asymmetrical in the frontal lobe of the brain. Specifically, there was a higher right-to-left ratio of PCr (Deicken, Fein, & Weiner, 1995).

Epidemiological differences between bipolar disorder type I and type II are well-known, so researchers realized that it would be beneficial to examine PCr in patients with these different subtypes of the disorder (Tadafumi Kato et al., 1994). A study by Kato et al. did just that and found reduced brain PCr levels in the patients with bipolar disorder type II (1994). Not only that, but this decrease was seen in the euthymic, depressive, and hypomanic states. The researchers concluded that the reduction in PCr was trait-dependent (upon the type of bipolar disorder) instead of state-dependent (euthymic vs. depressed vs. manic) (Tadafumi Kato et al., 1994).

These researchers went further and hypothesized why this decrease in PCr was observed (Tadafumi Kato et al., 1994). They indicated that there are four possible reasons why there is a decrease in PCr, all of which are clear when looking at the reaction

illustrated in Figure 2: (1) an increase in energy demand, (2) a decrease in CK activity, (3) a decrease of ATP synthesis, and (4) a decreased concentration of creatine.

Upon closer examination, a few of these hypothesized explanations were found to be untrue. First, an increase in energy demand cannot be applied to this situation due to the fact that brain glucose metabolism is reduced in the frontal lobes of patients with bipolar disorder. Second, a reduction in CK activity could not be feasible as this study showed that the reduction of PCr levels only occurred in the patients with bipolar disorder type II while intracellular pH and intracellular magnesium concentration, two regulators of CK, were shown to be altered in all patients with bipolar disorder. However, intracellular magnesium levels may be a possible indicator because serum magnesium concentration is correlated to the severity of depressive symptoms, although it must be confirmed as it was not assessed in this study.

The third possible explanation, a reduction in ATP synthesis, cannot be eliminated for two reasons: (1) mtDNA depletion has been seen in those with depressive disorders, and (2) a patient with mitochondrial myopathy was reported to show manic-depressive symptoms. In addition, those with migraines have been shown to have reduced brain PCr, mitochondrial dysfunction has been commonly linked to migraines, and bipolar type II patients have been frequently noted to have comorbid migraines (approximately 25%) (Tadafumi Kato et al., 1994).

Finally, in a study that examined the CSF of bipolar disorder patients (80% of which had type II), researchers noted a decrease in creatine in these subjects compared to those with major depression. Thus, the probable causes of lower PCr in bipolar disorder

type II patients are as followed: (1) decreased CK activity, (2) increased intracellular magnesium concentration, (3) reduction of brain creatine content, and (4) genetic mitochondrial abnormalities (Tadafumi Kato et al., 1994).

A later study took this further and examined CK mRNA expression in the dorsolateral prefrontal cortex (DLPFC) and hippocampus (HIP) of patients with bipolar disorder (MacDonald, Naydenov, Chu, Matzilevich, & Konradi, 2006). They examined two isoforms of CK that are known to be present in these brain regions: CKB, which is expressed in oligodendrocytes and astroglia, and CKMt1, which is expressed in the mitochondria of neurons. Both of these isoforms were found to be downregulated in the patients with bipolar disorder (MacDonald et al., 2006).

Although the aforementioned studies seem to suggest that decreased PCr levels are relevant to bipolar disorder, problems arise when the PCr levels are examined in adolescent bipolar disorder patients. In one study using subjects between the ages of 11 and 20, no difference was seen in the levels of PCr when comparing eight bipolar disorder patients with eight controls (Sikoglu et al., 2013). Furthermore, another study that examined participants of ages 13 to 18, showed an increase in PCr compared to the controls. Although this contradicted their hypothesis based on previous studies with adult patients, this was not altogether surprising as pediatric and adult bipolar disorder patients have been found to have different neurochemical characteristics, suggesting that the brain metabolism may vary with age (Shi et al., 2012).

Apoptosis

Apoptosis involves the self-destruction of cells (Tsujimoto, 1998). It is characterized by phenomena listed in Table 7 (Fries et al., 2014). Apoptosis is used for developmental sculpturing, homeostasis of tissues, and the removal of cells that are no longer needed or are damaged (Tsujimoto, 1998). In addition, neural apoptosis is an important part of neural development, but apoptotic dysfunction can causes "synaptic apoptosis", which is the atrophy of synapses or neurites (de Sousa et al., 2014).

Table 7. Phenomena that Characterize Apoptosis. Information taken from (Fries et al., 2014).

Rounding of the cell
Pyknosis (decrease in cell size)
Condensation of chromatin
A small amount of cytoplasmic ultrastructural organelle modifications
Nuclear fragmentation
Blebbing of the plasma membrane
Phagocyte engulfment

Mitochondria play a role in both the intrinsic and extrinsic apoptotic pathways (de Sousa et al., 2014). In the intrinsic pathway, certain stimuli such as increased levels of cytoplasmic calcium or reactive oxygen species, or even the activation of Bcl-2 family proteins, lead to caspase activation, which causes apoptosis. In the extrinsic pathway, a change in membrane permeability to leaked proapoptotic factors occurs due to the activation of extracellular death receptors.

Systemic changes seen in patients with bipolar disorder include increased inflammatory markers, reduced neurotrophic factors, oxidative stress, and DNA damage (Fries et al., 2014). All of which characterize systemic toxicity. These dysfunctional cellular resilience mechanisms lead to increased susceptibility to cell death when exposed to a stressful environment.

Certain apoptotic factors undergo changes in bipolar disorder, including DNA damage in peripheral blood, apoptotic serum activity increase, differential expression of cell death and survival molecules in peripheral blood mononuclear cells (PBMCs), and, of particular interest, mitochondrial dysfunction (Fries et al., 2014). Thus, cell death clearly plays a role in bipolar disorder, and this connection even involves mitochondrial dysfunction. Fries et al. hypothesized that specific transmembrane receptors in PBMCs identify peripheral molecules that act as extracellular stress signals, such as tumor necrosis factor (TNF-α), that induce extrinsic apoptosis. TNF-α is increased in the serum of bipolar disorder patients and induces apoptosis by activating caspases. Other research shows that bipolar disorder patients' lymphocytes have decreased expression of HSP70, an anti-apoptotic factor, and BAX levels in the cytoplasm. This suggests that they were relocated to the mitochondria, a movement that induces apoptosis (Fries et al., 2014).

Figure 3 details targets for drug development (de Sousa et al., 2014), one of which includes apoptotic mechanisms. These targets are promising, but mood stabilizers and atypical antipsychotics, which are used in the treatment of bipolar disorder, already regulate some of these abnormalities. For example, lithium has been shown to regulate apoptosis (de Sousa et al., 2014). When rotenone, a substance that induces complex I

dysfunction, was given to rats, an annexin V assay showed that the rotenone cause an increase in cells in early and late apoptosis, and lithium decreased the amount of apoptosis (Scola et al., 2014).

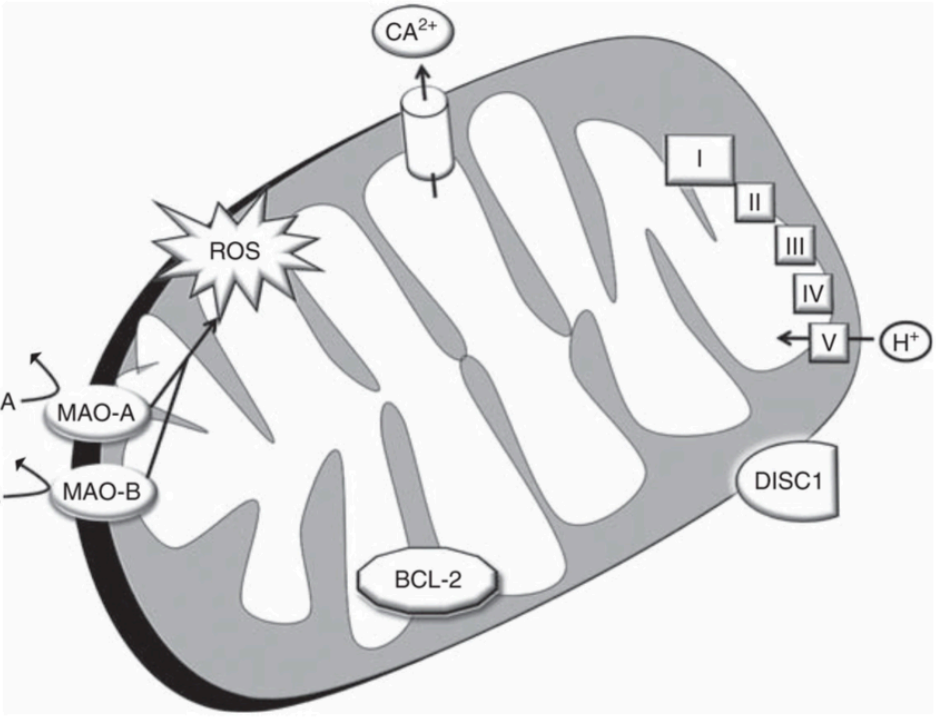

Figure 3: Targets for drug development include complex I of the ETC, monoamine oxidases, mitochondrial transport, apoptotic mechanisms, and calcium regulation. Note: Monoamines (MA) generate reactive oxygen species (ROS) via monoamine oxidase A (MAO-A) and monoamine oxidase B (MAO-B). Figure taken from (de Sousa et al., 2014).

Inositol

There are three sources of inositol: synthesis from glucose 6-phosphate via MIPS (1-D-*myo*-inositol-phosphate synthase), inositol phosphate dephosphorylation from inositol-containing membrane phospholipid breakdown, and *myo*-inositol transporters' uptake of extracellular fluid (Deranieh & Greenberg, 2009).

The high inositol levels in the neurons of patients with bipolar disorder is likely due to the high demand of membrane phospholipids, which are required for plasticity and formation of synapses in neurons (Deranieh & Greenberg, 2009). Most research examining inositol depletion has focused on the phosphoinositide cycle, but inositol also plays a role in various intracellular signaling molecules that relate to the following: phospholipid synthesis, the UPR (unfolded protein response), and protein secretion. Researchers have proposed that the impaired phospholipid metabolism in bipolar disorder is due to the changes in choline, *myo*-inositol, inositol monophosphate, and phosphomonoester levels. The decrease in energy due to mitochondrial dysfunction is responsible for the changes listed above. In addition, inositol plays a role in the expression of over 712 genes, and inhibition of inositol alters the expression of hundreds of these genes, which are involved in important pathways (Deranieh & Greenberg, 2009).

VPA (valproic acid), carbamazepine, and lithium, three bipolar disorder medications, all cause reduced levels of inositol, although by different mechanisms (Deranieh & Greenberg, 2009). The lack of a common mechanism leads to the following question: why does inositol depletion have therapeutic effects?

Research on VPA by Ju and Greenberg showed that treatment with VPA led to increased levels of inositol, which then led to an elevation in cardolipin (a phospholipid required for efficient mitochondrial function) (2003).

Though it is not entirely clear why, carbamazepine seems to lead to inositol depletion through uptake inhibition (Deranieh & Greenberg, 2009).

Research by Toker and Agam addressed the inositol depletion hypothesis and lithium. This was first described by Berridge et al., and it states that the inhibition of various enzymes in the PI (phosphatidylinositol cycle) causes the mood-stabilizing action of lithium (Toker & Agam, 2014). Specifically, it works through the inhibition (uncompetitive) of inositol monophosphatase (IMPase), which causes the down-regulation of the PI cycle. Normally, neurotransmitters (which are also thought to play a role in disorders such as bipolar disorder) are thought to play a role in the hydrolysis of PIs to second messenger molecules through specific subtypes of receptors as well as G-protein activation (van Calker & Belmaker, 2000). These second messenger molecules include DAG (diacylglercerol) and IP_3 (inositol-1,4,5-trisphosphate). The former is responsible for the activation of PKC (protein kinase C) and the latter is responsible for the activation of intracellular Ca^{2+} release. In addition, IP_3 is metabolized through serial phosphorylation and dephosphorylation to myo-inositol, which along with CDP-DAG, is used to resynthesize PIs. Hydrolysis of inositol monophosphate to myo-inositol is the final step in the IP_3 metabolism, and it is inhibited by lithium ions (if given at the therapeutic concentration). The inositol-depletion hypothesis suggests that the resulting decrease in myo-inositol leads to fewer PI-dependent second messenger molecules (van Calker & Belmaker, 2000).

Lithium leads to cellular signaling regulation, but the effects of lithium in the context of inositol have proven to relate to another process called autophagy (Toker & Agam, 2014). This process leads to the removal of misfolded proteins and protein aggregations. One study looked at lithium treatment versus knockouts of two genes

(separately) that play a role in the PI cycle (Toker et al., 2014). They found that the knockouts of the two genes mimicked the effect of lithium, and all three showed a favorable alteration in mitochondrial function. The mechanism behind the inositol depletion's impact on mitochondrial activity is unknown at this point, but the induced autophagy may play a role. Mitochondrial fission creates two daughter organelles, and this process often leads to an unequal ratio of functional to dysfunctional organelles. The latter can be degraded by autophagy (called mitophagy in this case), which leads to an increase in the proportion of healthy mitochondria, which is consistent with the experiments with lithium and the gene knockouts (Toker & Agam, 2014).

Homozygote *inositol monophophatase 1* (*IMPA1*) knockout mice are, by definition, unable to synthesize inositol, mimicking animals treated with lithium. In a study by Shtein et al., those mice, in addition to lithium-treated wild type mice, were fed an inositol-deficient diet. In this experiment, pilocarpine, a cholinergic agonist, is used to assess the effect of this inositol-deficient diet, which in conjunction with lithium treatment, is known to improve the function of the lithium. In other words, the pilocarpine is used to see if the inositol-deficient diet improves the action of lithium. The study showed that both experimental groups had increased cholinergic behavior, which indicates enhanced action of lithium. These results raise the possibility of a practical application in which an inositol-deficient diet is used to enhance lithium's action in a clinical setting (Shtein, Agam, Belmaker, & Bersudsky, 2015).

We cannot assume that lithium, VPA, and carbazemine work therapeutically because of inositol depletion, although it is certainly tempting as they all result in such a decrease.

DISCUSSION

This paper examined various types of mitochondrial dysfunction that correlate with bipolar disorder. Some of these forms of mitochondrial dysfunction may very well be underlying causes of bipolar disorder. However, almost every consulted study concludes that more research is needed as there were many caveats.

While it is useful to study the post-mortem brain, its examination comes with problems such as the pH variability, post-mortem interval, and the small sample sizes as noted by Scola et al. (2014). Another study that incorporated the use of post-mortem brains noted that the retrospective view of the patients' histories could lead to some errors. In addition, some of the participants were included even though they were known to have substance abuse problems and many were medicated, which could have potentially confounded the data (Che et al., 2010). A third study acknowledged storage conditions as another post-mortem brain limitation, but they controlled for post-mortem interval and pH (Andreazza et al., 2013). In addition, they acknowledged that they only looked at a specific brain region, the pre-frontal cortex, and further examination of alternative brain regions would be beneficial. The importance of examining different brain regions was an issue that was also raised in the mtDNA common deletion studies, in which the common deletion varies from region to region (Sequeira et al., 2012).

However, this study acknowledged that, although they looked at different brain regions, they did not look at specific differences between different parts of these regions nor did they examine specific differences between the cells. In the future, the paper suggested the use of laser capture microdissection to do the latter (Sequeira et al., 2012).

In the studies on lactate concentration and intracellular pH, it seems clear that there is an obvious trend that points to mitochondrial dysfunction as an underlying cause. However, the majority of studies focused on bipolar patients in the euthymic state and only one study looks at the different states within the same participants. In addition, almost every study looks at different brain regions. It would be interesting to see some of these studies replicated in the same brain regions but during different states.

Many of the studies that have illustrated a decrease in PCr in bipolar disorder patients have been conducted using non-medicated participants. Studies that used medicated patients have been unable to replicate this result (Frey et al., 2007). For example, one study showed that when comparing medicated to non-medicated bipolar disorder patients, the medicated subjects actually had lower PCr levels than those that were non-medicated (Shi et al., 2012). Further research is needed.

Finally, the papers on the comorbidity of bipolar disorder and mitochondrial disorders simply link mitochondrial disorders with psychiatric symptoms in general, not specifically bipolar disorder. In the study by Inczedy-Farkas et al., only three patients of the 19 presented were diagnosed with some form of bipolar disorder (2012). Similarly, in the study by Fattal et al., only one patient of the 18 listed was diagnosed with bipolar disorder (2006). While these studies illustrate a high prevalence of psychiatric problems

among individuals with mitochondrial diseases, more research must be conducted on the correlation with bipolar disorder exclusively.

In summary, there are many limitations to the studies that have been conducted on bipolar disorder and mitochondrial dysfunction. However, that does not undermine the relationship between the two. Through the examination of the published research, it seems clear that there is some correlation, but it is too soon to claim causation. Further research is needed and, based on the promise of these studies, warranted.

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