Chapter 9

Mitochondrial dysfunction in bipolar disorder

Tadafumi Kato
Department of Psychiatry & Behavioral Science, Juntendo University Graduate School of Medicine, Tokyo, Japan

9.1 Introduction

The facts clarified in the 20th century by the pathological study of bipolar disorder are that MRI shows subcortical hyperintensity regions and a decrease in gray matter volume in the anterior cingulate gyrus, lithium may increase the volume of gray matter, prefrontal cortex activation for emotional facial expressions is reduced and amygdala activation is increased, etc. Studies using blood cells have shown changes in intracellular calcium ion (Ca^{2+}) concentration.

However, these findings were fragmental and the connection between them was unclear. Thus it was difficult to synthesize these findings into a full picture (Kato, 2019).

9.2 Proposal of mitochondrial hypothesis

Under these circumstances, we proposed the mitochondrial dysfunction hypothesis (Kato & Kato, 2000). This was based on findings suggesting a change in mitochondrial function in the brain of patients with bipolar disorder by magnetic resonance spectroscopy [decreased intracellular pH (Kato, Takahashi, Shioiri, & Inubushi, 1993) and decreased creatine phosphate (Kato et al., 1994)], detection of mitochondrial DNA (mtDNA) deletions in the postmortem brain of a patient of mitochondrial disease with recurrent depression (Suomalainen et al., 1992), detection of mtDNA deletions in the postmortem brain of patients with bipolar disorder (Kato, Stine, McMahon, & Crowe, 1997), and a cytoprotective effect of lithium and valproic acid through upregulation of the mitochondrial protein, Bcl-2 (Chen et al., 1999).

The point of this hypothesis is that the individual differences in mtDNA and accumulation of secondary mtDNA mutations due to the mutations in

Biomarkers in Bipolar Disorders. DOI: https://doi.org/10.1016/B978-0-12-821398-8.00014-X

mitochondria-related nuclear genes causes mitochondrial Ca^{2+} dysregulation, which causes abnormalities in intracellular Ca^{2+} signaling and neuroplasticity and results in bipolar disorder (Kato & Kato, 2000). When I think about it now, the big question is what kind of neuroplasticity in which neural circuit in the brain should be important, but at the time of proposal, we had not reached such an awareness of the problem.

This mitochondrial dysfunction hypothesis has been recognized to some extent, not so much by our study, but by a series of findings in 2004, such as decreased expression of mitochondria-related genes in postmortem brains of bipolar patients (Konradi et al., 2004) and elevated brain lactate by proton magnetic resonance spectroscopy (Dager et al., 2004).

After presenting this hypothesis, the author was appointed as a team leader at the RIKEN Brain Science Institute and started a research project to test the mitochondrial hypothesis in parallel with a comprehensive analysis study that later became known as omics analysis.

This chapter is not a systematic review, but rather an introduction to the mitochondrial hypothesis and its subsequent development, focusing mainly on our research for 20 years at the Laboratory of Molecular Dynamics of Mental Disorders in RIKEN.

9.3 Cellular models

By the 20th century, studies using platelets and cultured lymphoblastoid cells to examine intracellular Ca^{2+} concentrations had been conducted (Fig. 9.1), and many studies had reported that the basal intracellular Ca^{2+} concentration or the intracellular Ca^{2+} response after stimulation was greater in bipolar disorder (Warsh, Andreopoulos, & Li, 2004).

In order to confirm this finding and to investigate the mechanism, the authors also conducted a study using cultured lymphoblastoid cells. As a result, we found that thapsigargin-stimulated intracellular Ca^{2+} response was enhanced (Kato et al., 2003).

To investigate whether these findings are related to individual differences in mtDNA, patient-derived platelets were fused with rho zero cells (cells without mtDNA) to form mitochondrial cybrids (hybrid cells), and cytoplasmic and mitochondrial Ca^{2+} concentrations in the cybrids were examined (Kazuno et al., 2006). The results showed no significant differences in cytoplasmic and mitochondrial Ca^{2+} concentrations between bipolar patients and controls. We sequenced the whole mtDNA to see if individual differences in mtDNA were related to these findings, and found that two linked polymorphisms, 8701 A/G and 10398 A/G, affected basal mitochondrial Ca^{2+} levels, and that cybrids with 8701 A/10398 A had higher mitochondrial Ca^{2+} levels. However, candidate gene analyses in such a small number of cases can cause false positive findings. As mtDNA is not a major subject of GWAS, there have been few large-scale studies of mtDNA, and further

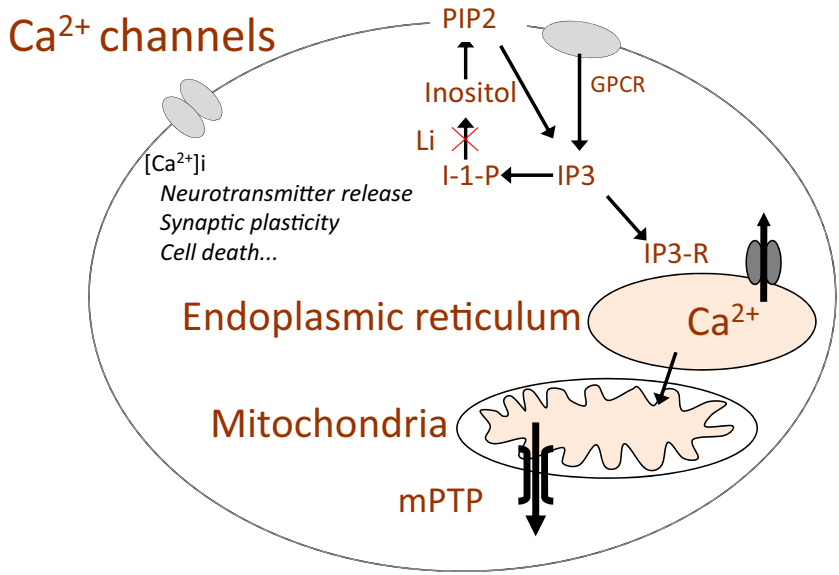

FIGURE 9.1 Calcium signaling in cells. Ca^{2+} channels, mitochondria, and endoplasmic reticulum play a role in intracellular Ca^{2+} signaling and lithium alters intracellular Ca^{2+} signaling. *Li*, lithium; *IP3*, inositol triphosphates; *mPTP*, mitochondrial permeability transition pore; *PIP2*, phosphatidylinositol 4,5-bis phosphate; *IP3-R*, IP3 receptor.

research is needed to determine whether this polymorphism is associated with bipolar disorder.

Gene expression analysis by DNA microarray using cultured lymphoblasts from two pairs of monozygotic twins, in which only one of the twins developed bipolar disorder, did not show mitochondrial dysfunction but showed that the endoplasmic reticulum (ER) stress-related genes, *XBP1* and *GRP78*, were decreased. We hypothesized that the ER stress response may be related to bipolar disorder. In cultured lymphoblasts from bipolar patients, *XBP1* induction by ER stress was decreased. Since it had been suggested that valproic acid might act through the endoplasmic reticulum (Bown, Wang, Chen, & Young, 2002), we investigated the effect of valproic acid, and found that valproic acid improved *XBP1* induction by ER stress in patient-derived cells. At that time, we focused on polymorphisms in the *XBP1* gene as a genetic factor involved in the ER stress-induced *XBP1* response, and showed an association with bipolar disorder by genetic association analysis. However, this association was not reproducible in a larger sample set (Cichon et al., 2004), and later GWAS did not show the association with this gene. On the other hand, the attenuation of XBP1-induced response to ER stress in cultured lymphoblastoid cells derived from bipolar disorder patients was reproduced by another group (So, Warsh, & Li, 2007) and confirmed by us in an independent sample set (Hayashi et al., 2009).

We then investigated the role of the transcription factor XBP1, and showed that XBP1 is involved in plastic changes in neurites, such as neurite outgrowth, which is induced by ER stress and requires protein synthesis (Hayashi et al., 2007). XBP1 induces the causative gene of Wolfram's disease, *WFS1*, in neuroblastoma cell lines (Kakiuchi, Ishiwata, Hayashi, & Kato, 2006). Wolfram's disease is a genetic disease that causes diabetes, optic atrophy, and depression (Swift, Perkins, Chase, Sadler, & Swift, 1991). It has also been reported that mtDNA deletions accumulate in the tissues of patients with Wolfram's disease (Barrientos et al., 1996), and Wfs1 knockout mice show behavioral changes such as retardation in emotional behavior (Kato et al., 2008).

Cataldo et al. (2010) examined the morphology of mitochondria in the leukocytes and postmortem brains of patients with bipolar disorder, and reported that mitochondria were clustered around the nucleus in leukocytes and the mitochondria in the brain were smaller.

9.4 Cerebrospinal fluid

Although we have little data from studies using cerebrospinal fluid, studies using cerebrospinal fluid have also produced findings suggestive of mitochondrial dysfunction.

Lactate levels in cerebrospinal fluid are reported to be increased (Kuang, Duong, Jeong, Zachos, & Andreazza, 2018). This is consistent with findings of increased lactate by proton magnetic resonance spectroscopy (Stork & Renshaw, 2005).

On the other hand, metabolome analysis of cerebrospinal fluid from bipolar patients has found significantly higher isocitrate levels in bipolar patients (Yoshimi et al., 2016). In the postmortem brains of bipolar patients, gene expression of a subtype of isocitrate dehydrogenase (*IDH*; *IDH3A*), an enzyme that degrades isocitrate in the TCA cycle, was decreased, and animal studies suggested that this was not due to medication. Therefore the elevation of isocitrate was considered to reflect a decrease in *IDH3A* and to be a finding suggestive of mitochondrial dysfunction.

9.5 Genetics

As mentioned above, linkage analysis was actively conducted in the 20th century, and candidate genes (tyrosine hydroxylase, monoamine oxidase A, etc.) located at linkage loci were examined from a pharmacological viewpoint, but the causative gene was not identified (Kato, 2007).

We also examined *NDUFV2* (Washizuka et al., 2004), a candidate gene at the linkage locus, as a candidate gene and reported an association, but as mentioned above, candidate gene polymorphism studies in several hundred

individuals did not yield reproducible results, and these findings were not supported by GWAS, which has become popular in the 21st century.

The most recent GWAS results from the Psychiatric Genomics Consortium in 41,917 bipolar patients and 371,549 controls showed an association with 64 loci (Mullins et al., 2020). Here, in addition to the L-type Ca^{2+} channel gene *CACNA1C*, one of the earliest genes found in GWAS for bipolar disorder, an association was also found with *CACNB2*. Overall, the risk genes for bipolar disorder were enriched in gene related to synapses, Ca^{2+} signaling, and neurogenesis.

On the other hand, rare mutations with a large effect size are likely to be missed by GWAS. We sequenced polymerase gamma (mtDNA polymerase, *POLG*), the most frequent causative gene of chronic progressive external ophthalmoplegia (CPEO), a mitochondrial disease, in 796 Japanese bipolar disorder patients and 767 healthy subjects, and compared them with 1070 healthy subjects from Tohoku Medical Megabank (Kasahara et al., 2017). Mutations were found at about the same frequency (about 3%) in both patients and healthy subjects, but computer prediction showed that deleterious mutations were more common in patients. Furthermore, when POLG proteins with each mutation were produced and enzyme activity was measured, the mutations found in patients showed significantly reduced activity. In addition, the mutations reported in CPEO patients were not found in healthy subjects, but were found in three patients with bipolar disorder, which was significantly more frequent. These results indicated that the *POLG* mutations carried by bipolar patients are often deleterious mutations.

ANT1 (*SLC25A4*) is also one of the genes responsible for CPEO, and a linkage between *ANT1* mutations and bipolar disorder has been reported in a family (Siciliano et al., 2003). In this family, all have been diagnosed with bipolar disorder before the onset of mitochondrial disease. Since there are no reports of CPEO cases due to *ANT1* mutations in the Japanese population, we sequenced 304 bipolar patients in NIMH pedigrees and found that 2 (0.61%) had loss-of-function mutations (Kato et al., 2018). This was significantly more than the frequency in the database (10 out of 128,632 patients, 0.000069%).

When combined with reports that around 20% of patients with mitochondrial disease were diagnosed with bipolar disorder as a result of structured interviews (Fattal, Link, Quinn, Cohen, & Franco, 2007; Inczedy-Farkas et al., 2012; Mancuso et al., 2013), it is possible that mitochondrial disease or having a mutation that causes mitochondrial disease is a risk factor for bipolar disorder.

However, no association with *POLG* or *ANT1* has been found in whole genome or whole exome analysis studies of bipolar patients (Goes et al., 2016; Kato, 2015). However, association studies of rare mutations would require a larger number of samples.

In our exome analysis of the trio families, genes with damaging mutations were enriched for genes encoding Ca^{2+}-binding proteins (Kataoka et al., 2016).

9.6 Postmortem brains

We have previously reported that common deletions of mtDNA are detected in the postmortem brains (cerebral cortex) of patients with bipolar disorder (Kato et al., 1997), but this was not replicated by the other group (Sequeira et al., 2015). They reported regional differences in the accumulation of partially deleted mtDNA, and it would be important to determine which brain regions should be examined.

Konradi et al. (2004) reported that the postmortem brains of bipolar patients in the Harvard Brain Bank showed globally decreased expression of mitochondria-related genes, which reflects mitochondrial dysfunction. In our study using samples from the Stanley Brain Bank, the first group (consortium collection) of 60 patients did not show these findings (Iwamoto, Kakiuchi, Bundo, Ikeda, & Kato, 2004), and the second group (microarray collection) showed similar findings, but the postmortem brains of bipolar patients had generally low pH, and note the fact that mitochondria-related gene expression was found in the low pH samples. Thus we discussed that the reduced expression of mitochondria-related genes was an artifact due to the agonal factor (Iwamoto, Bundo, & Kato, 2005). However, in the Harvard Brain Bank samples, no pH decrease was seen in the bipolar patient samples, and the initial finding was not considered to be an artifact. Sun, Wang, Tseng, and Young (2006) argued that the low pH in bipolar patients itself reflects mitochondrial dysfunction in bipolar disorder. Hagihara et al. (2018) pointed that animal models of mental disorders also show low brain pH, and low pH would be an endophenotype of mental disorders. The significance of decreased mitochondria-related gene expression in the postmortem brain of bipolar patients remains to be determined. It has also been reported that the expression of mitochondrial respiratory chain proteins is decreased in the postmortem brains of bipolar patients (Sun et al., 2006), but the effects of agonal factor would be similarly an issue to be considered.

On the other hand, in our study, the expression of mitochondria-related genes increased when samples with low pH were excluded (Iwamoto et al., 2005). The most upregulated mitochondria-related gene in the consortium collection was *LARS2* (mitochondrial leucyl-tRNA synthetase) (Munakata, Iwamoto, Bundo, & Kato, 2005). *LARS2* is a nuclear gene that encodes an enzyme involved in aminoacylation of $tRNA^{Leu}$. The 3243 A > G mutation (Goto, Nonaka, & Horai, 1990), which is the causative mutation of the major mitochondrial disease MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes), impairs aminoacylation of $tRNA^{Leu\ (UUR)}$. We hypothesized that the increased expression of *LARS2* might reflect the accumulation of the 3243 A > G mutation, and examined cybrids with the mtDNA 3243 A > G mutation, and found that *LARS2* expression was indeed increased in cells with the 3243 A > G mutation. We examined the 3243 A > G mutation in postmortem brains using a highly sensitive detection

method and found small amounts of this mutation in the postmortem brains of two patients with bipolar disorder and one with schizophrenia. These patients had significantly higher expression of *LARS2* and also had high levels of 3243 A > G mutation in the liver (Munakata et al., 2005). These results suggest that the accumulation of somatic mutations of mtDNA in the brain may have a pathophysiological role in bipolar disorder.

9.7 Animal models

To test the mitochondrial dysfunction hypothesis, we created a transgenic mouse that expresses a mutant of Polg with loss of exonuclease activity specifically in the brain (m*Polg* Tg mouse) (Kasahara et al., 2006). Knock-in mice of a mutant that loses the exonuclease activity of Polg have also been produced by two groups and used as a model of aging (Kujoth et al., 2005; Trifunovic et al., 2004). In such cases, mtDNA mutations accumulate throughout the body, making it difficult to determine if the brain is the cause of behavioral changes (Fuke et al., 2014). In the m*Polg* Tg mice, as expected, brain-specific accumulation of deleted mtDNA was observed (Kasahara et al., 2006). In addition, the brain-derived mitochondria of these mice showed enhanced Ca^{2+} uptake into mitochondria and attenuated intracellular Ca^{2+} responses mediated by metabolic glutamate receptors (Kubota et al., 2006).

In the initial analysis of m*Polg* Tg mice, it was found that the amount of wheel-running behavior at the beginning of the light period was high, and that the change in wheel-running behavior with the estrous cycle in females was large, which was improved by lithium (Kasahara et al., 2006). Subsequent analysis of long-term wheel running revealed that there were significantly more periods of reduced wheel-running behavior of about two weeks (Kasahara et al., 2016). To determine whether this was a depressive state or not, we examined six of the nine A items of the diagnostic criteria for the depressive episode in the DSM-5, excluding three items that are difficult to assess in animals (depressed mood, feelings of guilt, and suicidal ideation), and B item (impairment of social functioning). As a result, we considered that this hypoactivity period met the criteria for the depressive episode in DSM-5. This episode was less frequent during treatment with a selective serotonin reuptake inhibitor and lithium, a mood stabilizer. In addition, corticosterone excretion was elevated during this episode. Based on these findings, we interpreted that this episode was homologous to depressive episodes in humans.

For example, Parkinson's disease has also been reported to have an accumulation of mtDNA deletions in neurons in the substantia nigra (Bender & Alloy, 2011), and *Polg* mutations are also known to be a risk factor for Parkinson's disease (Luoma et al., 2004). Therefore the question is where is

the brain region that accumulates mtDNA deletions that cause depressive episodes. In order to determine the brain region that accumulates the mtDNA deletions in these mice, we developed a method for mapping the location of the deleted mtDNA and examined it (Kasahara et al., 2016) (Fig. 9.2). The results showed that mtDNA deletions accumulated most frequently in the paraventricular thalamic nucleus (PVT). In the postmortem brains of mitochondrial disease patients who exhibited depression, cells with loss of cytochrome oxidase (COX), a mtDNA-derived protein (COX-negative cells), also accumulated in the paraventricular region of the thalamus. Furthermore, when neurotransmission of neurons in the PVT of normal mice was inhibited by expressing tetanus toxin, hypoactivity episodes similar to depressive episodes in model mice appeared. These results suggest that the depressive state of m*Polg* Tg mice is caused by dysfunction of the PVT.

Detailed analysis using next-generation sequencing suggested that the mtDNA deletions observed in these mice may actually be a multimers, tandem duplications of the control region of mtDNA (Bagge, Fujimori-Tonou, Kubota-Sakashita, Kasahara, & Kato, 2020). In the m*Polg* Tg mice, point mutations were also accumulated in the PVT.

Brain-specific conditional knockout mice of *Ant1* were also analyzed (Kato et al., 2018). The brain-derived mitochondria of these mice showed functional changes in that they were less able to retain Ca^{2+}. We examined wheel-running behavior, but did not find similar depressive episodes.

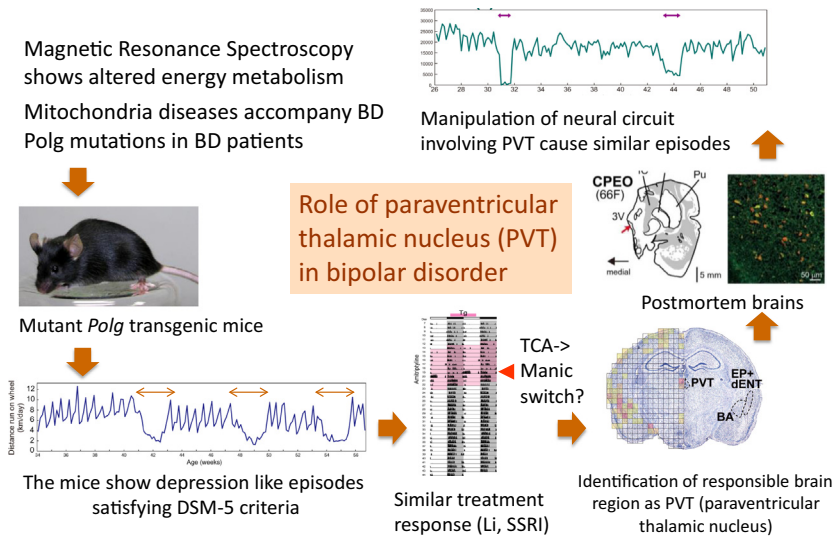

FIGURE 9.2 The process of how the study of mitochondrial dysfunction in bipolar disorder leads to the hypothetical role of paraventricular thalamic nucleus (PVT) in bipolar disorder. *BD*, bipolar disorder; *Polg*, polymerase γ; *TCA*, tricyclic antidepressant; *SSRI*, selective serotonin reuptake inhibitor; *CPEO*, chronic progressive external ophthalmoplegia.

Therefore we conducted a comprehensive behavioral analysis using IntelliCage, and found that the delay discounting of rewards (i.e., the reward obtained in the future is estimated to be lower than the reward obtained now) was attenuated. Normal mice stopped waiting when they had to wait 8 seconds for a reward (sweetened water), but *Ant1*-mutant mice continued to wait for the reward even after the 8-second interval. This reduction in delay discounting of reward was also observed in mice in which the activity of serotonin neurons was activated using optogenetics, suggesting that serotonergic function is altered in *Ant1*-mutant mice. In the search for COX-negative cells in the brains of *Ant1* mutant mice, we also found that the COX-negative cells were enriched in the dorsal raphe nucleus, where serotonin neurons are located, and increased with age. In these mice, serotonin metabolism in the brain was enhanced. Furthermore, electrophysiological studies revealed that the serotonin neurons in the dorsal raphe nucleus were in a hyperexcitable state.

9.8 Drug discovery research

In order to find drug targets for bipolar disorder, we searched for genes whose expression was commonly altered in m*Polg* Tg mice and postmortem brains of bipolar patients, and found changes in *Ppif*, a gene encoding cyclophilin D. Cyclophilin D is a component of the mitochondrial permeability transition pore (mPTP). mPTP is opened during apoptosis and cytochrome c and apoptosis-inducing factor (AIF) are released from mitochondria, resulting in cell death. Transient mPTP opening releases Ca^{2+} from mitochondria. Since cyclophilin D inhibitors have neuroprotective effects, and the phenotype of mouse models of neurodegenerative diseases is improved when crossed with cyclophilin D knockout mice (Du et al., 2008; Forte et al., 2007), compounds with mPTP inhibitory effects were expected to exert neuroprotective effects and could be useful for bipolar disorder.

We searched for drugs that inhibit mPTP using mitochondria isolated from mouse brain and found a compound that inhibits mPTP (Kato, Kubota-Sakashita, Kawakami, Kikusato, & Shirai, 2020). The compound exhibited neuroprotective effects in a middle cerebral artery occlusion model. The compound and its derivatives with mPTP inhibitory activity could be potential candidates for mood stabilizers.

9.9 iPS cell model

Mertens et al. (2015) established iPS cells from six bipolar disorder patients (three lithium responders and three nonresponders) and four control subjects and studied them by differentiating them into hippocampal dentate gyrus granular cells. The results showed increased expression of mitochondria-related genes and increased mitochondrial function; RNA sequencing showed

increased expression of PKA/PKC signaling-related genes and increased expression of action potential-related genes. Consistent with this finding, patient-derived neurons showed hyperexcitability. In cells from lithium responders, this finding was ameliorated by lithium.

We also generated iPS cells from a pair of monozygotic twins in which only one twin developed bipolar type schizoaffective disorder (Sawada et al., 2020). Brain organoids were generated from these cells, and single-cell RNA sequencing was performed. The results showed that the differentiation into GABAergic neurons was enhanced in patient-derived brain organoids compared to healthy twins. Two-dimensional cultured neurons also showed similar results. This result may indicate that the Wnt signaling pathway is impaired in bipolar schizoaffective disorder, resulting in increased differentiation into inhibitory neurons. Previous studies have reported that GABAergic neurons are decreased in the postmortem brains of patients with bipolar disorder and schizophrenia, and these results suggest that the decrease in GABAergic neurons seen in the postmortem brain might be a result of compensatory changes. In our study, however, we did not find hyperexcitability as shown by Mertens et al. and no findings suggestive of mitochondrial dysfunction.

9.10 Molecular and cellular pathogenesis in the paraventricular thalamic nucleus (PVT) and action mechanisms of antibipolar drugs

It remains unclear what pathology occurs at the cellular level in the PVT in m*Polg* Tg mice. We found that stimulation of the PVT by DREADD (designer receptors exclusively activated by designer drugs) in mice for a long period of time resulted in a repetitive hypoactive episodes similar to m*Polg* Tg mice (Kato et al., 2019). Together with the hyperexcitability in the dorsal raphe nucleus of *Ant1* mutant mice (Kato et al., 2018) and hyperexcitability in iPS cells derived from bipolar disorder patients (Mertens et al., 2015), it might be possible that the PVT in m*Polg* Tg mice also exhibits hyperexcitability.

In thalamic neurons, T-type Ca^{2+} channels are involved in the low-threshold spikes. A gene encoding T-type Ca^{2+} channel, CACNA1G, is strongly expressed in the thalamus, especially in the PVT. Valproic acid, one of the mood stabilizers, has an inhibitory effect on T-type Ca^{2+} channels, which may be involved as one of the mechanisms of action (Kato, 2019).

The mechanism of action of valproic acid in bipolar disorder has been focused on the inhibition of histone deacetylases (HDACs) (Chuang, 2005). However, considering the fact that the antiepileptic drugs lamotrigine and carbamazepine, in addition to valproic acid, are also effective for bipolar disorder and the genetic findings of an association with genes involved in cell excitability, including Ca^{2+} channels, it would be more reasonable to assume

that the anticonvulsive effect itself would be central to the antibipolar effect of these mood stabilizers.

9.11 Neural circuits around the paraventricular thalamic nucleus (PVT)

From the PVT, there are projections to the nucleus accumbens, amygdala, bed nucleus of stria terminalis, anterior cingulate gyrus, and insular cortex. There are projections from the suprachiasmatic nucleus of the hypothalamus, serotonergic nerves, etc., to the PVT (Kirouac, 2015).

Mass spectrometry imaging studies have identified the PVT as a site of high levels of both serotonin and noradrenaline (Sugiyama et al., 2019).

Several serotonin receptors are expressed in the PVT, among which the serotonin 7 receptor is the most highly expressed in the PVT among any other brain regions (Horisawa, Ishiyama, Ono, Ishibashi, & Taiji, 2013). Lurasidone that is effective for bipolar depression (Kato, Ishigooka, et al., 2020) is an inhibitor of the serotonin 7 receptor, and it is possible that the serotonin 7 receptor may be involved in its mechanism of action.

9.12 Conclusion

With regard to the mitochondrial dysfunction hypotheses that we originally proposed, we believe that we have proven that mtDNA mutations impair intracellular Ca^{2+} signaling. During the period when we are focusing on testing the mitochondrial dysfunction, genetic studies have ensured that bipolar disorder is associated with Ca^{2+} channels. Thus Ca^{2+} signaling is central to the molecular and cellular pathogenesis of bipolar disorder, and mitochondria should also be considered as one player in Ca^{2+} signaling. Disturbed Ca^{2+} regulation by dysfunction of endoplasmic reticulum should also play a role in bipolar disorder (Nakajima et al., 2021).

Even if we say that mitochondria and Ca^{2+} signaling are involved in bipolar disorder, it is no different from saying that cells are involved in bipolar disorder, because most cells have mitochondria, and Ca^{2+} signaling plays an important role in all cells. Thus there is a need to clarify which neural systems in the brain are altered by mitochondrial dysfunction and how they are related to the pathogenesis of bipolar disorder.

In our search for answers to this question, a neural circuit from the serotonin neurons to the PVT has emerged. The possibility that valproic acid and lurasidone exert their effects via the PVT was also considered.

Interest in the function of the PVT has increased over the past five years, and it has been reported to be involved in a variety of functions, including fear conditioning (Penzo et al., 2015), opiate withdrawal (Zhu, Wienecke, Nachtrab, & Chen, 2016), salience (Zhu et al., 2018), and arousal (Ren et al., 2018), among others, but the full picture of its function remains unclear.

Neurons in the PVT send collateral to the amygdala, which is involved in fear, and the nucleus accumbens, which is involved in reward (Dong, Li, & Kirouac, 2017). In other words, they may have an effect of intensifying emotions regardless of whether they are negative or positive, which makes sense if we think of the manic and depressive states of bipolar disorder as an extreme intensification of normal emotional responses.

The pathogenesis of bipolar disorder is still not completely understood, and core questions such as whether some lesions in the PVT are observed in the postmortem brains of bipolar patients and whether there are some changes in the neural activity of the PVT in bipolar patients have not yet been answered. However, we believe that we have reached a clue to understand the pathogenesis of bipolar disorder through our research over the past 20 years.

Acknowledgments

I would like to express my sincere gratitude to the patients and families who participated in the research and the members of the Laboratory of Molecular Dynamics of Mental Disorders for conducting these studies.

References

Bagge, E. K., Fujimori-Tonou, N., Kubota-Sakashita, M., Kasahara, T., & Kato, T. (2020). Unbiased PCR-free spatio-temporal mapping of the mtDNA mutation spectrum reveals brain region-specific responses to replication instability. *BMC Biology*, *18*(1), 150.

Barrientos, A., Volpini, V., Casademont, J., Genis, D., Manzanares, J. M., Ferrer, I., et al. (1996). A nuclear defect in the 4p16 region predisposes to multiple mitochondrial DNA deletions in families with Wolfram syndrome. *The Journal of Clinical Investigation*, *97*(7), 1570–1576.

Bender, R. E., & Alloy, L. B. (2011). Life stress and kindling in bipolar disorder: Review of the evidence and integration with emerging biopsychosocial theories. *Clinical Psychology Review*, *31*(3), 383–398.

Bown, C. D., Wang, J. F., Chen, B., & Young, L. T. (2002). Regulation of ER stress proteins by valproate: Therapeutic implications. *Bipolar Disorders*, *4*(2), 145–151.

Cataldo, A. M., McPhie, D. L., Lange, N. T., Punzell, S., Elmiligy, S., Ye, N. Z., et al. (2010). Abnormalities in mitochondrial structure in cells from patients with bipolar disorder. *The American Journal of Pathology*, *177*(2), 575–585.

Chen, G., Zeng, W. Z., Yuan, P. X., Huang, L. D., Jiang, Y. M., Zhao, Z. H., et al. (1999). The mood-stabilizing agents lithium and valproate robustly increase the levels of the neuroprotective protein bcl-2 in the CNS. *Journal of Neurochemistry*, *72*(2), 879–882.

Chuang, D. M. (2005). The antiapoptotic actions of mood stabilizers: Molecular mechanisms and therapeutic potentials. *Annals of the New York Academy of Sciences*, *1053*, 195–204.

Cichon, S., Buervenich, S., Kirov, G., Akula, N., Dimitrova, A., Green, E., et al. (2004). Lack of support for a genetic association of the XBP1 promoter polymorphism with bipolar disorder in probands of European origin. *Nature Genetics*, *36*(8), 783–784, author reply 4–5.

Dager, S. R., Friedman, S. D., Parow, A., Demopulos, C., Stoll, A. L., Lyoo, I. K., et al. (2004). Brain metabolic alterations in medication-free patients with bipolar disorder. *Archives of General Psychiatry*, *61*(5), 450–458.

Dong, X., Li, S., & Kirouac, G. J. (2017). Collateralization of projections from the paraventricular nucleus of the thalamus to the nucleus accumbens, bed nucleus of the stria terminalis, and central nucleus of the amygdala. *Brain Structure & Function*, *222*(9), 3927–3943.

Du, H., Guo, L., Fang, F., Chen, D., Sosunov, A. A., McKhann, G. M., et al. (2008). Cyclophilin D deficiency attenuates mitochondrial and neuronal perturbation and ameliorates learning and memory in Alzheimer's disease. *Nature Medicine*, *14*(10), 1097–1105.

Fattal, O., Link, J., Quinn, K., Cohen, B. H., & Franco, K. (2007). Psychiatric comorbidity in 36 adults with mitochondrial cytopathies. *CNS Spectrums*, *12*(6), 429–438.

Forte, M., Gold, B. G., Marracci, G., Chaudhary, P., Basso, E., Johnsen, D., et al. (2007). Cyclophilin D inactivation protects axons in experimental autoimmune encephalomyelitis, an animal model of multiple sclerosis. *Proceedings of the National Academy of Sciences of the USA*, *104*(18), 7558–7563.

Fuke, S., Kametani, M., Yamada, K., Kasahara, T., Kubota-Sakashita, M., Kujoth, G. C., et al. (2014). Heterozygous Polg mutation causes motor dysfunction due to mtDNA deletions. *Annals of Clinical and Translational Neurology*, *1*(11), 909–920.

Goes, F. S., Pirooznia, M., Parla, J. S., Kramer, M., Ghiban, E., Mavruk, S., et al. (2016). Exome sequencing of familial bipolar disorder. *JAMA Psychiatry*, *73*(6), 590–597.

Goto, Y., Nonaka, I., & Horai, S. (1990). A mutation in the tRNA(Leu)(UUR) gene associated with the MELAS subgroup of mitochondrial encephalomyopathies. *Nature*, *348*(6302), 651–653.

Hagihara, H., Catts, V. S., Katayama, Y., Shoji, H., Takagi, T., Huang, F. L., et al. (2018). Decreased brain pH as a shared endophenotype of psychiatric disorders. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, *43*(3), 459–468.

Hayashi, A., Kasahara, T., Iwamoto, K., Ishiwata, M., Kametani, M., Kakiuchi, C., et al. (2007). The role of brain-derived neurotrophic factor (BDNF)-induced XBP1 splicing during brain development. *The Journal of Biological Chemistry*, *282*(47), 34525–34534.

Hayashi, A., Kasahara, T., Kametani, M., Toyota, T., Yoshikawa, T., & Kato, T. (2009). Aberrant endoplasmic reticulum stress response in lymphoblastoid cells from patients with bipolar disorder. *The International Journal of Neuropsychopharmacology/Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *12*(1), 33–43.

Horisawa, T., Ishiyama, T., Ono, M., Ishibashi, T., & Taiji, M. (2013). Binding of lurasidone, a novel antipsychotic, to rat 5-HT7 receptor: Analysis by [3H]SB-269970 autoradiography. *Progress in Neuropsychopharmacology & Biological Psychiatry*, *40*, 132–137.

Inczedy-Farkas, G., Remenyi, V., Gal, A., Varga, Z., Balla, P., Udvardy-Meszaros, A., et al. (2012). Psychiatric symptoms of patients with primary mitochondrial DNA disorders. *Behavioral and Brain Functions*, *8*, 9.

Iwamoto, K., Bundo, M., & Kato, T. (2005). Altered expression of mitochondria-related genes in postmortem brains of patients with bipolar disorder or schizophrenia, as revealed by large-scale DNA microarray analysis. *Human Molecular Genetics*, *14*(2), 241–253.

Iwamoto, K., Kakiuchi, C., Bundo, M., Ikeda, K., & Kato, T. (2004). Molecular characterization of bipolar disorder by comparing gene expression profiles of postmortem brains of major mental disorders. *Molecular Psychiatry*, *9*(4), 406–416.

Kakiuchi, C., Ishiwata, M., Hayashi, A., & Kato, T. (2006). XBP1 induces WFS1 through an endoplasmic reticulum stress response element-like motif in SH-SY5Y cells. *Journal of Neurochemistry*, *97*(2), 545–555.

Kasahara, T., Ishiwata, M., Kakiuchi, C., Fuke, S., Iwata, N., Ozaki, N., et al. (2017). Enrichment of deleterious variants of mitochondrial DNA polymerase gene (POLG1) in bipolar disorder. *Psychiatry and Clinical Neurosciences*, *71*(8), 518−529.

Kasahara, T., Kubota, M., Miyauchi, T., Noda, Y., Mouri, A., Nabeshima, T., et al. (2006). Mice with neuron-specific accumulation of mitochondrial DNA mutations show mood disorder-like phenotypes. *Molecular Psychiatry*, *11*(6), 577−593, 23.

Kasahara, T., Takata, A., Kato, T. M., Kubota-Sakashita, M., Sawada, T., Kakita, A., et al. (2016). Depression-like episodes in mice harboring mtDNA deletions in paraventricular thalamus. *Molecular Psychiatry*, *21*(1), 39−48.

Kataoka, M., Matoba, N., Sawada, T., Kazuno, A. A., Ishiwata, M., Fujii, K., et al. (2016). Exome sequencing for bipolar disorder points to roles of de novo loss-of-function and protein-altering mutations. *Molecular Psychiatry*, *21*(7), 885−893.

Kato, T. (2019). Current understanding of bipolar disorder: Toward integration of biological basis and treatment strategies. *Psychiatry and Clinical Neurosciences*, *73*(9), 526−540.

Kato, T. (2007). Molecular genetics of bipolar disorder and depression. *Psychiatry and Clinical Neurosciences*, *61*(1), 3−19.

Kato, T. (2015). Whole genome/exome sequencing in mood and psychotic disorders. *Psychiatry and Clinical Neurosciences*, *69*(2), 65−76.

Kato, T., Ishigooka, J., Miyajima, M., Watabe, K., Fujimori, T., Masuda, T., et al. (2020). Double-blind, placebo-controlled study of lurasidone monotherapy for the treatment of bipolar I depression. *Psychiatry and Clinical Neurosciences*, *74*(12), 635−644.

Kato, T., Ishiwata, M., Mori, K., Washizuka, S., Tajima, O., Akiyama, T., et al. (2003). Mechanisms of altered Ca^{2+} signalling in transformed lymphoblastoid cells from patients with bipolar disorder. *The International Journal of Neuropsychopharmacology/Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*, *6*(4), 379−389.

Kato, T., Ishiwata, M., Yamada, K., Kasahara, T., Kakiuchi, C., Iwamoto, K., et al. (2008). Behavioral and gene expression analyses of Wfs1 knockout mice as a possible animal model of mood disorder. *Neuroscience Research*, *61*(2), 143−158.

Kato, T., & Kato, N. (2000). Mitochondrial dysfunction in bipolar disorder. *Bipolar Disorders*, *2* (3 Pt 1), 180−190.

Kato, T., Kubota-Sakashita, M., Kawakami, H., Kikusato, K., & Shirai, F. (2020). Mitochondrial permeability transition pore (mPTP) inhibitors, novel compounds inhibiting mPTP and their use. Patent application, WO/2020/255983.

Kato, T., Stine, O. C., McMahon, F. J., & Crowe, R. R. (1997). Increased levels of a mitochondrial DNA deletion in the brain of patients with bipolar disorder. *Biological Psychiatry*, *42* (10), 871−875.

Kato, T., Takahashi, S., Shioiri, T., & Inubushi, T. (1993). Alterations in brain phosphorous metabolism in bipolar disorder detected by in vivo 31P and 7Li magnetic resonance spectroscopy. *Journal of Affective Disorders*, *27*(1), 53−59.

Kato, T., Takahashi, S., Shioiri, T., Murashita, J., Hamakawa, H., & Inubushi, T. (1994). Reduction of brain phosphocreatine in bipolar II disorder detected by phosphorus-31 magnetic resonance spectroscopy. *Journal of Affective Disorders*, *31*(2), 125−133.

Kato, T. M., Fujimori-Tonou, N., Mizukami, H., Ozawa, K., Fujisawa, S., & Kato, T. (2019). Presynaptic dysregulation of the paraventricular thalamic nucleus causes depression-like behavior. *Scientific Reports*, *9*(1), 16506.

Kato, T. M., Kubota-Sakashita, M., Fujimori-Tonou, N., Saitow, F., Fuke, S., Masuda, A., et al. (2018). Ant1 mutant mice bridge the mitochondrial and serotonergic dysfunctions in bipolar disorder. *Molecular Psychiatry*, *23*(10), 2039−2049.

Kazuno, A. A., Munakata, K., Nagai, T., Shimozono, S., Tanaka, M., Yoneda, M., et al. (2006). Identification of mitochondrial DNA polymorphisms that alter mitochondrial matrix pH and intracellular calcium dynamics. *PLOS Genetics*, *2*(8), e128.

Kirouac, G. J. (2015). Placing the paraventricular nucleus of the thalamus within the brain circuits that control behavior. *Neuroscience and Biobehavioral Reviews*, *56*, 315–329.

Konradi, C., Eaton, M., MacDonald, M. L., Walsh, J., Benes, F. M., & Heckers, S. (2004). Molecular evidence for mitochondrial dysfunction in bipolar disorder. *Archives of General Psychiatry*, *61*(3), 300–308.

Kuang, H., Duong, A., Jeong, H., Zachos, K., & Andreazza, A. C. (2018). Lactate in bipolar disorder: A systematic review and meta-analysis. *Psychiatry and Clinical Neurosciences*.

Kubota, M., Kasahara, T., Nakamura, T., Ishiwata, M., Miyauchi, T., & Kato, T. (2006). Abnormal Ca^{2+} dynamics in transgenic mice with neuron-specific mitochondrial DNA defects. *The Journal of Neuroscience*, *26*(47), 12314–12324.

Kujoth, G. C., Hiona, A., Pugh, T. D., Someya, S., Panzer, K., Wohlgemuth, S. E., et al. (2005). Mitochondrial DNA mutations, oxidative stress, and apoptosis in mammalian aging. *Science*, *309*(5733), 481–484.

Luoma, P., Melberg, A., Rinne, J. O., Kaukonen, J. A., Nupponen, N. N., Chalmers, R. M., et al. (2004). Parkinsonism, premature menopause, and mitochondrial DNA polymerase gamma mutations: Clinical and molecular genetic study. *Lancet*, *364*(9437), 875–882.

Mancuso, M., Orsucci, D., Ienco, E. C., Pini, E., Choub, A., & Siciliano, G. (2013). Psychiatric involvement in adult patients with mitochondrial disease. *Neurological Sciences: Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, *34*(1), 71–74.

Mertens, J., Wang, Q. W., Kim, Y., Yu, D. X., Pham, S., Yang, B., et al. (2015). Differential responses to lithium in hyperexcitable neurons from patients with bipolar disorder. *Nature*, *527*(7576), 95–99.

Mullins, N., Forstner, A., O'Connell, K., Coombes, B., Coleman, J., Qiao, Z., et al. (2020). Genome-wide association study of over 40,000 bipolar disorder cases provides novel biological insights. *medRxiv*.

Munakata, K., Iwamoto, K., Bundo, M., & Kato, T. (2005). Mitochondrial DNA 3243A > G mutation and increased expression of LARS2 gene in the brains of patients with bipolar disorder and schizophrenia. *Biological Psychiatry*, *57*(5), 525–532.

Nakajima, K., Ishiwata, M., Weitemier, A., Shoji, H., Monai, H., Miyamoto, H., et al. (2021). Brain-specific heterozygous loss-of-function of ATP2A2, endoplasmic reticulum Ca^{2+} pump responsible for Darier's disease, causes behavioral abnormalities and a hyperdopaminergic state. *Human Molecular Genetics*.

Penzo, M. A., Robert, V., Tucciarone, J., De Bundel, D., Wang, M., Van Aelst, L., et al. (2015). The paraventricular thalamus controls a central amygdala fear circuit. *Nature*, *519*(7544), 455–459.

Ren, S., Wang, Y., Yue, F., Cheng, X., Dang, R., Qiao, Q., et al. (2018). The paraventricular thalamus is a critical thalamic area for wakefulness. *Science*, *362*(6413), 429–434.

Sawada, T., Chater, T. E., Sasagawa, Y., Yoshimura, M., Fujimori-Tonou, N., Tanaka, K., et al. (2020). Developmental excitation-inhibition imbalance underlying psychoses revealed by single-cell analyses of discordant twins-derived cerebral organoids. *Molecular Psychiatry*, *25*(11), 2695–2711.

Sequeira, A., Rollins, B., Magnan, C., van Oven, M., Baldi, P., Myers, R. M., et al. (2015). Mitochondrial mutations in subjects with psychiatric disorders. *PLOS ONE*, *10*(5), e0127280.

Siciliano, G., Tessa, A., Petrini, S., Mancuso, M., Bruno, C., Grieco, G. S., et al. (2003). Autosomal dominant external ophthalmoplegia and bipolar affective disorder associated with a mutation in the ANT1 gene. *Neuromuscular Disorders*, *13*(2), 162–165.

So, J., Warsh, J. J., & Li, P. P. (2007). Impaired endoplasmic reticulum stress response in B-lymphoblasts from patients with bipolar-I disorder. *Biological Psychiatry*, *62*(2), 141–147.

Stork, C., & Renshaw, P. F. (2005). Mitochondrial dysfunction in bipolar disorder: Evidence from magnetic resonance spectroscopy research. *Molecular Psychiatry*, *10*(10), 900–919.

Sugiyama, E., Guerrini, M. M., Honda, K., Hattori, Y., Abe, M., Kallback, P., et al. (2019). Detection of a high-turnover serotonin circuit in the mouse brain using mass spectrometry imaging. *iScience*, *20*, 359–372.

Sun, X., Wang, J. F., Tseng, M., & Young, L. T. (2006). Downregulation in components of the mitochondrial electron transport chain in the postmortem frontal cortex of subjects with bipolar disorder. *Journal of Psychiatry & Neuroscience*, *31*(3), 189–196.

Suomalainen, A., Majander, A., Haltia, M., Somer, H., Lonnqvist, J., Savontaus, M. L., et al. (1992). Multiple deletions of mitochondrial DNA in several tissues of a patient with severe retarded depression and familial progressive external ophthalmoplegia. *The Journal of Clinical Investigation*, *90*(1), 61–66.

Swift, R. G., Perkins, D. O., Chase, C. L., Sadler, D. B., & Swift, M. (1991). Psychiatric disorders in 36 families with Wolfram syndrome. *The American Journal of Psychiatry*, *148*(6), 775–779.

Trifunovic, A., Wredenberg, A., Falkenberg, M., Spelbrink, J. N., Rovio, A. T., Bruder, C. E., et al. (2004). Premature ageing in mice expressing defective mitochondrial DNA polymerase. *Nature*, *429*(6990), 417–423.

Warsh, J. J., Andreopoulos, S., & Li, P. P. (2004). Role of intracellular calcium signaling in the pathophysiology and pharmacotherapy of bipolar disorder: Current status. *Clinical Neuroscience Research*, *4*, 201–213.

Washizuka, S., Iwamoto, K., Kazuno, A., Kakiuchi, C., Mori, K., Kametani, M., et al. (2004). Association of mitochondrial complex I subunit gene NDUFV2 at 18p11 with bipolar disorder in Japanese and the NIMH pedigrees. *Biological Psychiatry*, *56*(7), 483–489.

Yoshimi, N., Futamura, T., Bergen, S. E., Iwayama, Y., Ishima, T., Sellgren, C., et al. (2016). Cerebrospinal fluid metabolomics identifies a key role of isocitrate dehydrogenase in bipolar disorder: Evidence in support of mitochondrial dysfunction hypothesis. *Molecular Psychiatry*, *21*(11), 1504–1510.

Zhu, Y., Nachtrab, G., Keyes, P. C., Allen, W. E., Luo, L., & Chen, X. (2018). Dynamic salience processing in paraventricular thalamus gates associative learning. *Science*, *362*(6413), 423–429.

Zhu, Y., Wienecke, C. F., Nachtrab, G., & Chen, X. (2016). A thalamic input to the nucleus accumbens mediates opiate dependence. *Nature*, *530*(7589), 219–222.