Review

Biological Psychiatry

What Can Mitochondrial DNA Analysis Tell Us About Mood Disorders?

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ABSTRACT

Variants in mitochondrial DNA (mtDNA) and nuclear genes encoding mitochondrial proteins in bipolar disorder, depression, or other psychiatric disorders have been studied for decades, since mitochondrial dysfunction was first suggested in the brains of patients with these diseases. Candidate gene association studies initially resulted in findings compatible with the mitochondrial dysfunction hypothesis. Many of those studies, however, were conducted with modest sample sizes ($N < 1000$), which could cause false positive findings. Furthermore, the DNA samples examined in these studies, including genome-wide association studies, were generally derived from peripheral tissues. One key unanswered question is whether there is an association between mood disorders and somatic mtDNA mutations (deletions and point mutations) in brain regions that accumulate a high amount of mtDNA mutations and/or are involved in the regulation of mood. Two lines of robust evidence supporting the importance of mtDNA mutations in brain tissues for mood disorders have come from clinical observation of mitochondrial disease patients who carry primary mtDNA mutations or accumulate secondary mtDNA mutations due to nuclear mutations and an animal model study. More than half of mitochondrial disease patients have comorbid mood disorders, and mice with neuron-specific accumulation of mtDNA mutations show spontaneous depression-like episodes. In this review, we first summarize the current knowledge of mtDNA and its genetics and discuss what mtDNA analysis tells us about neuropsychiatric disorders based on an example of Parkinson's disease. We also discuss challenges and future directions beyond mtDNA analysis toward an understanding of the pathophysiology of "idiopathic" mood disorders.

Keywords: Chronic progressive external ophthalmoplegia, Mitochondrial disease, Mitochondrial DNA, Mitochondrial dysfunction, Paraventricular thalamus, POLG

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Mitochondrial DNA (mtDNA) is the genetic material of mitochondria, the intracellular organelles that are present in almost all cells of the body. There are vast differences in patterns of genetic inheritance and variations between mtDNA and the nuclear genome. mtDNA was a target of genetic analysis before the nuclear genome, because mtDNA is overwhelmingly smaller (1.7×10^4 base pairs [bp] vs. 3.2×10^9 bp) (Figure 1), and there are hundreds or thousands of copies of mtDNA in each cell (1,2). The complete sequence of human mtDNA was determined more than 20 years before completion of the Human Genome Project (3).

The more we understand mtDNA and mitochondria, however, the more difficult a target mtDNA turns out to be in genetics. mtDNA is maternally transmitted and does not appear to undergo recombination, and therefore conventional, family-based association studies are not applicable, and mtDNA polymorphisms cannot be treated as independent polymorphisms. Thus, the entire mtDNA sequence should be analyzed as if it is one genotype, which is called a haplogroup. Because haplogroups vary greatly across human populations (4), genetic association studies of mtDNA are subject to a larger degree of population stratification than those on the nuclear genome. A simulation test predicted that approximately 6000 cases and a similar number of control subjects would be required to achieve 90% power to detect a 10% change in frequency, even when the most common haplogroup is associated (5). A case-control association study of mtDNA polymorphisms in approximately 50,000 individuals from 11 common diseases and control subjects identified several polymorphisms associated with eight diseases, including schizophrenia, after multiple testing correction (6). However, the analysis of functional effect of each mtDNA polymorphism would be hampered by other polymorphisms within the same haplogroup. For example, we showed that mitochondrial calcium levels were lower in cybrids (transmitochondrial hybrid cells) with m.10398A>G (7) that is protective for multiple diseases (6). However, m.10398A>G was closely linked to another polymorphism, m.8701A>G, and it was difficult to assess the functional effects of the two polymorphisms separately (7). Rare variants, which are present sporadically across haplotypes, also interfere with association studies and functional analyses. Another complicating factor is heteroplasmy (8), which refers to the presence of two (or more) different sequences of mtDNA in a single individual cell. In patients with a mitochondrial disease caused by a maternally inherited heteroplasmic mtDNA mutation, such as m.3243A>G in individuals with mitochondrial

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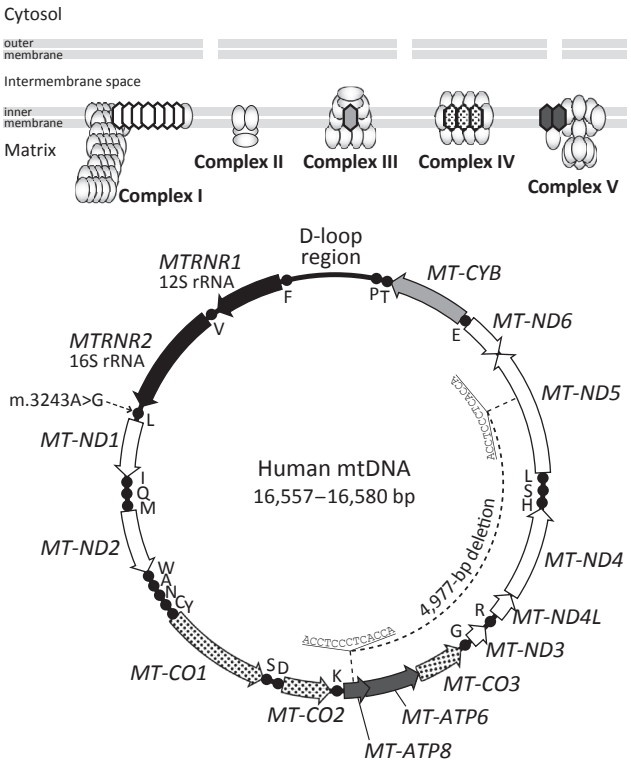

Figure 1. Human mitochondrial DNA (mtDNA) and complexes for respiratory chain/oxidative phosphorylation system. The 37 mtDNA genes include two ribosomal RNA genes (12S and 16S), 22 transfer RNA genes (shown by one-letter codes), and 13 genes encoding structural subunits of the complexes for respiratory chain/oxidative phosphorylation (7 subunits of complex I [white], one of complex III [light gray], three of complex IV [polka dot], and two of complex V [dark gray]). Complexes I, III, IV, and V contain both mtDNA-encoded subunits (represented by hexagonal shapes) and nuclear gene–encoded subunits (ovoid shapes), whereas complex II is comprised of only nuclear gene–encoded subunits. The standard gene symbols of mtDNA genes are italicized. Replication and transcription are usually initiated within the displacement loop (D-loop) region, where no genes are located. The most common pathogenic mutation in MELAS, m.3243A>G, is shown in the direction of 10 o'clock. Since an identical sequence stretch of 13 base pairs (bp) (ACCTCCCTCACCA) is located in *MT-ATP8* and *MT-ND5*, the region of 4799 bp between the two sites is prone to be deleted in human cells. The human mtDNA genome comprises about 16.6 kb. The first published sequence of human mtDNA (3), referred to as the Cambridge reference sequence, was 16,569 bp. Resequencing of the mtDNA belonging to a European haplogroup confirms that there are 11 incorrect nucleotides in the Cambridge Reference Sequence. One of them is the CC doublet at positions 3106 and 3107, which is actually a single cytosine residue. In the revised Cambridge Reference Sequence (GenBank NC_012920.1) (103), nucleotide numbers are maintained by insertion of 'N' at position 3107. The length of human mtDNA varies generally depending on haplogroup background.

myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (Online Mendelian Inheritance in Man #540000), the ratio of mutant mtDNA compared with the wild-type varies among cells and tissues, and the clinical manifestations vary among individuals (8–10). Modifier genes in the nuclear genome have been suggested to account for the individual difference (11). Further complicating matters is the fact that the somatic mutation rate of mtDNA is much higher than that of the nuclear genome (12–14). An inability to detect mutations in blood cells cannot exclude the possibility that the brain (or other organs) of a patient carries somatic mutations. To clarify the relationship between mtDNA mutations and mood disorders and to test the mitochondrial dysfunction hypothesis of the diseases (15–17), it is of great significance to study mental symptoms in mitochondrial disease patients and in animal models with mtDNA mutations (16).

We review the latest knowledge on the unique characteristics of mtDNA and the relationship between mtDNA mutations and Parkinson's disease (PD) as a model case of mtDNA mutation–associated nervous system disease. We then briefly summarize knowledge from research on polymorphisms and deletions of mtDNA in patients with mood disorders and review the research on psychiatric symptoms of mitochondrial disease patients and those of animal models. The challenges and future directions after mtDNA analysis will also be discussed.

THE CURRENT UNDERSTANDING OF mtDNA

Over a period of a billion years since commencement of symbiosis, the origin of mitochondria, most of the symbiotic genome has been transferred to the host genome. SAR11, the Rickettsiales (Alphaproteobacteria) that are closest to the inferred symbiotic bacteria (18,19), have a genome of about 1000 kb, whereas the human mtDNA is no longer than 16.6 kb (Figure 1). It carries 13 protein-coding genes, two mitochondrial ribosomal RNAs, and 22 mitochondrial transfer RNAs. All 13 proteins are involved in mitochondrial oxidative phosphorylation (Figure 1). The other genes essential for mitochondrial biology are encoded by the nuclear genome; of the approximately 20,000 genes in the human nuclear genome, at least 1100 genes are estimated to encode proteins that are transported to and function in the mitochondria (20). According to the endosymbiotic theory, transferring of mtDNA genes to the nuclear genome has been beneficial for mtDNA (the endosymbiont genome) because the mutation rate of mtDNA is much higher than that of the nuclear DNA (21,22). Mitochondria generate a lot of reactive oxygen species (ROS) as byproducts of the mitochondrial respiration process, and the repair system of mtDNA is less efficient than that of nuclear DNA (23). In addition, humans and other eukaryotes have a diploid genome and the sex system offering large gene pools, which robustly protect endosymbiont-derived genes in the nuclear genome. The main mutagenic product of base damage in mtDNA is 8-oxoguanine, causing G→T transversion upon replication (24). This is the first step of a vicious cycle of ROS generation and mtDNA mutations. ROS produced by respiration cause mtDNA mutations, which lead to dysfunction in the electron transport chain and to a further increase in ROS generation due to this dysfunction (25,26).

Multiple deletions or a single deletion in mtDNA can also occur as somatic mutations. Small truncated mtDNAs with large deletions (for example, 30% of the total length) can be maintained in mitochondria if the region involved in replication (mtDNA control region or the D-loop region) is intact. Replication of deleted mtDNAs can be completed in a shorter time and they may increase in abundancy preferentially (27,28). Probably by chance, human mtDNA has two identical 13–bp sequences separated by 4977 bp (Figure 1), and the

approximately 5-kb DNA stretch between the two sites is likely to be lost (called "common deletion"). This suggests that a repair system working on mtDNA is similar to microhomology-mediated end joining, one of the nuclear DNA repair systems (29), and a mtDNA replication-dependent repair pathway (30), although mtDNA was previously thought to lack efficient DNA repair mechanisms.

Our sensitive quantitative polymerase chain reaction method for assaying deleted mtDNA in mutant mtDNA polymerase (*Polg*) knock-in mice showed that multiple deleted mtDNAs were more abundantly accumulated in the neural tissues (28). Although neurons are in a postmitotic state, mtDNAs in the cells are actively metabolized (i.e., replicated and degraded). Pulse labeling of mtDNA using 5-bromo-2-deoxyuridie incorporation revealed that the time to reach the half maximum levels of 5-bromo-2-deoxyuridie incorporation was shortened in the brain (approximately 4 hours) compared to the liver (28). The small truncated mtDNAs are able to be replicated and transcribed, and thus produce mutant mitochondrial proteins, which accelerate the vicious cycle of ROS in mitochondria (16).

New problems have emerged in the era of next-generation sequencing: next-generation sequencing helps us to examine mtDNA, but it is difficult to distinguish between heteroplasmy and sequence errors. The presence of mtDNA-like sequences in the nuclear genome (nuclear mitochondrial sequences) is also a major obstacle (31). Human mtDNA genes are still being transferred into the nuclear DNA, and thereby a number of nuclear mitochondrial sequences exist across the nuclear genome. During the human lineage of evolution, mutations have occurred in the nuclear mitochondrial sequences, which are likely to produce spurious variant calls. To reduce the risk, a bioinformatics pipeline, MToolBox, will be helpful, which filters out nuclear mitochondrial sequences and also reconstructs the mtDNA sequence from next-generation sequencing data (32).

In addition to point mutations and deletions, depletion of mtDNA should also be considered a predisposing factor for mitochondrial diseases and related disorders (33). In most cases of mtDNA depletions, nuclear mutations in the enzymes for mtDNA replication are involved (33). To detect depletion of mtDNA or to measure mtDNA copy number, quantitative polymerase chain reaction can be performed using primers targeting specific regions of mtDNA (for example, the D-loop region, *MT-CO1* genes) (Figure 1). Primers and probes should be carefully designed and validated because many nuclear mitochondrial sequences are almost identical to the mtDNA sequences.

THE LESSON FROM PD

There are many diseases in which mitochondrial dysfunction is involved (34–36), such as diabetes mellitus and PD, because of the presence of mitochondria in almost all cells in humans. Before we discuss psychiatric disorders and mtDNA mutations, we review the mtDNA genetics in PD as a good precedent of neuronal diseases with the potential involvement of mtDNA mutations. The link between PD and mitochondrial dysfunction (37–39) first came from the observation that accidental exposure to 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine resulted in parkinsonism. In the brain, 1-methyl-4-phenyl-1,2,3,4-tetrahydropyridine is converted into 1-methyl-4-phenylpyridinium, an inhibitor of mitochondrial complex I. 1-Methyl-4-phenylpyridinium is selectively taken up by dopaminergic neurons in the substantia nigra (SN), resulting in their degeneration. Genetics of familial PD has also suggested mitochondrial dysfunction to play a role in PD (40,41). Briefly, PINK1 (encoded by *PARK6*) detects damaged mitochondria with reduced membrane potential, and PINK1 kinase activity initiates Parkin (*PARK2*) translocation to mitochondria and mitophagy. In addition, DJ-1 (*PARK7*) is a mitochondrial peroxiredoxin-like peroxidase scavenging mitochondrial ROS.

Despite the solid link between PD and mitochondrial dysfunction, association with mtDNA polymorphisms in case-control association studies was not reproducible (42). This was due to the small number of subjects, the lack of identification of haplogroups, a failure to control population stratification, and other factors. An increase in sample size may resolve this problem in part (6). In contrast, the role of somatic mtDNA mutations has been strongly suggested in PD. Patients with chronic progressive external ophthalmoplegia (CPEO) frequently have comorbid Parkinsonism (39,43,44). CPEO is a Mendelian inheritance mitochondrial disease (Online Mendelian Inheritance in Man #157640) in which mutations in the *POLG* gene and others cause an increase in multiple deletions in mtDNA. Importantly, mtDNA deletions are accumulated in neurons microdissected from the SN of postmortem brains from patients with idiopathic PD compared to age-matched control subjects (45).

What the lesson from PD tells is to focus not on mtDNA polymorphisms in peripheral tissues but on somatic mtDNA mutations in tissues responsible for the disease. For brain disorders, studying patients with mitochondrial diseases and comorbid mood disorders will ultimately be informative and lead to drug targets and understanding the mental illnesses (16).

mtDNA VARIANTS IN MOOD DISORDERS

In the past 20 years, a number of studies have explored mtDNA variants in patients with psychiatric diseases (46,47). The mtDNA variants explored included polymorphisms, rare variants, heteroplasmic mutations causative for mitochondrial diseases, such as the mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes mutation m.3243A>G (48), and other somatic mutations, including the common deletion (49–53).

Most of the reported genetic association studies of mtDNA polymorphisms and mood disorders were performed in small samples and cannot be considered robust findings at the current standard. Hundreds of mtDNA polymorphisms can be tested using recent single nucleotide polymorphism arrays that are used for genome-wide association study analyses. A modest association of mtDNA polymorphisms m.3666G>A and m.15784T>C with bipolar disorder (BD) was detected in genome-wide association study (965 cases and 3938 control subjects) (52). Even if in a larger-scale association study some mtDNA polymorphism associations will be found to be significant after correcting for multiple testing, they are not likely to bring us closer to connecting the polymorphisms and

Table 1. Comorbid Mood Disorders in Mitochondrial Disease Patients

Medical Condition	Subjects, *n*	Diagnosis Method	Prevalence of Comorbid Psychiatric Disorders	Reference
Mitochondrial diseases	36	MINI	MDD 54%, BD 17%	Fattal *et al.* (72)
Mitochondrial diseases (adolescents)	35	DSM-IV	MDD 14%, suspected depression 9%	Koene *et al.* (73)
CPEO	19	SCID	Depressive state 32%	Smits *et al.* (74)
Mitochondrial diseases	15	CIDI	MDD 67%, BD 7%	Anglin *et al.* (75)
Mitochondrial diseases	24	MINI	MDD 37%, BD 21%[a]	Mancuso *et al.* (76)
Primary mtDNA mutation disease	19	SCID	BD 16%, other mood disorders 32%[b]	Inczedy-Farkas *et al.* (77)

BD, bipolar disorder; CIDI, Composite International Diagnostic Interview; CPEO, chronic progressive external ophthalmoplegia; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; MDD, major depressive disorder; MINI, Mini-International Neuropsychiatric Interview; SCID, Structured Clinical Interview for DSM.

[a]Calculated from the results of MINI.

[b]Other mood disorders include MDD, dysthymia, and adjustment disorder with depressed mood.

neuroscience because the odds ratios are expected to be low. Contrary to polymorphisms (common variants), rare variants of mtDNA, which are detected using whole-genome sequencing or mtDNA resequencing array, could provide explicit cues to the involvement of aberrant mtDNA or mitochondrial dysfunction in the disease. Vawter *et al.* (54) sequenced entire mtDNAs (14 BD cases, 15 major depressive disorder cases, and 20 control subjects) and identified two rare variants, m.114C>T and m.16300A>G, in patients with BD (the worldwide frequencies of these variants were <.004) (54). "Private mutations" that had not been found in controls so far were not identified in the study. The same is equally true of other mtDNA variants, which were once identified as private mutations in patients with mood disorders (52,55–57), but almost all of them now turn out to be polymorphisms or rare variants with one exception: m.4564G>A found in a patient with BD (55).

Another line of study is the measurement of mtDNA copy number in the brain (58,59), peripheral cells (60,61), and plasma (62). Quantification of the mtDNA copy number in the brain is particularly important in the study of mood disorders, as the copy number has implications for biogenesis and the turnover of mitochondria (63), and the activity and the structural change of mitochondrial in neurons are crucial for proper synaptic functioning (64,65). Although the regulation of mtDNA copy number is complex and the sampling methods affect the apparent mtDNA copy number, it should be investigated in a large number of samples in a refined way.

In order to investigate the link between mtDNA variants and psychiatric diseases, it is undoubtedly reasonable to examine somatic mtDNA mutations in the tissues responsible for the diseases, just as mtDNA mutations in neurons of the SN should be examined in PD (45). The common deletion was quantified in candidate brain regions, such as the prefrontal cortex, anterior cingulate cortex, amygdala, caudate nucleus, dorsolateral prefrontal cortex, hippocampus, nucleus accumbens, and others, which have been implicated by magnetic resonance imaging and other methods (66), and found an increase in the level of the common deletion in BD in brain (49–53,67). To focus on somatic mtDNA mutations, however, brings another question: Are the somatic mutations observed an effect of medication (68–70) or the cause or the consequence of the disease (16,71)? To investigate such questions, an alternative approach based on animal models would be useful.

There has been an alternative approach that focuses on the mental condition of mitochondrial disease patients since the publication by Fattal *et al.* (72). Although sample sizes were small, they gave a substantially consistent result—namely, that major depressive disorder is present in about half of mitochondrial disease patients and BD is present in 7% to 21% (Table 1) (72–77). The comorbidity with mood disorders has been reported in patients with primary mtDNA mutations, such as m.3243A>G, m.3271T>C, m.3460G>A, m.8344A>G, m.9035C>T, and the common deletion, as well as in patients with CPEO harboring somatic mtDNA deletions due to mutations in nuclear genes encoding mitochondrial proteins involved in mtDNA replication, such as mtDNA polymerase (*POLG*), mtDNA helicase, and adenine nucleotide transporter (73–79). Importantly, some literature has described that at least some of the mitochondrial disease patients suffered from mood disorders before the onset or diagnosis of mitochondrial disease (80–82).

In addition to clinical observation of mitochondrial disease patients, another important strategy is animal model research, in which behaviors of mice with mtDNA abnormalities are examined. There have been reported "mito-mice" carrying a heteroplasmic single mtDNA deletion (83) or a heteroplasmic pathogenic mutation (84) and knock-in mice carrying the proofreading-deficient mutation in the *Polg* gene (85,86). These mutant mice modeled mitochondrial diseases with primary mtDNA mutations or secondary mtDNA mutations led by nuclear genome mutations, respectively. In these mutant mice, mitochondrial dysfunction occurred in the whole body, which would impede behavioral analyses because of weakness of skeletal muscles, cardiomyopathy, motor abnormalities, and/or premature aging (83–86). Thus, we generated transgenic (Tg) mice expressing proofreading-deficient POLG in a neuron-specific manner; in other words, the mutant mice are a model of central nervous system manifestation of CPEO (78). mtDNAs in the skeletal muscles and the heart remained intact in the aged Tg mice, and accumulation of point mutations and multiple deletions in mtDNA was profound only in the forebrain (78).

The greatest problem of animal models for psychiatric disorders is how to evaluate the psychiatric symptoms of the models. Since diagnosis based on the DSM-5 by an interview is currently used both in clinical setting and in research fields, we considered that behaviors of the mutant *Polg* Tg mice

should be assessed based on the DSM-5 (87). The inner experience/feelings (such as depressed mood and feeling of worthlessness) assessed by verbal communication that were part of the diagnostic criteria are unable to be evaluated in mice. However, other items that define a major depressive episode, such as changes in appetite, sleep disturbance, fatigability, loss of interest, retardation, and concentration, could be operationally defined, even in mice. The mutant *Polg* Tg mice showed hypoactivity episodes lasting 2 to 3 weeks at a frequency of about once half a year, and the behavioral and physiological phenotypes during the episodes satisfied the diagnostic criteria of major depressive episode of the DSM-5 (88). The episodes were also accompanied with biological changes, including elevated levels of corticosteroid and altered body temperature (88). Furthermore, the episodes responded to treatment by a selective serotonin reuptake inhibitor (escitalopram) and a mood stabilizer (lithium) (88). The mutant *Polg* Tg mice clearly demonstrated that accumulation of mtDNA mutations (multiple deletions and point mutations) in the brain triggered mood disorder–like phenotypes (88).

BEYOND THE mtDNA VARIANTS

To proceed to the next stage, at least two major directions are possible. One is to clarify what happens in the neurons with accumulation of mtDNA mutations. It will likely lead to drug development. With mtDNA defect, the respiratory chain activity declines and the mitochondrial membrane potential decreases. Since the mitochondrial membrane potential is important for translocation of mitochondrial proteins encoded by the nuclear genome (89), almost all functions of mitochondria (for example, adenosine triphosphate synthesis, Ca^{2+} regulation, and metabolic processes, such as beta oxidation) will be impaired. If dysfunction of a particular mitochondrial pathway is linked to the onset of a psychiatric symptom, drugs that improve the dysfunction or bypass the pathway will be potent against mood symptoms. Mitochondria in the neurons could possibly have neuron-specific functions, as several genes encoding mitochondrial proteins are specifically expressed in neurons (Figure 2). Another possibility is that when the mtDNA defect causes mitochondrial dysfunction and cell damage, the contents of mitochondria may leak out of the cells and act as damage-associated molecular patterns, which initiate the innate immune response (90,91). This may explain the enigmatic inflammation often seen in psychiatric disorders (62,92,93). Alternatively, if it is the same as in the case of PD, neurons in a particular nucleus or a brain region may be degenerated owing to mtDNA mutations and consequent mitochondrial dysfunction. Such a neuronal loss, however, in a specific brain region, which corresponds to the SN in the case of PD, has not been found yet in postmortem brains of patients with mood disorders (16).

To identify the unknown brain region(s) with neurons with severe mitochondrial dysfunction is of paramount importance and a possible future step toward the understanding of mood disorders themselves. A comprehensive search for brain region(s) with higher levels of somatic mtDNA mutations can identify the pathological or responsible site(s) of mood disorders. It is also possible to investigate mitochondrial respiratory chain activity using activity staining methods (94,95). Although

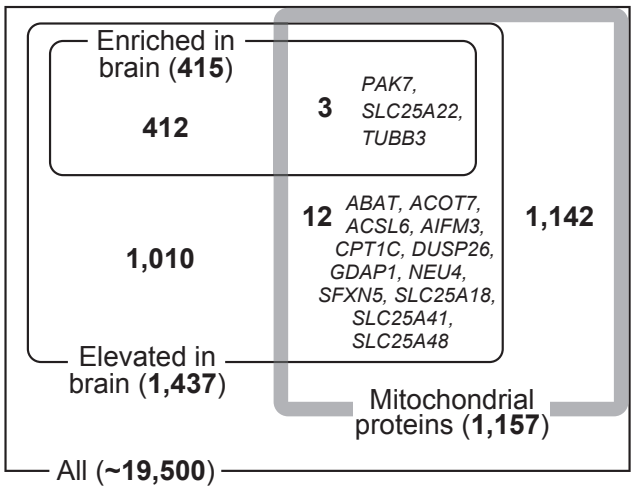

Figure 2. Genes encoding brain-specific mitochondrial proteins in the human genome. This Venn diagram shows an overlap of two human gene sets identified by the brain-specific transcriptome (104) and Human MitoCarta2.0 (20), which provides genes expressed highly in the brain and genes encoding mitochondrial proteins, respectively. The numbers in parentheses indicate numbers of genes in each set: approximately 19,500, all genes in the genome; 1437, genes showing a more than fivefold increase in messenger RNA levels in the brain compared with average levels in all tissues; 415, genes showing a more than fivefold increase in messenger RNA levels in the brain compared with all other tissues tested (104). The numbers that are not denoted by parentheses indicate numbers of genes in each region of the Venn diagram. Genes encoding brain-specific mitochondrial proteins are *PAK7*, *SLC25A22*, and *TUBB3*, all of which are expressed in neurons (105–107).

the human brain is too huge to search comprehensively, such studies become feasible with the rapid development of digital microscope slide scanners, brain clearing technology, machine learning for image analysis, and other technologies. We searched for regions with a higher level of multiple deletions in the brains of mutant *Polg* mice. The mouse brain slices were divided into small pieces of 200 × 200 μm^2 by laser microdissection, and quantitative polymerase chain reaction was performed to specifically amplify mtDNA deletions on extracted DNA from each piece. We found that mtDNA deletions were accumulated most in the paraventricular nucleus of the thalamus (88). Activity staining of mitochondrial complexes revealed that there were a number of neurons deficient in the activity of complex IV (cytochrome *c* oxidase; COX), which includes three mtDNA-encoded subunits, but the neurons retained the activity of complex II, which consists of only nuclear genome–encoded subunits. We found a higher level of mtDNA deletions in the COX-negative neurons in the paraventricular nucleus of the thalamus (88), just like COX-deficient neurons in the SN of PD patients (45). In addition, we used anti-MTCO1 (a mtDNA-encoded subunit of COX) and anti-SDHA (a subunit of complex II) antibodies, performed immunofluorescent staining on fixed brain sections, and obtained a similar result: significantly increased number of COX-negative (MTCO1-negative, SDHA-positive) neurons in the paraventricular nucleus of the thalamus of mutant *Polg* mice than in control mice (88). Using the immunofluorescent staining method, we examined postmortem brains of two CPEO

patients with comorbid mood symptoms, and both contained high levels of COX-negative cells in paraventricular thalamus compared with age-matched controls (88).

However, not all patients with mtDNA mutations have mood disorders or other psychiatric diseases (72–77). In addition, mtDNA mutations or mitochondrial dysfunction do not explain the etiology of all patients with mood disorders. We searched for variants in *POLG* gene in patients with "idiopathic" BD, who were not diagnosed with mitochondrial disease, and comprehensively investigated the deleterious effects of identified nonsynonymous variants (96). As a result, deleterious *POLG* variants were significantly enriched in patients; however, the frequency of carriers of the deleterious variants was low in patients with BD: variants predicted as deleterious, 2.4%; deleterious variants biochemically defined, 2.3%; and CPEO-related mutations, 0.38%.

Genetic and clinical studies point out that idiopathic mood disorders are heterogeneous (87,97–99), in which causative genetic factors, endophenotypes, and psychiatric manifestations vary (but the core symptoms of mood disorders are present). This suggests that multiple brain regions, circuits, or neurons are affected in individual patients and also in the patient population. We are starting on the road to identify each of the affected sites as genetic risk factors have been emerging (98–100). In such a situation, to study mitochondrial disease patients and of animal models with mtDNA mutations will provide a powerful clue to identify the affected sites in brains of patients with idiopathic mood disorders because they undergo a variety of symptoms, including the core symptoms of mood disorders, and they have damaged neurons in the brain (10,88,101,102).

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