Peripheral and Central Glucose Utilizations Modulated by Mitochondrial DNA 10398A in Bipolar Disorder

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Peripheral and central glucose utilizations modulated by mitochondrial DNA 10398A in bipolar disorder

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Summary Bipolar disorder (BD) is highly heritable and associated with dysregulation of brain glucose utilizations (GU). The mitochondrial DNA (mtDNA) 10398A polymorphism, as a reported BD risk factor, leads to deficient glycolytic energy production by affecting mitochondrial matrix pH and intracellular calcium levels. However, whether mtDNA-10398A has functional effects on the brain and how our body responds remain elusive. We compared peripheral and central glucose-utilizing patterns between mtDNA A10398G polymorphisms in BD and their unaffected siblings (BDsib). Since siblings carry identical mtDNA, we hypothesized that certain characteristics co-segregate in BD families. We recruited twenty-seven pairs of non-diabetic BD patients and their BDsib and 30 well-matched healthy control subjects (HC). The following were investigated: mtDNA, fasting plasma glucose/insulin, cognitive functions including Montreal Cognitive Assessment (MoCA), and brain GU at rest. Insulin resistance was rechecked in sixty-one subjects (19-BD, 18-BDsib, and 24-HC) six months later. We found that BD-pairs (BD+BDsib) carried more mtDNA-10398A and had higher fasting glucose, even after controlling for many factors.
covariates. BD-pairs had abnormally lower dorso-prefrontal-GU and higher cerebellar-GU, but only BD demonstrated lower medio-prefrontal-GU and MoCa. Subjects carrying mtDNA-10398A had significantly lower prefrontal-GU (FWE-corrected p < 0.05). An abnormal inverse pattern of insulin-GU and insulin-MoCa correlation was found in BD-pairs. The insulin-MoCa correlation was particularly prominent in those carrying mtDNA-10398A. mtDNA-10398A predicted insulin resistance 6 months later. In conclusion, mtDNA-10398A was associated with impaired prefrontal-GU. An up-regulation of glucose utilizations was found in BD-pairs, probably compensating for mtDNA-10398A-related energy loss.

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1. Introduction

Bipolar disorder (BD) is a highly heritable disorder (McGuffin et al., 2003), characterized by mood swings between extremely high-energy (mania) and low-energy (depression) states. A wide range of drugs available for the treatment of BD (e.g., valproate, lithium, and atypical antipsychotics) are able to elicit these symptoms (Kato and Kato, 2000), leaving some other symptoms (e.g., cognitive dysfunction) untreated. Such insufficient therapeutic effects of the available medications imply that the underlying pathophysiology of BD remains obscure and new therapeutic target is warranted.

Several lines of evidence indicate that mitochondrial dysfunction play a critical role in the pathophysiology of BD (Kato, 2006; de Sousa et al., 2014). For example, studies investigating in vivo brain chemistry by magnetic resonance spectroscopy (MRS) revealed that BD involves impaired oxidative phosphorylation, altered phospholipid metabolism, increased intracellular pH, a resultant shift toward glycolytic energy production, and a decrease in total cerebral energy production (Stork and Renshaw, 2005).

Mitochondria are the most important organelles in most eukaryotic cells that regulate cellular metabolism and convert glucose to ATP energy which organisms need. Glucose is the main energy source for the human brain. Impaired brain glucose utilizations have been reported in functional studies of positron emission tomography (PET) investigating BD patients, such as hypo-utilizations in the prefrontal cortex (Buchsbaum et al., 1986; Baxter et al., 1989).

The observed hypofrontality was left untreated even during patients’ euthymic state and also accountable for the cognitive deficits in remission (Li et al., 2012). Impaired cognitive function (e.g., executive dysfunction) has been also reported in the unaffected relatives of BD patients (Arts et al., 2008; Balanza-Martinez et al., 2008; Bora et al., 2009). However, whether the mitochondrial dysfunction hypothesis of BD could also explain the altered cerebral glucose utilizations (e.g., impaired prefrontal utilization) remains controversial.

Another piece of evidence supporting BD as a heritable disorder is from mitochondrial genetic studies. For example, mitochondria DNA (mtDNA) 10398A polymorphism is one of the reported mtDNA polymorphisms associated with BD (Kato and Kato, 2000). BD patients carrying mtDNA-10398A have been reported to have lower GU (Washizuka et al., 2003). Given the polymorphism plays a role in glycolytic energy production by affecting mitochondrial matrix pH and intracellular calcium levels, the resulting mitochondrial dysfunction would lead to an ineffective production of ATP energy from glucose and might have an impact on human brains. However, whether mtDNA-10398A has functional effects on brain glucose utilizations (GU) and long-term glucose homeostasis remains elusive.

Therefore, in the present study, we investigated mtDNA A10398G polymorphism, fasting plasma glucose and insulin, cognitive functions, and brain GU as measured by resting 18F-FDG PET, and their relationships among three groups of subjects without a history of diabetes mellitus: (1) BD patients, (2) unaffected siblings of the BD patients (BDsib), and (3) healthy control subjects (HC). We hypothesized that, in response to insufficient glycolytic energy production, some compensatory glucose-utilizing characteristics might be observed and co-segregate in BD families.

Since siblings carry identical mtDNA, we thus investigated central and peripheral glucose-utilizing patterns in BD and BDsib and the effects from mtDNA-10398 polymorphisms were compared. To further investigate whether long-term mitochondrial dysfunction might affect the glucose homeostasis, homeostasis model assessment for insulin resistance (HOMA-IR) (Matthews et al., 1985) was tested at baseline and re-tested six months later between subjects with and without the mtDNA-10398A.

2. Methods and materials

2.1. Participants

Eighty-four subjects were recruited, including twenty-seven pairs of stable BD subtype I patients and their unaffected BDsib (age differences < 5 years in the BD pairs) and 30 age-, gender-, and ethnicity-matched HC (Table 1). Subtype I of BD patients were selected mainly due to its high heritability (Gersten et al., 1982), and the diagnoses were established by structured history-taking and administration of the Mini International Neuropsychiatric Interview (MINI) based on the Fourth Edition of the Diagnostic and Statistical Manual system criteria. The recruited BDsib and HC were all free of axis I disorders, and all subjects were free of major medical and neurological illness, a lifetime alcohol or substance abuse history. HC subjects with a family history of an axis I disorder were also excluded. To reduce potential confounding effects from medications, all recruited patients were either clinically stable off medications or had stop taking medications for at least five days before the enrollment. Furthermore, sixty-one subjects (19-BD, 18-BDsib, and 24-HC) had fasting plasma sugar and insulin rechecked six months...
Table 1  Demographic data, clinical variables, and mitochondrial DNA (mtDNA) polymorphisms related to bipolar disorders (BD) among three age-, gender-, education-, and ethnicity-matched groups: BD, unaffected siblings of BD patients (BDsib), and healthy control subjects (HC).

<table>
<thead>
<tr>
<th></th>
<th>BD (n = 27)</th>
<th>BDsib (n = 27)</th>
<th>HC (n = 30)</th>
<th>F/t value</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demographic data</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>15/12</td>
<td>13/14</td>
<td>16/14</td>
<td>χ² = 0.314</td>
<td>0.855</td>
</tr>
<tr>
<td>Age (y/o)</td>
<td>39.7 (9.8)</td>
<td>39.6 (11.1)</td>
<td>39.0 (10.6)</td>
<td>0.044</td>
<td>0.957</td>
</tr>
<tr>
<td>Education (N/N/N)</td>
<td>13/11/13</td>
<td>7/17/3</td>
<td>9/19/2</td>
<td>χ² = 4.25</td>
<td>0.373</td>
</tr>
<tr>
<td>BMI</td>
<td>25.7 (4.5)</td>
<td>24.1 (2.6)</td>
<td>22.6 (3.9)</td>
<td>1.218</td>
<td>0.304</td>
</tr>
<tr>
<td>Clinical variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDRS-17</td>
<td>6.8 (6.4)</td>
<td>1.1 (1.8)</td>
<td>0.8 (1.4)</td>
<td>15.609</td>
<td>0.001</td>
</tr>
<tr>
<td>YMRS</td>
<td>3.5 (3.5)</td>
<td>0.6 (1.7)</td>
<td>0.1 (0.2)</td>
<td>10.335</td>
<td>0.001</td>
</tr>
<tr>
<td>Duration of illness (y)</td>
<td>13.4 (9.3)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>Manic episodes (number)</td>
<td>5.1 (5.3)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Major depressive episodes (number)</td>
<td>4.1 (3.9)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospitalizations (number)</td>
<td>2.2 (2.7)</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mitochondria DNA (mtDNA) gene polymorphism</td>
<td>20/7</td>
<td>20/7</td>
<td>14/16</td>
<td>6.310</td>
<td>0.043</td>
</tr>
</tbody>
</table>

Note: HDRS-17, 17-item Hamilton Depression Rating Scale; YMRS, Young Mania Rating Scale; BMI, Body Mass Index (=weight (kg)/height² (m²)).  
* p < 0.05.  
† N/N/N: ≤12 years/12–16 years/ > 16 years of education.

2.2. DNA extraction and analysis for mtDNA polymorphisms

Blood samples were collected by venipuncture into EDTA-2Na vacutubes. Total DNA from peripheral blood cells was extracted with the QIAamp DNA Mini kit (QIAGEN) according to the manufacturer’s instructions. The final DNA was dissolved in doubly distilled water and frozen at −30 °C until use. The mtDNA polymorphism site at A10398G was genotyped using the polymerase chain reaction—restriction fragment length polymorphism (PCR-RFLP) method. Other details please refer to the supplementary material.

2.3. Fasting plasma glucose (FPG), insulin, and HOMA-IR

The fasting blood samples for glucose and insulin were collected from 8:00 to 10:00 am. Subjects fasted for at least 8 h before the blood exam. Insulin was assayed by enzyme-linked immunosorbent assay (ELISA) kits (Millipore, Billerica, MA, USA). The lowest level of insulin that can be detected by this assay is 1 μU/ml. The assay was performed following the vendor’s instructions. The final absorbance of the mixture was measured and analyzed at 450 nm using an ELISA plate reader by Bio-Tek PowerWave XS and Bio-Tek’s KC junior software (Winooski, VT, USA). A linear regression R-square value of 0.95 and above was a reliable standard curve. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the following formula: HOMA-IR = FPG (mg/dl) × fasting insulin (μU/ml)/405 (Matthews et al., 1985). Higher HOMA-IR values represented greater degrees of insulin resistance.

2.4. Cognitive measurement

The Wisconsin card sorting test (WCST) (Heaton et al., 1993) was adopted for evaluating executive functions which better reflected activity of the prefrontal cortex (PFC) (Li et al., 2012). In addition, the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005) were used to detect global cognitive functions in BD patients. While the MoCA was more sensitive than the MMSE in detecting mild cognitive impairment (Nasreddine et al., 2005), both demonstrated good agreement with the executive function in the subjects with cognitive impairments (e.g., post-stroke) (Cumming et al., 2013). Additional details were available in the online supplemental materials.

2.5. Imaging studies

2.5.1. Structural magnetic resonance imaging (MRI)

MR images were acquired with a 3.0 GE Discovery 750 whole-body high-speed imaging device. High-resolution structural T1-weighted images were acquired, for improving coregistration of the PET images, in the sagittal plane using a high resolution sequence (repetition time (TR), 2530 ms; echo spacing, 7.25 ms; echo time (TE), 3 ms; flip angle 7°) with isotropic 1 mm voxels and FOV = 256 mm × 256 mm.

after the recruitment. Other details please refer to the supplementary material.

The study was performed in accordance with the Declaration of Helsinki and was approved by the Ethics Review Committee of Taipei Veterans General Hospital (2013-06-028B).
2.5.2. Positron emission tomography (PET)

2.5.2.1. PET data acquisition. 18F-FDG PET scans of at rest glucose utilizations were acquired on a PET/CT scanner (Discovery VCT; GE Healthcare, USA) with the 3D brain mode. All subjects, except three subjects in the HC, completed the PET scans. In total, 81 subjects (27-BD, 27-BDsib, and 27-HC) were included in the PET analysis. All subjects fasted for at least 8 h before the PET examination. PET images were acquired within 45 min after an intravenous injection of approximately 370 MBq per 70 kg of 18F-FDG (adjusted by their weight). Other details of acquisitions were identical and mentioned in our previous study (Li et al., 2012).

2.5.2.2. PET analysis. PET data were analyzed using Statistical Parametric Mapping version 8 software (SPM8; Wellcome Department of Cognitive Neurology, Institute of Neurology, University College London, London, England) implemented in Matlab 7.1 (The Mathworks Inc., Sherborn, MA, USA). A group-specific MRI-aided 18F-FDG template was created (Signorini et al., 1999; Gispert et al., 2003) and used to normalize each subject’s PET images, followed by smoothing with a 3D Gaussian kernel (FWHM = 8 mm). Other details please referred to the online supplemental materials.

We first calculated and compared global utilizations among the three groups. Then, the overall grand mean from the PET scans was centered and normalized to 100, and global variance across the scans was removed by analysis of covariance (ANCOVA) (Lee et al., 2008).

To assess potential group differences of normalized brain GU, an ANCOVA with age, gender (Willis et al., 2002), mood symptoms, BMI, and global gray matter values as covariates of no interest was used for between-group comparisons. The significance thresholds for 3-group comparison (F-map) were set at a voxel-level $p < 0.05$ [FWE(family-wise errors) – corrected for multiple comparisons]. We further inspected the between-group differences (BD vs. HC; BDsib vs. HC; BD vs. BDsib) on the identified F-map, and the significance threshold for the subsequent between-group t-test analyses was set at $p = 0.0167$ (=0.05/3, corrected for 3 group comparisons). A two-way ANCOVA with mtDNA and group as the two independent factors was used to compare GU between subjects carrying mtDNA-10398A and mtDNA-10398G and interactions between mtDNA and group. Since a priori knowledge about the abnormal brain network of BD involved brain regions such as PFC (Buchsbaum et al., 1986; Baxter et al., 1989; Li et al., 2012), we aimed to explore potential influences of mtDNA on brain GU by setting the significance thresholds at voxel-level uncorrected $p < 0.001$ and cluster-level corrected $p < 0.05$. Here we focused on medial PFC and dorsolateral PFC, since these PFC subregions were the most significant areas found to be hypoactive in euthymic BD patients (Li et al., 2012). Finally, multivariate regression analyses, with controlling for age, sex, mood symptoms, BMI, and global glucose utilizations, were performed to study the correlations between insulin (i.e., the predictor) and brain GU (i.e., the outcome variable) in each group. Then, a t-test was used to test whether the slope of a regression line differs significantly from 0. A cluster-level corrected $p$-value less than 0.05 in the correlation analyses was deemed to be statistically significant.

2.6. Statistical analysis for other variables

For analysis of the demographic data and clinical variables, SPSS 16.0 software (SPSS Inc., Chicago, IL) was used. One-way ANOVA (or Student’s $t$ tests) and chi-square tests were applied to compare the continuous and categorical variables among groups, respectively. Pearson’s correlation was used to investigate relationships between continuous variables (e.g., insulin and cognitive function). A $p$-value less than 0.05 was considered statistically significant.

3. Results

3.1. Demographic data, clinical variables and mtDNA gene polymorphism

There was no difference in the age, gender, educational levels, and BMI among the three groups. BD subjects were in clinically stable condition but had statistically more severe mood symptoms (i.e., HDRS-17 and YMRS scores) (Table 1). Most of the patients were in remission at baseline (i.e., 81.8% of all patients had a score of $<7$ on the HDRS-17 and $<7$ on the YMRS for at least two weeks), while 89.4% of the patients at the 6-month follow-up ($n = 17$) were also euthymic. The recruited BD patients ranged widely in the distribution of the duration of illness (1–35 years), with a mean (SD) of 13.4 (9.3) years (Table 1). The distribution of the mtDNA-10398A, the known mitochondrial risk polymorphism gene, was statistically significant among three groups ($p = 0.043$) (Table 1). More specifically, the mtDNA-10398A was as frequent in the BD patients and the BDsib subjects and was more frequent in the BD pairs than in the HC.

3.2. Fasting plasma glucose (FPG), insulin, HOMA-IR, and cognitive functions

The BD pairs, including BD and BDsib, had significantly higher FPG than the HC, even after controlling out covariates such as age and BMI (Table 2) (Fig. S1A). Direct comparisons among three groups demonstrated that the BD had a higher HOMA-IR level than the HC (Table 2) (Fig. S2). However, after adjusting for covariates, no significant differences existed in the insulin and HOMA-IR. The BD cognitive performance was significantly worse than the BDsib and HC in many domains including the MMSE, MoCA, and the executive functions as measured by WCST. However, after adjusting for covariates, BD performed significantly worse than the other two groups only on the MoCA (Table 2) (Fig. S1B).

Since BMI is a well-known factor for glucose levels, we excluded subjects who were severely underweight (BMI < 18.5) and obese (BMI > 26) in the subsequent analyses. If the analyses were done in all the subjects ($n = 50$, BD/BDsib/HC = 15/18/17), subjects with mtDNA-10398A had non-significantly higher levels of insulin, HOMA-IR, and FPG than those carrying mtDNA-10398G after controlling for age, sex, education, mood symptoms, and BMI. However, if the analysis was done in separate groups, only the BD group demonstrated that subjects with mtDNA-10398A had higher levels of insulin ($p = 0.040$), HOMA-IR ($p = 0.029$), and
FPG (p = 0.076, in a trend significance) than those carrying mtDNA-10398G (Table S1).

No statistical significance existed on cognitive functions.

### 3.3. Regional GU and correlations with insulin

If analyzed in all study subjects, subjects with mtDNA-10398A polymorphism had lower GU than those carrying mtDNA-10398G in a big cluster (3960 voxels, peak = [16, 28, 50], t = 4.30) covering the mPFC and dPFC (boxed in red, Fig. 1). Subsequent analyses showed that the finding of lower mPFC- and dPFC-GU associated with mtDNA-10398A existed for subjects across BD, BDsib, or HC groups (Fig. 1). No interaction between group and mtDNA was found. The global GU among the three groups was not statistically significant (F = 1.264, p = 0.295). The ANCOVA analysis demonstrated that the most significant brain regions among 3 groups included two large clusters [PFC (1091 voxels, peak MNI coordinates [x, y, z] = [−26, 54, 18], F = 8.27) and cerebellum (3659 voxels, peak = [−20, 48, 28], F = 9.11)] (voxel-level p < 0.05, FWE-corrected for multiple comparisons) (boxed in red, Fig. S3). These clusters covered medial PFC (mPFC), dorsal PFC (dPFC), ante- rior cingulate cortex (ACC), cerebellar vermis and anterior lobe and posterior lobe of cerebellum. The subsequent between-group analysis revealed that BD pairs all had lower GU in the dPFC (BD < BDsib < HC) and higher GU in the cerebellum (BD > BDsib > HC) (Fig. S3). A contrasting pattern between BD and BDsib were in the mPFC, in which BD had lower GU and BDsib had higher GU than HC (Fig. S3).

Regarding the insulin-brain GU correlations, the BD pairs all demonstrated a significantly negative correlation in the mPFC and dPFC (cluster-level corrected p < 0.05) (marked in solid arrows, Fig. 2). No such correlations existed in the HC. On the other hand, BDsib and HC demonstrated a similar pattern of a positive correlation between insulin and cerebellar GU (Fig. 2). However, BD failed to demonstrate this correlation (Fig. 2, in a dashed line), but instead showed a negative correlation in the cerebellum.

### 3.4. Insulin-MoCA correlations in those carrying mtDNA-10398A and mtDNA-10398G

We compared the correlations among the three groups with well-matched BMI, by excluding subjects who were severely underweight (BMI < 18.5) and obese (BMI > 26). The results demonstrated that HC had significantly negative correlations of insulin and MoCA, whether they carried mtDNA-10398A (r = −0.776, p < 0.05) or 10398G (r = −0.727, p < 0.05) (Fig. 3). In contrast, such negative correlations disappeared in BDsib and BD, especially in those carrying mtDNA-10398A. BD patients carrying mtDNA-10398A had a strong positive correlation between insulin and MoCA (r = 0.526, p < 0.05) (Fig. 3). Insulin and related glucose metabolism may have a different role in BD families as compared to HC. Higher insulin in HC was related to worse global cognition, while higher insulin in BD pairs was more associated with better cognitive functions (Fig. 3).
3.5. mtDNA A10398G polymorphism and long-term insulin resistance

Linear regression adopting age, sex, BMI, FPG at baseline, fasting insulin at baseline, and mtDNA as independent variables revealed that mtDNA A10398G polymorphism (beta = −0.240, t = −2.125, p = 0.038) and BMI (beta = 0.400, t = 3.159, p = 0.003) predicted the HOMA-IR at month-6th. Age (beta = −0.221, t = −1.769, p = 0.083), fasting insulin (beta = 0.236, t = 1.862, p = 0.068), fasting plasma glucose (beta = −0.087, t = −0.649, p = 0.519), and sex (beta = 0.083, t = 0.755, p = 0.454) had no predicting values. If the group of the BD-pairs was entered as another variable in the regression analysis, mtDNA A10398G still predicted the 6th-month HOMA-IR (beta = −0.234, t = −2.016, p = 0.048), but the group (beta = 0.030, t = 0.240, p = 0.812) failed to predict.

4. Discussion

The strengths of the study included a combination of mtDNA, fasting insulin/glucose, brain glucose utilizations (GU), and cognitive functions to directly investigate the role of mitochondrial dysfunction in the pathophysiology of BD, allowing comparison of the findings among BD, BDsib, and HC to study potential endophenotypes. It is notable that mtDNA is maternally inherited, so siblings have identical mtDNA.

The most important finding was that mtDNA-10398A had functional effects on brain GU. The mtDNA-10398A polymorphism was reported to affect mitochondrial calcium levels...
by a cybrid assay and altered calcium signaling has been reported in the peripheral blood cells of BD patients (Kato and Kato, 2000; Kato et al., 2003). These findings suggested that the polymorphism could affect energy metabolism. For the first time, we demonstrated that subjects with mtDNA-10398A had lower brain GU in the pPFC and dPFC.

The second important finding was that the BD and BDsib shared several trait-like glucose-utilizing abnormalities. The common abnormal features in the BD pairs included (1) a significantly higher prevalence of mtDNA-10398A which was found to be associated with lower GU in PFC, (2) lower GU in dPFC, (3) an up-regulation of fasting plasma glucose, (4) the correlations of lower PFC GU and higher fasting insulin, and (5) a ‘de novo’ pattern of positive correlations between fasting insulin and PFC GU and between insulin and MoCA (esp. in BD carrying mtDNA-10398A). These findings replicated the findings of the decreased PFC GU in a separate cohort of BD (Li et al., 2012), and were also in line with a large body of evidence proposing a critical role of mitochondrial dysfunction involved in the pathophysiology of BD (Stork and Renshaw, 2005; Kato, 2006).

Since mitochondrial dysfunction would lead to an ineffective production of ATP energy from glucose, the observed increases of the basal plasma glucose levels in the BD pairs might act as a compensatory mechanism for the mitochondria-related energy loss, including insufficient CNS energy supply to the brain. The supportive evidence was the presence of the positive insulin-PFC GU and insulin-MoCA in the BD pairs. In the BD pairs, higher fasting insulin was associated with better MoCA (particularly in those with more mitochondrial deficits — i.e., those with mtDNA-10398A), in contrast to the negative insulin-MoCA finding in the HC. In healthy subjects without diabetes and dementia, higher insulin levels were not beneficial and had been consistently reported to be associated with more cognitive decline (Young et al., 2006). However, in subjects with impaired glucose tolerance or insulin resistance, insulin stimulates ‘global’ cerebral GU (Hirvonen et al., 2011). We speculated that, in order to compensate for the impaired GU related to mtDNA-10398A, brains of subjects with BD diathesis may become insulin-dependent. Energy utilized by brain cells such as neurons and glia is generated from plasma glucose in the mitochondria and the process needs insulin. Insulin plays an important role in transporting extra-cellular glucose into cells, and basal insulin facilitates global GU including cortical regions and cerebellum (Bingham et al., 2002). However, whether other mtDNA genes play a role in the regulation of brain GU remains to be determined.

These findings on the compensatory up-regulation in the peripheral glucose level and central GU co-segregate within BD and BDsib and may thus serve as an endophenotype for BD. Furthermore, we speculated that the maintained cognitive function could be a result from compensatory up-regulation of brain GU by increasing glucose levels that we found in the BD pairs (Table 2). It has been reported that when brains desperately need energy, e.g., during extreme fasting states, the strongest effect of insulin on brain glucose utilizations is in the cerebellum (Bingham et al., 2002). The

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Figure 3  Correlations of fasting insulin and MoCA in subjects carrying mtDNA-10398A (circles) and mtDNA-10398G (squares). HC had significantly negative correlations of insulin and MoCA, for both polymorphisms mtDNA-10398A (r = −0.776) and 10398G (r = −0.727). In contrast, such negative correlations disappeared in BDsib and BD, especially in those carrying mtDNA-10398A. BD patients carrying mtDNA-10398A had a strong positive correlation between insulin and MoCA (r = 0.526). Pearson’s correlation was used to investigate relationships between insulin and MoCA. A p-value less than 0.05 was considered statistically significant. An asterisk (*) represents p < 0.05, and asterisks (**) represents p < 0.01. (For interpretation of the references to color in figure legend, the reader is referred to the web version of the article.)
common finding of the abnormally increased cerebellar GU in the BD pairs (Fig. S3, BDsib > HC and BD > HC) could thus be regarded as a reflection of the compensatory result from long-term insufficient CNS glucose supply to the brains of BD pairs. Our result was not only in line with previous studies showing cerebellar abnormalities in BD patients (Womer et al., 2009), but also in line with structural and neurochemical studies revealing that unaffected relatives of BD had enlarged cerebellum (Kempton et al., 2009) and relative decreases in myo-inositol and choline in the cerebellum (Singh et al., 2011). Given that a functional relationship exists between PFC and cerebellum, the cerebellar abnormalities in unaffected relatives at risk of BD have been proposed to be resulted from a compensatory regulation in the frontocerebellar circuit (Singh et al., 2011). Our study provided more direct evidence for this and such compensatory up-regulation of GU in cerebellum seemed to be activated by insulin as a result of the decreased GU in the PFC. Insulin resistance (i.e., reduced insulin sensitivity) mostly occurs due to unhealthy lifestyle (Phelix et al., 2011), which is often observed in patients with mood disorders and could lead to fat accumulation in the peripheral such as muscles, which in turn impedes insulin signaling, affects oxidative capacity of muscular mitochondria, and results in hyperglycemia. The observed hyperglycemia in our BD pairs may be also resulted from an increased need of glucose supply in the ‘brains with mitochondrial dysfunction’, in addition to the impaired ‘muscular’ mitochondrial oxidative capacity. The notion of an increased risk of insulin resistance in those with mitochondrial dysfunction was corroborated by our follow-up results, which revealed that both of the presence of risk allele (i.e., mtDNA-10398A) and higher BMI predicted higher HOMA-IR six months later. The prevalence of diabetes mellitus was reported to be higher in BD patients than in the general population (Cassidy et al., 1999).

The interpretation of our findings needs to be tempered by some limitations of this study. First, the potential effects from medication cannot be totally removed. However, we had tried to avoid the confounding from medications by recruiting un-medicating patients or those free from medications for a period of at least five days. Furthermore, it is almost impossible to recruit euthymic BD patients who are never treated by medications in clinical situations. Second, the link between mtDNA-10398A polymorphism and BD was not statistically strong, despite its significantly higher prevalence among the BD pairs. However, this study was not a genetic study and our results replicated the findings that mtDNA-10398A may represent a BD risk polymorphism (Kazuno et al., 2006, 2008). Third, MMSE and MoCA were not frequently used in non-dementia populations. Although we found MoCA sensitive for the detection of mild cognitive impairment in the BD patients, further studies are needed to confirm its application in BD.

Finally, although the difference of the PFC GU between the single-stranded mtDNA subgroups (i.e., 10398A vs. 10398G) seemed to be greatest in the BDsib (Fig. 2), this may have occurred due to unbalanced sample sizes. Unbalanced sample sizes in subgroups defined by genetic polymorphisms are almost inevitable in genetic imaging studies and could influence imaging statistics. However, to what degree the extent of the between-genotype difference did not change our main results, since all groups consistently demonstrated that subjects carrying mtDNA-10398A had lower GU in the mPFC and dPFC (Fig. 2), which are energy-consuming brain regions responsible for attention, working memory, planning, decision-making, and moderating social behaviors.

5. Conclusion

This first study provided direct evidence to support that mtDNA-10398A had functional effects on brain GU. An up-regulation of glucose utilisations was found in both the BD and BDs, probably compensating for mtDNA-10398A-related energy loss.

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Conflict of interest statement

None declared.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.psyneuen.2015.02.003.

References


