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•LETTER TO THE EDITOR•

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Decreased peripheral mtDNA in methamphetamine use disorder

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Dear Editor,

Methamphetamine (METH) is the most prevalent addictive psychostimulant in China. METH use disorder (MUD) and its associated disorders constitute a significant burden to the affected individuals and their families (Paulus and Stewart, 2020). Neurotransmitter system aberrations, mitochondrial dysfunction, oxidative stress, and inflammasome activation have been involved in METH-induced neurotoxicity (Shin et al., 2018; Wang et al., 2015; Wei et al., 2020). However, it is unclear whether peripheral mitochondrial DNA (mtDNA) changes are associated with MUD.

Herein, we analyzed serum samples from 76 male individuals with MUD (aged 19–51 years, mean age 32.1±7.0 years) and 29 drug-free healthy volunteers (aged 24–55 years, mean age 30±8.1 years) as the control group. All subjects participated in this study voluntarily and signed a written informed consent form prior to the study. The institutional review board of Kunming Institute of Zoology and the local hospital approved this study. Serum samples were collected and stored at −80°C until further use. Genomic DNA was extracted using Genomic DNA Miniprep Kit (Axygen, USA). mtDNA copy number and damage ratio in peripheral blood were measured using real-time quantitative PCR (qPCR) as described in our previous study (Feng et al., 2013). The mtDNA copy number was normalized to the nuclear *β-globin* gene. The mtDNA damage was defined as the mtDNA damage ratio, which is the relative level of damage measured using qPCR of mtDNA fragments of different lengths (Rothfuss et al., 2010). qPCR was performed on the platform of the iQ5 system (BioRad Laboratories, USA) with SYBR® Premix Ex Taq™ II kit (BioRad Laboratories). Serum concentrations of melatonin (Immuno-Biological Laboratories, USA), interleukin-1 beta (IL-1β; R&D Systems, USA), interleukin-6 (IL-6; Neobioscience, Shenzhen, China), tumor necrosis factor alpha (TNF-α; Neobioscience), 5-hydroxytryptamine (5-HT; Elabscience, Wuhan, China), brain derived neurotrophic factor (BDNF; IBL, USA), dopamine (DA; Elabscience, Wuhan, China) and cortisol (Alpha Diagnostic Intl, USA) were measured by

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respective enzyme-linked immunosorbent assay (ELISA) kits.

We found that individuals with MUD had a lower mtDNA copy number (Figure 1A) and increased mtDNA damage (Figure 1B) than healthy individuals in the peripheral blood. Similarly, the level of serum melatonin, which can salvage

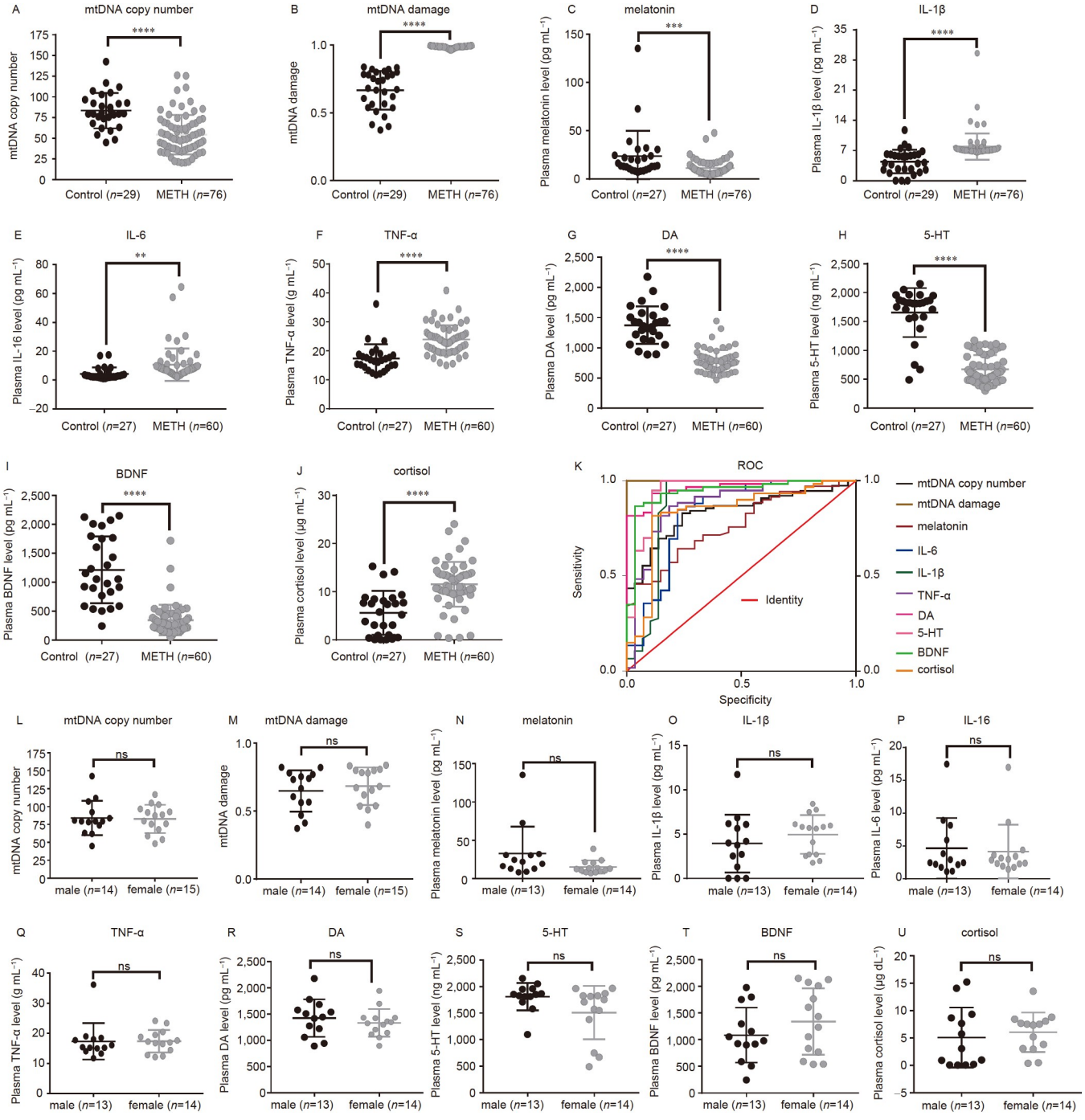

Figure 1 Changes of mtDNA, serum melatonin, pro-inflammatory cytokines, and neurotransmitters in peripheral blood in individuals with methamphetamine use disorder (METH) and healthy individuals (Control). A and B, Altered levels of mtDNA copy number (A) and mtDNA damage (B) in peripheral blood of healthy donors and individuals with methamphetamine use disorder. C–J, Altered serum levels of melatonin (C), IL-1β (D), IL-6 (E) and TNF-α (F), DA (G), 5-HT (H), BDNF (I) and cortisol (J) in individuals with methamphetamine use disorder and healthy individuals. K, The ROC curve of potential biomarkers. L–U, The levels of mtDNA copy number (L), mtDNA damage (M), serum melatonin (N), IL-1β (O), IL-6 (P), TNF-α (Q), DA (R), 5-HT (S), BDNF (T) and cortisol (U) in male and female healthy individuals. We used SPSS 17.0 software to get the ROC results. The AUC values were used to evaluate the diagnostic value of each marker combination. Statistical analysis was performed using GraphPad Prism version 7.0. We used the unpaired *t*-test to quantify the difference in levels of each marker between healthy individuals and individuals with methamphetamine use disorder or between male and female healthy individuals. All results are presented as mean±standard deviation. A *P*-value<0.05 was regarded as statistically significant. ***, *P*<0.001; ****, *P*<0.0001; ns, not significant.

the decreased mtDNA content induced by morphine treatment (Feng et al., 2013), was significantly decreased (Figure 1C) in individuals with MUD. In addition, there were significantly increased levels of IL-1β (Figure 1D), IL-6 (Figure 1E), and TNF-α (Figure 1F) in individuals with MUD than in healthy individuals. Last but not least, serum levels of DA (Figure 1G), 5-HT (Figure 1H), and BDNF (Figure 1I) were significantly lower in MUD individuals than in healthy participants, whereas the level of cortisol (Figure 1J) showed the opposite pattern.

The receiver operating characteristic (ROC) curve of these serum markers showed a reasonable sensitivity and specificity for recognizing MUD from controls (Figure 1K). The area under curve (AUC) values of mtDNA damage (AUC=1.00), DA (AUC=0.96), 5-HT (AUC=0.95), and BDNF (AUC=0.94) showed an excellent accuracy (ranges in value from 0.90 to 1.00), followed by those of mtDNA copy number (AUC=0.83), IL-1β (AUC=0.88), IL-6 (AUC=0.83), TNF-α (AUC=0.88), and cortisol (AUC=0.83). Among these serum markers, only melatonin (AUC=0.77) showed a fair accuracy. These findings indicate that mtDNA alterations, serum melatonin, pro-inflammatory cytokines, and neurotransmitters might be used as potential biomarkers for MUD. Given that all the individuals with MUD were male in this study, we performed analysis to exclude potential gender-biased effects. There was no gender-biased effect on the levels of mtDNA alterations, inflammatory cytokines, and neurotransmitters in healthy individuals though the sample size was modest (Figure 1L–U), suggesting the altered levels of these factors in individuals with MUD were unlikely to be caused by male-only sampling.

The present study, to our knowledge, is the first to report the alterations in peripheral mtDNA in individuals with MUD. We previously found reduced levels of mtDNA copy number and serum melatonin, along with increased levels of mtDNA damage and serum IL-1β in patients with heroin addiction (Feng et al., 2013; Liu et al., 2020). Consistent with our results, previous studies also demonstrated that METH can induce mitochondrial dysfunction, which includes a decreased mtDNA copy number in cell lines and rodent models (Shin et al., 2018). The possible mechanism of METH neurotoxicity is the induction of oxidative stress, which is an important link between mitochondrial damage and METH-induced neurotoxicity and neuroinflammation (Shin et al., 2018). On the other hand, the METH-induced blood-brain barrier breaks down by oxidative stress (Zhao et al., 2020); therefore, it might lead to the alterations of mtDNA damage and copy number, serum melatonin, pro-inflammatory cytokines, and neurotransmitters in peripheral blood in individuals with MUD.

A limitation of this study is the small sample size of the healthy individuals. In the future, it will be important to carry out a longitudinal analysis for the change of these markers in a large number of healthy individuals and individuals with MUD during the treatment and rehabilitation period, and to further justify the usage of peripheral mtDNA as a potential molecular marker for MUD and other substance use disorders.

Compliance and ethics *The author(s) declare that they have no conflict of interest.*

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