



## CLINICAL RESEARCH ARTICLE

## Mitochondrial gene mutations in pediatric septic shock

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**BACKGROUND:** There has been a growing interest in the association between mitochondrial dysfunction and sepsis. However, most studies have focused on mitochondrial structural damage, functional aspects, or the clinical phenotypes in sepsis. The purpose of this study was to evaluate mitochondrial DNA (mtDNA) gene mutations in critically ill pediatric patients with septic shock.

**METHOD:** Thirteen patients with severe sepsis or septic shock admitted to the pediatric intensive care unit (PICU) of a tertiary children's hospital were enrolled in this prospective observational study. Clinical data from electronic medical records were obtained. Whole-blood samples were collected within 24 h of PICU admission to perform PBMC isolation, mtDNA extraction, and mtDNA sequencing using next-generation sequencing.

**RESULTS:** mtDNA sequencing revealed mutations in 9 of the 13 patients, presenting 27 point mutations overall, with 15 (55.6%) located in the locus related to adenosine triphosphate production and superoxide metabolism, including electron transport.

**CONCLUSION:** In this pilot study, significant numbers of mtDNA point mutations were detected in critically ill pediatric patients with septic shock. These mutations could provide promising evidence for mitochondrial dysfunction in sepsis and a basis for further large-scale studies.

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**IMPACT:**

- This study is the first to examine mitochondrial DNA mutations in pediatric patients with septic shock using next-generation sequencing.
- A high frequency of mitochondrial DNA mutations was detected in these patients indicating an association with septic shock.
- This pilot study may provide a potential explanation for the association between mitochondrial dysfunction and septic shock on a genetic basis.

**INTRODUCTION**

Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection.<sup>1</sup> The complex interaction of uncontrolled cellular and molecular pathways result in abnormal host responses to infection, damaged tissues, and consequently, multiple organ dysfunction, a leading cause of morbidity and mortality in the pediatric population.<sup>2,3</sup> The pathophysiology of sepsis and sepsis-induced multiple organ failure (MOF) is not fully understood; however, a putative explanation is changes in mitochondrial function.<sup>4–7</sup>

Mitochondria is an essential subcellular organelle that supplies energy by oxidative phosphorylation via adenosine triphosphate (ATP) production. It is also involved in the maintenance of homeostasis including thermoregulation, hormone metabolism, and regulation of intracellular second messengers like calcium and reactive oxygen species (ROS) and their cell signaling.<sup>8–12</sup>

With the growing interest in mitochondrial dysfunction associated with sepsis, studies indicate that mitochondrial function in sepsis may be influenced by inflammatory cytokines, tissue hypoxia and oxidative stress changing mitochondrial permeability, inhibition of oxidative phosphorylation, ATP

depletion, pro-apoptotic protein release, or increased ROS generation.<sup>6,13–15</sup> In addition, mitochondrial ultrastructural changes, biogenesis alteration, fission/fusion, and mitochondrial DNA (mtDNA) damage may be possible causes of mitochondrial dysfunction in sepsis.<sup>4,16–19</sup>

However, most of these findings result from experimental studies focusing mainly on mitochondrial functional aspects or their role in clinical phenotypes of sepsis.<sup>16,20</sup> Especially in pediatric patients, there are only a few studies.<sup>21–23</sup> Furthermore, few studies explored mitochondrial genotypes to determine the association between mitochondrial genes and septic shock.<sup>24–26</sup>

Therefore, in this study, we aimed to identify mtDNA mutations by sequencing the mitochondrial genome using next-generation sequencing (NGS) and to determine the frequency and locus of mtDNA mutations in critically ill pediatric patients with septic shock.

**METHOD****Study population**

This prospective, observational study was approved by the institutional review board of the Asan Medical Center, Seoul,

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Korea (IRB number: 2019–1526). All patients or respective parents provided formal written informed consent before study participation. Pediatric patients with a diagnosis of septic shock, admitted to a 14-bed multidisciplinary pediatric intensive care unit (PICU) of a tertiary academic referral hospital, were eligible for enrollment. Exclusion criteria for the study were patients <1 month and >18 years of age; inability to provide informed consent; cases of inappropriate samples; alleged mitochondrial disorders; treatment with coenzyme Q10 (CoQ10), arginine, or carnitine; and patients with a do-not-resuscitate order.

Septic shock was defined as a severe infection leading to cardiovascular dysfunction (including hypotension, need for treatment with vasoactive therapeutic agents, or impaired perfusion) and organ dysfunction, according to definitions for sepsis and organ dysfunction in pediatrics.<sup>27</sup>

#### Data collection

Data was collected from electronic medical records. Variables included demographics; underlying disease; use of mechanical ventilation; supportive high flow nasal cannula oxygen therapy; use of vasoactive-inotropic agents and antibiotics; length of PICU and hospital stay; duration of recovery following organ dysfunction and shock status; mortality; laboratory findings, including routine complete blood count, electrolytes, chemical profile, C-reactive protein, serum lactate; acid–base analysis; battery of coagulation tests; and microbiological data. Additionally, the Vasoactive–Inotropic Score [dopamine dose ( $\mu\text{g}/\text{kg}/\text{min}$ ) + dobutamine dose ( $\mu\text{g}/\text{kg}/\text{min}$ ) +  $100 \times$ epinephrine dose ( $\mu\text{g}/\text{kg}/\text{min}$ ) +  $10 \times$ milrinone dose ( $\mu\text{g}/\text{kg}/\text{min}$ ) +  $10,000 \times$  vasopressin dose (unit/ $\text{kg}/\text{min}$ ) +  $100 \times$  norepinephrine dose ( $\mu\text{g}/\text{kg}/\text{min}$ )] was calculated. For outcome prediction and evaluation of organ dysfunction, the Pediatric Risk of Mortality (PRISM) III<sup>28</sup> and pediatric Sequential Organ Failure Assessment (pSOFA) scores<sup>29</sup> were calculated using the worst documented values within the first 24 h of PICU admission.

#### mtDNA analysis

*Peripheral blood mononuclear cell (PBMC) isolation from whole blood.* A 2-mL blood sample was collected from each of the 13 participants into EDTA-containing tubes. For mtDNA extraction, PBMCs were isolated from these 2-mL samples using Lymphoprep and SepMate (STEMCELL Technologies, Vancouver, BC, Canada), according to the manufacturer's protocol. Briefly, peripheral blood sample was diluted with an equal volume of 2% fetal bovine serum and centrifuged in a density gradient medium in 15-mL tubes at  $1200 \times g$  for 15 min at room temperature. Further, the white buffy coat (middle, white layer) was isolated and stored in liquid nitrogen until further use. In total, 300 cells of PBMCs were collected and tested three times, with 100 PBMCs tested each time.

*mtDNA sequencing by MiSeq.* DNA extraction from PBMCs was performed using the Gentra DNA Extraction Kit (QIAGEN, Venlo, Netherlands). The entire mtDNA was amplified by a single polymerase chain reaction (PCR) using the following primers: F-2120 GGACACTAGGAAAAACCTTGTAGAGAGAG and R-2119 AAA-GAGCTGTTCTCTTTGGACTAACA.<sup>30</sup> These primers specifically recognize genuine mtDNA but not nuclear mitochondrial pseudo-genes. The PCR conditions were as follows:  $94^\circ\text{C}$  for 1 min,  $98^\circ\text{C}$  for 10 s, and  $68^\circ\text{C}$  for 16 min, for 30 cycles, and then  $72^\circ\text{C}$  for 10 min (TaKaRa LA Taq, Catalog No. RR02AG). The concentration of PCR products was measured using the Qubit 2.0 Fluorometer (Invitrogen, Waltham, MA). The DNA was used for library preparation with the Nextera XT DNA Kit (Illumina, San Diego, CA). Sequencing was performed on the Illumina MiSeq platform (DNA Core Facility, Cincinnati Children's Hospital Medical Center), and the data were analyzed using the NextGENe software (SoftGenetics, LLC, State College, PA). Briefly, sequence reads

ranging from 100 to 200 bp were quality filtered and processed using the NextGENe software and an algorithm similar to BLAT (BLAST-like alignment tool). The sequence error correction feature (condensation) was performed to reduce false-positive variants and produce sample consensus sequences and variant calls. Alignment without sequence condensation was utilized to calculate the percentage of mitochondrial genome with a coverage depth of 1000. Starting from quality FASTQ reads, the reads were quality filtered and converted to FASTA format. Filtered reads were then aligned to the human mitochondrial sequence reference NC\_012920.1, followed by variant calling. Variant heteroplasmy was calculated using the NextGENe software as follows: base heteroplasmy (mutant allele frequency %) = mutant allele (forward + reverse)/total coverage of all alleles C, G, T, and A (forward + reverse)  $\times 100$ .<sup>31</sup> The clinical significance of the variants was analyzed using MitoMaster (<http://www.mitomap.org/MITOMASTER/WebHome>)<sup>32</sup>

*Data analysis.* For mtDNA analysis, we determined the absence of mutations among read sequences matching the reference sequence. Sequences with variations not match the reference were searched in GenBank, the big data of mtDNA mutations in the general population. The reference used was GenBank number NC\_012920.1, with the GenBank frequency data derived from 50,175 human mtDNA sequences presenting a size >15.4 kbp. The sequences collected from GenBank on Jan 1, 2020 were aligned with the Revised Cambridge Reference Sequence (rCRS) using BLASTN and haplotyped using Haplogrep via the Mitomaster web service.<sup>33</sup> A mutation was defined as new after searching and confirming its absence in the general population. However, if all three samples showed similar levels of heteroplasmy, representing the proportion of mutant cells in the sample of an individual, mosaicism due to de novo mutation in the embryonic stage could not be excluded. Conversely, when heteroplasmy was <100% and presented a variation that could be observed in the general population, it was deemed as contamination by sperm mtDNA or donor mtDNA during blood transfusion.<sup>34</sup> We also demonstrated conservation and haplogroup percents of the identified mutations.

## RESULTS

Thirteen patients were included in this study, and the demographics and clinical data are summarized in Table 1. Overall, 5 patients (patient nos. 2, 3, 6, 7, 11) presented nadir levels of absolute neutrophil count after chemotherapy owing to hematological disease, 2 patients (patient nos. 5, 9) used immunosuppressive agents following solid organ transplantation, and 1 patient (patient 12) had received haplogenic hematopoietic stem cell transplantation without chimerism due to aplastic anemia; the remaining patients (patient nos. 1, 4, 13) presented no underlying immunocompromising conditions. The median [interquartile range] level of PRISM III and pSOFA scores were 17.5 [13–21.3] and 11 [7–12.3], respectively. In total, 4 patients died (patient nos. 2, 3, 9, 10).

The results of the mitochondrial genetic test including the heteroplasmy proportion of each mutation, gene locus in the rCRS, coding gene product, amino acid change, and conservation and haplogroup percents are summarized in Table 2. Among the many mutations found in an individual, only those mutations not found in the general population following the GenBank search were selected and described. A total of 27 mutations were identified in 9 out of the 13 (69.2%) patients, and each variant is summarized in Table 2. The heteroplasmy proportions were found to range from 3.45 to 24.68%. The mutations were located in the locus of the following genes: MT-RNR1, MT-RNR2, MT-ND6, MT-CYB, MT-CO1, MT-CO2, MT-CO3, and MT-AP6, expressing the following gene products:

**Table 1.** Demographic and clinical data.

| Patient no. | Age (years) | Weight (kg) | Underlying disease  | Days after chemotherapy | WBC ( $\times 10^3/\mu\text{L}$ ) | Cytotoxic agent use | Proven pathogen             | VIS | Duration of shock (day) | PRISM III | pSOFA | Mortality |
|-------------|-------------|-------------|---|-------------------------|-----------------------------------|---------------------|-----------------------------|-----|-------------------------|-----------|-------|-----------|
| 1           | 6           | 18.3        | Hemophagocytic lymphohistiocytosis  | —                       | 6.7                               | —                   | Tsutsugamushi               | 2   | 3                       | 13        | 12    | Expired   |
| 2           | 1.5         | 14.4        | Rhabdoid tumor  | 39                      | 8                                 | —                   | <i>E. coli</i>              | 16  | 2                       | 19        | 11    | Expired   |
| 3           | 6           | 34.1        | Acute lymphoid leukemia   | 25                      | 0.1                               | —                   | <i>Trichosporon asahii</i>  | 6.5 | 0.2                     | 22        | 14    | Expired   |
| 4           | 4.2         | 18          | Weaver syndrome   | —                       | 19.8                              | —                   | CRPA                        | 7.5 | 2                       | 13        | 12    |           |
| 5           | 3.3         | 11.2        | Heart transplantation due to DCMP   | —                       | 12.4                              | TAC, MMF            | CMV                         | 5   | 2                       | 8         | 6     |           |
| 6           | 1.5         | 14          | Acute myeloid leukemia  | 14                      | 4                                 | —                   | MRSE                        | 3   | 2                       | 16        | 7     |           |
| 7           | 15          | 60          | Burkitt's lymphoma  | 9                       | 8.5                               | —                   | <i>Candida parapsilosis</i> | 2.5 | 3                       | 12        | 10    |           |
| 8           | 0.2         | 3.4         | Primary pulmonary hypertension  | —                       | 7.4                               | —                   | MRSA                        | 1.8 | 4.5                     | 23        | 11    |           |
| 9           | 7           | 13          | Lung transplantation due to chemotherapy-induced interstitial pneumonitis | —                       | 14.9                              | TAC                 | <i>K. pneumoniae</i>        | 10  | 1                       | 14        | 7     | Expired   |
| 10          | 0.3         | 3.9         | DiGeorge syndrome, TOF, PA  | —                       | 29                                | —                   | <i>K. pneumoniae</i>        | 3   | 8                       | 21        | 13    | Expired   |
| 11          | 6           | 32.25       | Acute lymphoid leukemia   | 7                       | 0.1                               | —                   | <i>S. mitis</i>             | 5   | 2                       | 21        | 6     |           |
| 12          | 11          | 45.4        | Aplastic anemia S/P haploPBSCT  | —                       | 4.8                               | MMF                 | MRSA                        | 15  | 7                       | 24        | 13    |           |
| 13          | 1.8         | 11.7        | Congenital megacolon S/P pull through operation                           | —                       | 4.7                               | —                   | <i>C. difficile</i>         | 17  | 2                       | 27        | 9     |           |

WBC white blood cell, VIS Vasoactive-Inotropic Score, PRISM III pediatric risk of mortality, pSOFA pediatric sequential organ failure assessment, DCMP dilated cardiomyopathy, TOF tetralogy of Fallot, PA pulmonary atresia, S/P status post, PBSCT peripheral blood stem cell transplantation, TAC tacrolimus, MMF mycophenolate mofetil, MRSE methicillin-resistant *Staphylococcus epidermidis*, CMV cytomegalovirus, CRPA cabapenem-resistant *Pseudomonas aeruginosa*, MRSA methicillin-resistant *Staphylococcus aureus*, *E. coli* *Escherichia coli*, *K. pneumoniae* *Klebsiella pneumoniae*, *S. mitis* *Streptococcus mitis*, *C. difficile* *Clostridium difficile*.

**Table 2.** Mitochondrial mutations in patients

| Patient number | CRS position | Heteroplasmy percent (%) | Locus      | Gene product                                 | Translation effect | GB frequency | Conservation percent (%) | Haplogroup percentage (%) |
|----------------|--------------|--------------------------|------------|--|--------------------|--------------|--------------------------|---------------------------|
| 1              | m.955A>C     | 24.68                    | 12S        | rRNA   | rRNA               | 0            | 11.11                    | 0                         |
|                | m.2806T>A    | 3.92                     | 16S        | rRNA   | rRNA               | 0            | 86.67                    | 0                         |
|                | m.3096T>C    | 3.45                     | 16S        | rRNA   | rRNA               | 0            | 100.00                   | 0                         |
|                | m.6020C>A    | 5.43                     | MT-CO1     | Cytochrome c oxidase, subunit 1 (complex IV) | COI:A39A           | 0            | 93.33                    | 0                         |
| 2              | m.7379G>A    | 3.77                     | MT-CO1     | Cytochrome c oxidase, subunit 1 (complex IV) | COI:L492L          | 0            | 71.11                    | 0                         |
| 3              | m.3054G>A    | 12.1                     | 16S        | rRNA   | rRNA               | 0            | 95.56                    | 0                         |
|                | m.6649C>T    | 6.25                     | MT-CO1     | Cytochrome c oxidase, subunit 1 (complex IV) | COI:P249L          | 0            | 97.78                    | 0                         |
| 4              | m.8606C>T    | 3.51                     | MT-AT6     | ATP synthase, Fo subunit 6 (complex V)       | ATPase6P27L        | 0            | 93.33                    | 0                         |
|                | m.6367T>C    | 5.41                     | MT-CO1     | Cytochrome c oxidase, subunit 1 (complex IV) | COI:V155A          | 0            | 71.11                    | 0                         |
|                | m.9403C>A    | 6.31                     | MT-CO3     | Cytochrome c oxidase, subunit 3 (complex IV) | COIII:T68K         | 0            | 100.00                   | 0                         |
|                | m.9415A>G    | 5.41                     | MT-CO3     | Cytochrome c oxidase, subunit 3 (complex IV) | COIII:H70R         | 0            | 97.78                    | 0                         |
| 5              | m.7749T>C    | 4.12                     | MT-CO2     | Cytochrome c oxidase, subunit 2 (complex IV) | COII:H55T          | 0            | 22.22                    | 0                         |
|                | m.16184C>CC  | 9.68                     | Non-coding | —  | Non-coding         | 0            | —                        | 0                         |
|                | m.2492G>A    | 4.24                     | 16S        | rRNA   | rRNA               | 0            | 100.00                   | 0                         |
|                | m.2647G>A    | 3.48                     | 16S        | rRNA   | rRNA               | 0            | 88.89                    | 0                         |
|                | m.2806T>A    | 7.55                     | 16S        | rRNA   | rRNA               | 0            | 86.67                    | 0                         |
|                | m.3098T>C    | 5.08                     | 16S        | rRNA   | rRNA               | 0            | 100.00                   | 0                         |
|                | m.7065G>A    | 4.26                     | MT-CO1     | Cytochrome c oxidase, subunit 1 (complex IV) | COI:A388T          | 0            | 100.00                   | 0                         |
|                | m.7943T>C    | 4.55                     | MT-CO2     | Cytochrome c oxidase, subunit 2 (complex IV) | COII:S120P         | 0            | 100.00                   | 0                         |
|                | m.15350G>A   | 3.54                     | MT-CYB     | Cytochrome b (complex III)                   | Cytb:E202K         | 0            | 97.78                    | 0                         |
| 7              | m.15200G>T   | 4.76                     | MT-CYB     | Cytochrome b (complex III)                   | Cytb:A152S         | 0            | 100.00                   | 0                         |
|                | m.16184C>CC  | 6.9                      | Non-coding | —  | Non-coding         | 0            | —                        | 0                         |
| 8              | —            | —                        | —          | —  | —                  | —            | —                        | —                         |
| 9              | —            | —                        | —          | —  | —                  | —            | —                        | —                         |
| 10             | —            | —                        | —          | —  | —                  | —            | —                        | —                         |
| 11             | m.773T>C     | 9.91                     | 12S        | rRNA   | rRNA               | 0            | 22.22                    | 0                         |
|                | m.2151A>AA   | 9.15                     | 16S        | rRNA   | rRNA               | 0            | 100.00                   | 0                         |
|                | m.6544A>C    | 4.31                     | MT-CO1     | Cytochrome c oxidase, subunit 1 (complex IV) | COI:N214T          | 0            | 100.00                   | 0                         |
|                | m.9266G>T    | 4.55                     | MT-CO3     | Cytochrome c oxidase, subunit 3 (complex IV) | COIII:G20G         | 0            | 100.00                   | 0                         |
| 12             | —            | —                        | —          | —  | —                  | —            | —                        | —                         |
| 13             | m.14342C>A   | 6.41                     | MT-ND6     | NADH dehydrogenase (complex I)               | ND6:G111V          | 0            | 17.78                    | 0                         |

CRS Cambridge Reference Sequence, GB GenBank, rRNA ribosomal RNA, ATP adenosine triphosphate, NADH nicotinamide adenine dinucleotide.

mitochondrial-derived peptide MOTS-c; Humanin; NADH-ubiquinone oxidoreductase chain 6 (Complex I); cytochrome b (complex III); cytochrome c oxidase, subunit 1 (complex IV); cytochrome c oxidase, subunit 2 (complex IV), cytochrome c oxidase, subunit 3 (complex IV) ATP synthase, and the F<sub>O</sub> subunit 6 (complex V), in the electron transport chain. That is, 15 (55.6%) of the mtDNA mutations located in loci is related to adenosine triphosphate production and superoxide metabolism, including electron transport. Except for a few mutations, over 70% (19/27) loci showed >85% conservation in humans. This finding shows that the mutations were detected in relatively conserved sequences compared to the background mutation rate. None of the detected mutations were found in the human haplogroups, suggesting that the mutations identified in this study are not identified in the data of human mtDNA mutations accumulated over a long period and are hence novel mutations.

## DISCUSSION

This study revealed a high incidence of mtDNA mutations by NGS of the complete mitochondrial genome in critically ill pediatric patients with septic shock.

NGS is a sensitive, cost-effective, and high-throughput method, rendering it a suitable tool for detection of DNA variants even at low levels of DNA modifications and can hence identify low levels of heteroplasmy.<sup>32,35</sup> Sufficient coverage depth was obtained by inspecting 100 PBMCs in triplicate. The use of NGS allowed determination of all low-level mutations that are difficult to detect using traditional methodologies. Consequently, we detected 27 point mutations in mtDNA in 9 of the 13 patients and most of them were on loci encoding respiratory chain complexes.

Mammalian mitochondrial genome contains 37 genes and 13 of mtDNA genes encode polypeptide components of electron transport chain complexes.<sup>36,37</sup> Point mutations in these genes affect mitochondrial oxidative phosphorylation complexes, which disrupt electron transport, respiratory chain inhibition, and consequently prevents mitochondrial ATP production.<sup>38</sup> Therefore, the identified mutations were located in genes directly or indirectly involved in ATP formation, which could provide a possible explanation for the association between mitochondrial dysfunction in sepsis and sepsis-induced MOF.

Further, we elaborate the protein-coding loci containing the point mutation and the associated role. NADH-ubiquinone oxidoreductase chain 6 (Complex 1), encoded by the MT-ND6 gene, catalyzes the transfer of electrons from NADH to CoQ10 and translocates protons across the inner mitochondrial membrane. Mutations in MT-CYB were detected in some patients, and the gene encodes cytochrome b, which is fundamental for the assembly and function of complex III, that catalyzes electron transfer from coenzyme Q to cytochrome c in the mitochondrial respiratory chain.<sup>39</sup> Mutations in cytochrome b influence the levels of superoxide generation, which can lead to progressive exercise intolerance and cardiomyopathy.<sup>40,41</sup> Mutations in MT-CO1, MT-CO2, and MT-CO3, observed in some patients, encode cytochrome c oxidase subunit I, II, and III respectively, which constitute respiratory complex IV. Complex IV is the third and final enzyme of the electron transport chain of mitochondrial oxidative phosphorylation. There are reports on an increase in the liability of sepsis associated with several mutations in this complex.<sup>26,42</sup> ATP synthase, encoded by MT-AT6, is an enzyme generating the energy storage molecule ATP from ADP and inorganic phosphate. Mutations in this gene are associated with cardiomyopathy, energy depletion, and ROS overproduction.<sup>43–45</sup>

It is reported that inflammatory response of sepsis leads to an overproduction of ROS. Imbalance between overproduction and inadequate elimination of ROS by insufficient antioxidant results in oxidative stress. Sepsis is known to be frequently accompanied by

increased oxidative stress, which was suggested to disrupt electron transport and interrupt mitochondrial oxidative phosphorylation and respiratory chain inhibition, leading to impairment of mitochondrial function.<sup>6,13,46–49</sup> Moreover, mitochondrial genome is especially vulnerable to oxidative stress and highly susceptible to ROS with prolonged exposure resulting in irreversible inhibition of respiratory complexes.<sup>50–52</sup> These data could support our findings that mtDNA mutations in respiratory chain complexes could be associated with septic shock.

However, the interpretation of the results requires some cautions. A confounding factor is the contamination of the recipient's blood with the donor's mtDNA in blood transfusion. mtDNA is absent in red blood cell transfusion; however, contamination remains a possibility in platelet transfusion.<sup>53,54</sup> In this study, patient nos. 2 and 6 received platelet transfusion 2 days prior to sample collection; however, the possibility of contamination was minimized by isolation of PBMCs instead of using the whole blood. In spite of the use of PBMCs, the possibility of contamination persists as platelets or extracellular mtDNA may not be completely removed during the PBMC isolation process.<sup>55,56</sup> In such cases, comparison of the study data with GenBank data is recommended to differentiate if the heteroplasmy identified is a contamination from the donor haplogroup or a new mutation in the patient.

Besides, cytotoxic agents, like chemotherapeutic drugs and immunosuppressants, could directly influence mtDNA.<sup>57</sup> In this study, five patients with mutations received chemotherapy (two presented nadir levels), and three patients received tacrolimus or mycophenolate mofetil as immunosuppressant therapy after allogeneic transplantation. Thus it should be considered while interpreting the gene analysis results whether the mutations were caused by other possible affecting factors or associated with sepsis. Therefore, further large-scale, well-designed, case-control studies including measure of by-products and enzyme activity, gene-protein quantitation, genotype-phenotype correlation resulting from mutations are required to verify the association between the identified mutations and sepsis.

To the best of our knowledge, this study is the first to perform mitochondrial whole-genome sequencing in critically ill pediatric patients with septic shock. An additional strength is the use of NGS technique, as it detected somatic mutations at crucial mitochondrial loci present at extremely low rates of heteroplasmy. As a pilot study in this field, our findings could shed a light on the association between mitochondrial dysfunction and sepsis, providing possible details for mitochondrial dysfunction.

The current study has a few limitations; it included a small sample size of pediatric patients with septic shock. Due to the relatively low incidence of septic shock, the included population was heterogeneous and no further subgroup analysis could be performed, with a limited generalization of these findings. As this was an exploratory preliminary pilot study, there could be several unrevealed confounding factors in the gene analysis results. Owing to the small number of patients, many factors like genotype-phenotype association, clinical and functional association, and sequential or causal relationship assessment of sepsis susceptibility due to mutations or increase in mutations due to sepsis, could not be investigated. Finally, search of the detected mutations in GenBank confirmed their absence in the general population and their occurrence in highly conserved positions (>90%). They are not usually found in the entire human haplogroup. However, considering the characteristics of the included patients, it is a limitation that there was no appropriate control group corresponding to the patient group. Thus further well-designed, large-scale, case-control studies are required with better adjustment of confounding factors to elucidate the role of these mutations in sepsis and septic shock.



## CONCLUSION

Overall, 9 of the 13 pediatric patients admitted to PICU for septic shock presented mitochondrial gene mutations. The detected mutation loci were mainly associated with respiratory chain complexes involved in ATP formation. It may provide a potential explanation for the mitochondrial dysfunction in septic shock. The results of the current pilot study may encourage the assessment of association between septic shock and mitochondrial dysfunction on a genetic basis. In the future, additional well-designed, large-scale, case-control studies are required to elucidate the results.

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## AUTHOR CONTRIBUTIONS

J.P.: drafting the article and revising it critically for important intellectual content, and substantial contributions to analysis and interpretation of data; E.K.: substantial contributions to analysis and interpretation of data; S.K., Deokhoon Kim, and Dahyun Kim: substantial contributions to acquisition of data; S.J.P. and W.K.J.: revising article critically for important intellectual content and final approval of the version to be published.

## ADDITIONAL INFORMATION

**Competing interests:** The authors declare no competing interests.

**Consent statement:** Written informed consent was obtained from all patients or their respective parents.

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