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Mitochondrial DNA mutations in extremely preterm infants with bronchopulmonary dysplasia

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ABSTRACT

Abstract: Bronchopulmonary dysplasia (BPD) is a serious chronic lung disease affecting extremely preterm infants. While mitochondrial dysfunction has been investigated in various medical conditions, limited research has explored mitochondrial DNA (mtDNA) gene mutations, specifically in BPD. This study aimed to evaluate mitochondrial mtDNA gene mutations in extremely preterm infants with BPD. In this prospective observational study, we enrolled a cohort of extremely preterm infants diagnosed with BPD. Clinical data were collected to provide comprehensive patient profiles. Peripheral blood mononuclear cells were isolated from whole-blood samples obtained within a defined timeframe. Subsequently, mtDNA extraction and sequencing using next-generation sequencing technology were performed to identify mtDNA gene mutations. Among the cohort of ten extremely preterm infants with BPD, mtDNA sequencing revealed the presence of mutations in seven patients, resulting in a total of twenty-one point mutations. Notably, many of these mutations were identified in loci associated with critical components of the respiratory chain complexes, vital for proper mitochondrial function and cellular energy production. This pilot study provides evidence of mtDNA point mutations in a subset of extremely preterm infants with BPD. These findings suggest a potential association between mitochondrial dysfunction and the pathogenesis of BPD. Further extensive investigations are warranted to unravel the mechanisms underlying mtDNA mutations in BPD.

1. Introduction

Bronchopulmonary dysplasia (BPD) is a prevalent and severe chronic neonatal lung disease that poses respiratory complications for premature infants (Jobe, 2011). Despite advancements in neonatal care, BPD remains a challenging condition with long-term consequences (Landry et al., 2011). Immature alveolar and vascular structures in the developing lungs are adversely affected by various pathogenic factors, such as perinatal infections, mechanical trauma from positive pressure ventilation, and oxygen toxicity (Abman et al., 2017). Although efforts have been made to minimize oxygen exposure as a preventive measure for BPD, extremely premature infants are unavoidably exposed to high oxygen levels after birth, transitioning from a hypoxic uterine environment to an oxygen-rich outside world (Chess, 2006).

Recent research has focused on mitochondrial dysfunction and its relation to oxidative stress in BPD (Kandasamy et al., 2017).

Abbreviations: BPD, bronchopulmonary dysplasia; ROS, reactive oxygen species; mt, mitochondria; NGS, next-generation sequencing; NICU, neonatal intensive care unit; FiO2, Fraction of inspired oxygen; NICHD, National institute of Child Health and Human Development; PMA, postmenstrual age; RDS, respiratory distress syndrome; PDA, patent ductus arteriosus; NEC, necrotizing enterocolitis; ROP, retinopathy of prematurity; BSID-II, Bayley Scales of Infant Development II; MDI, mental developmental index; PDI, psychomotor developmental index; PBMC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; IRB, Institutional Review Board; rCRS, revised Cambridge Reference Sequence; NADH, nicotinamide-adenine dinucleotide and its reduced form; UCB, umbilical cord blood.

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Mitochondria, responsible for energy production, also generate reactive oxygen species (ROS) and play a vital role in cellular defense against oxidative stress (Tang et al., 2014). Animal models have shown that hyperoxia, excessive oxygen exposure, impairs pulmonary mitochondrial function and disrupts alveolar development (Das, 2013; Ratner et al., 2013). The proximity of mitochondrial DNA (mtDNA) to ROS generation sites, the absence of a protective histone shield, and limited DNA repair mechanisms make it highly susceptible to oxygen-induced damage (Yakes and Van Houten, 1997). High oxygen concentrations have been linked to mitochondrial damage, leading to lung structural abnormalities and contributing to the development of BPD (Ratner et al., 2009; Narala et al., 2018).

While previous research has primarily focused on mitochondrial function in BPD, somatic mtDNA mutations have received limited attention. This study aims to use next-generation sequencing (NGS) to investigate the occurrence and extent of somatic mtDNA mutations induced by oxygen exposure in extremely preterm infants, shedding light on the relationship between oxygen exposure and mtDNA alterations in BPD.

2. Materials and methods

2.1. Study population

This observational pilot study enrolled patients admitted to a tertiary hospital's neonatal intensive care unit (NICU) between March and December 2020. The inclusion criteria were infants with gestational age below 28 + 0 weeks, requiring either oxygen supplementation or mechanical/noninvasive ventilation. The initiation of these interventions was based on clinical judgment, particularly when noninvasive respiratory support was insufficient for adequate gas exchange. The specific indicators for initiating invasive mechanical ventilation included: a pH of less than 7.20 with a $PaCO_2$ greater than 65 mmHg; a requirement for FiO_2 exceeding 0.4 to 0.5 to achieve a target SpO_2 of 90–95 %; the occurrence of multiple apneic episodes per hour that caused desaturations and bradycardia, or more than one episode necessitating positive pressure ventilation within a few hours. Our protocol for the provision of supplemental oxygen was guided by a target SpO_2 range of 90–95 %. Patients with major congenital anomalies or those unable to provide informed consent were excluded from the study. Exclusions also applied to cases of inappropriate sample collection, which encompassed non-sterile collection leading to contamination, samples obtained through incorrect techniques, improper handling or storage of samples, collection made at an inappropriate time point, or any cases not complying with ethical guidelines.

2.2. Definitions for perinatal morbidities

BPD was diagnosed according to the National Institute of Child Health and Human Development (NICHD) criteria and Jensen's criteria, with severity determined at a postmenstrual age (PMA) of 36 weeks or at the time of discharge (Jobe and Bancalari, 2001; Jensen et al., 2019). Respiratory distress syndrome (RDS) was defined as a condition requiring the administration of surfactant therapy. Patent ductus arteriosus (PDA) was defined as the need for prostaglandin inhibitor injection or surgical ligation. Necrotizing enterocolitis (NEC) was a condition with Bell's stage IIa or higher (Walsh and Kliegman, 1986). Retinopathy of prematurity (ROP) was defined as the need for therapeutic operative intervention (International Committee for the Classification of Retinopathy of Prematurity, 2005). Sepsis was defined as a positive blood culture accompanied by clinical signs indicative of sepsis.

2.3. Data collection

Demographic characteristics, BPD severity, other morbidities, mechanical ventilation use, and data on oxygen therapy, systemic steroid treatment, NICU/hospital stays, laboratory findings, and somatic mtDNA mutations were collected. The Bayley Scales of Infant Development II (BSID-II) assessment was conducted at 18–24 months of age. A mental developmental index (MDI) or psychomotor developmental index (PDI) < 70 was considered abnormal.

2.4. Sample collection and mtDNA analysis

2.4.1. Isolation of peripheral blood mononuclear cells (PBMCs) from whole blood and collection of endotracheal and nasopharyngeal samples

Blood samples (0.5 mL) and endotracheal or nasopharyngeal samples were collected from each of the 10 participants into EDTA-containing tubes on Days 0, 7, 28, and PMA 36 weeks, respectively. Day 0 samples were collected to verify congenital mtDNA mutations in the absence of oxidative stress, while Day 7, Day 28, and PMA 36-week samples were collected to assess acquired mtDNA mutations under oxidative stress conditions. If necessary, additional analysis was planned after discharge to determine whether the observed mutations were temporary or permanent. PBMCs were isolated from the samples to extract DNA, which was then diluted and subjected to centrifugation. The DNA extracted from PBMCs was used for mtDNA sequencing. Additionally, DNA was also extracted from the endotracheal and nasopharyngeal samples and used for mtDNA sequencing.

2.4.2. mtDNA sequencing by Miseq

mtDNA sequencing was performed with modifications to a previous protocol (Kang et al., 2016). The whole mtDNA was amplified using the primers as follows: first segment— mt7272F-GGCTCATTCATTTCTC TAACAGC and mt11599R-TGTTTGTCGTAGGCAGATGG; second segment— mt11506F-TCTCAACCCCCTGACAAAAC and mt15712R-TTGGCTTAGTGGGCGAAATA; third segment— mt15635F-TCCATC CTCATCCTAGCAAT and mt3259R-TATGCGATTACCGGGCTCT; and fourth segment— mt3163F- GCCTTCCCCCGTAAATGATA and mt74 01R-GGGGGCATCCATATAGTCAC. The polymerase chain reaction (PCR) program with 2X PCRBIO Ultra Mix (PCRBIO) was set at the following conditions: 95 °C for 2 min, then 35 cycles of 95 °C for 15 s, 56 °C for 15 s, and 72 °C for 2 min, followed by 72 °C for 3 min. The concentration of PCR products was measured using a Qubit 2.0 Fluorometer (Invitrogen). The library was prepared using the amplified DNA from a Nextera XT DNA kit (Illumina). Sequencing was performed on the Illumina MiSeq platform (Asan Medical Center), and the data was analyzed with NextGENe software. Concisely, sequence readings ranging from 100 to 200 bp were quality filtered and processed using NextGENe software and BLAT-like algorithms. The sequence error correction feature (condensation) was used to reduce false positive responses and generate sample consensus sequences and variant calls. Starting with quality FASTQ reads, the reads were quality filtered and converted to the FASTA format. The filtered readings were aligned with human mitochondrial sequence reference NC_012920.1, followed by a variant call. Variant heteroplasmy was calculated using the NextGENe software as follows: base heteroplasmy (% of mutant allele frequency) = mutant allele (forward + reverse) / total coverage of all alleles C, G, T, and A (forward + reverse) × 100 (Kang et al., 2016). The clinical significance of the variants was analyzed by MitoMaster (https://www.mitomap.org/MITOMASTER/WebHome) (Tang and Huang, 2010).

2.4.3. Ethics approval and consent from participants

This study received ethical approval from the Institutional Review Board (IRB) of Asan Medical Center, Seoul, South Korea (IRB approval number: [2020–0285]). Informed consent was obtained from the legal guardians or parents of the infants prior to the commencement of the study.

3. Results

Ten infants were enrolled in this study, with a mean gestational age of 25 + 4 weeks and a mean birth weight of 767 g. The study population comprised five males and five females, resulting in a gender ratio of 1:1.

A summary of the demographics and clinical data for the ten patients included in the study is provided in Table 1. According to the NICHD criteria, four patients (Nos. 1, 6, 7, and 9) exhibited mild BPD, while the remaining six (Nos. 2, 3, 4, 5, 8, and 10) were classified as having moderate to severe BPD. In contrast, in classifications utilizing Jensen's criteria, two patients (Nos. 1 and 6) were determined to have no BPD, three (Nos. 5, 7, and 9) were categorized as BPD grade 1, four (Nos. 2, 3, 4, and 8) were classified as BPD grade 2, and patient No. 10 was assessed as having BPD grade 3. All patients successfully survived and showed improved clinical outcomes upon discharge. Nine out of the ten patients underwent the BSID-II test between the corrected ages of 18–24 months. Regarding the MDI score, six patients (Nos. 1,5,6,7,8,9) were classified within the normal range of development, while three patients (Nos. 2,3,10) showed delayed development. Based on the PDI score, two patients (Nos. 6 and 9) were categorized as having normal development, while seven patients (Nos. 1, 2, 3, 5, 7, 8, and 10) showed mild delay. Notably, one patient (No. 3) had an abnormal PDI score of 61. Table 2 presents the results of the mitochondrial genetic test conducted in this study. The table includes detailed information on the heteroplasmy proportion, gene locus in the rCRS, coding gene product, amino acid change, conservation, and haplogroup percentages for each detected mutation. Only mutations not found in the general population, as indicated by a GenBank search, were selected and described. Among the ten patients, a total of twenty-one mutations were identified in seven infants (70 %). These mutations were located in genes such as MT-CO1, MT-ND6, MT-ND4, MT-ND5, MT-W, MT-ND1, MT-ND2, MT-I, MT-ATPase6, expressing cytochrome *c* oxidase subunit 1 (complex IV, for MT-CO1), NADH dehydrogenase subunit 6 (complex I), NADH dehydrogenase subunit 4 (complex I), NADH dehydrogenase subunit 5 (complex I), MT-TK gene(which produces the mitochondrial transfer RNA for lysine (tRNA-Lys)), NADH dehydrogenase subunit 1 (complex I), NADH dehydrogenase subunit 2 (complex I), MT-I gene(which encodes a core subunit of NADH: ubiquinone oxidoreductase (complex I)), and ATP synthase subunit 6 (complex V), respectively.

Approximately 95 % of the mtDNA mutations (20 out of 21) were associated with loci related to ATP production, superoxide metabolism, and electron transport. Most of these loci (17 out of 21) exhibited over 85 % conservation in humans, suggesting that the mutations were detected in relatively conserved sequences. Interestingly, only one mutation was found in the human haplogroups, indicating that most of the identified mutations are novel findings not previously reported in extensive datasets of human mtDNA mutations.

Patients with mtDNA mutations tended to experience a longer duration of exposure to oxygen and ventilatory support, alongwith pro-inflammatory conditions such as histologic chorioamnionitis.

Most infants in the study exhibited an average of 0–1 mutations, except patient No. 10, who had more than 2 mutations in their PBMCs. A detailed analysis was conducted for patient No. 10 on day 206 after birth. This analysis focused on the number of detected mtDNA mutations, excluding the D-loop region, known for its high variability. The results revealed that the total number of mutations at PMA 36 weeks was higher (n = 38) compared to day 0 (n = 19), but it decreased to n = 33 by day 206 (Fig. 1). Moreover, the shared mutation heteroplasmy in the 25 PBMCs of patient No. 10 decreased from 3.81 % at PMA 36 weeks to 3.33 % at day 206, coinciding with the discontinuation of mechanical ventilation and/or oxygen supply (Fig. 2). Additionally, the mtDNA copy number was assessed through quantitative PCR (qPCR) in pooled PBMC samples from patient No. 10, and it showed a decrease from day 0 (n = 415) to PMA 36 weeks (n = 406) and day 206 (n = 311), indicating a recovery in the patient's condition (Fig. 3). Importantly, no deletions were observed in any of the analyzed mtDNA samples.

4. Discussion

This study revealed a notable prevalence and significance of somatic mtDNA mutations in extremely preterm infants with BPD. The utilization of NGS allowed for the detection of a high incidence of mtDNA mutations, providing critical insights into the potential role of mitochondrial dysfunction in the pathogenesis of BPD (Tang and Huang, 2010; Huang, 2011).

A key finding of this study is the identification of specific gene loci affected by mtDNA mutations, particularly those encoding respiratory chain complexes involved in energy production, such as MT-ND1, MT-ND6, MT-ND4, MT-ND5, MT-W, MT-ND2, MT-I, and MT-ATPase6. The majority of mutations reported in this study were labeled as 'not reported' in the GenBank database, potentially attributable to the lack of functional evidence, which contributes to their absence from the database. The mutations identified in the study patients were explored within publicly available sequencing databases. Specifically, two mutations, NC_012920.1(MT-CO1):m.7191 T > C and NC_012920.1(MT-ND1):m.3492A > C, have been previously reported to be associated with Leigh syndrome. However, their clinical significance remains uncertain as they do not correlate with the patients' clinical symptoms. Confirming such associations and understanding the underlying mechanisms requires larger-scale studies and more in-depth analysis. Careful consideration is necessary when discussing functional annotations. However, when considering the gene loci affected by mtDNA mutations, these mutations can potentially impair mitochondrial respiration and electron transfer, leading to compromised energy production and contributing to the development of lung inflammation (Tang et al., 2014; Tuppen et al., 2010; Szczepanowska et al., 2012), consistent with previous research linking mitochondrial dysfunction to oxidative stress and respiratory chain inhibition. Considering the interdependent and interconnected nature of these dysfunctional states is essential due to the evident complexity of mitochondrial damage in the context of BPD. Mitochondrial dysfunction, oxidative stress, and altered energy metabolism are intricately related and can influence each other, affecting developmental outcomes (Kowalczyk et al., 2021). This study aligns with the consensus view of neonatologists that hyperoxia, a hallmark of BPD treatment, inhibits angiogenesis, stimulates alveolar epithelial cell expansion, and promotes the recruitment of inflammatory cells into the lung (Thekkeveedu et al., 2017). However, the response of different cell types to hyperoxia remains poorly understood and warrants further investigation.

The broader implications of mtDNA damage have been observed in sepsis, where hyperoxia-induced mtDNA damage leads to increased cellular oxidative stress, senescence, and apoptosis (Roper et al., 2004; Dobson et al., 2002). Mitochondrial DNA lacks repair enzymes and is more susceptible to structural and functional damage due to its proximity to the source of ROS production, rendering it vulnerable to damage (Aravamudan et al., 2013). Damaged mtDNA, released as danger-associated molecular patterns, activates fibroblasts and induces inflammatory and immune responses (Yang et al., 2020; Schumacker et al., 2014).

Studies in neonatal rats and mice exposed to hyperoxia have shown that targeted repair of mtDNA using specific enzymes can mitigate cellular damage and suggest the potential for therapeutic interventions (Ruchko et al., 2005; Dylag et al., 2019). These findings raise the intriguing possibility that targeting mtDNA repair pathways could be a promising approach to mitigate mtDNA damage in BPD, thereby improving clinical outcomes and potentially reducing the long-term risk of neurologic and cardiopulmonary diseases in survivors of preterm birth (Hwang and Rehan, 2018).

The intriguing dynamics of mtDNA mutations observed in patient No. 10 provide valuable insights into the potential reversibility of mtDNA damage in response to changes in clinical management. Over time, as illustrated in Fig. 1, a compelling scenario emerges, particularly for Patient 10, who exhibited 19 mutations on day 0, 38 mutations at a

Table 1
Demographic and clinical findings of study patients.

Patient No.	GA (weeks)	Birth weight (g)	Sex	Total hospital days	BPD (NICHD criteria)	BPD (Jensen's criteria)	Systemic steroids for BPD treatment	RDS	PDA	Pneu-monia	NEC	ROP	Chorio-amnionitis	Sepsis	Duration of Total oxygen exposure	MV	NIV	Supportive O2	Bayley Score 18–24 months
1	26 + 0	1010	Male	72	Mild	No BPD	–	+	–	–	–	–	–	–	63	0	59	4	MDI 117 PDI 73
2	25 + 6	670	Female	97	Moderate	Grade 2	+	+	+	–	+	+	–	+	96	41	46	9	MDI 84 PDI 80
3	25 + 6	670	Female	112	Severe	Grade 2	+	+	+	–	–	–	–	–	112	39	52	21	MDI 84 MDI 61
4	25 + 1	510	Male	129	Severe	Grade 2	–	+	+	+	–	–	–	–	135	60	45	30	
5	25 + 3	930	Male	86	Moderate	Grade 1	–	+	+	–	–	–	–	–	140	39	41	60	MDI 133 PDI 84
6	26 + 4	870	Female	66	Mild	No BPD	–	+	+	–	–	–	+	–	77	0	49	28	MDI 88 PDI 88
7	26 + 4	840	Male	66	Mild	Grade 1	–	+	–	–	–	–	+	–	77	0	52	25	MDI 114 PDI 80
8	26 + 1	923	Male	88	Severe	Grade 2	+	+	–	–	–	–	+	–	104	0	84	20	MDI 128 PDI 82
9	25 + 1	609	Female	93	Mild	Grade 1	+	+	–	–	+	–	+	–	141	21	76	44	MDI 92 PDI 92
10	23 + 2	640	Female	157	Severe	Grade 3	+	+	+	+	–	+	+	+	175	68	71	36	MDI 82 PDI 82

GA; gestational age, BPD; bronchopulmonary dysplasia, RDS; respiratory distress syndrome, PDA; patent ductus arteriosus, NEC; necrotizing enterocolitis, ROP; retinopathy of prematurity, MV; mechanical ventilation, NIV; non-invasive ventilator.

Table 2
Mitochondrial DNA mutations identified in patients.

Patient No.	Tissue sample	Day point	CRS position	Heteroplasmy percent (%)	Locus	Translation effect	GB frequency	GB frequency percent (%)	Conservation percent (%)	Haplogroup percentage (%)
1	Nasopharynx	D28	m.7191T > C	8.00	MT-CO1	COI:F430L	5	0.01	100	0
	Whole blood-1	PMA36	m.14308T > C	0.96	MT-ND6	ND6:G122G	1017	2.03	71.11	99.68
2	–	–	–	–	–	–	–		–	–
3	–	–	–	–	–	–	–		–	–
4	–	–	–	–	–	–	–		–	–
5	Whole blood-2	D0	m.11377G > A	7.23	MT-ND4	ND4:K206K	644	1.26	100	0
	Nasopharynx	D28	m.14336A > C	19.05	MT-ND6	ND6:V113G	0	0	64.44	0
6	Whole blood-2	D0	m.12618G > A	3.7	MT-ND5	ND5:L94L	701	1.37	100	0
		D7		3.6	MT-ND5	ND5:L94L	701	1.37	100	0
	Nasopharynx	D7	m.12618G > A	3.67	MT-ND5	ND5:L94L	701	1.37	100	0
	Whole blood-2		m.12618G > A							
7	Whole blood-1	D7	m.6943T > C	6.41	MT-CO1	COI:L347P	0	0	100	0
	Nasopharynx	D28	m.3492A > C	10.64	MT-ND1	ND1:K62N	1	0.002	22.22	0
8	Whole blood-1	PMA36	m.5547A > G	4.12	MT-W		0	0	100	0
9	Nasopharynx	D7	m.3492A > C	7.81	MT-ND1	ND1:K62N	1	0.002	22.22	0
10	Nasopharynx	D7	m.6498C > T	5.61	MT-CO1	COI:L199F	0	0	100	0
	Whole blood-1	D28		6.8	MT-ND2	ND2:E117E	1219	2.38	97.78	0
		D28	m.4820G > A	6.42	MT-CO1	COI:L199F	0	0	100	0
	Whole blood-1	D28		4.76	MT-I		0	0	95.56	0
		D28	m.6498C > T	3.81	MT-CO1	COI:L36F	1	0.002	100	0
	Whole blood-2	D28		5.41	MT-CO1	COI:L199F	0	0	100	0
		PMA36	m.4319C > T	6.36	MT-CO1	COI:L199F	0	0	100	0
	Whole blood-2	PMA36		4.72	MT-CO1	COI:I280I	16	0.031	100	0
		PMA36	m.6009C > T	5.49	MT-CO1	COI:Y304N	0	0	100	0
	Whole blood-2	PMA36	m.6498C > T	9.09	MT-ATPase6	ATPase6: S148C	0	0	100	0
	Whole blood-1		m.6498C > T							
	Whole blood-1		m.6743T > C							
	Whole blood-1		m.6813T > A							
	Whole blood-2		m.8968A > T							

CRS; Cambridge Reference Sequence, GB; GenBank.

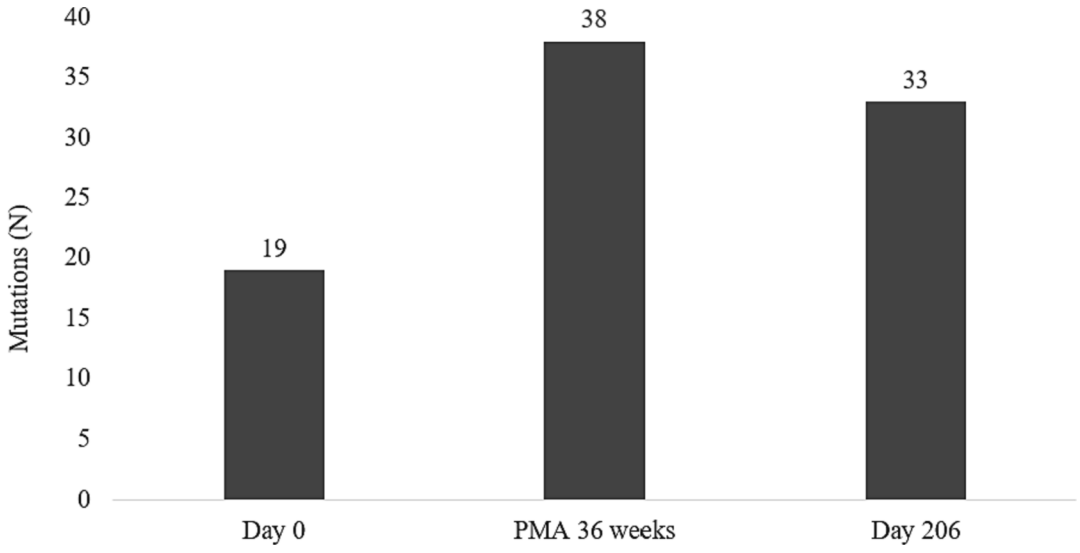

Fig. 1. Number of mitochondrial DNA mutations in the PBMC samples from patient No. 10.

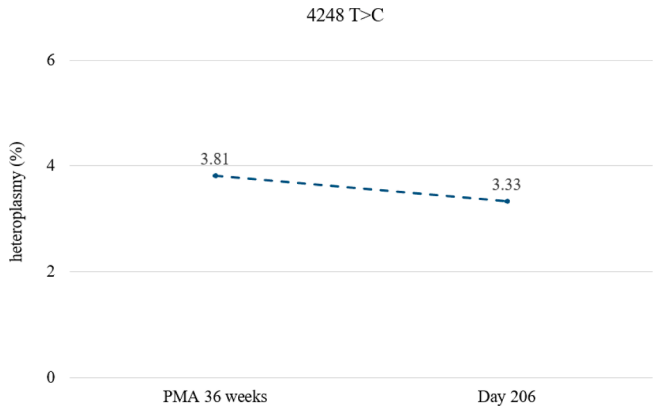

Fig. 2. Shared mutation heteroplasmy change in the PBMC samples from patient No. 10.

PMA of 36 weeks, and 33 mutations on day 206. The mutations detected at birth are theorized to originate not solely from hyperoxia but rather from new mutations arising during embryonic developmental stages. We posit that the increase in mutations between days 0 and a PMA of 36 weeks correlates with hyperoxia-induced oxidative stress and the severity of lung disease, while the subsequent decrease between a PMA of 36 weeks and day 206 is associated with a reduced incidence of hyperoxia-induced stress, wherein a diminished occurrence of new mutations transpires. This phenomenon is concomitant with an elevated distribution of mutation-free cells, resulting in a reduced detection rate of mutations. The reduction in mtDNA mutations and increased mtDNA copy number coinciding with clinical improvement suggest a correlation between the resolution of oxidative stress and the potential recovery of mitochondrial function. mtDNA mutations and copy numbers could serve as valuable biomarkers for assessing the progression and prognosis of BPD patients.

Additionally, altenating between hyperoxia and hypoxia can impact mtDNA and its role in oxygen sensing. Preterm infants with BPD exhibit hypoxic conditions characterized by phenomena like desaturation, apnea, bradycardia, and increased oxygen requirements. In these scenarios, supplemental oxygen is administered, assuming oxygen deficiency. Paradoxically, Hadanny et al. suggested that during these hypoxic states, mtDNA mutations can occur, and providing additional oxygen does not necessarily result in recovery. Recent research has shifted the focus to mitochondrial oxygen sensing and signal transduction during hypoxia instead of hyperoxia (Handanny and Efrati, 2020). Oxygen level fluctuations induced by intermittent positive pressure ventilation prompt cells to shift from a hyperoxic to a non-hypoxic stage, eliciting responses akin to those observed during hypoxia.

The diagnosis and classification of BPD are pivotal for interpreting mtDNA mutation results. Although the NICHD and Jensen's criteria are widely recognized for diagnosing BPD, they inadequately classify the presence and severity of BPD across all very preterm infants. This limitation highlights a research gap requiring a more precise characterization of BPD phenotypes. The NICHD criteria can assess BPD in infants born before 28 weeks of gestation by evaluating the level of respiratory support and oxygen requirement ($FiO2 < 30$ % vs. ≥ 30 %) at a PMA of 36 weeks or upon discharge, categorizing BPD into mild, moderate, and severe based on the need for supplemental oxygen (Jobe and Bancalari, 2001). However, these criteria may not fully capture contemporary neonatal respiratory care practices, including the prevalent use of high-flow nasal cannulae (Poindexter et al., 2015). In contrast, Jensen's criteria offer a more nuanced classification by considering the type and extent of respiratory support required at a PMA of 36 weeks, thus providing a more comprehensive assessment of BPD severity (Jensen et al., 2019). From a clinical perspective, the diagnosis of BPD is based on pragmatic criteria rather than pathophysiology alone. Clinicians, when assisting an infant's respiration, must determine whether the patient requires support with oxygenation, which may lead to hyperoxia, needs positive pressure due to inadequate ventilation, or requires backup ventilation for conditions such as apnea of prematurity. Given the variability in respiratory support options, it is critical to consider their impact when correlating mtDNA mutation results with BPD severity. This complexity accentuates the intricate relationship between respiratory support strategies and the risk of mtDNA mutations in preterm infants, ultimately underscoring the need for an expanded understanding of the BPD pathophysiology and its implications for patient management and outcomes.

Another critical aspect in interpreting the results of mtDNA mutations is the presence of multiple additional comorbidities. Extremely preterm infants are not only exposed to high oxygen concentrations, but they also suffer from inflammation, leading to an increase in ROS production. The concept of 'oxygen radical disease of the neonatology,' proposed by Saugstad, suggests that oxidative stress simultaneously affects multiple organs, resulting in diverse symptoms depending on the most affected organ (Saugstad, 2005). Free radicals are known to play a

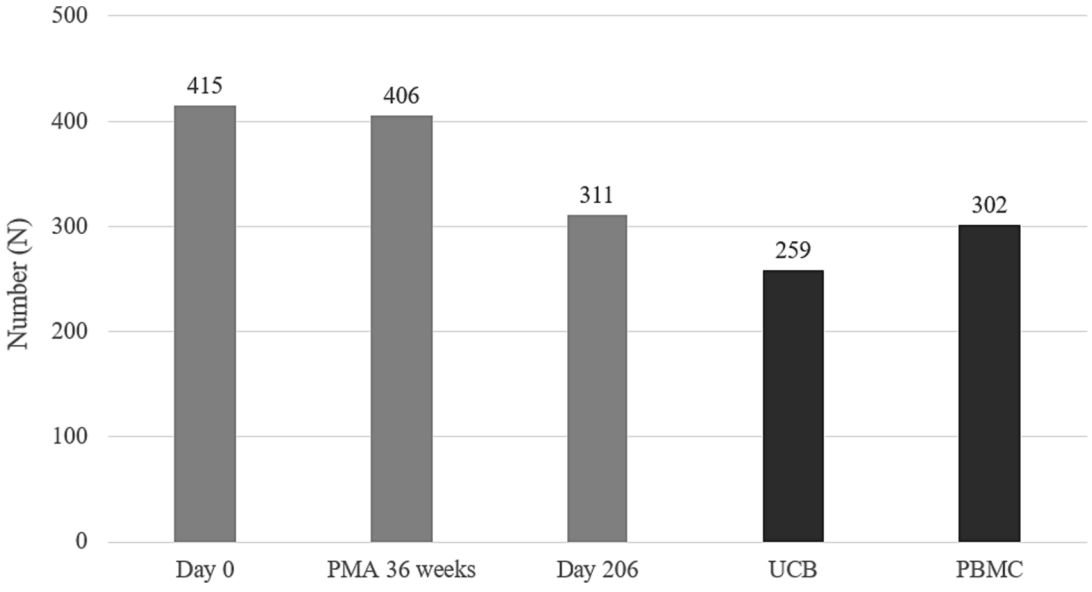

Fig. 3. Mitochondrial DNA copy numbers in patient No. 10.

role in BPD, chronic lung disease, ROP, NEC, periventricular leukomalacia, and PDA (Saugstad, 2005; Scarpato et al., 2020). Moreover, in situations where these comorbidities are present concurrently, the clinical condition of patients may deteriorate, potentially requiring increased oxygen and greater mechanical ventilation support for infants. Mechanical ventilation induces a rapid onset of oxidative stress, and the amount of generated oxygen free radicals depends on the duration of lung ventilation. Therefore, when interpreting gene analysis results, it is crucial to consider whether the mutations were caused by other potential influencing factors or are specifically associated with BPD. Hence, further large-scale, well-designed, case-control studies are necessary, including the measurement of by-products and enzyme activity, gene-protein quantitation, and genotype-phenotype correlations resulting from mutations. These studies are essential to validate the association between the identified mutations and BPD.

Despite the compelling findings, it is essential to acknowledge the limitations of this study, including the small sample size and lack of a suitable control group. This is particularly challenging given the high prevalence of BPD in infants born before 28 weeks, as reflected in data from various studies, coupled with the necessity for oxygen therapy, which complicates the identification of a control group not exposed to similar oxidative stress (Stoll et al., 2015; Geetha et al., 2021). Additionally, confounding factors such as blood transfusion and sample processing may influence the interpretation of results. Future studies with larger cohorts and well-controlled designs are warranted to validate and extend the current findings. In addition, the impact of mtDNA mutations on other clinical factors, such as the severity of BPD, respiratory support requirements, and long-term neurodevelopmental outcomes, should be further investigated. Understanding the functional consequences of these mtDNA mutations in relation to disease progression and patient outcomes will provide a deeper understanding of the molecular mechanisms underlying BPD. Furthermore, investigating the interaction between mtDNA mutations and other genetic and environmental factors may provide valuable insights into personalized approaches for managing and treating BPD. The identification of specific mtDNA mutations associated with increased susceptibility to BPD could enable the development of targeted interventions aimed at preserving mitochondrial function and mitigating the detrimental effects of oxidative stress.

5. Conclusion

In conclusion, this study provides novel insights into somatic mtDNA mutations in extremely preterm infants with BPD. Identifying specific gene loci affected by these mutations highlights the role of mitochondrial dysfunction in the pathogenesis of BPD. The dynamics of the mtDNA mutations observed in patient No. 10 suggest the potential for spontaneous repair or that these mutations are lost over time. Further research is warranted to elucidate the underlying mechanisms and clinical implications of mtDNA mutations in BPD, paving the way for targeted therapeutic strategies and personalized medicine approaches to managing this complex neonatal lung disease.

Author Contributions

JJ and YL drafted the article and revised it critically for important intellectual content and substantially contributed to the analysis and interpretation of data; EK substantially contributed to the analysis and interpretation of data; YL, JH, DK, KSK, EARK, BSL, and EJ substantially contributed to the acquisition of data; EK and EJ conceptualized the study, revised the manuscript critically for important intellectual content, and approved the final version to be published.

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CRediT authorship contribution statement

Jiyoon Jeong: Writing – review & editing, Writing – original draft, Formal analysis, Data curation. **Yeonmi Lee:** Writing – review & editing, Writing – original draft, Resources, Formal analysis, Data curation. **Jongsuk Han:** Resources. **Eunju Kang:** Writing – review & editing, Supervision, Project administration, Formal analysis, Conceptualization. **Deokhoon Kim:** Resources. **Ki-soo Kim:** Resources. **Ellen Ai-Rhan Kim:** Resources. **Byong Sop Lee:** Resources. **Euiseok Jung:** Writing – review & editing, Supervision, Resources, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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