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# Investigation of human paternal mitochondrial DNA transmission in ART babies whose fathers with male infertility

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ABSTRACT

*Objective:* To investigate the paternal mitochondrial DNA's effect on assisted reproductive technology (ART) applications and possible paternal mitochondrial DNA transmission in male factor infertility diagnosed fathers.

*Study design:* Study group was designed according to the families all of which applied to assisted reproductive technologies as a result of male infertility. A total of 16 trios (48 mother-father-child samples) which contain 7 newborns and 9 infants born by *in vitro* fertilization method (IVF-ICSI) were studied using "Illumina, MiSeq" next-generation sequencing platform (Case-parent trio study). The study has been conducted between February 2017 and May 2018.

*Result(s):* Sequencing analysis results were investigated on the basis of "mother-father-child", "mother-child" and "father-child" mitochondrial DNA whole genome sequence data, respectively. In 14 "*trios*" of 16; maternal mitochondrial DNA haplotype were detected for children, the remaining 2 "*trios*" had different mitochondrial DNA haplotypes when compared to their mother and fathers. Also; "father-child" sharing same genetic variants (SNP ("*Single nucleotide polymorphism*") / MNP ("*Multiple nucleotide polymorphism*") / INDEL ("*Insertion/Deletion*") were found in 8 "*trios*". In 5 "*trios*" of 16; 98–99% paternal mitochondrial DNA genome sequence similarity were obtained by alignment of "father-child" mitochondrial DNA genome.

*Conclusion(s):* This study is the first whole mitochondrial genome investigation for paternal mitochondrial DNA contribution in human IVF / ICSI applied trio cases. Our findings for paternally derived variants could be the result of intermolecular recombination between maternal and paternal mitochondrial DNA.



## Introduction

Mitochondria exist in almost all eukaryotic cells. They are semiautonomous, having their own genome together with their transcriptional and protein synthesizing machinery [1]. Mitochondria play an important role in numerous cellular functions including calcium signaling, programmed cell death (apoptosis), cellular aging and energy generation. They generate cellular adenosine triphosphate (ATP) and control the machinery for cellular differentiation, cell death and cell cycle [2,3].

In humans, mitochondrial DNA (mtDNA) encodes 13 polypeptide subunits essential for the process of oxidative phosphorylation, 22 transfer RNAs and 2 ribosomal RNAs [4].

It is widely accepted that in the cells of most animals, mtDNA is inherited solely from the mitochondria of the oocyte the animal develops [5–7]. This pattern of inheritance is generally referred to as "uniparental inheritance" and in case, it's specifically called "maternal inheritance" [8]. Although this phenomenon is widespreadly accepted, its advantages and the evolutionary factors remain as an unknown [9].

In mammals, sperm-derived paternal mitochondria generally enter the oocyte cytoplasm after fertilization and temporarily coexist in the zygote alongside an excess of maternal mitochondria [7].

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Two hypotheses have been proposed to explain the mechanism underlying the maternal inheritance of mtDNA. The paternal mtDNA which is present at a much lower copy number, is simply diluted away by the excess of oocyte mtDNA according to the "simple dilution model" and consequently detectable in the offspring [10]. On the other hand, in the "active degradation model" the paternal mtDNA or mitochondria are thought to be selectively degraded, either before or after fertilization to actively prevent the transmission of paternal mtDNA to the next generation [8].

However, the role of sperm mitochondria and its final fate after fertilization are still controversial. Although this may not be true for several mammalian species including mice and humans, the viewpoint of sperm mtDNA has a better fertilizing capability and that sperm mtDNA is actively eliminated during early embryogenesis is widely accepted [11]. Reviewing current literature on paternal mitochondrial inheritance suggest that the elimination of paternal mtDNA can be accomplished by multiple mechanisms according to the species-specific and also tissue-specific manner.

Schwartz and Vissing had reported a case for paternal inheritance in humans. It was demonstrated on a 28-year-old patient with a muscle specific mitochondrial disease. The 90% of the mtDNA was paternally derived from the muscle of this patient. He had a novel 2 bp deletion in *ND2* (*MTND2*) "*Mitochondrially Encoded NADH Dehydrogenase 2*" gene which encodes a subunit of the enzyme complex I of the mitochondrial respiratory chain. They suggested that this unusual mtDNA inheritance resulted from a failure to eliminate the low levels of mtDNA normally present in human sperm [12].

In 2004, Kraytsberg et al. published a brief report describing recombination between paternally and maternally inherited mtDNA that solved the question of whether modern human mtDNA may recombine [13]. Until recently, paternal or biparental inheritance of mtDNA in humans had not been observed [14]. Kraytsberg and colleagues carried out a series of experiments to identify potentially recombinant molecules and eliminate the risk of experimental error. It was found that 7% of single mtDNA molecules in the patient's muscle cells contained alternating paternal and maternal mtDNA segments providing the first direct evidence for recombination.

Nevertheless, the mechanism underlying the maternal inheritance of mitochondrial DNA has not been completely explained. Recently, several lines of evidence suggest that different species seem to employ distinct mechanisms to prevent the inheritance of paternal mitochondrial DNA.

Therefore, the aim of this study is to investigate the paternal mitochondrial DNA's effect on assisted reproductive technology applications working on the molecular basis of sperm mitochondrial DNA elimination and paternal mitochondrial DNA transmission in human.

## Materials and methods

### *Patients and DNA extraction*

Study group was designed according to the families all of which applied to assisted reproductive technologies as a result of male infertility. 48 mother-father-child samples which contain 16 children (15 boys and 1 daughter) born by *in vitro* fertilization techniques (IVF – ICSI) were included in the study. Totally 13 families were included in this study which comprised of three childs for Family 4 and two childs for Family 13, respectively. However, each child was studied and evaluated within his / her own parent trio. So, 16 different trios for mother-father-child mtDNA genome sequence comparisons were performed in this study.

Our study population consists of infertile men with impaired sperm parameters, which were all an indication for assisted reproductive treatments including IVF or ICSI. While azoospermia was the most commonly encountered spermiogram abnormality, severe teratozoospermia and asthenozoospermia were also present in the other patients.

All the couples were living in Istanbul, the largest city of Turkey, and all couples were Caucasian. The study population was collected from the largest tertiary care center in Istanbul, providing care for practically most of the region's infertile population.

Ethical approval for the study was obtained from the Ethics Committee of Istanbul Faculty of Medicine (Istanbul, Turkey; Project No: 26.06.2015-1331). The analyses were performed according to the institutional review board-approved protocols for human study participants at the Istanbul University. All participants provide written informed consent for this research study.

Blood samples were obtained from either peripheral blood lymphocytes (fathers, mothers and children at 10 months-4 years of age) or cord blood (at delivery) collected in EDTA-containing tubes from Istanbul University, Istanbul Faculty of Medicine, Department of Obstetrics and Gynecology, Division of Reproductive Endocrinology and Infertility (Istanbul, Turkey). Clinical details of the families were identified in Table 1.

Total genomic DNA was isolated and purified following the manufacturer's instructions from 200 μL blood samples by using QIAamp® DNA Mini Kit (QIAGEN GmbH, Hilden, Germany, #51304). The quantity and quality of DNA samples were measured by NanoPhotometer, P330 (Implen GmbH, Munich, Germany).

### *MtDNA genome amplification*

Whole genome mtDNA was amplified using two special primer pairs (10 μM concentration of each) that generate two amplicons approximately ~9.1 kb and ~11.2 kb in length (Sentromer, Istanbul, Turkey) (Table 2). PCR was performed using TaKaRa LA Taq® DNA Polymerase Kit (TaKaRa, Kusatsu, Shiga, Japan, #RR002 M) and 10 μL (100 pg/ μL) of purified total genomic DNA as template in a 50 μL PCR reaction. The pre-heat lid option was set to 100 °C on BIO-RAD, T100 Thermal Cycler (Bio-Rad, CA, USA). PCR was performed by one cycle of enzyme activation at 94 °C for 5 min, followed by 30 cycles of denaturation at 98 °C for 15 s and annealing/extension at 68 °C for 10 s (slow ramp from 68 °C to 60 °C at 0.2 °C per second), 60 °C for 15 s, 68 °C for 11 min, respectively. The reaction was completed by 1 cycle of final extension at 72 °C for 10 min and was hold at 10 °C. After whole mtDNA amplification, 5 μL of each MTL1 (9.1 kb) and MTL2 (11.2 kb) PCR amplicons were analyzed on 1% agarose gel with 1 kb DNA ladder (Hibrigen, Istanbul, Turkey). Amplified products were quantified using a Qubit 3.0 Fluorometer (Life Technologies, UK) and Qubit™ dsDNA HS Assay Kit (Invitrogen, Eugene, OR, USA) according to the manufacturer's protocol. Each mitochondrial amplicons were diluted to 0.2 ng/μL final concentration with ultrapure nuclease-free water and pooled in equimolar concentrations.

### *MtDNA library preparation and sequencing*

Human mtDNA genome sequencing procedure of Illumina sequencing platform (Document # 15037958, v01) was applied for whole mitochondrial genome sequencing on the MiSeq system (Illumina, San Diego, CA, USA). Nextera XT DNA Library Prep Kit (Illumina, San Diego, CA, USA, #FC-131-1024) and Nextera XT DNA Library Prep Index Kit (24 Indices, 96 Samples, Illumina, San Diego, CA, USA, #FC-131-1001) were used for the library preparation, template preparation and sequencing reactions, respectively. Amplicons were tagmented (fragmented and tagged) and indexed according to the Nextera XT protocol. Amplified DNA templates were size selected, cleaned and normalized using "Ampure XP" beads with Agencourt AMPure XP Kit (Beckman Coulter,

**Table 1**
Clinical details for study participants.

| Family | Relationship | Familial relation | Age | Gender (F/M) | IVF / ICSI | Male factor diagnosis | Blood sample type |
|---|---|---|---|---|---|---|---|
| Trio 1 | Mother 1 | No | 22 | | | | Peripheral blood |
| | Father 1 | | 31 | | | Azoospermia | Peripheral blood |
| | Child 1 | | Newborn | Male | ICSI | | Cord blood |
| Trio 2 | Mother 2 | No | 38 | | | | Peripheral blood |
| | Father 2 | | 37 | | | Azoospermia | Peripheral blood |
| | Child 2 | | Newborn | Male | ICSI | | Cord blood |
| Trio 3 | Mother 3 | No | 34 | | | | Peripheral blood |
| | Father 3 | | 42 | | | Azoospermia | Peripheral blood |
| | Child 3 | | Newborn | Male | ICSI | | Cord blood |
| Trio 4.1[+] | Mother 4 | No | 26 | | | | Peripheral blood |
| | Father 4 | | 27 | | | Asthenoteratospermia | Peripheral blood |
| | Child 4.1 | | Newborn | Female | IVF | | Cord blood |
| Trio 4.2[+] | Mother 4 | No | 26 | | | | Peripheral blood |
| | Father 4 | | 27 | | | Asthenoteratospermia | Peripheral blood |
| | Child 4.2 | | Newborn | Male | IVF | | Cord blood |
| Trio 4.3[+] | Mother 4 | No | 26 | | | | Peripheral blood |
| | Father 4 | | 27 | | | Asthenoteratospermia | Peripheral blood |
| | Child 4.3 | | Newborn | Male | IVF | | Cord blood |
| Trio 5 | Mother 5 | No | 31 | | | | Peripheral blood |
| | Father 5 | | 35 | | | Oligoteratozoospermia | Peripheral blood |
| | Child 5 | | 2 | Male | ICSI | | Peripheral blood |
| Trio 6 | Mother 6 | No | 33 | | | | Peripheral blood |
| | Father 6 | | 37 | | | Asthenoteratospermia | Peripheral blood |
| | Child 6 | | Newborn | Male | IVF | | Cord blood |
| Trio 7 | Mother 7 | No | 35 | | | | Peripheral blood |
| | Father 7 | | 44 | | | Teratozoospermia | Peripheral blood |
| | Child 7 | | 2 | Male | ICSI | | Peripheral blood |
| Trio 8 | Mother 8 | Yes[1] | 32 | | | | Peripheral blood |
| | Father 8 | (paternal side) | 32 | | | Asthenozoospermia | Peripheral blood |
| | Child 8 | | 4 | Male | ICSI | | Peripheral blood |
| Trio 9 | Mother 9 | Yes[2] | 27 | | | Oligozoospermia and Teratozoospermia | Peripheral blood |
| | Father 9 | (paternal side) | 37 | | | | Peripheral blood |
| | Child 9 | | 22 months | Male | ICSI | | Peripheral blood |
| Trio 10 | Mother 10 | No | 37 | | | Oligozoospermia and Teratozoospermia | Peripheral blood |
| | Father 10 | | 43 | | | | Peripheral blood |
| | Child 10 | | 4 | Male | ICSI | | Peripheral blood |
| Trio 11 | Mother 11 | No | 36 | | | | Peripheral blood |
| | Father 11 | | 38 | | | Asthenozoospermia | Peripheral blood |
| | Child 11 | | 4 | Male | ICSI | | Peripheral blood |
| Trio 12 | Mother 12 | No | 31 | | | Asthenozoospermia and Teratozoospermia | Peripheral blood |
| | Father 12 | | 35 | | | | Peripheral blood |
| | Child 12 | | 10 months | Male | ICSI | | Peripheral blood |
| Trio 13.1 | Mother 13.1 | No | 29 | | | | Peripheral blood |
| ++ | Father 13.1 | | 36 | | | Azoospermia | Peripheral blood |
| | Child 13.1 | | 2 | Male | ICSI | | Peripheral blood |
| Trio 13.2[+] | Mother 13.2 | No | 29 | | | | Peripheral blood |
| | Father 13.2 | | 36 | | | Azoospermia | Peripheral blood |
| | Child 13.2 | | 2 | Male | ICSI | | Peripheral blood |

[+] Child 4.1, Child 4.2 and Child 4.3 are triplets., ++: Child 13.1 and Child 13.2 are twins.
[1] "Mother 8" is daughter of "Father 8"s paternal uncle.
[2] "Mother 9" and "Father 9"s paternal grandfathers are paternal cousin.

**Table 2**
List of primers used in whole genome mtDNA amplification (MTL1 amplicon (9.1 kb): MTL-F1 + MTL-R1; MTL2 amplicon (11.2 kb): MTL-F2 + MTL-R2).

| Oligo Name | Oligo Sequence (5'-3') | Base number | Purity | Scale (nmol) | Tm (ºC) |
|---|---|---|---|---|---|
| MTL-F1 | AAA GCA CAT ACC AAG GCC AC | 20 | HPLC | 200 | 57.3 |
| MTL-R1 | TTG GCT CTC CTT GCA AAG TT | 20 | HPLC | 200 | 55.3 |
| MTL-F2 | TAT CCG CCA TCC CAT ACA TT | 20 | HPLC | 200 | 55.3 |
| MTL-R2 | AAT GTT GAG CCG TAG ATG CC | 20 | HPLC | 200 | 57.3 |

Indianapolis, IN, USA, #A63880). Bead-based normalized libraries were pooled in a single tube with PhiX Control v3 (Illumina, San Diego, CA, USA, #FC-110-3001) as a sequencing control. Libraries were diluted and heat-denatured with regard to the MiSeq System Denature and Dilute Libraries Guide (document #15039740). Multiplex pools were sequenced using MiSeq Reagent Kit v2 (Illumina, San Diego, CA, USA, #MS-102-2002) in paired-end, $2 \times 251$-cycle run with 2 index reads on the MiSeq system (Illumina, San Diego, CA, USA) (Fig. 1A).

*Bioinformatics analysis*

Mitochondrial DNA genome sequencing data analysis was done by using web-based bioinformatics tools: "mtDNA Variant

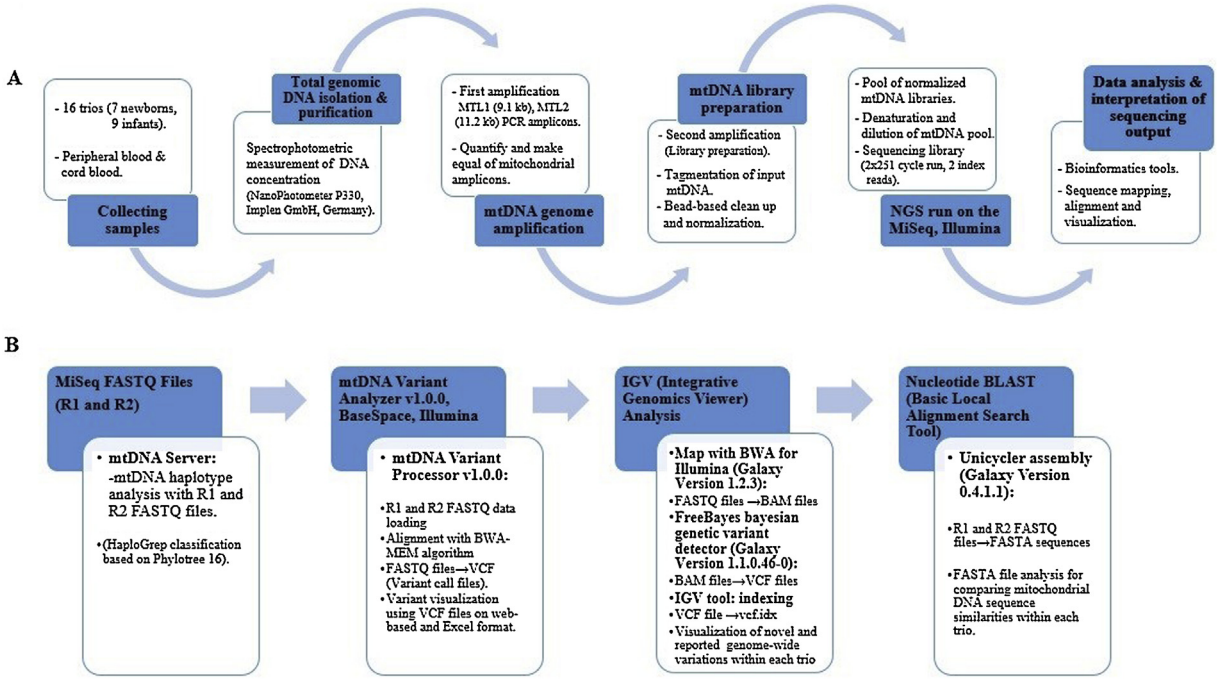


**Fig. 1.** NGS application steps and study workflow.

Processor", "mtDNA Variant Analyzer", "Integrative Genomics Viewer (IGV)", "mtDNA Server" and "BLASTN". FASTQ files from the sequencer were processed into VCF files using the "mtDNA Variant Processor v1.0.0" application (https://basespace.illumina.com/apps/2382380/mtDNA-Variant-Processor?preferredversion). The VCF outputs were then displayed in the web-based "mtDNA Variant Analyzer v1.0.0" application for visualization and report generation in an Excel file format including single nucleotide variants (SNVs), insertions, deletions compared by revised Cambridge reference sequence (rCRS, NC_012920) (https://basespace.illumina.com/apps/2381379/mtDNA-Variant-Analyzer?preferredversion) (Fig. 1B).

IGV was used to visualize the sequence alignment data from mitochondrial genome data sets of each trio (http://software.broadinstitute.org/software/igv/). FASTQ reads were aligned to the "Homo_sapiens_nuHg19_mtrCRS (hg19 with mtDNA replaced with rCRS)" reference genome using "Map with BWA for Illumina (Galaxy Version 1.2.3)" and BAM files were generated for each sequenced sample (https://usegalaxy.org; NGS:Variant Analysis). After the preferred file format for viewing alignments in IGV was generated, all VCF files were indexed using "igv tool" and "mother-father-child" alignment data were compared with rCRS (NC_012920) to detect genome-wide variations within each trio.

Mitochondrial DNA haplotypes were identified from FASTQ reads of each sample on "mtDNA-Server" free service for the analysis of human mitochondrial DNA data (https://mtdna-server.uibk.ac.at/index.html). Detected haplogroups were generated using HaploGrep classification (http://haplogrep.uibk.ac.at) based on Phylotree 16.

"Nucleotide BLAST" (BLASTN-Basic Local Alignment Search Tool) was used to compare mtDNA nucleotide sequences of each trio and to find the sequence similarities between "mother-child" and "father-child". FASTQ files were converted to FASTA file format to create assemblies with "Unicycler (Galaxy Version 0.4.1.1)" tool (https://usegalaxy.org; NGS:Assembly). FASTA sequences were used to compare mitochondrial DNA sequences for each trio sample data set (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch).

## Results

Study group was designed according to the case-parent trio study. Our primary aim was to investigate the paternal mitochondrial DNA transmission on children borned by IVF or ICSI. Literature data has been suggested that male infertility factors could have unfavourable effects on paternal mitochondria degradation and normal embryo development. In addition to this, mutations in mtDNA have been described in subfertile men. Therefore, we have selected the fathers with male factor infertility to increase the possibility to pick up the children whose sharing paternal mtDNA variants as distinct from maternal mitochondrial DNA sequence.

A total of 16 trios (48 individuals) which contain 7 newborns and 9 infants born by *in vitro* fertilization method (ICSI-IVF) and their parents were studied using "Illumina, MiSeq" next-generation sequencing platform. Total genomic DNA was isolated from either peripheral blood lymphocytes or cord blood samples. Mitochondrial DNA genome was amplified using two special primer pairs by polymerase chain reaction method to generate the entire human mitochondrial genome. Sequencing reads were analyzed in mitochondrial DNA databases and the results were evaluated within each family. Bioinformatics analysis of whole mtDNA genome sequence data allowed determination of novel and reported variations for each individual. In bioinformatics analysis, we aligned whole mtDNA genome sequence of 48 individuals against to the revised Cambridge Reference Sequence (rCRS, NC_012920) using "mtDNA Variant Analyzer" and "Integrative Genomics Viewer" analysis tools, respectively.

Sequencing results were investigated on the basis of "mother-father-child", "mother-child" and "father-child" data reads. Mitochondrial DNA haplogroups and haplotypes were identified by using "mtDNA-Server" program in accordance with HaploGrep classification. In 14 "*trios*" of 16; maternal mitochondrial DNA haplotype was detected for child, the remaining 2 "*trios*" had

different mitochondrial DNA haplotypes when compared to their mother and fathers (Table 3,Fig. 2). In Trio 2, mtDNA haplotype of child 2 (B4c1a1) was detected different from his mother's (H13a2b1) and father's (U4b1b1). In Trio 4.3, child 4.3 had maternal mtDNA haplogroup, but different mtDNA haplotype (U4b1a1a) from mother 4 (U4b1a1a1) and father 4 (G2a2a).

It was detected four different mitochondrial variations at nucleotides m.73 A > G, m.11719 G > A, m.14766 C > T, m.16519 T > C in Trio 2 and two variations at nucleotides m.2706 A > G, m.4769 A > G in Trio 10 which were observed as shared variants between paternal and offspring mtDNA according to the "mtDNA Variant Analyzer" results (Table 4). In addition to that, the detailed NGS data output was added as Supplementary Table 1.

We investigated the paternal mitochondrial DNA transmission by genome-scale IGV analysis tool. In this context, we evaluated the genetic variants that observed on maternal mtDNA without paternal and child or paternal-child shared mtDNA variants without maternal. Also; "father-child" sharing same genetic variants (SNP ("*Single nucleotide polymorphism*") / MNP ("*Multiple nucleotide polymorphism*") / INDEL ("*Insertion/Deletion*") were detected in 8 trios. In Trio 1, it was found two distinct variations between mother and child. Although "mother 1" had INDEL variation at nucleotide m.5467–5471 in *ND2 "Mitochondrially Encoded NADH Dehydrogenase* 2" gene, "child 1" and "father 1" had normal allele sequence for reference genome. Also; "mother 1" had heteroplasmic INDEL variation at position m.16292–16294 (D-Loop) and "child 1 – father 1" had homoplasmic INDEL variation on the same mitochondrial DNA position (Figs. 3A, 4 A). It was the first case report for paternal mtDNA transmission of a 28 years old patient with mitochondrial myopathy due to a novel 2 bp deletion in the *ND2* gene (known as *MT-ND2*) [12].

While "child 2 – father 2" had novel homoplasmic mitochondrial variants at position m.7028 (T/T), m.11719 (A/A), m.14133 (G/G), m.14766 (T/T), m.16519 (C/C), "mother 2" had normal allelic sequence for rCRS reference genome in Trio 2 (Fig. 3B). The mitochondrial variation m.7028 C > T was in the *COX1* (*MT-CO1*) *"Mitochondrially Encoded Cytochrome C Oxidase I"* gene, m.11719 G > A was in the *MT-ND4 "Mitochondrially Encoded NADH Dehydrogenase* 4" gene, m.14133 A > G was in the *MT-ND5 "Mitochondrially Encoded NADH Dehydrogenase* 5" gene, m.14133 A > G was in the *MT-ND5* gene, m.14766 (C > T) was in the *MT-CYB*

**Table 3**
Comparing the "mother-father-child" mtDNA haplotypes obtained by mtDNA-Server analysis tool, List of mtDNA haplotypes within each trio (based on Phylotree 16).

| Trio Number | Mother mtDNA Haplotype | Father mtDNA Haplotype | Child mtDNA Haplotype |
|---|---|---|---|
| Trio 1 | T2c1 | T1a1 | T2c1 |
| Trio 2* | H13a2b1 | U4b1b1 | B4c1a1 |
| Trio 3 | H26a1 | J1b5b | H26a1 |
| Trio 4.1 | U4b1a1a1 | G2a2a | U4b1a1a1 |
| Trio 4.2 | U4b1a1a1 | G2a2a | U4b1a1a1 |
| Trio 4.3** | U4b1a1a1 | G2a2a | U4b1a1a |
| Trio 5 | W4d | G2a2 | W4d |
| Trio 6 | U8b1a2 | K1a | U8b1a2 |
| Trio 7 | K1a4f | J1b1b | K1a4f |
| Trio 8 | I5a2 | H15 | I5a2 |
| Trio 9 | I5a | H13a2c1 | I5a |
| Trio 10 | R0a1a | U4a | R0a1a |
| Trio 11 | J1b3b | U2b1 | J1b3b |
| Trio 12 | H5e1 | K1a19 | H5e1 |
| Trio 13.1 | U4b1a1a1 | U1a1a | U4b1a1a1 |
| Trio 13.2 | U4b1a1a1 | U1a1a | U4b1a1a1 |

* In trio 2, child mtDNA haplogroup was detected different from his mother's and father's.
** In trio 4.3, mtDNA haplogroup was the same for "mother-child", but mtDNA haplotype was different.

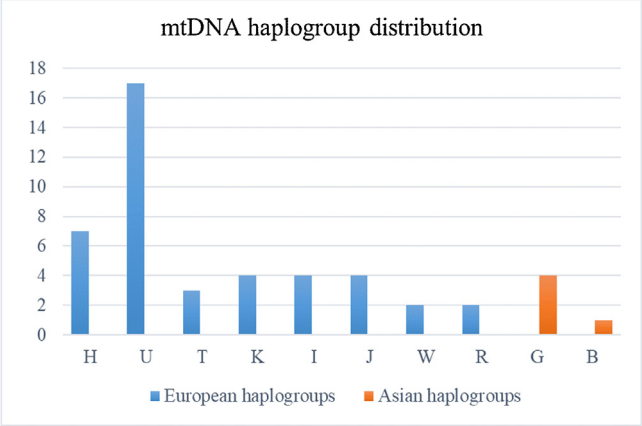


**Fig. 2.** Distribution of mtDNA haplogroup lineages according to the rCRS (GenBank NC_012920, Mitomap).

*"Mitochondrially Encoded* Cytochrome B" gene, m.14766 (C > T) was in the *MT-CYB* gene and m.16519 T > C was in the *MT-DLOOP1 "Mitochondrial Displacement Loop"* region.

In Trio 3; "mother 3" had a polymorphism at nucleotides m.8563 A > C (SNP), but "child 3 – father 3" had wild type allel on m.8563 (A/A) region. However; "mother 3" had a MNP variation at nucleotides m.8909–8914 (TCTTAC / CCTAAA), "child 3" did not have any variation at this location which was near the *COX3 "Mitochondrially Encoded Cytochrome C Oxidase III"* gene (Fig. 3C). In Trio 4.1; as "mother 4" had a heteroplasmic MNP variant at nucleotides m.8269–8279 (GT / GTACCCCCTCT), "child 4.1" had a homoplasmic variant at position m.8269–8279 (GT/GT) (Fig. 3D).

It was determined two novel variations at nucleotides m.8896 G > C, m.8909–8914 (TCTTAC / CCTAAA, MNP) for "child 5 – father 5" without "mother 5" in Trio 5. These variations were in the *ATP6 "Mitochondrially Encoded ATP Synthase* 6" gene (Fig. 4B).

In Trio 8; "child 8 – father 8" had three different SNP variations at nucleotides m.3483 G > C, m.3488 T > A, m.3492 A > C which was in the *MT-ND1"Mitochondrially Encoded NADH Dehydrogenase* 1" gene, but "mother 8" had wild type genotype at that mitochondrial region (Fig. 4C).

In Trio 9; as "child 9 – father 9" had a novel variation at nucleotides m.8896 G > C in the *ATP6* gene, "mother 9" had wild type genotype (Fig. 4D).

In Trio 10; while "mother 10" had two SNP variations within *MT-ND2* gene at nucleotides m.5192 A > C, m.5208 A > C, "child 10" and "father 10" did not have any variation in that location (Fig. 4E). In relation to those of shared genetic variants, "mother-father-child" and "father-child" comparative mtDNA nucleotide BLAST sequence alignment results were added as Supplementary Fig. 1 and Supplementary Table 2, respectively.

Bioinformatics analysis results confirmed by different programmes, simultaneously and mitochondrial genetic variations were compared within each trio. Paternal / maternal polymorphisms, insertion / deletion variations were evaluated and common shared variants were summarized in Table 5.

MiSeq NGS workflow was completed by 93.5% Q-Score value for whole experiment. Adapter sequences were removed from the forward and reverse reads until there were no more than 3 adapter bases on the end of the sequence read. BWA-MEM (Burrows-Wheeler Alignment algorithm) which has a significant positive impact on detection of variants [15] was performed for the alignment. Analysis parameters were setted as Q30 (Minimum Basecall Quality Score for a Call), 10% (Analysis Threshold), 25% (Interpretation Threshold) and 10 (Minimum Read Count) for variant calling analysis.

**Table 4**
mtDNA Variant Analyzer (v1.0.0) Results (Father-child shared variants).

| Family | Relationship | Position | rCRS | A | C | G | T | Ins | Del | Variant Detail |
|---|---|---|---|---|---|---|---|---|---|---|
| Trio 2 | Mother | m.73 | A | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| | Father | m.73 | | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | A>G |
| | Child | m.73 | | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | A>G |
| Trio 2 | Mother | m.11719 | G | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | |
| | Father | m.11719 | | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | G>A |
| | Child | m.11719 | | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | G>A |
| Trio 2 | Mother | m.14766 | C | 0.0 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | |
| | Father | m.14766 | | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | C>T |
| | Child | m.14766 | | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | C>T |
| Trio 2 | Mother | m.16519 | T | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | |
| | Father | m.16519 | | 0.0 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | T>C |
| | Child | m.16519 | | 0.0 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | T>C |
| Trio 10 | Mother | m.2706 | A | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| | Father | m.2706 | | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | A>G |
| | Child | m.2706 | | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | A>G |
| Trio 10 | Mother | m.4769 | A | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | |
| | Father | m.4769 | | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | A>G |
| | Child | m.4769 | | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | A>G |

rCRS: revised Cambridge Reference Sequence (rCRS, NC_012920), Ins: Insertion, Del: Deletion, A: Adenine, G: Guanine, C: Cytosine, T: Thymine.

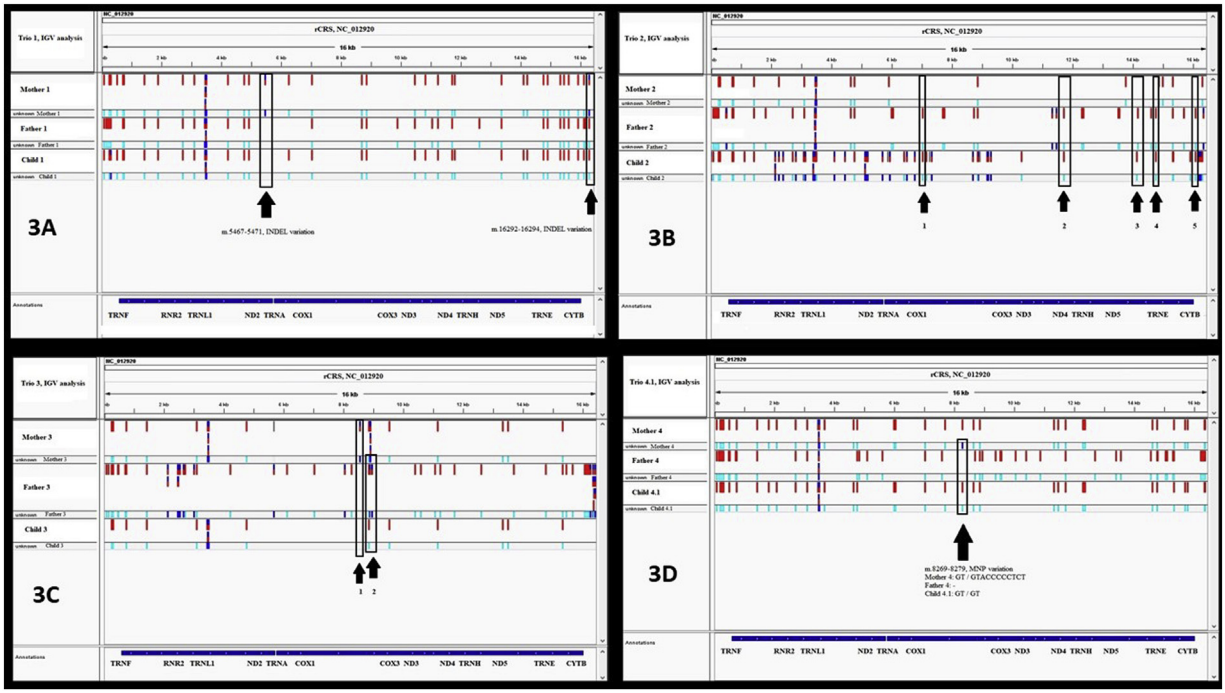


**Fig. 3.** IGV analysis results for mtDNA genome comparison.
**A.** Trio 1: m.5467–5471, INDEL variation, Ref: CCACG (CCACG / ACCT, Mother 1: +, Father 1: -, Child 1: -), m.16292–16294, INDEL variation, Ref: CAC (CAC / TAT) (Mother 1: Heteroplasmic mtDNA variant, Father 1: Homoplasmic mtDNA variant, Child 1: Homoplasmic mtDNA variant).
**B.** Trio 2: Mother 2: -, Father 2: +, Child 2: + variants. 1; m.7028 (T/T), 2; m.11719 (A/A), 3; m.14133 (G/G), 4; m.14766 (T/T), 5; m.16519 (C/C).
**C.** Trio 3: 1; m.8563 (A/C, SNP, Mother 3: +, Father 3-Child 3: -), 2; m.8909–8914 (TCTTAC/CCTAAA, MNP, Reference: TCTTAC, Mother 3-Father 3: +, Child 3: -).
**D.** Trio 4.1: m.8269–8279, MNP variation (Ref seq. GCACCCCCTCT) (Mother 4: GT / GTACCCCCTCT -Heteroplasmic variant, Father 4: No variation, Child 4.1: GT / GT-Homoplasmic variant).
rCRS: revised Cambridge Reference Sequence (mtDNA).

## Discussion

The paternal transmission of mtDNA to the offspring has been proved by scientific findings in human and animals. However, it is still a huge question how often it is occurring in human. Because only one case had been reported up to now, maternal inheritance which is the general accepted rule for mtDNA inheritance would not have been refused for now and a few case in the future. It would be opened the scientific debate related to mtDNA recombination when human researches on paternal mtDNA inheritance have been increased. Also, it would be reconsidered as anthropological research based on mtDNA analysis of modern human migration and evolution.

In mammals, sperm-derived paternal mitochondria and their mtDNA usually enter the oocyte cytoplasm after fertilization. It is thought that some mechanisms such as simple dilution model, active degradation model including ubiquitination, autophagy and proteasomal or lysosomal pathways have function to prevent the inheritance of paternal mtDNA to the


**Fig. 4.** Paternal derived variation carried childs unlike maternal mtDNA sequence.
**A.** Trio 1: m.5467–5471, INDEL variation (Mother 1: CCACG / ACCT, Father 1: -, Child 1: -).
**B.** Trio 5: 1; m.8896 (G/C, Mother 5: -, Father 5: +, Child 5: +), 2; m.8909–8914, MNP variation Ref: TCTTAC, (TCTTAC / CCTAAA, Mother 5: -, Father 5: +, Child 5: +).
**C.**Trio 8: 1; m.3483 (G/C, SNP, Mother 8: -, Father 8: +, Child 8: +), 2; m.3488 (T/A, SNP, Mother 8: -, Father 8: +, Child 8: +), 3; m.3492 (A/C, SNP, Mother 8: -, Father 8: +, Child 8: +).
**D.** Trio 9: m.8896 (G/C, Mother 9: -, Father 9: +, Child 9: +).
**E.** Trio 10: 1; m.5192 (A/C, SNP, Mother 10: +, Father 10: -, Child 10: -), 2; m.5208 (A/C, SNP, Mother 10: +, Father 10: -, Child 10: -).

next generation. The mechanisms for the recognition and exclusion of paternal mtDNA differ between organisms. In *Drosophila melanogaster*, mtDNA is removed from the spermatozoa during their development [16]. In fertilized primate and cow eggs, sperm mitochondria are tagged with ubiquitin targeting the organelles for destruction by the ubiquitin proteasome system [17]. There is also evidence about active degradation of paternal mtDNA in fertilized eggs of several organisms like Japanese medaka fish *Oryzias latipes* [18], *Caenorhabditis elegans* [19,20]. In humans, sperm mtDNA is eliminated at the stage of two or four cell embryos [7,17] and sperm loss is observed during embryogenesis in mice [21].

There is no direct evidence showing the destruction of sperm mtDNA and the mechanism for the recognition of paternal mitochondria is not precisely known [11]. There are several examples where paternal mtDNA has escaped from this process [22]. In mammals, the "leakage" of paternal mtDNA has been observed in atypical situations such as in the interspecies hybrids in mice (between *Mus musculus* and *Mus spretus* strain) [10], the persistence of human sperm-derived mtDNA when introduced into somatic cells [23] and in the abnormal fertilised human polyploid embryos from the four-cell to the eight-cell stage [24].

Schwartz and Vissing reported a sporadic patient with mitochondrial myopathy due to a novel 2-bp mtDNA deletion in *ND2* gene which encodes a subunit of the respiratory chain. In muscle from this patient, it was shown that 90% of the mtDNA was paternally derived and it was the first case of paternal inheritance in humans [12]. Kraytsberg and colleagues confirmed that 7% of single mtDNA molecules in the same patient's muscle cells contained alternating paternal and maternal mtDNA segments providing the first direct evidence for recombination [13]. It was suggested that this unusual mtDNA inheritance likely resulted from a failure to eliminate the low levels of mtDNA normally present in human sperm [9].

The mechanism underlying the maternal inheritance of mitochondrial DNA has not been completely explained. Recently, several lines of evidence suggest that different species seem to employ distinct mechanisms to prevent the inheritance of paternal mitochondrial DNA. Therefore, the aim of this study is to investigate the paternal mitochondrial DNA's effect on assisted reproductive technology applications working on the molecular basis of sperm mitochondrial DNA elimination and paternal mitochondrial DNA transmission in human. Studies on the next generation sequencing of mitochondrial DNA (mtDNA) have been carried out in the families (16 trios) to which assisted reproductive technologies were applied as a result of male infertility. The mitochondrial dysfunction might also be a feature of human male infertility and mutations in mtDNA have been described in subfertile men [25,26]. Regarding this, the fathers clinically diagnosed for azospermia, asthenozoospermia, oligozoospermia and teratozoospermia were selected in this study.

Mitochondria are thought to be the most important organelles for the evaluation of sperm quality including sperm DNA integrity and motility by means of their own DNA and membrane potential [27,28]. Mitochondrial oxidative phosphorylation and glycolysis are the energy production systems within sperm cells. It is mentioned that sperm cells can modulate glycolysis and oxidative phosphorylation functions to satisfy their energy need based on different conditions and fertilization stages [29]. In some asthenozoospermic sperm samples, abnormalities in mitochondrial number and morphology were also found [30,31]. Luo and colleagues suggest that although oxidative phosphorylation and

**Table 5**
Comparison of mitochondrial genome sequencing data analysis results.

| Family | Relationship | mtDNA-Server Haplotype | mtDNA Variant Analyzer Paternal shared variant | rCRS | Integrative Genomics Viewer Paternal shared variant | BLASTN mtDNA sequence similarity (Identity) | (Query cover) |
|---|---|---|---|---|---|---|---|
| Trio 1 | Mother | T2c1 | | | m.5467-5471 (INDEL), m.16292-16294 (INDEL/Het.) | 99% | 100% |
| | Father | T1a1 | | | –, m.16292-16294 (INDEL/Hom.) | 98-100% | 100% |
| | *Child | T2c1 | | | –, m.16292-16294 (INDEL/Hom.) | | |
| Trio 2 | Mother | H13a2b1 | m.73A, m.11719 G, m.14766C, m.16519 T | A/G/ C/T | –, –, –, | 99% | 100% |
| | Father | U4b1b1 | m.A73G, m.G11719A, m.C14766T, m. T16519C | A/G/ C/T | m.7028 (T/T), m.11719 (A/A), m.14133 (G/G), m.14766 (T/T), m.16519 (C/C) | 99-100% | 1-53% |
| | *Child | B4c1a1 | m.A73G, m.G11719A, m.C14766T, m. T16519C | A/G/ C/T | m.7028 (T/T), m.11719 (A/A), m.14133 (G/G), m.14766 (T/T), m.16519 (C/C) | | |
| Trio 3 | Mother | H26a1 | | | m.A8563C (SNP), m.8909-8914 (TCTTAC / CCTAAA, MNP) | 100% | 100% |
| | Father | J1b5b | | | –, m.8909-8914 (TCTTAC / CCTAAA, MNP) | 99-100% | 8-24% |
| | *Child | H26a1 | | | –, – | | |
| Trio 4.1 | Mother | U4b1a1a1 | | | m.8269-8279 (GT / GTACCCCCTCT, Heteroplasmic variant) | 100% | 100% |
| | Father | G2a2a | | | – | 99% | 100% |
| | *Child | U4b1a1a1 | | | m.8269-8279 (GT / GT, Homoplasmic variant) | | |
| Trio 4.3 | Mother | U4b1a1a1 | | | | 100% | 100% |
| | Father | G2a2a | | | | 95-99% | 1-44% |
| | *Child | U4b1a1a | | | | | |
| Trio 5 | Mother | W4d | | | –, – | 93-100% | 1-44% |
| | Father | G2a2 | | | m.G8896C, m.8909-8914 (TCTTAC / CCTAAA, MNP) | 87-99% | 1-30% |
| | *Child | W4d | | | m.G8896C, m.8909-8914 (TCTTAC / CCTAAA, MNP) | | |
| Trio 8 | Mother | I5a2 | | | –, –, – | 99-100% | 1-14% |
| | Father | H15 | | | m.G3483C (SNP), m.T3488A (SNP), m.A3492C (SNP) | 99% | 100% |
| | *Child | I5a2 | | | m.G3483C (SNP), m.T3488A (SNP), m.A3492C (SNP) | | |
| Trio 9 | Mother | I5a | | | – | 100% | 100% |
| | Father | H13a2c1 | | | m.8896 (G/C) | 99% | 100% |
| | *Child | I5a | | | m.8896 (G/C) | | |
| Trio 10 | Mother | R0a1a | m.2706, m.4769 | A/G | m.5192 (A/C, SNP), m.5208 (A/C, SNP) | 99% | 99% |
| | Father | U4a | m.A2706G, m.A4769G | A/G | –, – | 98-100% | 1-19% |
| | *Child | R0a1a | m.A2706G, m.A4769G | A/G | –, – | | |

glycolysis may have different roles in the spermatozoa from diverse species, oxidative phosphorylation is not dispensable for the sperm normal functions and any defect of it may also affect the sperm cell's fertilization ability [11]. Palanichamy and Zhang suggest that mtDNA plays a crucial role in sperm dysfunction and it serves as a potential diagnostic marker in infertile men, especially in cases of idiopathic oligoasthenozoospermia [32]. So, our study group was designed according to the male infertility subgroups which include azospermia (Father 1, 2, 3, 13), asthenozoospermia (Father 8, 11), asthenoteratozoospermia (Father 4, 6, 12), oligoteratozoospermia (Father 5, 9, 10) and teratozoospermia (Father 7), respectively.

Almost half of cases of infertility may be associated with male infertility. A semen analysis that measures sperm concentration, motility and morphology is the gold standard test for determining a person's fertility. There are also other tests that investigate male infertility; one being sperm DNA fragmentation test. It is known that DNA fragmentation is significantly higher in infertile men and men with poor semen parameters tend to have high sperm DNA fragmentation. Possibly, high DNA fragmentation may also be associated with decreased live birth rates in both natural and ART pregnancies. However, its utility is highly questionable due to its cost. Most of the causes of DNA fragmentation can not be treated. Damage caused by oxidative stress can be treated with lifestyle and diet modifications. But, it is well known that risk factors for oxidative stress should be emphasized to be stopped regardless of DNA fragmentation ratio in every couple. So we do not routinely investigate the sperm DNA fragmentation ratio, we do not have this result for all patients, thereby can not give this data.

Kumar and colleagues have observed a high frequency of nucleotide changes in the mitochondrial genes *COII* (*MT-CO2*) *"Mitochondrially Encoded Cytochrome C Oxidase II"*, *ATPase 6* (*MT-ATP6*) *"Mitochondrially Encoded ATP Synthase* 6", *ATPase 8* (*MT-ATP8*) *"Mitochondrially Encoded ATP Synthase* 8", *ND2*, *ND3 "Mitochondrially Encoded NADH Dehydrogenase* 3", *ND4* and *ND5* in the semen/blood cells of infertile men [33,34]. However, we have found father-child shared variants in mitochondrial genes *COX1* (*MT-CO1*) (Trio 2), *ATPase 6* (Trio 5, Trio 9), *ND1* (Trio 8), *ND4* (Trio 2), *ND5* (Trio 2), *MT-CYB* (Trio 2), *MT-DLOOP1* region (Trio 2). Besides these findings, we have also observed maternal mitochondrial variants within or near mitochondrial genes *COX3* (Trio 3), *ND2* (Trio 10) without detected on child-father mtDNA.

The identified novel and reported variations were in the *ND1*, *ND2*, *ND4*, *ND5*, *COX1*, *COX3*, *CYTB*, *ATP6* genes and *DLOOP* non-coding mitochondrial control region. These genes are encoded by mtDNA and they play an important role in oxidative phosphorylation process as a member of respiratory chain enzyme complex subunits.

In our population, the previously reported m.73 A > G and m.16519 T > C in non-coding MT-CR region were detected in Trio 2 (father 2 – child 2). These variations were reported as a homoplasmic point mutation related to oral cancer and cyclic vomiting syndrome with migraine / metastasis risk (m.16519 T > C), respectively [35–38]. Three kinds of single nucleotide polymorphism (SNP) variations within *MT-ND1* gene including m.3483 G > C, m.3488 T > A, m.3492 A > C were determined in Trio 8. In recent studies, m.3481 G > A was reported to be related with the diseases of Mitochondrial Encephalomyopathy, Lactic Acidosis and Stroke-like episodes (MELAS) and progressive encephalomyopathy [39–41]. The other *MT-ND1* variations for m.3488 T > C [42] and m.3496 G > T [43,44] were reported for Leber Hereditary Optic Neuropathy (LHON) disease.

The same "father-child" shared variation for m.8896 G > C within *ATP6* gene was observed in Trio 5 and Trio 9. Also, father 5 and father 9 had same male infertility factor which was diagnosed for oligoteratozoospermia. However, m.8890 A > G variation in *ATP6* gene was also reported in Juvenile-onset metabolic syndrome [45]. The same MNP variation at nucleotides m.8909–8914 within *ATP6* gene was detected in Trio 3 of mother 3-father 3 and in Trio 5 of father 5-child 5. It was reported as prostate cancer/neuromuscular disorder risk associated variation (m.8932 C > T) adjacent to m.8909–8914 [46–48].

In this study, 48 mitochondrial genome of 16 trio samples were analyzed for mtDNA haplotype assessment by mtDNA-Server program. It has mostly observed Europian derived haplogroups (H, I, J, K, T, U, W and R0) except two Asian haplogroup B and G in our samples. In Trio 2 and Trio 4.3 child had different mtDNA haplotype from their parents, in the remaining 14 trios their maternal mtDNA haplotype was detected. Although there has been reported interpretations associated with mtDNA polymorphisms and mitochondrial haplogroups in infertile groups, we could not find any evidence supporting this.

Homologous and non-homologous DNA recombination activity has been reported in human mitochondria and intramolecular / intermolecular mtDNA recombination has also been detailed in fungi, plants, animals [49,14]. Our findings for paternal transmission of shared variants could be the result of intermolecular recombination between maternal and paternal mitochondrial DNA.

In recently published article, Luo and colleagues have demonstrated biparental transmission of mtDNA in three unrelated multigeneration families with a high level of mtDNA heteroplasmy (ranging from 24 to 76%) in a total of 17 individuals [50]. It would appear as an important evidence that supports our demonstrated results after Schwartz and Vissing's published report in 2002.

## Conclusion

As a conclusion, paternal inheritance would appear to have importance on genetic consultancy especially for complex mitochondrial diseases. Further detailed evaluation of molecular genetic analysis data would provide evidence about paternal inheritance transmission forms especially in assisted reproduction techniques.

## Authors' roles

CE and TG conceived and designed the experiments. HGC and BKB conducted blood sampling from trios and medical assistance for study. CE performed the NGS experiments, data analysis and wrote the paper. TG, HGC, BKB and CE carried out manuscript drafting and editing. All authors read and approved the final version of the manuscript.

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## Conflict of interest

The authors report no conflicts of interest in connection with this article.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejogrb.2019.02.011.

## References

[1] Saccone C, Gissi C, Lanave C, Larizza A, Pesole G, Reyes A. Evolution of the mitochondrial genetic system: an overview. Gene 2000;1:153–9.
[2] Boore JL. Animal mitochondrial genomes. Nucleic Acids Res 1999;8:1767–80.
[3] Song WH, Ballard JWO, Yi YJ, Sutovsky P. Regulation of mitochondrial genome inheritance by autophagy and ubiquitin-proteasome system: implications for health, fitness and fertility. Biomed Res Int 2014;2014:1–16.
[4] Anderson S, Bankier AT, Barrell BG, de Bruijn MHL, Coulson AR, Drouin J, et al. Sequence and organization of the human mitochondrial genome. Nature 1981;5806:457–65.
[5] Giles RE, Blanc H, Cann HM, Wallace DC. Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci U S A 1980;11:6715–9.
[6] Birky Jr. CW. Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. Proc Natl Acad Sci U S A 1995;25:11331–8.
[7] Ankel-Simons F, Cummins JM. Misconceptions about mitochondria and mammalian fertilization: implications for theories on human evolution. Proc Natl Acad Sci U S A 1996;24:13859–63.
[8] Sato M, Sato K. Maternal inheritance of mitochondrial DNA by diverse mechanisms to eliminate paternal mitochondrial DNA. Biochim Biophys Acta 2013;8:1979–84.
[9] Chan DC, Schon EA. Eliminating mitochondrial DNA from sperm. Dev Cell 2012;3:469–70.
[10] Gyllensten U, Wharton D, Josefsson A, Wilson AC. Paternal inheritance of mitochondrial DNA in mice. Nature 1991;6332:255–7.
[11] Luo SM, Ge ZJ, Wang ZW, Jiang ZZ, Wang ZB, Ouyang YC, et al. Unique insights into maternal mitochondrial inheritance in mice. Proc Natl Acad Sci U S A 2013;32:13038–43.
[12] Schwartz M, Vissing J. Paternal inheritance of mitochondrial DNA. N Engl J Med 2002;8:576–80.
[13] Kraytsberg Y, Schwartz M, Brown TA, Ebralidse K, Kunz WS, Clayton DA, et al. Recombination of human mitochondrial DNA. Science 2004;5673:981.
[14] Slate J, Gemmell NJ. Eve 'n' Steve: recombination of human mitochondrial DNA. Trends Ecol Evol 2004;11:561–3.
[15] Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. Quantitative Biology Genomics arXiv: 1303.3997 [q-bio.GN]. New York, USA: Cornell University Library Press; 2013.
[16] DeLuca SZ, O'Farrell PH. Barriers to male transmission of mitochondrial DNA in sperm development. Dev Cell 2012;3:660–8.
[17] Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C, Schatten G. Development: ubiquitin tag for sperm mitochondria. Nature 1999;6760:371–2.
[18] Nishimura Y, Yoshinari T, Naruse K, Yamada T, Sumi K, Mitani H, et al. Active digestion of sperm mitochondrial DNA in single living sperm revealed by optical tweezers. Proc Natl Acad Sci U S A 2006;5:1382–7.
[19] Al Rawi S, Louvet-Vallée S, Djeddi A, Sachse M, Culetto E, Hajjar C, et al. Postfertilization autophagy of sperm organelles prevents paternal mitochondrial DNA transmission. Science 2011;6059:1144–7.
[20] Sato M, Sato K. Degradation of paternal mitochondria by fertilization-triggered autophagy in C. elegans embryos. Science 2011;6059:1141–4.
[21] Shitara H, Kaneda H, Sato A, Inoue K, Ogura A, Yonekawa H, et al. Selective and continuous elimination of mitochondria microinjected into mouse eggs from spermatids, but not from liver cells, occurs throughout embryogenesis. Genetics 2000;3:1277–84.
[22] Pyle A, Hudson G, Wilson IJ, Coxhead J, Smertenko T, Herbert M, et al. Extreme-depth Re-sequencing of mitochondrial DNA finds No evidence of paternal transmission in humans. PLoS Genet 2015;5:1–11.
[23] Manfredi G, Thyagarajan D, Papadopoulou LC, Pallotti F, Schon EA. The fate of human sperm-derived mtDNA in somatic cells. Am J Hum Genet 1997;4:953–60.
[24] St John JC, Schatten G. Paternal mitochondrial DNA transmission during nonhuman primate nuclear transfer. Genetics 2004;2:897–905.
[25] Folgero T, Bertheussen K, Lindal S, Torbergsen T, Oian P. Mitochondrial disease and reduced sperm motility. Hum Reprod 1993;11:1863–8.
[26] Marchington DR, Scott Brown MSG, Lamb VK, van Golde RJT, Kremer JAM, Tuerlings JHAM, et al. No evidence for paternal mtDNA transmission to offspring or extra-embryonic tissues after ICSI. Mol Hum Reprod 2002;11:1046–9.
[27] Carra E, Sangiorgi D, Gattuccio F, Rinaldi AM. Male infertility and mitochondrial DNA. Biochem Biophys Res Commun 2004;1:333–9.

[28] Amaral A, Ramalho-Santos J. Assessment of mitochondrial potential: implications for the correct monitoring of human sperm function. Int J Androl 2010;1:e180–6.
[29] John JS, John B. Sperm mitochondrial DNA. In: Zini A, Agarwal A, editors. Sperm chromatin 2011. New York, USA: Springer; 2019. p. 81–94.
[30] Wilton LJ, Temple-Smith PD, de Kretser DM. Quantitative ultrastructural analysis of sperm tails reveals flagellar defects associated with persistent asthenozoospermia. Hum Reprod 1992;4:510–6.
[31] Rawe VY, Olmedo SB, Benmusa A, Shiigi SM, Chemes HE, Sutovsky P. Sperm ubiquitination in patients with dysplasia of the fibrous sheath. Hum Reprod 2002;8:2119–27.
[32] Palanichamy MG, Zhang YP. Identifying potential pitfalls in interpreting mitochondrial DNA mutations of male infertility cases. Indian J Med Res 2011;4:447–51.
[33] Kumar R, Bhat A, Bamezai RN, Shamsi MB, Kumar R, Gupta NP, et al. Necessity of nuclear and mitochondrial genome analysis prior to assisted reproductive techniques/intracytoplasmic sperm injection. Indian J Biochem Biophys 2007;6:437–42.
[34] Kumar R, Venkatesh S, Kumar M, Tanwar M, Shasmsi MB, Kumar R, et al. Oxidative stress and sperm mitochondrial DNA mutation in idiopathic oligoasthenozoospermic men. Indian J Biochem Biophys 2009;2:172–7.
[35] Boles RG, Zaki EA, Lavenbarg T, Hejazi R, Foran P, Freeborn J, et al. Are pediatric and adult-onset cyclic vomiting syndrome (CVS) biologically different conditions? Relationship of adult-onset CVS with the migraine and pediatric CVS-associated common mtDNA polymorphisms 16519T and 3010A. Neurogastroenterol Motil 2009;9: 936-e72.
[36] Zaki EA, Freilinger T, Klopstock T, Baldwin EE, Heisner KR, Adams K, et al. Two common mitochondrial DNA polymorphisms are highly associated with migraine headache and cyclic vomiting syndrome. Cephalalgia 2009;7:719–28.
[37] Ebner S, Lang R, Mueller EE, Eder W, Oeller M, Moser A, et al. Mitochondrial haplogroups, control region polymorphisms and malignant melanoma: a study in middle European Caucasians. PLoS One 2011;12:e27192.
[38] Venkatesan T, Zaki EA, Kumar N, Sengupta J, Ali M, Malik B, et al. Quantitative pedigree analysis and mitochondrial DNA sequence variants in adults with cyclic vomiting syndrome. BMC Gastroenterol 2014;181:1–7.
[39] Malfatti E, Bugiani M, Invernizzi F, de Souza CF, Farina L, Carrara F, et al. Novel mutations of ND genes in complex I deficiency associated with mitochondrial encephalopathy. Brain 2007;7:1894–904.
[40] Moslemi AR, Darin N, Tulinius M, Wiklund LM, Holme E, Oldfors A. Progressive encephalopathy and complex I deficiency associated with mutations in MTND1. Neuropediatrics 2008;1:24–8.
[41] Valente L, Piga D, Lamantea E, Carrara F, Uziel G, Cudia P, et al. Identification of novel mutations in five patients with mitochondrial encephalomyopathy. Biochim Biophys Acta 2009;5:491–501.
[42] Ji Y, Liang M, Zhang J, Zhu L, Zhang Z, Fu R, et al. Mitochondrial ND1 variants in 1281 chinese subjects with Leber's hereditary optic neuropathy. Invest Ophthalmol Vis Sci 2016;(6):2377–789.
[43] Matsumoto M, Hayasaka S, Kadoi C, Hotta Y, Fujiki K, Fujimaki T, et al. Secondary mutations of mitochondrial DNA in Japanese patients with Leber's hereditary optic neuropathy. Ophthalmic Genet 1999;3:153–60.
[44] Mitchell AL, Elson JL, Howell N, Taylor RW, Turnbull DM. Sequence variation in mitochondrial complex I genes: mutation or polymorphism? J Med Genet 2006;2:175–9.
[45] Ye W, Chen S, Jin S, Lu J. A novel heteroplasmic mitochondrial DNA mutation, A8890G, in a patient with juvenile-onset metabolic syndrome: a case report. Mol Med Rep 2013;4:1060–6.
[46] Petros JA, Baumann AK, Ruiz-Pesini E, Amin MB, Sun CQ, Hall J, et al. mtDNA mutations increase tumorigenicity in prostate cancer. Proc Natl Acad Sci U S A 2005;3:719–24.
[47] Arnold RS, Sun CQ, Richards JC, Grigoriev G, Coleman IM, Nelson PS, et al. Mitochondrial DNA mutation stimulates prostate cancer growth in bone stromal environment. Prostate 2009;1:1–11.
[48] Felhi R, Mkaouar-Rebai E, Sfaihi-Ben Mansour L, Alila-Fersi O, Tabebi M, Ben Rhouma B, et al. Mutational analysis in patients with neuromuscular disorders: detection of mitochondrial deletion and double mutations in the MT-ATP6 gene. Biochem Biophys Res Commun 2016;1:61–6.
[49] Rokas A, Ladoukakis E, Zouros E. Animal mitochondrial DNA recombination revisted. Trends Ecol Evol 2003;8:411–7.
[50] Luo S, Valencia CA, Zhang J, Lee NC, Slone J, Gui B, et al. Biparental inheritance of mitochondrial DNA in humans. Proc Natl Acad Sci U S A 2018;51:13039–44.