Comprehensive analysis of mitochondrial DNA variants, mitochondrial DNA copy number and oxidative damage in psoriatic arthritis

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Abstract. Growing evidence suggests that abnormalities in mitochondrial DNA (mtDNA) are involved in the pathogenesis of various inflammatory and immuno-mediated diseases. The present study analysed the entire mitochondrial genome by next-generation sequencing (NGS) in 23 patients with psoriatic arthritis (PsA) and 20 healthy controls to identify PsA-related variants. Changes in mtDNA copy number (mtDNAcn) were also evaluated by quantitative polymerase chain reaction (qPCR) and mtDNA oxidative damage was measured using an 8-hydroxy-2'-deoxyguanosine assay. NGS analysis revealed a total of 435 variants including 187 in patients with PsA only and 122 in controls only. Additionally, 126 common variants were found, of which 2 variants differed significantly in their frequencies among patients and controls ($P<0.05$), and may be associated with susceptibility to PsA. A total of 33 missense variants in mtDNA-encoded genes for complexes I, III, IV and V were identified only in patients with PsA. Of them, 25 variants were predicted to be deleterious by affecting the functions and structures of encoded proteins, and 13 variants were predicted to affect protein's stability. mtDNAcn analysis revealed decreased mtDNA content in patients with PsA compared with controls ($P=0.0001$) but the decrease in mtDNAcn was not correlated with patients' age or inflammatory biomarkers ($P>0.05$). Moreover, a higher level of oxidative damage was observed in patients with PsA compared with controls ($P=0.03$). The results of the present comprehensive analysis of mtDNA in PsA revealed that certain mtDNA variants may be implicated in the predisposition/pathogenesis of PsA, highlighting the importance of NGS in the identification of mtDNA variants in PsA. The current results also demonstrated that decreased mtDNAcn in PsA may be a consequence of increased oxidative stress. These data provide valuable insights into the contribution of mtDNA defects to the pathogenesis of PsA. Additional studies in larger cohorts are needed to elucidate the role of mtDNA defects in PsA.

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Introduction

Psoriatic arthritis (PsA) is a heterogeneous chronic immune-mediated disease characterized by musculoskeletal inflammation. Numerous patients develop PsA on the background of psoriasis, a skin condition of scaly erythematous plaques that commonly affects the extensor surfaces of the elbows and knees, and other parts of the body (1-3). The onset of PsA often occurs between the age of 30 and 50 years but may arise at any point throughout a patient's lifetime. The clinical manifestations of PsA vary greatly between patients and range from relatively mild to severe disease, and disease flares can alternate with periods of remission (4). Due to the shared similarities in the clinical presentation of PsA and other arthritic diseases such as rheumatoid arthritis (RA) and osteoarthritis (OA), PsA is frequently undiagnosed and/or misdiagnosed (5). However, six clinical domains are involved in PsA including peripheral arthritis, enthesitis, dactylitis, psoriasis, psoriatic nail disease and axial disease (6).

Although the aetiology of PsA is not fully understood, genetics, epigenetics and environmental factors contribute to abnormal immune responses and disease expression (7). At the genetics level, both human leukocyte antigen (HLA) and non-HLA genes have been associated with PsA (7). Moreover, 33-50% of patients with PsA have at least one first-degree relatives who are also affected by psoriasis or PsA (8). Previous studies have shown that mitochondrial dysfunction contributes significantly to the pathogenesis of PsA by modulating innate immunity via redox-sensitive inflammatory pathways (9,10). Oxidative stress can disrupt redox signalling and cause molecular damage, which impacts angiogenesis, inflammation and immune cell function (11). Mitochondria produce most of the cellular energy through the process of oxidative

phosphorylation (OXPHOS) and are also the major site of reactive oxygen species (ROS). Besides their central role in cellular metabolism, mitochondria also participate in other important cellular processes such as innate immune, inflammatory and stress responses (12). Mitochondria have their own genome called mitochondrial DNA (mtDNA), which is a double-stranded molecule encoding 37 genes. In total, 13 of the mtDNA genes are involved in the OXPHOS and the remaining genes are essential in assembling amino acids into functional proteins (13). mtDNA presents in multiple copies (1,000-10,000 copies) per cell, resulting in both homoplasmic and heteroplasmic mtDNA variants. Moreover, the mtDNA copy number (mtDNAcn) is regulated in a tissue-specific manner (14) and correlates positively with the number of mitochondria, and thus is considered an indicator of mitochondrial function (15). Numerous factors make mtDNA particularly vulnerable to ROS and oxidative damage, including its proximity to the site of the electron transport chain (ETC), absence of protective histones and inadequate DNA repair capacity (16). Oxidative stress is an important source of mitochondrial genomic instability and can induce mtDNA variations and copy number changes, which may lead to abnormalities in mitochondrial function (17,18). Both mtDNA variants and copy number alterations have been implicated in human aging and various pathological conditions including mitochondrial disorders, cancer, and neurodegenerative diseases (19,20).

Harty *et al* (21) evaluated the total mtDNA mutational load in PsA/RA using a mitochondrial random capture assay, which revealed a significant increase in the frequency of mtDNA variants in synovial tissue from patients with RA and PsA compared with controls. However, mitochondrial random capture assay has limitations such as low sensitivity and inability to detect heteroplasmy, which is an important characteristic of numerous mtDNA-related diseases. Previously, next-generation sequencing (NGS) has emerged as a robust technique for screening the mitochondrial genome. It enables comprehensive analysis of the entire mitochondrial genome for the detection of common and rare mtDNA variants, mtDNA disease-associated variants, and accurate measurement of heteroplasmy (22). Since no previous studies have analysed the entire mitochondrial genome or evaluated changes in mtDNAcn and oxidative stress in PsA, the present study aimed to investigate mtDNA variants related to PsA and/or associated with the risk of PsA via NGS. The present study also aimed to examine changes in mtDNAcn as well as evaluate mtDNA oxidative damage in patients with PsA and healthy controls.

Materials and methods

Study subjects. A total of 43 subjects including 23 patients with PsA and 20 healthy controls were enrolled in the present study. Patients with PsA were recruited from the out-patient clinic at the Department of Rheumatology of Mubarak Hospital, (City of Kuwait, State of Kuwait). The patients fulfilled the classification criteria for PsA (CASPAR). Patients with other inflammatory or autoimmune diseases were excluded from study (23). Clinicopathological characteristics of patients (including sex and age distribution) are presented in Table I.

Healthy control individuals without inflammatory dermatoses or autoimmune diseases were recruited from the Central Blood Bank, State of Kuwait. Basic clinical characteristics and laboratory data were obtained from the medical and electronic records of patients and controls. Written informed consent was obtained from all participants under protocols approved by Kuwait University and Ministry of Health (City of Kuwait, State of Kuwait) (approval no. 2018/496).

Extraction of genomic DNA. Blood samples (5 ml) were collected in EDTA tubes from the participants and centrifuged at 4˚Cn 1,000 x g for 15 min to separate the buffy coat which was subjected to genomic DNA extraction using QIA amp DNA Mini kit (cat. no. 51304; Qiagen GmbH) according to the manufacturer's instructions as previously described (24). Briefly, a mixture of 200 μl buffy coat, 20 μl protease and 200 μl lysis buffer was incubated at 56˚C for 10 min and then centrifuged at 20,000 x g for 1 min at 4˚C. Next, absolute ethanol (200 μl) was added and centrifuged at 6,000 x g for 1 min followed by washing with 500 μl washing buffer. The mixture was then centrifuged at room temperature at 6,000 x g for 1 min and then at 20,000 x g for 3 min. Genomic DNA was eluted with 200 μl elution buffer after incubation at room temperature for 1 min and centrifugation at 6,000 x g for 1 min at room temperature. The DNA samples were quantified and assessed for purity using a NanoDrop ND-1000 ultraviolet-visible light spectrophotometer (Thermo Fisher Scientific, Inc.).

Mitochondrial genome sequencing. The entire mitochondrial genome was sequenced using the S5™XL NGS system (Applied Biosystems; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol, as previously described (25). After library preparation and purification, the raw data were automatically transferred from the Ion Torrent S5 XL sequencer to the Torrent Suite software version 5.0 (Applied Biosystems; Thermo Fisher Scientific, Inc.), which allowed the conversion of the raw voltage semiconductor sequencing data into DNA base calls. For identification of variants, the Ion Torrent Variant Caller plug-in, Ion Reporter software version 5.2. and Torrent Variant Caller version 5.2 (Applied Biosystems; Thermo Fisher Scientific, Inc.) were used. For alignment, the Revised Cambridge Reference Sequence of the Human mtDNA (NC_012920.1) was applied as a reference mitochondrial sequence (26). The average throughput of the Ion 520 chip was 3.5 Mb. The sequence data sets were registered in the Sequence Read Archive repository (reference no. PRJNA 928743).

Bioinformatics analysis. The impact of nonsynonymous mtDNA variants on protein function and structure was determined using three *in-silico* prediction tools used: i) Combined Annotation Dependent Depletion (CADD): Incorporates multiple annotations including conservation and functional information into one tool and categorizes variants as benign or deleterious using a machine learning approach. Variants with scores ≥20 were predicted to be deleterious (27); ii) CONsensus DELeteriousness (Condell): Integrates the output of five algorithms including Pfam *E-value* (Logre), MAPP, Mutation Assessor, Polyphen2, and SIFT to assess the outcome of nonsynonymous single nucleotide variants (SNVs) on protein function. Variants with scores ≥0.5 were predicted to

Table I. Demographic and clinical data of patients with psoriatic arthritis and controls.

Characteristics	PsA	Controls	P-value
Number of subjects	23	20	
Sex			0.6
Male, n (%)	11 (48)	8 (50)	
Female, n (%)	12 (52)	12 (50)	
Age, years (mean ± SD)	39±3	30±1.3	0.01
C-Reactive protein, mg/dl	5±0.9	0.1±0.05	0.02
Rheumatoid factor, U/ml	21±2	0.4±0.02	<0.001
Erythrocyte sedimentation rate, mm/h	28±9	2.6±1	0.003
Medications			
Topical treatment	4		
Systemic treatment	19		
Methotrexate	4		
Adalimumab	8		
Etanercept	3		
Secukinumab	2		
Ixekizumab	2		

be deleterious (28); and iii) Protein Variation Effect Analyzer (PROVEAN) predicts the impact of an amino acid substitution or indel on protein function. PROVEAN performance is comparable to SIFT or PolyPhen-2 and it can process a large number of protein variants. Variants with scores ≤-2.5 were predicted to be deleterious, while those with scores >-2.5 were considered neutral (29).

Analysis of protein stability. Analysis of the impact of nonsynonymous mtDNA variants on protein stability was conducted using Site-Directed Mutator (SDM), which is a statistical potential energy function that uses environment-specific amino acid substitution frequencies within the family of homologous proteins of known (3-D) structures to calculate a stability score, which is analogous to the free energy difference between the wild-type and mutant protein (30). A change in the Gibbs free energy for protein stability is expressed as ΔΔG (30).

Determination of relative mtDNAcn. Quantitative polymerase chain reaction (qPCR) was used to determine the mtDNAcn relative to nuclear DNA (nDNA). Mitochondrial NADH dehydrogenase subunit 2 (*ND2*) was used as a target gene for the amplification of mtDNA with the following primers: forward, 5'-CAC AGA AGC TGC CAT CAA GTA-3' and reverse, 5'-CCG GAG AGT ATA TTG TTG AAG AG-3'; while nuclear b2-microglobulin (β2M) was used as a reference gene for the amplification of nDNA with the following primers: forward, 5'-CCA GCA GAG AAT GGA AAG TCA A-3' and reverse, 5'-TCT CTC TCC ATT CTT CAG TAA GTC AAC T-3'. The PCR mixture contained 10 ng genomic DNA, 1X SYBR1 Green PCR Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.), forward and reverse primers (50 nM each), and nuclease-free water to a final volume of 10 μl. PCR was performed in a 7900HT real-time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) using the following thermocycling conditions: Initial denaturation at 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec, 60˚C for 30 sec and 72˚C for 30 sec. The experiments were performed in duplicate and non-template control was included in each run. Relative quantitation of mtDNAcn was performed using the $2^{-\Delta\Delta Cq}$ method (31) by obtaining the Cq values of the *ND2* and *β2M* genes, and then the ΔCq (Cq *ND2*-Cq β2M) values for cases and controls were calculated.

Determination of mtDNA oxidative damage. 8-Hydroxyl 2'-deoxyguanosine (8-OHdG) is one of the most common markers of oxidative DNA lesions and is widely applied for the measurement of oxidative DNA damage (32,33). In the detection of oxidative damage with 8-OHdG, the marker does not deform the DNA structure but causes inhibition of *Taq* DNA polymerase during PCR. Therefore, the presence of 8-OHdG in a certain region of mtDNA can be digested by formamidopyrimidine [fapy]-DNA glycosylase (FPG) which breaks the DNA fragment at the lesion site and reduces further amplification of this particular region. In the present study, 8-OHdG assay was conducted to measure mtDNA oxidative damage by qPCR. A total of 100 ng DNA was incubated for 1 h at 37˚C in a 10 μl of reaction mixture containing 1 U FPG enzyme (New England Bio Labs, Inc), 1X NEB buffer and 0.1 mg/ml bovine serum albumin (New England Bio Labs, Inc). Next, the digested DNA was amplified by PCR under the same aforementioned cycling protocol and conditions. DNA damage was measured as ΔCq (ΔCq=Cq treated-Cq untreated). The presence of oxidative damage in DNA after treatment of FPG reduces the PCR efficiency and increases the Ct value.

Statistical analysis. Statistical analysis of data was performed using SPSS software (version 23; IBM Corp.). The normal distribution of data was first evaluated using the Kolmogorov-Smirnov test. Accordingly, the comparisons of variables between patients and controls were conducted using the χ^2 test for categorical variables and the Mann-Whitney test for normally distributed variables. The Fisher's exact test was used to assess differences in the frequency of variants between patients and controls and the odds ratio (OR) and 95% confidence interval (CI) were reported. P≤0.05 was considered to indicate a statistically significant difference.

Results

Characteristics of the study subjects. The demographic and clinical data of patients with PsA (n=23) and healthy control individuals (n=20) are presented in Table I. The sex ratio (male: female) was 11:12 for patients and 8:12 for controls. There was no significant difference in sex distribution between PsA and controls (P=0.6), but there was a significant difference in mean age between the two groups (P=0.01). The clinical inflammatory marker C-reactive protein (CRP) (0-0.8 mg/dl), rheumatoid factor (RF) (0-20 U/ml), and erythrocyte sedimentation rate (ESR) (0-20 mm/hr) were all higher than the normal range in patients with PsA. The mean value of CRP, RF and ESR were significantly higher in PsA patients compared with controls (P<0.05). Patients were under the following medications: Topical treatment with corticosteroid cream (n=4),

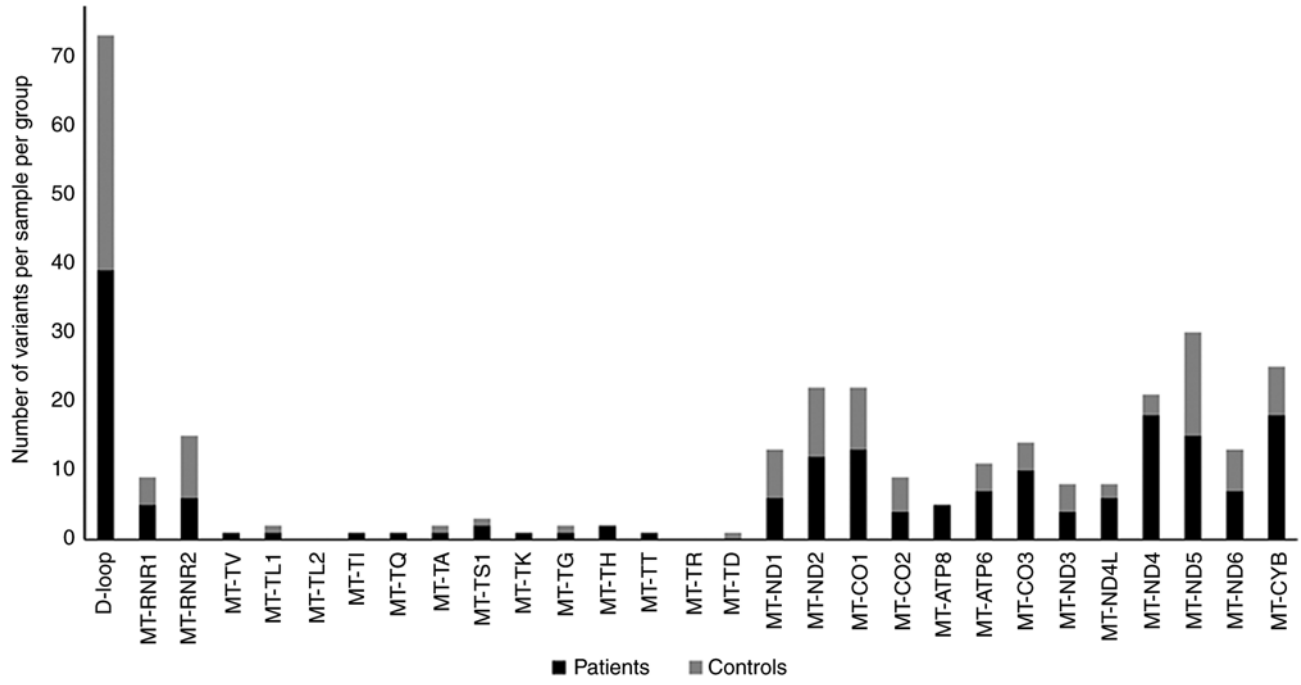

Figure 1. Distribution of mitochondrial DNA sequence variants in patients with psoriatic arthritis and controls.

or systemic treatment (n=19) including Methotrexate (n=4), Adalimumab (n=8), Etanercept (n=3), Secukinumab (n=2) and Ixekizumab (n=2).

mtDNA sequence variants identification. A total of 435 mtDNA sequence variants were identified in 43 samples. Among them, 187 (43%) variants were exclusive for patients with PsA, and 122 (28%) variants were found in control individuals only (Fig. 1). In both patients and controls, the highest number of variants was observed in the D-loop region, while the lowest number of variants was observed in the tRNAs genes. In protein-coding genes of patients with PsA, there were 152 variants including 33 nonsynonymous and 92 synonymous silent variants. By contrast, in protein-coding genes of control individuals, there were 76 variants, including 18 non-synonymous and 58 synonymous silent variants.

Particularly, a higher number of variants were observed in the MT-ND4, MT-ND5 and MT-CYB genes. A higher number of variants in the MT-ND4, and MT-CYB genes was also observed in patients than in controls. Moreover, the majority of mtDNA variants in patients and controls were homoplasmy accounting for 183 and 118, respectively, compared with the small number of heteroplasmy variants (n=4) in each group. Details of the identified variants in patients or controls as well as their localization in different mtDNA regions are shown in Table II. Notably, of non-synonymous variants in controls only, 4640C>A in the MT-ND2 gene was reported as a pathogenic variant causing Leber optic atrophy according to the Mito Map (https://www.mitomap.org/MITOMAP) and ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases.

In addition, 126 (28.9%) mtDNA variants were common for both patients with PsA and controls (Appendix I). The frequency of two common variants differed significantly between the two groups. The substitution variant m.152T>C in the D-loop region was found in 26% of patients and 55% of controls (OR=0.3, 95% CI=0.1-0.5, P=0.02), whereas the silent variant m.15301G>A in the *MT-CYB* gene was found in 30% of patients and 10% of controls (OR=3.8, 95% CI=1-8, P=0.04). The remaining variants showed no significant differences in their prevalence among patients or controls (P>0.05).

Variants with amino acid substitutions in patients with PsA and their impact on protein function and protein stability. Out of 187 (43%) mtDNA variants detected only in patients with PsA, 33 missense variants in mtDNA-encoding genes of complexes I, III, IV, and V resulted in amino acid substitutions.

Bioinformatics analysis using CADD, Condel, and PROVEAN was conducted to determine the potential impact of 33 missense variants that detected in PsA patients, on protein function and structure. The analysis predicted 25 variants to be deleterious by at least one bioinformatics tool (Table III). The highest impact on protein function and structure (by all three *in-silico* algorithms) was predicted for 2 variants, namely the m.11172A>G variant (referred to as rs2853489 polymorphism) in the MT-*ND4* gene and the m.15257G>A variant (referred to as rs41518645 polymorphism) in the *MT-CYB* gene. The allelic frequencies of these variants in the gnome AD database were 0.0007. and 0.01, respectively.

In total, 5 other variants were predicted to be deleterious by two *in-silico* algorithms tools: 3 variants in the *MT-ATP6* gene namely m.8860A>G (referred to as rs2001031 polymorphism), m.9007A>G (referred to as rs1603221973 polymorphism) and m.9103T>C (referred as rs1603222077 polymorphism); and 2 variants in the *MT-CYB* gene namely m.15431G>A (referred to as rs193302993 polymorphism) and m.15735C>T (referred to as rs1603225446 polymorphism). With the exception of the *MT-ATP6* variant m.8860A>G with an allelic frequency of 0.99, all other variants had allelic frequencies of <0.01 in the gnome AD database (https://gnomad.broadinstitute.org/).

Table II. Details of mtDNA variants exclusive for patients with psoriatic arthritis or controls.

Non-coding region										
	Patients					Controls				
Locus	Variant	Type of variant	Amino acid change	rs ID	Homoplasmy/ Heteroplasmy	Variant	Variant type	Affected amino acid	rs ID	Homoplasmy/ Heteroplasmy
D-loop	m.57T>TC	Insertion		rs21245905	Heteroplasmy	m.143G>A	Substitution		rs375589100	Homoplasmy
D-loop	m.72A>G	Substitution		rs869183622	Heteroplasmy	m.178A>G	Substitution		Not provided	Homoplasmy
D-loop	m.93A>G	Substitution		rs369034419	Homoplasmy	m.182C>T	Substitution		rs41473347	Homoplasmy
D-loop	m.153A>G	Substitution		rs370716192	Homoplasmy	m.185 G>T	Substitution		rs879015046	Homoplasmy
D-loop	m.189A>G	Substitution		rs371543232	Homoplasmy	m.199T>C	Substitution		rs72619362	Homoplasmy
D-loop	m.200A>G	Substitution		rs372099630	Homoplasmy	m.250 T>C	Substitution		Not provided	Homoplasmy
D-loop	m.207G>A	Substitution		rs369669319	Homoplasmy	m.285C>T	Substitution		rs201801609	Homoplasmy
D-loop	m.215A>G	Substitution		rs879219259	Homoplasmy	m.357A>G	Substitution		rs28678375	Homoplasmy
D-loop	m.217T>C	Substitution		rs41531144	Homoplasmy	m.385A>G	Substitution		rs201801609	Homoplasmy
D-loop	m.235A>G	Substitution		rs3937037	Homoplasmy	m.497C>T	Substitution		rs28660704	Homoplasmy
D-loop	m.236T>C	Substitution		rs375896687	Homoplasmy	m.16093T>C	Substitution		rs2853511	Heteroplasmy
D-loop	m.340C>T	Substitution		rs117394573	Homoplasmy	m.16150C>T	Substitution		rs879004379	Homoplasmy
D-loop	m.508A/G	Substitution		rs113683159	Homoplasmy	m.16163 A>G	Substitution		rs41479950	Homoplasmy
D-loop	m.524 C>CAC	Insertion		Not provided	Heteroplasmy	m.16185C>T	Substitution		rs1556424787	Homoplasmy
D-loop	m.16041A>G	Substitution		rs369904200	Homoplasmy	m.16186C>T	Substitution		rs879166752	Homoplasmy
D-loop	m.16051A>G	Substitution		rs117565943	Homoplasmy	m.16224T>C	Substitution		rs386420031	Homoplasmy
D-loop	m.16067C>T	Substitution		rs1556424732	Homoplasmy	m.16232C>A	Substitution		rs1603225749	Homoplasmy
D-loop	m.16111C>T	Substitution		rs35315169	Homoplasmy	m.16249T>C	Substitution		rs372301309	Homoplasmy
D-loop	m.16124T>C	Substitution		rs386829272	Homoplasmy	m.16264C>G	Substitution		rs878922147	Homoplasmy
D-loop	m.16148C>T	Substitution		rs201893071	Homoplasmy	m.16288T>C	Substitution		rs386829301	Homoplasmy
D-loop	m.16178T>C	Substitution		rs1603225705	Homoplasmy	m.16293A>G	Substitution		rs878890610	Homoplasmy
D-loop	m.16179CA-AAA>CAA§	Deletion		rs371240719	Heteroplasmy	m.16296C>T	Substitution		rs879138789	Homoplasmy
D-loop	m.16183A>T	Substitution		rs28671493	Homoplasmy	m.16318A>T	Substitution		rs879067317	Homoplasmy
D-loop	m.16192C>T	Substitution		rs879025248	Homoplasmy	m.16319G>A	Substitution		rs35105996	Homoplasmy
D-loop	m.16201C>T	Substitution		Not provided	Homoplasmy	m.16343A>G	Substitution		rs374065731	Homoplasmy
D-loop	m.16214C>T	Substitution		rs368055283	Homoplasmy	m.16380C>T	Substitution		rs878952395	Homoplasmy
D-loop	m.16217T>C	Substitution		rs35134837	Homoplasmy	m.16381T>C	Substitution		rs1556424876	Homoplasmy
D-loop	m.16219A>G	Substitution		rs878960666	Homoplasmy	m.16526G>A	Substitution		rs386829315	Homoplasmy
D-loop	m.16230A>G	Substitution		rs2853514	Homoplasmy					
D-loop	m.16234C>T	Substitution		rs368259300	Homoplasmy					
D-loop	m.16289A>G	Substitution		rs1603225781	Homoplasmy					

Table II. Continued.

	Patients					Controls				
Locus	Variant	Type of variant	Amino acid change	rs ID	Homoplasmy/ Heteroplasmy	Variant	Variant type	Affected amino acid	rs ID	Homoplasmy/ Heteroplasmy
D-loop	m.16290C>T	Substitution		rs386828866	Homoplasmy					
D-loop	m.16295C>T	Substitution		rs878874012	Homoplasmy					
D-loop	m.16300A>G	Substitution		rs879082592	Homoplasmy					
D-loop	m.16301C>T	Substitution		rs879194775	Homoplasmy					
D-loop	m.16304T>C	Substitution		rs386829305	Homoplasmy					
D-loop	m.16320C>T	Substitution		rs62581338	Homoplasmy					
D-loop	m.16324T>C	Substitution		rs1556424863	Homoplasmy					
D-loop	m.16399A>G	Substitution		rs139001869	Homoplasmy					
rRNA genes										
MT-RNR1 (12sRNA)	m.961T>C	Substitution		rs3888511	Homoplasmy	m.954C>T	Substitution		Not provided	Homoplasmy
MT-RNR1 (12sRNA)	m.1008A>G	Substitution		rs727504505	Homoplasmy	m.980T>C	Substitution		rs397515731	Homoplasmy
MT-RNR1 (12sRNA)	m.1048C>T	Substitution		rs2000974	Homoplasmy	m.1018G>A	Substitution		rs2856982	Homoplasmy
MT-RNR1 (12sRNA)	m.1442G>A	Substitution		rs28358573	Homoplasmy	m.1189T>C	Substitution		rs28358571	Homoplasmy
MT-RNR1 (12sRNA)	m.1598G>A	Substitution		rs3135027	Homoplasmy					
MT-RNR2 (16sRNA)	m.2028G>A	Substitution		rs2124591154	Homoplasmy	m.1733C>T	Substitution		rs878868960	Homoplasmy
MT-RNR2 (16sRNA)	m.2245A>G	Substitution		rs3020600	Homoplasmy	m.1738T>C	Substitution		rs28358574	Homoplasmy
MT-RNR2 (16sRNA)	m.2259C>T	Substitution		rs201336470	Homoplasmy	m.2218C>T	Substitution		rs200813159	Homoplasmy
MT-RNR2 (16sRNA)	m.2283C>T	Substitution		rs200131896	Homoplasmy	m.2380C>T	Substitution		rs1556422622	Homoplasmy
MT-RNR2 (16sRNA)	m.2484C>T	Substitution		rs2124591301	Homoplasmy	m.2768A>G	Substitution		rs3895615	Homoplasmy
MT-RNR2 (16sRNA)	m.2626T>C	Substitution		rs879158835	Homoplasmy	m.2772C>T	Substitution		rs200221487	Homoplasmy

Table II. Continued.

Locus	Patients					Controls				
	Variant	Type of variant	Amino acid change	rs ID	Homoplasmy/ Heteroplasmy	Variant	Variant type	Affected amino acid	rs ID	Homoplasmy/ Heteroplasmy
MT-RNR2 (16sRNA)	m.3221A>G	Substitution		rs1556422691	Homoplasmy	m.2833A>G	Substitution		rs3928312	Homoplasmy
MT-RNR2 (16sRNA)						m.3158A>AT	Insertion		rs1556422679	Homoplasmy
MT-RNR2 (16sRNA)						m.3221A>G	Substitution		rs1556422691	Homoplasmy
tRNA genes										
MT-TV (tRNA)	m.1676A>G	Substitution		rs1603218612	Homoplasmy					
MT-TL1 (tRNA)	m.3387T>C	Substitution		rs1569483877	Homoplasmy	m.3277G>A	Substitution		rs386828902	Homoplasmy
MT-TQ (tRNA)	m.4454T>C	Substitution		rs11510098	Homoplasmy					
MT-TA (tRNA)	m.5603C>T	Substitution		rs369496446	Homoplasmy	m.5655T>C	Substitution		rs1556423019	Homoplasmy
MT-TS1 (tRNA)	m.7476C>T	Substitution		rs201950015	Homoplasmy	m.7474G>C	Substitution		rs2068703713	Heteroplasmy
MT-TS1 (tRNA)	m.7570A>G	Substitution		rs1556423311	Homoplasmy					
MT-TD (tRNA)						m.7581T>C	Substitution		rs201582552	Homoplasmy
MT-TK (tRNA)	m.8292G>A	Substitution		rs1556423422	Homoplasmy					
MT-TG (tRNA)	m.10042A>G	Substitution		rs1603222643	Homoplasmy	m.10034T>C	Substitution		rs41347846	Homoplasmy
MT-TH (tRNA)	m.12171A>G	Substitution		rs1603223589	Homoplasmy					
MT-TH(tRNA)	m.12175T>C	Substitution		rs1057520099	Homoplasmy					
MT-TT (tRNA)	m.15907A>G	Substitution		rs41383248	Homoplasmy					
MT-ND1	m.3516C>A	Silent	p.leu70=	rs2854132	Homoplasmy	m.3438G>A	Silent	p.Gly44=	rs377699338	Homoplasmy
MT-ND1	m.3720A>G	Silent	p.Gln138=	rs41355750	Homoplasmy	m.3546C>A	Silent	p.Thr80=	rs1556422747	Homoplasmy
MT-ND1	m.3834G>A	Silent	p.Leu176=	rs372080842	Homoplasmy	m.3591G>A	Silent	p.Leu95=	rs1556422757	Homoplasmy
MT-ND1	m.3873A>G	Silent	p.Thr189=	rs386828925	Homoplasmy	m.3666G>A	Silent	p.Gly120=	rs28357968	Homoplasmy
MT-ND1	m.4104A>G	Silent	p.Leu266=	rs1117205	Homoplasmy	m.3693G>A	Silent	p.Leu129=	rs193303027	Homoplasmy
MT-ND1	m.4188A>G	Silent	p.Leu294=	Not provided	Homoplasmy	m.3741C>T	Silent	p.Thr145=	rs878907222	Homoplasmy
MT-ND2	m.4688T>C	Silent	p.Ala73=	rs878853056	Homoplasmy	m.4640C>A	Missense	p.Ile57Met	rs387906426	Homoplasmy
MT-ND2	m.4695T>C	Missense	p.Phe76Leu	rs1556422885	Homoplasmy	m.4774T>TG	Insertion		Not provided	Heteroplasmy
MT-ND2	m.4703T>C	Silent	p.Asn78=	rs386828949	Homoplasmy	m.4991G>A	Silent	p.Gln174=	rs386828958	Homoplasmy
MT-ND2	m.4742T>C	Silent	p,Asn91=	rs1553139137	Homoplasmy	m.5036A>G	Silent	p.Trp189=	rs28357982	Homoplasmy
MT-ND2	m.5075T>C	Silent	p,IIe202=	rs1603219767	Homoplasmy	m.5046G>A	Missense	p.Val193Ile	rs878927053	Homoplasmy
MT-ND2	m.5090T>C	Silent	p.IIe207=	Not provided	Homoplasmy	m.5120A>G	Silent	p.Leu217=	rs1603219790	Homoplasmy

Table II. Continued.

	Patients					Controls				
Locus	Variant	Type of variant	Amino acid change	rs ID	Homoplasmy/ Heteroplasmy	Variant	Variant type	Affected amino acid	rs ID	Homoplasmy/ Heteroplasmy
MT-ND2	m.5165C>T	Silent	p.Arg232	rs1556422959	Homoplasmy	m.5333T>C	Silent	p.Leu288=	rs1603219906	Homoplasmy
MT-ND2	m.5300C>T	Silent	p.IIe277=	rs376259646	Homoplasmy	m.5360C>T	Silent	p.IIe297=	rs879217723	Homoplasmy
MT-ND2	m.5390A>G	Silent	p. Met307=	rs41333444	Homoplasmy	m.5393T>C	Silent	p.Ser308=	rs28357987	Homoplasmy
MT-ND2	m.5442T>C	Missense	p.Phe325Leu	rs3020601	Homoplasmy	m.5480A>G	Silent	p.Leu337=	rs1603219977	Homoplasmy
MT-ND2	m.5492T>C	Silent	p.Pro341=	rs377109345	Homoplasmy					
MT-ND2	m.5493T>C	Missense	p.Phe342Leu	rs1603219983	Homoplasmy					
MT-CO1	m.5981T>C	Silent	p.Ala26=	rs1603220211	Homoplasmy	m.6026G>A	Silent	p.Leu41=	rs879112886	Homoplasmy
MT-CO1	m.6045C>T	Silent	p.Leu48=	rs879061193	Homoplasmy	m.6216T>C	Silent	p.Leu105=	rs367837524	Homoplasmy
MT-CO1	m.6179G>A	Silent	p.Met92=	rs374303341	Homoplasmy	m.6261G>A	Missense	p.Ala120Thr	rs201262114	Homoplasmy
MT-CO1	m.6185T>C	Silent	p.Phe94=	rs1029272	Homoplasmy	m.6446G>A	Silent	p.Thr181=	rs386420010	Homoplasmy
MT-CO1	m.6257G>A	Silent	p.Val118=	rs2856983	Homoplasmy	m.6521C>T	Silent	p.IIe 206=	Not provided	Homoplasmy
MT-CO1	m.6366G>A	Missense	p.Val155Ile	rs370673798	Homoplasmy	m.6548C>T	Silent	p.Leu215=	rs28358870	Homoplasmy
MT-CO1	m.6497T>C	Silent	p.Ser198=	rs1556423143	Homoplasmy	m.6680T>C	Silent	p.Thr259=	rs41352249	Homoplasmy
MT-CO1	m.6515T>C	Silent	p.Ala204=	rs878998677	Homoplasmy	m.6989A>G	Silent	p.Ser362=	rs1978001	Homoplasmy
MT-CO1	m.6546C>T	Missense	p.Leu215Phe	rs1603220531	Homoplasmy	m.7325A>G	Silent	p.Glu474=	rs1556423269	Homoplasmy
MT-CO1	m.6599A>G	Silent	p.Gln232	rs879012660	Homoplasmy					
MT-CO1	m.6962G>A	Silent	p.Leu353=	rs1970771	Homoplasmy					
MT-CO1	m.7028C>T	Silent	p.Ala375=	rs2015062	Homoplasmy					
MT-CO1	m.7193T>C	Silent	p.Phe430=	rs1603220829	Homoplasmy					
MT-CO2	m.7861T>C	Silent	p.Asp92=	rs368623956	Homoplasmy	m.7673A>G	Missense	p.Ile30Val	rs1569484167	Homoplasmy
MT-CO2	m.8014A>T	Silent	p.Val143=	rs879223416	Homoplasmy	m.7711T>C	Silent	p.Leu42=	rs372012410	Homoplasmy
MT-CO2	m.8053A>G	Silent	p.Ser156=	rs56041322	Homoplasmy	m.7867C>T	Silent	p.Ser94=	rs9783079	Homoplasmy
MT-CO2	m.8179A>G	Silent	p.Glu198=	rs1603221317	Homoplasmy	m.8137C>T	Silent	p.Phe148=	rs879043235	Homoplasmy
MT-CO2						m.8155G>A	Silent	p.Gly190=	rs374052533	Homoplasmy
MT-ATP8	m.8386C>T	Silent	p.Thr7=	rs1603221443	Homoplasmy					
MT-ATP8	m.8428C>T	Silent	p.Phe21=	rs1116905	Homoplasmy					
MT-ATP8	m.8460A>G	Missense	p.Asn32Ser	rs1116906	Homoplasmy					
MT-ATP8	m.8472T>C	Silent	p.Pro36=	rs386829037	Homoplasmy					
MT-ATP8	m.8554A>G	Missense	p.Ile10Val	rs1603221583	Homoplasmy					
MT-ATP6	m.8618T>C	Missense	p.Ile31Thr	rs28358885	Homoplasmy	m.8655C>T	Silent	p.IIe43=	rs2853822	Homoplasmy
MT-ATP6	m.8705T>C	Missense	:p.Met60Thr	rs878959404	Homoplasmy	m.8684C>T	Missense	p.Thr53Ile	rs201336180	Homoplasmy
MT-ATP6	m.8860A>G	Missense	p.Thr112Ala	rs2001031	Homoplasmy	m.8978T>C	Missense	p.Ile151Thr	rs1603221954	Homoplasmy
MT-ATP6	m.8958C>T	Silent	p.IIe144=	rs1603221942	Homoplasmy	m.9157G>A	Missense	p.Ala211Thr	rs1556423625	Homoplasmy

Table II. Continued.

	Patients					Controls				
Locus	Variant	Type of variant	Amino acid change	rs ID	Homoplasmy/ Heteroplasmy	Variant	Variant type	Affected amino acid	rs ID	Homoplasmy/ Heteroplasmy
MT-ATP6	m.9007A>G	Missense	p.Thr161Ala	rs1603221973	Homoplasmy					
MT-ATP6	m.9042C>T	Silent	p.His172=	rs3020605	Homoplasmy					
MT-ATP6	m.9103T>C	Missense	p.Thr161Ala	rs1603222077	Homoplasmy					
MT-CO3	m.9336A>G	Missense	p.Met44Leu	rs28474779	Homoplasmy	m.9302C>T	Silent	p.Ala32=	rs878986141	Homoplasmy
MT-CO3	m.9347A>G	Silent	p.Leu47=	rs2853824	Homoplasmy	m.9656T>C	Silent	p.Ser150=	rs1556423706	Homoplasmy
MT-CO3	m.9494A>G	Silent	p.Gly96=	rs1556423680	Homoplasmy	m.9899T>C	Silent	p.His231=	rs41345446	Homoplasmy
MT-CO3	m.9509T>C	Silent	p.Phe101=	rs375478739	Homoplasmy	m.9956A>G	Silent	p.Leu250=	rs1603222594	Homoplasmy
MT-CO3	m.9545A>G	Silent	p.Gly113=	rs878853022	Homoplasmy					
MT-CO3	m.9575G>A	Silent	p.Pro123=	rs372078920	Homoplasmy					
MT-CO3	m.9755G>A	Silent	p.Glu183=	rs2856985	Homoplasmy					
MT-CO3	m.9776C>T	Silent	p.Asp190=	Not provided	Homoplasmy					
MT-CO3	m.9818C>T	Silent	p.His204	rs2854139	Homoplasmy					
MT-CO3	m.9948G>A	Missense	p.Val248Ile	rs1556423747	Homoplasmy					
MT-ND3	m.10101T>C	Silent	p.Leu15=	rs1603222669	Homoplasmy	m.10084T>C	Missense	p.Ile9Thr	rs41487950	Homoplasmy
MT-ND3	m.10143G>A	Missense	p.Gly29Ser	rs202131419	Homoplasmy	m.10142C>T	Silent	p.Asn28=	rs878969753	Homoplasmy
MT-ND3	m.10184C>T	Silent	p.Asp42=	Not provided	Homoplasmy	m.10238T>C	Silent	p.Ile60=	rs193302927	Homoplasmy
MT-ND3	m.10275T>C	Silent	p.Leu73=	rs373277477	Homoplasmy	m.10289A>G	Silent	p.Trp77=	rs1556423796	Homoplasmy
MT-ND4L	m.10499A>G	Silent	p.Leu10=	rs1057520074	Homoplasmy	m.10550A>G	Silent	p.Met27=	rs28358280	Homoplasmy
MT-ND4L	m.10556C>T	Silent	p.Ser29=	rs1603222890	Homoplasmy	m.10586G>A	Silent	p.Ser39=	rs28358281	Homoplasmy
MT-ND4L	m.10589G>A	Silent	p.Leu40=	rs2853487	Homoplasmy					
MT-ND4L	m.10628C>T	Silent	p. Ser53=	Not provided	Homoplasmy					
MT-ND4L	m.10632T>C	Silent	p.Leu55=	rs878888873	Homoplasmy					
MT-ND4L	m.10664C>T	Silent	p.Val65=	rs193302933	Homoplasmy					
MT-ND4	m.10819A>G	Silent	p.Lys20=	rs28358283	Homoplasmy	m.10822C>T	Silent	p.His21=	rs879041592	Homoplasmy
MT-ND4	m.10876A>G	Silent	p.Leu39=	rs879036391	Homoplasmy	m.11299T>C	Silent	p.Thr180=	rs28358285	Homoplasmy
MT-ND4	m.10915T>C	Missense	p.Cys52Trp	rs2857285	Homoplasmy	m.11476C>T	Silent	p.Gly239=	rs386829131	Homoplasmy
MT-ND4	m.11002A>G	Silent	p.Gln81=	rs386829114	Homoplasmy					
MT-ND4	m.11016G>A	Missense	p.Ser86Thr	rs28594904	Homoplasmy					
MT-ND4	m.11050T>C	Silent	p.Ser97=	rs1603223077	Homoplasmy					
MT-ND4	m.11143C>T	Silent	p.Pro128=	rs1556423898	Homoplasmy					
MT-ND4	m.11172A>G	Missense	p.Asn138Ser	rs2853489	Homoplasmy					
MT-ND4	m.11176G>A	Silent	p.Gln139=	rs2853490	Homoplasmy					
MT-ND4	m.11287T>C	Silent	p.Ile176=	rs386829125	Homoplasmy					

Table II. Continued.

	Patients					Controls				
Locus	Variant	Type of variant	Amino acid change	rs ID	Homoplasmy/ Heteroplasmy	Variant	Variant type	Affected amino acid	rs ID	Homoplasmy/ Heteroplasmy
MT-ND4	m.11377G>A	Silent	p.Lys206=	rs193302938	Homoplasmy					
MT-ND4	m.11440G>A	Silent	p.Gly227=	rs386829130	Homoplasmy					
MT-ND4	m.11590A>G	Silent	p.Leu277=	rs370318850	Homoplasmy					
MT-ND4	m.11641A>G	Silent	p.Met294=	rs2853494	Homoplasmy					
MT-ND4	m.11776T>C	Silent	p.Ser339=	rs28396842	Homoplasmy					
MT-ND4	m.11935T>C	Silent	p.Thr392=	rs1603223480	Homoplasmy					
MT-ND4	m.12007G>A	Silent	p.Trp416=	rs2853497	Homoplasmy					
MT-ND4	m.12061C>T	Silent	p.Asn434=	rs1556424043	Homoplasmy					
MT-ND5	m.12570A>G	Silent	p.Leu78=	rs1603223816	Homoplasmy	m.12403C>T	Missense	p.Leu23Phe	rs879096684	Homoplasmy
MT-ND5	m.12720A>G	Silent	p.Met128=	rs2853500	Homoplasmy	m.12501G>A	Silent	p.Met55=	rs28397767	Homoplasmy
MT-ND5	m.12771G>A	Silent	p.Glu145=	rs878865822	Homoplasmy	m.12633C>A	Silent	p.Ser99=	rs3926883	Homoplasmy
MT-ND5	m.12876C>T	Silent	p.IIe180=	rs1603223952	Homoplasmy	m.12879T>C	Silent	p.Gly181=	rs1556424182	Homoplasmy
MT-ND5	m.13020T>C	Silent	p.Gly228=	rs75577869	Homoplasmy	m.12950A>C	Missense	p.Asn205Thr	rs201361958	Homoplasmy
MT-ND5	m.13116C>T	Silent	p.Leu260=	rs1603224049	Homoplasmy	m.13104A>G	Silent	p.Gly256=	rs878871104	Homoplasmy
MT-ND5	m.13158A>G	Silent	p.Gln274=	rs1556424229	Homoplasmy	m.13422A>G	Silent	p.Leu362=	rs386829180	Homoplasmy
MT-ND5	m.13276A>G	Missense	p.Met314Leu	rs2853502	Homoplasmy	m.13500T>C	Silent	p.Gly388=	rs879066842	Homoplasmy
MT-ND5	m.13419A>G	Silent	p.Gly361=	rs1603224182	Homoplasmy	m.13530C>T	Silent	p.Thr398=	rs2068736572	Homoplasmy
MT-ND5	m.13734T>C	Silent	p.Phe466=	rs41421644	Homoplasmy	m.13780A>G	Missense	p.Ile482Val	rs41358152	Homoplasmy
MT-ND5	m.13759G>A	Missense	p.Ala475Thr	rs386420024	Homoplasmy	m.13789T>C	Missense	p.Tyr485His	rs28359179	Homoplasmy
MT-ND5	m.13813G>A	Missense	p.Val493Ile	rs1556424332	Homoplasmy	m.13880C>A	Missense	p.Ser515Tyr	rs28359181	Homoplasmy
MT-ND5	m.13886T>C	Missense	p.Leu517Pro	rs28359182	Homoplasmy	m.14070A>G	Silent	p.Ser578=	rs879201732	Homoplasmy
MT-ND5	m.13967C>T	Missense	p.Thr544Met	rs386829197	Homoplasmy	m.14110T>C	Missense	p.Phe592Leu	rs371451099	Homoplasmy
MT-ND5	m.14053A>G	Missense	p.Thr573Ala	rs200134839	Homoplasmy	m.14139A>G	Silent	p.Leu601=	rs878918283	Homoplasmy
MT-ND6	m.14212T>C	Silent	p.Val154=	rs28357672	Homoplasmy	m.14178T>C	Missense	p.Ile166Val	rs28357671	Homoplasmy
MT-ND6	m.14284C>T	Silent	p.Glu130=	rs28357673	Homoplasmy	m.14203A>G	Silent	p.Gly157=	rs1569484633	Homoplasmy
MT-ND6	m.14308T>C	Silent	p.Gly122=	rs28357674	Homoplasmy	m.14287T>C	Silent	p.Gly129=	rs1603224652	Homoplasmy
MT-ND6	m.14494T>C	Silent	p.Leu60=	rs879250748	Homoplasmy	m.14364G>A	Silent	p Leu104=	rs879086798	Homoplasmy
MT-ND6	m.14562C>T	Missense	p.Val38Ile	rs1603224791	Homoplasmy	m.14497A>G	Silent	p.Tyr59=	rs1556424454	Homoplasmy
MT-ND6	m.14587A>G	Silent	p.Gly29=	rs1556424469	Homoplasmy	m.14560G>A	Silent	p.Val38=	rs28357676	Homoplasmy
MT-ND6	m.14634T>C	Missense	p.Met14Val	rs1603224816	Homoplasmy					
MT-CYB	m.14755A>G	Silent	p.Pro3=	rs1603224856	Homoplasmy	m.14769A>G	Missense	p.Asn8Ser	rs28357679	Homoplasmy
MT-CYB	m.14839A>G	Silent	p.Trp31=	rs1603224921	Homoplasmy	m.14774C>T	Silent	p.Leu10=	rs1556424490	Homoplasmy
MT-CYB	m.14862C>T	Missense	p.Ala39Val	rs1603224933	Homoplasmy	m.15077G>A	Missense	p.Glu111Lys	rs201943501	Homoplasmy

Table II. Continued.

	Patients					Controls				
Locus	Variant	Type of variant	Amino acid change	rs ID	Homoplasmy/ Heteroplasmy	Variant	Variant type	Affected amino acid	rs ID	Homoplasmy/ Heteroplasmy
MT-CYB	m.14872C>T	Silent	p.Ile42=	rs878879194	Homoplasmy	m.15115T>C	Silent	p.Thr123=	rs879035822	Homoplasmy
MT-CYB	m.15136C>T	Silent	p.Gly130=	rs2854124	Homoplasmy	m.15217G>A	Silent	p.Gly157=	rs193302989	Homoplasmy
MT-CYB	m.15172G>T	Silent	p.Gly142=	rs367572771	Homoplasmy	m.15454T>C	Silent	p.Leu236=	rs879015290	Homoplasmy
MT-CYB	m.15218A>G	Missense	p.Thr158Ala	rs2853506	Homoplasmy	m.15865A>G	Silent	p.Glu373=	rs879154157	Homoplasmy
MT-CYB	m.15257G>A	Missense	p.Asp171Asn	rs41518645	Homoplasmy					
MT-CYB	m.15314G>A	Missense	p.Ala190Thr	rs527236176	Homoplasmy					
MT-CYB	m.15403C>T	Silent	p.Thr219=	rs1603225258	Homoplasmy					
MT-CYB	m.15431G>A	Missense	p.Ala229Thr	rs193302993	Homoplasmy					
MT-CYB	m.15468C>T	Missense	p.Thr241Met	rs1603225301	Homoplasmy					
MT-CYB	m.15490C>T	Silent	p.Asp248=	rs1603225311	Homoplasmy					
MT-CYB	m.15530T>C	Silent	p.Leu262=	rs1556424600	Homoplasmy					
MT-CYB	m.15646C>T	Silent	p.Ile300=	rs879113411	Homoplasmy					
MT-CYB	m.15679A>G	Silent	p.Lys311=	rs1603225420	Homoplasmy					
MT-CYB	m.15735C>T	Missense	p.Ala330Val	rs1603225446	Homoplasmy					
MT-CYB	m.15799A>G	Silent	p.Gly351=	rs1603225506	Homoplasmy					

Table III. mtDNA variants with amino acid substitutions in patients with psoriatic arthritis and their impact on protein function and structure.

Gene	Variant	Variant type	Amino acid change	rs ID	MAF	Prediction		
						CADD/score	Condel/ score	PROVEAN/score
MT-ND2	m.4695T>C	Missense	p.Phe76Leu	rs1556422885	0.0003	Neutral/5.35	Neutral/0.07	Neutral/1,2
MT-ND2	m.5442T>C	Missense	p.Phe325Leu	rs3020601	0.00478	Neutral/0.03	Neutral/0.28	Neutral/0.7
MT-ND2	m.5493T>C	Missense	p.Phe342Leu	rs1603219983	0.007	Neutral/12.34	Neutral/0.31	Neutral/0.52
MT-CO1	m.6366G>A	Missense	p.Val155Ile	rs370673798	0.0018	Neutral/0.01	Deleterious/1	Neutral/-1.1
MT-CO1	m.6546C>T	Missense	p.Leu215Phe	rs1603220531	0.0002	Neutral/6.11	Deleterious/0.84	Neutral/-0.45
MT-ATP8	m.8460A>G	Missense	p.Asn32Ser	rs1116906	0.00094	Neutral/12	Neutral/0.27	Neutral/-1.6
MT-ATP8	m.8554A>G	Missense	p.Ile10Val	rs1603221583	0.0001	Neutral/7.68	Deleterious/0.54	Neutral/0.18
MT-ATP6	m.8618T>C	Missense	p.Ile31Thr	rs28358885	0.009	Neutral/5.55	Deleterious/0.5	Neutral/2
MT-ATP6	m.8705T>C	Missense	p.Met60Thr	rs878959404	0.0043	Neutral/0.5	Deleterious/0.75	Neutral/0.32
MT-ATP6	m.8860A>G	Missense	p.Thr112Ala	rs2001031	0.99	Neutral/6.13	Deleterious/0.81	Deleterious/-3.9
MT-ATP6	m.9007A>G	Missense	p.Thr161Ala	rs1603221973	0.0058	Deleterious/23	Neutral/0.06	Deleterious/-3.5
MT-ATP6	m.9103T>C	Missense	p.Thr161Ala	rs1603222077	0.00045	Neutral/9.74	Deleterious/1	Deleterious/-2.5
MT-CO3	m.9336A>G	Missense	p.Met44Leu	rs28474779	0.0004	Neutral/0.01	Deleterious/0.68	Neutral/-0.32
MT-CO3	m.9948G>A	Missense	p.Val248Ile	rs1556423747	0.0012	Neutral/9.11	Deleterious/0.65	Neutral/-0.84
MT-ND3	m.10143G>A	Missense	p.Gly29Ser	rs202131419	0.0015	Neutral/5.51	Deleterious/0.96	Neutral/1.9
MT-ND4	m.10915T>C	Missense	p.Cys52Trp	rs2857285	0.02	Neutral/16.8	Neutral/0.36	Neutral/0.37
MT-ND4	m.11016G>A	Missense	p.Ser86Thr	rs28594904	0.004	Neutral/0.11	Deleterious/0.68	Neutral/0.21
MT-ND4	m.11172A>G	Missense	p.Asn138Ser	rs2853489	0.0007	Deleterious/20	Deleterious/0.69	Deleterious/-3.5
MT-ND5	m.13276A>G	Missense	p.Met314Leu	rs2853502	0.00169	Neutral/14	Neutral/0.12	Neutral/0.2
MT-ND5	m.13759G>A	Missense	p.Ala475Thr	rs386420024	0.0143	Neutral/0.69	Deleterious/0.73	Neutral /1.6
MT-ND5	m.13813G>A	Missense	p.Val493Ile	rs1556424332	0.0013	Neutral/6.86	Deleterious/0.74	Neutral/-0.37
MT-ND5	m.13886T>C	Missense	p.Leu517Pro	rs28359182	0.0018	Neutral/9.6	Deleterious/0.6	Neutral/0.23
MT-ND5	m.13967 C>T	Missense	p.Thr544Met	rs386829197	0.0011	Neutral/1.23	Deleterious/0.61	Neutral/0.85
MT-ND5	m.14053 A>G	Missense	p.Thr573Ala	rs200134839	0.002	Neutral/0.17	Deleterious/0.76	Neutral/1.8
MT-ND6	m.14562 C>T	Missense	p.Val38Ile	rs1603224791	0.0002	Neutral/1.7	Deleterious/0.8	Neutral/0.06
MT-ND6	m.14634 T>C	Missense	p.Met14Val	rs1603224816	0.0011	Neutral/12	Neutral/0.17	Neutral/0.001
MT-CYB	m.14862 C>T	Missense	p. Ala39Val	rs1603224933	0.0007	Neutral/17.5	Deleterious/0.65	Neutral/1.4
MT-CYB	m.15218 A>G	Missense	p.Thr158Ala	rs2853506	0.03	Neutral/13.3	Neutral/0.29	Neutral/-1.6
MT-CYB	m.15257 G>A	Missense	p.Asp171Asn	rs41518645	0.01	Deleterious/23.5	Deleterious/0.67	Deleterious/-3.5
MT-CYB	m.15314 G>A	Missense	p.Ala190Thr	rs527236176	0.002	Neutral/16.4	Deleterious/0.61	Neutral/-1.6
MT-CYB	m.15431 G>A	Missense	p.Ala229Thr	rs193302993	0.002	Deleterious/24.4	Deleterious/0.61	Neutral/-0.22
MT-CYB	m.15468 C>T	Missense	p.Thr241Met	rs1603225301	0.0004	Deleterious/20.8	Neutral/0.41	Neutral/-0.81
MT-CYB	m.15735 C>T	Missense	p.Ala330Val	rs1603225446	0.0001	Deleterious/22	Deleterious/0.78	Neutral/-1.7

Table IV. Deleterious mtDNA variants in patients with psoriatic arthritis and their impact on protein stability.

Gene	Variant	Variant type	Amino acid change	rs ID	SDM	
					DDG	Stability outcome
MT-CO1	m.6366G>A	Missense	p.Val155Ile	rs370673798	0.68	Increased
MT-CO1	m.6546C>T	Missense	p.Leu215Phe	rs1603220531	-0.09	Decreased
MT-ATP8	m.8554A>G	Missense	p.Ile10Val	rs1603221583	NP	NP
MT-ATP6	m.8618T>C	Missense	p.Ile31Thr	rs28358885	NP	NP
MT-ATP6	m.8705T>C	Missense	p.Met60Thr	rs878959404	NP	NP
MT-ATP6	m.8860A>G	Missense	p.Thr112Ala	rs2001031	NP	NP
MT-ATP6	m.9007A>G	Missense	p.Thr161Ala	rs1603221973	NP	NP
MT-ATP6	m.9103T>C	Missense	p.Thr161Ala	rs1603222077	NP	NP
MT-CO3	m.9336A>G	Missense	p.Met44Leu	rs28474779	-0.44	Decreased
MT-CO3	m.9948G>A	Missense	p.Val248Ile	rs1556423747	-0.94	Decreased
MT-ND3	m.10143G>A	Missense	p.Gly29Ser	rs202131419	-0.41	Decreased
MT-ND4	m.11016G>A	Missense	p.Ser86Thr	rs28594904	-0.69	Decreased
MT-ND4	m.11172A>G	Missense	p.Asn138Ser	rs2853489	-0.64	Decreased
MT-ND5	m.13759G>A	Missense	p.Ala475Thr	rs386420024	-0.66	Decreased
MT-ND5	m.13813G>A	Missense	p.Val493Ile	rs1556424332	-0.38	Decreased
MT-ND5	m.13886T>C	Missense	p.Leu517Pro	rs28359182	-1.5	Decreased
MT-ND5	m.13967C>T	Missense	p.Thr544Met	rs386829197	0.014	Increased
MT-ND5	m.14053A>G	Missense	p.Thr573Ala	rs200134839	1.53	Increased
MT-ND6	m.14562C>T	Missense	p.Val38Ile	rs1603224791	0.35	Increased
MT-CYB	m.14862C>T	Missense	p.Ala39Val	rs1603224933	-0.409	Decreased
MT-CYB	m.15257G>A	Missense	p.Asp171Asn	rs41518645	0.39	Increased
MT-CYB	m.15314G>A	Missense	p.Ala190Thr	rs527236176	-1.53	Decreased
MT-CYB	m.15431G>A	Missense	p.Ala229Thr	rs193302993	-2.05	Decreased
MT-CYB	m.15468C>T	Missense	p.Thr241Met	rs1603225301	1.19	Increased
MT-CYB	m.15735C>T	Missense	p.Ala330Val	rs1603225446	-0.84	Decreased

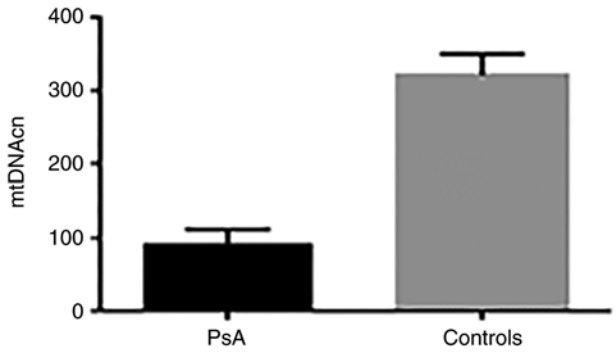

Figure 2. Leukocyte mitochondrial DNAcopy number in of patients with psoriatic arthritis and controls.

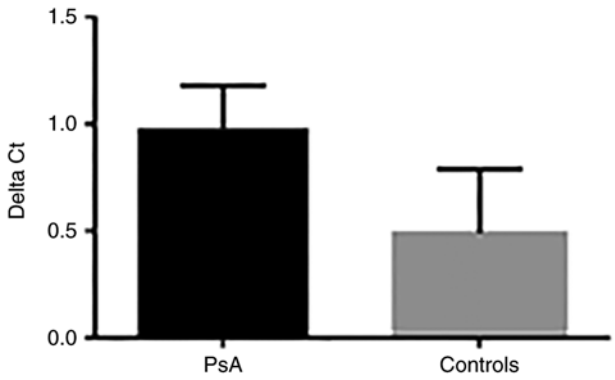

Figure 3. Mitochondrial DNA oxidative damage in patients with psoriatic arthritis and controls.

In addition, 18 other variants were predicted to be deleterious by one of the *in-silico* algorithms tools. These included 2 variants in the *MT-CO1* gene, 1 variant in the *MT-ATP8* gene, 2 variants in the *MT-ATP6* gene, 2 variants in the *MT-CO3*, 1 variant in the *MT-ND3* gene, 1 variant in the *MT-ND4* gene, 5 variants in the *MT-ND5* gene, 1 variant in the *MT-ND6* gene and 3 variants in the *MT-CYB* gene. The majority of these variants also had low allelic frequencies (<0.01) in the gnome AD database.

In subsequent analysis, the deleterious mtDNA variants were further evaluated for their effect on protein stability using SDM. In SMD, a stability score is calculated using environment-specific amino acid substitution frequencies within the family of homologous proteins of known 3-D structures (30). All variants in mtDNA-encoded genes of complexes I, III and IV were examined at the level of protein stability. However, the effect of variants in the *MT-ATP6* and *MT-ATP8* genes

of complex V could not be demonstrated as the human 3-D structures were not available for complex V proteins.

As shown in Table IV, SDM predicted 19 destabilizing variants in mtDNA-encoded genes of complexes I, III and IV. Of them, 13 variants were predicted to decrease the stability of encoded proteins, including 1 variant in the *MT-CO1* gene (m.6546C>T), 2 variants in the *MT-CO3* gene (m.9336A>G and m.9948G>A), 1 variant in the *MT-ND3* gene (m.10143G>A), 2 variants in the *MT-ND4* gene (m.11016G>A and m.11172A>G), 3 variants in the *MT-ND5* gene (m.13759G>A, m.13813G>A and m.13886T>C), and 4 variants in the *MT-CYB* gene (m.14862C>T, m.15314G>A, m.15431G>A, and m.15735C>T). Furthermore, 6 variants were found to increase the stability of encoded proteins: 1 variant in the *MT-CO1* gene (m.6366G>A), 2 variants in the *MT-ND5* gene (m.13967C>T and m.14053A>G), 1 variant in the *MT-ND6* gene (m.14562C>T) and 2 variants in the *MT-CYB* gene (m.15257G>A and m.15468C>T).

Relative leukocyte mtDNAcn. Using qPCR, the relative mtDNAcn was determined in the leukocytes of patients with PsA (n=23) and healthy controls (n=20). The results showed a 3.44-fold reduction in mtDNAcn in patients with PsA compared with controls. The mean ± SD mtDNAcn was 93.3±10 in patients vs. 321±29 in controls (P=0.0001) (Fig. 2).

mtDNA oxidative damage. mtDNA oxidative damage was assessed using qPCR by measuring the 8-OHdG content in patients with PsA and controls. The ΔCq value of the difference between the Cq value of DNA samples treated with FPG and the Cq value of DNA samples without FPG treatment was calculated. The results showed a 2-fold increase in the level of mtDNA oxidative damage in patients with PsA compared with controls. The ΔCq value was 0.98±0.29 in patients vs. 0.49±0.3 in controls (P=0.03) (Fig. 3).

Discussion

PsA is a chronic inflammatory disease, which presents in a significant number of individuals with psoriasis (1-3). PsA is widely regarded as a multi-factorial disease with underlying autoimmune mechanisms including infiltration of plasma cells and mononuclear cells that are observed in both psoriatic plaque and PsA articular space (7). Mitochondrial dysfunction plays an important role in the pathogenesis of PsA by modulating innate immunity via redox-sensitive inflammatory pathways or directly through activation of the inflammatory response (9,10). An imbalance in oxidant-antioxidant mitochondrial system results in oxidative stress, which can induce mtDNA variations and copy number changes leading to mitochondrial functional impairment (17,18). Currently, there is limited knowledge on whether abnormalities in mtDNA a possible factor could be involved in PsA.

The present study sequenced the entire mitochondrial genome using NGS, a high-throughput and sensitive method with the ability to detect any variants (22), investigated changes in mtDNAcn and evaluated mtDNA oxidative damage in patients with PsA and healthy controls.

Analysis of the entire mitochondrial genome revealed a total of 435 variants, distributed across all regions of the mtDNA. These included 187 (43%) variants exclusively found in patients with PsA, and 122 (28%) only present in control individuals. A higher number of variants were observed in the D-loop region in both patients and controls, whereas a higher number of variants in mtDNA-coding genes was found in patients compared with controls, particularly in the MT-ND4, MT-ND5, and MT-CYB genes. In addition, common mtDNA variants (126, 28.9%) were identified among patients and controls. The frequency of two specific variants differed significantly (P<0.05) between the two groups and may be linked with the susceptibility to PsA. Namely, the D-loop m.152T>C variant occurred in 26% of patients and 55% of controls (OR=0.3, 95% CI=0.1-0.5, P=0.02) and may confer a protective role against PsA. The D-loop (non-coding or control region) contains essential regulatory sequences for replication and transcription (34). Although the majority of harmful variants are removed by natural selection, some of these variants are introduced in certain populations and may influence the risk of developing certain disorders (35), whereas other variants such as m.152T>C may be selectively beneficial on some genetic backgrounds. Additionally, the silent m.15301G>A variant in the MT-CYB gene occurred in 30% of patients and 10% of controls (OR=3.8, 95% CI=1-8, P=0.04) and may be associated with the risk of developing PsA. Synonymous variants in protein-coding genes are generally considered to be silent with no effect on protein function. However, previous studies have shown that silent variants can significantly alter gene expression by affecting the stability and folding of mRNA (36,37), and can also influence the rate of translation and posttranslational modification of proteins (38).

mtDNA variants that cause changes in amino acid sequences of protein-coding genes have been implicated in the pathogenicity of numerous diseases such as rheumatoid arthritis (RA) (39). In the present study, analysis of mtDNA in patients with PsA only revealed 33 missense variants in mtDNA-encoded genes of the ETC. In total, 25 of these variants were predicted to have deleterious effects on encoded proteins by 1-3 bioinformatics tools (CADD, Condel and PROVEAN).

Specifically, 2 missense transition variants in *MT-ND4* gene and *MT-CYB* gene were predicted to have the highest impact on protein function and structure by all three *in silico* algorithms tools. Moreover, 5 mtDNA variants were predicted to be deleterious by two *in silico* algorithms tools, including 3 variants in the *MT-ATP6* gene and 2 variants in the *MT-CYB* gene. A total of 18 other variants in all mtDNA-encoded complexes of the ETC were predicted to be deleterious by one of the *in silico* algorithms tools. The majority of these variants are considered rare with low allelic frequencies <0.01 in the gnome AD database.

Protein function and stability are closely related to protein structure. Variants that cause amino acid alterations can markedly change protein structure (30,40). Particularly, the unique amino acid sequence of a protein is reflected in its folded structure, which is important to perform proper biological function. Changes in the hydration status of a protein also affect protein folding (41). Hydrophobic interactions are important for protein folding, and changes in hydrophobicity can lead to a collapse of the protein chain in an aqueous environment (42). The polarity of amino acids also promotes

appropriate folding by interacting with the water solvent, thus affecting protein stability (41). All the identified deleterious missense variants in the current study produce amino acids that are different from the usual amino acids at that position and alter the function of proteins. For instance, the MT-ND4 m.11172A>G variant causes changes in amino acid from Asparagine to Serine (p.Asn138Ser), while the MT-CYB m.15257G>A variant causes changes in amino acid from Asparagine to Aspartic acid (p.Asp171Asn). Asparagine is a hydrophilic uncharged polar amino acid, whereas serine is a neutral uncharged polar amino acid, and aspartic acid is a hydrophilic negatively charged polar amino acid.

Previous studies have demonstrated that protein flexibility is a key factor for its catalytic activity (43), and increased stability of thermophilic proteins has been shown to be associated with loss of protein flexibility and reduced enzymatic activity at low temperatures (44,45). Furthermore, high stability of proteins leads to increased proteolytic resistance, which make them difficult to regulate, particularly during cell signalling (46). It has been also shown that variants causing increased protein stability can lead to protein malfunction in human diseases, such as the stabilizing homozygous variant S37A in patients with parathyroid adenomas (47) and the Parkinson disease-associated A30P stabilizing variant in human neuroblastoma cells (48).

Analysis of the impact of deleterious variants on protein stability in present study revealed 19 destabilizing variants in mtDNA-encoded genes of complexes I, III and IV. Of them, 13 variants in different mtDNA-encoded complexes of the ETC were predicted to decrease protein stability. Moreover, 6 variants were found to increase the stability of encoded proteins. These variants may affect the stabilizing interaction within folded proteins, leading to protein instability and malfunction.

The mitochondrial OXPHOS system comprises four multi-enzymatic respiratory complexes (namely I-IV) and ATP synthase and is embedded in the inner mitochondrial membrane. A total of 4 of these complexes (I, III, IV and V) are encoded by both nDNA and mtDNA genes. Complex I (NADH: ubiquinone oxidoreductase) is one of the main contributors to ROS production within the mitochondrial matrix, which is a major cause of cellular oxidative stress and is associated with neuromuscular diseases and aging (49-52). mtDNA-encoded genes of complex I are hotspots for pathological variants (19). Such variants affect complex I assembly and activity leading to complex I deficiency with increased ROS production (51-55). Particularly, variants in mitochondrial complex I genes have been previously reported to be associated with severe erosive arthritis and to be implicated in the pathogenesis of RA (39). Complex III (bc1 complex; ubiquinol cytochrome *c* reductase) has been also identified as a main producer of superoxide within the mitochondrial respiratory chain (56,57). Previous studies have revealed that deleterious variants in the MT-CYB gene caused isolated complex III deficiency, leading to a variety of human diseases such as cardiomyopathy, encephalomyopathy, and Leber hereditary optic neuropathy (58-61). Complex IV or cytochrome *c* oxidase (COX) is one of the major regulation sites for OXPHOS and deficiency in the activity of COX has been linked to a variety of diseases (62). ATP6 and ATP8 are mtDNA-encoded subunits of the ATP synthase of complex V, which utilizes the energy provided by the proton electrochemical gradient across the inner membrane during OXPHOS and synthesizes ATP from ADP (63). Variants in MT-ATP8 gene can disturb the stability and function of complex V, affecting ATP production (64). In a previous study by Du *et al* (39), a higher rate of mtDNA variants in complex V was found in patients with RA compared with controls, suggesting that these variants may be associated with susceptibility to RA. Variants in complex V were also linked to ROS production and apoptosis pathways, and associated with RA progression (39).

Integrity of mtDNA which encodes essential proteins of the ETC subunits is mandatory for normal mitochondrial function (13). Improper function of ETC can enhance ROS production, which is implicated in psoriatic inflammation and PsA (9,65). Improper function of ETC can enhance ROS production, which is implicated in psoriatic inflammation and PsA (9,65). The deleterious mtDNA variants in patients with PsA identified in the present study were not detected in control individuals; were located in functionally/structurally important sites of mtDNA-encoded subunits of the ETC; and resulted in protein instability. Thus, these variants may be disease-related and could play a role in the pathogenicity of PsA.

There are several copies per cell of mtDNA, resulting in both homoplasmy (identical mtDNA) and heteroplasmy (mixture of mutated and wild-type mtDNA). In the present study, a higher rate of homoplasmic variants was detected compared with heteroplasmic variants. While most pathogenic mtDNA variants are heteroplasmic, the clinical expression of diseases is determined by the level of heteroplasmy. In this context, mitochondrial dysfunction becomes clinically apparent when the percentage of mutant mtDNA exceeds a certain threshold level. Instead, some mtDNA diseases such as Leber hereditary optic neuropathy are caused by homoplasmic variants (66). Pathogenic homoplasmic variants have been also reported in diseases such as Leigh syndrome (67) and multiple sclerosis (68), and may increase the risk of type 2 diabetes (69) and neurodegenerative diseases (70). Importantly, the phenotypic expression of homoplasmic variants can be tissue-specific, suggesting that incomplete penetrance and unidentified nuclear genetic and/or environmental factors are likely to contribute to the disease phenotype (66).

The present results also revealed a significant reduction in mtDNAcn in leukocytes of patients with PsA compared with healthy controls. mtDNAcn is an important indicator of mitochondrial biogenesis and function (15). Consequently, changes in mtDNA content could contribute to various pathological conditions (20). Low mtDNAcn was previously reported in numerous diseases such as OA (71). In our previous study, decreased mtDNAcn in patients with psoriasis was found compared with controls (24). Decreased mtDNAcn is associated with oxidative stress-induced mtDNA damage and poor oxidative capacity which can lead to a reduction in mitochondrial function and subsequent disruption of cellular functions which could affect several tissues (24,72).

The present study also found a higher level of mtDNA oxidative damage in patients with PsA compared with controls. It is well established that mtDNA is more susceptible to oxidative damage and has a higher mutational rate than nDNA (16). Oxidative damage to mtDNA can impair mitochondrial

bioenergetics and causes defective mitochondrial ATP generation, which leads to further mitochondrial dysfunction and increased ROS production. Therefore, decreased mtDNAcn in patients with PsA may be a consequence of mtDNA oxidative damage.

In conclusion, the present study identified a number of unique variants in patients with PsA only or healthy controls only, as well as common variants in patients and controls, two of which may be associated with the susceptibility to PsA, and also identified various missense variants that were present only in patients with PsA and were predicted to be deleterious with important effect on the function and stability of encoded proteins. In addition, lower mtDNAcn and higher levels of mtDNA oxidative damage were found in patients with PsA compared with controls. Taken together, the present findings suggested that impaired mitochondrial function due to deleterious mtDNA variants, low mtDNAcn, and oxidative damage may be contributory factors in the pathogenesis of PsA. To the best of our knowledge, the present study is the first comprehensive analysis of mtDNA in PsA. However, the present study is limited by the small number of subjects enrolled; thus, additional large-scale studies are warranted to further elucidate the role of mtDNA defects in PsA. Moreover, the possible effect of nuclear genes and environmental factors on mitochondrial genetics in PsA should be considered in future studies. In addition, to provide an improved estimate of mtDNA damage, several regions within the mtDNA genome including the D-loop region should be targeted in future studies.

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Availability of data and materials

The datasets generated and/or analysed during the current study are available in the Sequence Read Archive (SRA) repository with reference PRJNA 928743 (accession no. https://www.ncbi.nlm.nih.gov/sra/PRJNA928743).

Authors' contributions

MSA was the project administrator, was responsible for the conceptualization, methodology, investigation, acquisition of resources/funding and the data curation of the present study. MSA, GAK and MA implemented formal analysis. MSA and GAK confirm the authenticity of all raw data. MSA and GAK wrote the original draft, reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was conducted according to the guidelines of the Declaration of Helsinki and approved (approval no. 2018/496) by the Health Science Center Ethics Committee at Kuwait University and Health and Medical Research Committee in the Ministry of Health (City of Kuwait, State of Kuwait). Informed consent was obtained from all subjects involved in the study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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