

# Next-generation sequencing of the whole mitochondrial genome identifies novel and common variants in patients with psoriasis, type 2 diabetes mellitus and psoriasis with comorbid type 2 diabetes mellitus

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**Abstract.** Recent studies have shown the role of mitochondrial DNA (mtDNA) variants in the pathogenesis of both psoriasis (Ps) and type 2 diabetes (T2D) amongst different ethnicities. However, no studies have investigated the mtDNA variants present in patients with Ps, T2D, and both Ps and T2D (Ps-T2D) in the Arab population. The entire mitochondrial genomes of Kuwaiti subjects with Ps, T2D, Ps-T2D and healthy controls were sequenced using Ion Torrent next-generation sequencing. A total of 36 novel mutations and 51 previously reported mutations were identified in the patient groups that were absent in the controls. Amongst the novel mutations, eight were non-synonymous and exhibited amino acid changes. Of these, two missense mutations (G5262A and A12397G) in the *ND* genes were detected in the Ps group and a C15735T missense mutation in the *CYB* gene was detected in Ps-T2D. Other known sequence variations were seen more frequently in all or certain patient groups compared with the controls ( $P < 0.05$ ). Additionally, the A8701G missense mutation in the *ATPase 6* gene missense mutation was also observed in a higher frequency in the Ps group compared with the control. The present study is the first to perform a complete mitochondrial genome sequence analysis of Kuwaiti subjects with Ps, T2D and Ps-T2D, and both novel and known mtDNA variants were discovered. The patient-specific novel non-synonymous mutations may be co-responsible in the determination of these diseases. The higher frequency of certain mtDNA variants in the patients compared with the controls may suggest a role

in predisposing patients to these diseases. Further functional analyses are required to reveal the role of the identified mutations in these disease conditions.

## Introduction

Mitochondria are the primary site of energy production via the process of oxidative phosphorylation (OXPHOS). This process involves the transfer of electrons from reduced nicotinate adenine dinucleotide (NADH) or flavin adenine dinucleotide (FADH<sub>2</sub>) to oxygen through highly conserved mitochondrial membrane-bound enzyme complexes (I-V) of the electron transport chain (ETC) to create ATP (1). Mitochondria are also an essential source of reactive oxygen species (ROS) generation as by-products of normal mitochondrial metabolism (2).

One of the mitochondria's unique features is that it contains its own genome (mtDNA), separate and distinct from the nuclear genome of the cell. Human mtDNA is a double-stranded and circular molecule of 16,569 bp and contains two regions (3). The coding region encompasses 37 genes, which encode 13 crucial protein subunits of the ETC, two ribosomal (r)RNAs, and 22 transfer (t)RNAs. The control or regulatory (D-loop) region consists of sites for replicating and transcribing of the mtDNA. Except for complex II subunits, which are entirely encoded by the nuclear DNA (nDNA), subunits of complex I, III, IV and V are encoded by both nDNA and mtDNA. Specifically, mtDNA codes for seven subunits (ND1, ND2, ND3, ND4, ND4L, ND5 and ND6) of NADH-ubiquinone oxidoreductase of complex I, cytochrome *b* (CYTB) subunit of ubiquinol-cytochrome *c* oxidoreductase of complex III, three subunits (CO1, CO2 and CO3) of cytochrome *c* oxidase of complex IV and two subunits (ATPase 6 and 8) of ATP synthase of the complex.

The mtDNA is particularly susceptible to oxidative damage and has a high mutational rate due to its proximity to the site of ROS production, the lack of protective histones, and low DNA repair capacity (4,5). Since mtDNA encodes essential components of the ETC, these mutations can disrupt

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the mitochondria's ability to generate energy for the cell (6). Indeed, mtDNA mutations are linked with a wide range of human diseases (6).

Although primary mutations in the mtDNA have been observed in diseases of mitochondrial origin, secondary mutations and new variants are also involved in aging (7,8) and may underlie the predisposition of several common diseases, such as neurodegenerative, metabolic and inflammatory conditions (8-10). It is therefore useful to sequence the complete mitochondrial genome to explore disease-related variants in the mtDNA (11,12).

Psoriasis (Ps) is a chronic immune-mediated inflammatory skin disease characterized by hyperproliferative keratinocytes and the infiltration of the dermis by various immune cells (13,14). Ps affects ~3% of the population worldwide (15), and its incidence is also high in the Gulf countries, including Kuwait, where it affects around ~3% of people (16-18). Several studies have shown an association between Ps and metabolic syndrome (19-21), particularly type 2 diabetes (T2D), in which T2D was found to be twice as prevalent in patients with Ps (22). T2D is a progressive metabolic disease characterized by hyperglycaemia due to inadequate insulin secretion from the  $\beta$ -cells and insulin resistance. T2D is a leading cause of severe vascular complications, including cardiovascular disease (23,24), which is frequently observed in Ps patients (25,26).

Whilst the nature of the relationship between Ps and T2D remains ambiguous, both of these diseases are multifactorial, involving an interplay between genetic and environmental factors (27). Amongst the genetic factors that may explain the co-occurrence of Ps and T2D, variations in mtDNA have been suggested. In this context, studies have shown a potential role of mtDNA variants in the susceptibility or risk of T2D in different populations, including in Asian (28), European (29) and other populations (30,31). Similarly, the role of mtDNA variants in Ps has been observed in a European population (32). However, these studies have demonstrated ethnic diversity in the distribution or the implications of mtDNA variants in Ps and T2D.

To date, there are no studies that have investigated variations in the mtDNA in patients with Ps alone or in patients with Ps and T2D (Ps-T2D) in the Arab population, to the best of our knowledge. Therefore, this study aimed to sequence and compare whole mitochondrial genomes from Kuwaiti subjects with Ps, T2D, Ps-T2D and healthy controls to identify mtDNA variants in Arab individuals in Kuwait.

## Materials and methods

**Study subjects.** In the present study, a total of 98 subjects were enrolled, including 34 patients with Ps without T2D (male age range 34-76, median age 54; female age range 24-64, median age 37), 15 T2D patients with no history of skin diseases (male age range 35-60, median 54; female age range 35-57, median age 50), and 29 patients with Ps-T2D (male age range 43-73, median age 56; female age range 38-65 and median 51), as well as 20 healthy controls (male age range 24-57; median age 28; female age range 23-40, median age 27). T2D patients were diagnosed according to the World Health Organization criteria (33); fasting glucose level >7.0 mmol/l and glycated haemoglobin (HbA1c) levels of >6.5%. Patients diagnosed with plaque Ps with and without T2D were recruited from the

dermatology clinics of Abdul Kareem Al-Saeed and Suaid Al-Subah Dermatology Centres in the State of Kuwait. Healthy controls were free from inflammatory dermatoses or autoimmune diseases and without a history of T2D. Demographic and clinical parameters were obtained from the medical reports of all participants. Written informed consent was obtained from all participants under the protocols of the Joint Committee for the Protection of Human Subjects in Research in Kuwait. The study was approved by the Health Science Centre Ethics Committee at Kuwait University and the Health and Medical Research Committee in the Ministry of Health in Kuwait.

**Blood sampling and genomic DNA extraction.** Whole blood samples (5 ml) were collected from participants in EDTA tubes. Genomic DNA was extracted from whole blood using a QIAamp DNA Blood Mini kit (Qiagen GmbH) according to the manufacturer's protocol, and as previously described (9,34). The purity of the DNA samples were assessed using a NanoDrop 1000 system (Thermo Fisher Scientific, Inc.) and the concentration was measured using a Qubit 3.0 Fluorometer (Thermo Fisher Scientific, Inc.).

**Amplification of the mitochondrial genome.** The mitochondrial genome from the extracted DNA was amplified by PCR using a Precision ID mtDNA Whole Genome Panel (Applied Biosystems; Thermo Fisher Scientific, Inc.), which consisted of a 2-pool multiplex assay that targets the entire human mitochondrial genome. Amplification was performed according to the manufacturer's protocol. Each pool contained 81 primer pairs, with minimal primer overlap between pools. The mtDNA tiling approach was also used to construct the Precision ID mtDNA Control Region Panel which targets only the genome's control region, and was according to the manufacturer's protocol.

**Mitochondrial genome sequencing.** The whole mitochondrial genome was sequenced using the Ion Torrent S5™ XL Next Generation Sequencing system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Library preparation and purity were performed according to the manufacturer's protocol. Raw signal data from the Ion Torrent S5 XL sequencing were automatically transferred to the Torrent Server Hosting the Torrent Suite Software, which converted the raw voltage semiconductor sequencing data into DNA base calls. The pipeline included processing, base calling, quality score assignment, adapter trimming, read mapping to 19 reference human genomes, quality control of mapping quality, coverage analysis with down sampling and variant calling ([thermofisher.com/kw/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-data-analysis-workflow/ion-reporter-software.html](http://thermofisher.com/kw/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-data-analysis-workflow/ion-reporter-software.html)). Identification of variants was performed using the Ion Torrent Variant Caller plug-in and Ion Reporter Software version 5.2. Torrent Variant Caller version 5.2 was used for alignment and variant detection according to the revised Cambridge Reference Sequence of the human mitochondrial genome (35). The samples were multiplexed and sequenced on an Ion 520 chip (3-6 Mb throughput). The average throughput of the Ion 520 chip was 3.5 Mb. The datasets have been registered in the Sequence Read Archive (SRA) repository with reference PRJNA699142 (Table SI).

Table I. Characteristics of study subjects.

Characteristics	Ps	T2D	Ps-T2D	Controls
Sex, %				
Male	56	47	59	50
Female	44	53	41	50
Age range (median), year				
Male	34-76 (54)	35-60 (54)	43-73 (56)	24-57 (28)
Female	24-64 (37)	35-57 (50)	38-65 (51)	23-40 (27)
Fasting glucose, mmol/l <sup>c</sup>	5.4±0.68	10.0±3.0 <sup>a,b</sup>	10.6±4 <sup>a,b</sup>	5.0±0.4
Triglyceride, mmol/l <sup>c</sup>	1.7±1.2 <sup>a</sup>	2.1±1.1 <sup>a</sup>	2.5±1.6 <sup>a</sup>	0.8±0.2
Total cholesterol, mmol/l <sup>c</sup>	5.0±0.8 <sup>a</sup>	4.5±0.9	4.8±1.6	4.3±1.0

<sup>a</sup>P<0.05 vs. controls; <sup>b</sup>P<0.05 vs. Ps; <sup>c</sup>Data are presented as the mean ± standard deviation. Ps, psoriasis; T2D, type 2 diabetes; Ps-T2D, psoriasis with type 2 diabetes.

Table II. Novel mitochondrial DNA mutations present in the psoriasis patients.

Gene	Nucleotide change	Amino acid change	Type of mutation	Nature of mutation
<i>ND1</i>	A3711G	No change	Synonymous	Homoplasmic
<i>ND2</i>	T5093C	No change	Synonymous	Homoplasmic
<i>ND2</i>	C5303T	No change	Synonymous	Homoplasmic
<i>ND3</i>	A10286G	No change	Synonymous	Homoplasmic
<i>ND4</i>	A10816G	No change	Synonymous	Homoplasmic
<i>ND4L</i>	T10667C	No change	Synonymous	Homoplasmic
<i>ND5</i>	A13101C	No change	Synonymous	Homoplasmic
<i>CO1</i>	T6524C	No change	Synonymous	Homoplasmic
<i>ND2</i>	G5262A	Ala265Thr	Missense	Homoplasmic
<i>ND5</i>	A12397G	Thr21Ala	Missense	Homoplasmic

*ND*, NADH dehydrogenase subunit of complex I; *CO1*, cytochrome oxidase subunit 1 of complex IV.

**Statistical analysis.** SPSS version 15.0 (SPSS, Inc.) was used for statistical analysis. Comparisons of demographic and clinical parameters of multiple groups were performed using ANOVA followed by a post hoc Tukey's LSD test. Pearson's  $\chi^2$  was used to assess differences in the mtDNA variants distribution between cases and control. The results were evaluated with 95% confidence intervals (CIs), and P<0.05 was considered to indicate a statistically significant difference. mtDNA variants were interpreted for disease association using the data from the MITO synopsis (36), human mitochondrial database (hmtdb.uniba.it) and CLINVAR database (ncbi.nlm.nih.gov/clinvar/).

## Results

**Characteristics of the study subjects.** The study included 98 subjects, 34 patients with Ps, 15 patients with T2D, 29 patients with Ps-T2D and 20 healthy controls. Table I shows the characteristics of the study subjects. There was no significant difference in the mean age between the study subjects. Additionally, there was no significant difference in the sex distribution amongst the study subjects. The mean value of

fasting glucose differed significantly between patients and controls and was higher in the T2D patients and the Ps-T2D patients compared to the Ps patients and controls (P<0.001).

A significant difference in the mean triglyceride levels amongst the subject groups was observed (P≤0.001). The triglyceride levels were normal in the Ps patients, but were borderline high in the T2D patients and high in the Ps-T2D patients. In contrast, a significant difference in the mean value of total cholesterol was found between Ps patients compared with controls (P<0.05), but not between any of the other groups (P>0.05).

**Novel mtDNA mutations in patients.** Whole mitochondrial genome sequence analysis revealed several novel mutations that were not previously reported, were not associated with disease in the MitoMap, and are not listed in the Single Nucleotide Polymorphism Database (36). These included synonymous and non-synonymous mutations detected in patients with Ps, T2D and Ps-T2D, which were not present in the controls. The identified mutations and their characteristics are displayed in Tables II-IV. The majority of the non-synonymous mutations

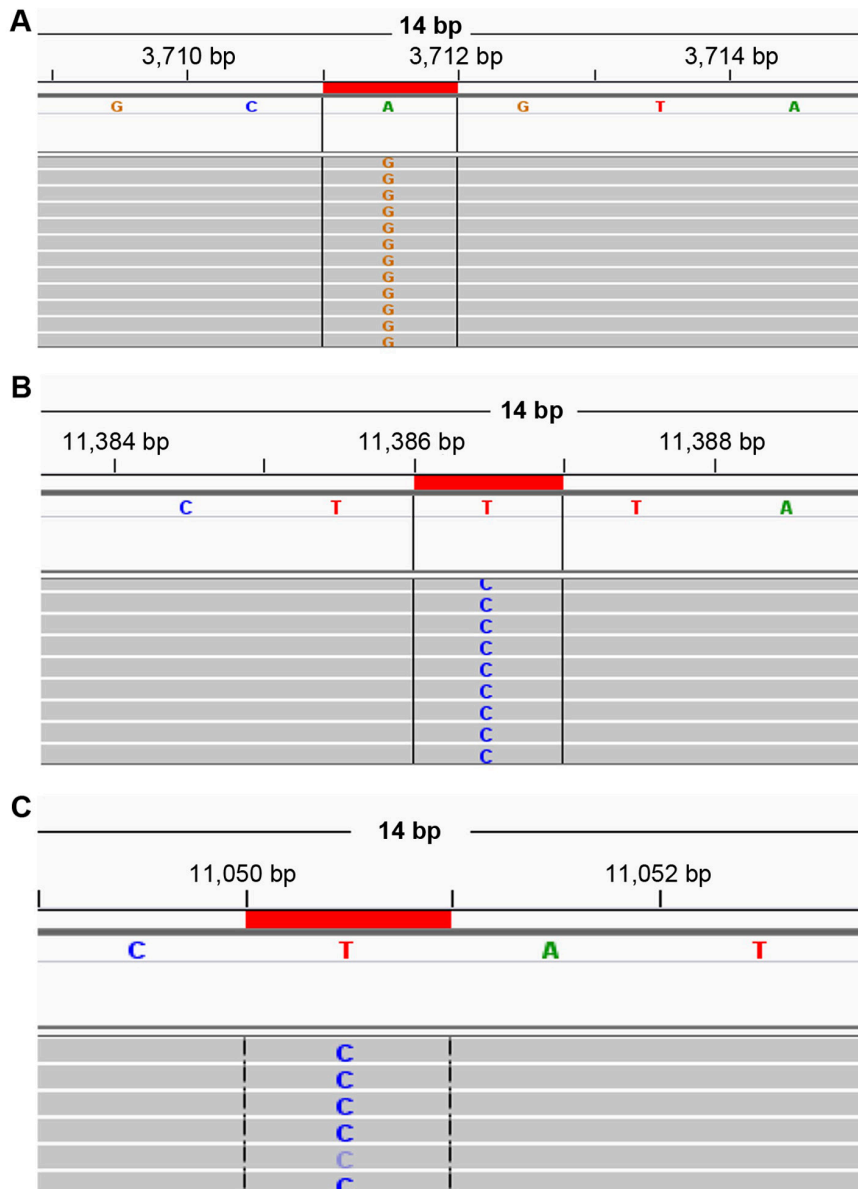


Figure 1. Histograms showing some of the mitochondrial DNA mutations that were identified in the patient groups. (A) A synonymous A3711G mutation in the Ps group. (B) A synonymous T11386C mutation in the T2D group. (C) A synonymous T11050C mutation in the Ps-T2D group.

were found in the mtDNA coding region. Most of these were observed in subunit genes of complex I, including *ND2*, *ND4* and *ND5*. The remaining mutations were found in the *CYB* gene of complex III and *ATP8* subunit gene of complex V. Additionally, the synonymous mutations were found in the mtDNA coding and control regions.

In the Ps group (Table II), two missense mutations were found in the *ND* genes. These included G5262A in the *ND2* gene and A12397G in the *ND5* gene. The identified synonymous mutations in Ps patients (Table II) were A3711G in the *ND1* gene, T5093C and C5303T in the *ND2* gene, A10286G in the *ND3* gene, A10816G in the *ND4* gene, T10667C in the *ND4L* gene, A13101C in the *ND5* gene and T6524C in the *COI* gene. (Fig. 1A; histograms show the A3711G synonymous mutation in the Ps group).

In the T2D (Table III), five missense mutations were detected. The missense mutations included, C12084T, G4959A and A11930G in the *ND* genes, C14751T in the *CYB* gene, as

well as T8951C in the *ATP6* gene. Moreover, the T1822C and T2226TA insertion mutations were found in the 16S *rRNA* gene. The identified synonymous mutations in the group of T2D patients (Table III) were T11386C, G11887A, T12136C, C13077A, C13680T, T5196C, T14020C and A14500G in the *ND* genes, C7648T and T7783C in the *CO2* gene, and T15310C in the *CYB* gene as well as the A16316G variant in the D-loop control region (Fig. 1B.; histograms show the T11386C synonymous mutation in the T2D group).

In the Ps-T2D (Table IV), the C15735T missense mutation was found in the *CYB* gene. The identified synonymous mutations in the Ps-T2D were T5090C, T11050C, C10556T, C10628T, A13419T and A3720G in the *ND* genes (Fig. 1C; histograms show the T11050C synonymous mutation in PsT2D group).

Several other variants that were previously reported as either missense polymorphisms or synonymous mutations were also found in all the patient groups and are shown in Table V.

Table III. Novel mitochondrial DNA mutations present in the type 2 diabetes patients.

Gene	Nucleotide change	Amino acid change	Type of mutation	Nature of mutation
<i>ND4</i>	T11386C	No change	Synonymous	Homoplasmic
<i>ND4</i>	G11887A	No change	Synonymous	Homoplasmic
<i>ND4</i>	C12084T	Ser 442 Pro	Missense	Homoplasmic
<i>ND4</i>	T12136C	No change	Synonymous	Homoplasmic
<i>ND5</i>	C13077A	No change	Synonymous	Homoplasmic
<i>ND5</i>	C13680T	No change	Synonymous	Homoplasmic
<i>CYB</i>	C14751T	Thr2Ile	Missense	Homoplasmic
<i>CYB</i>	T15310C	No change	Synonymous	Homoplasmic
<i>CO2</i>	C7648T	No change	Synonymous	Homoplasmic
<i>CO2</i>	T7783C	No change	Synonymous	Homoplasmic
<i>ND2</i>	G4959A	Ala164Thr	Missense	Heteroplasmic
<i>ND2</i>	T5196C	No change	Synonymous	Homoplasmic
<i>ND4</i>	A11930G	Ile391Val	Missense	Homoplasmic
<i>ND5</i>	T14020C	No change	Synonymous	Homoplasmic
<i>ND6</i>	A14500G	No change	Synonymous	Homoplasmic
<i>ATP6</i>	T8951C	Val142Ala	Missense	Homoplasmic
<i>16S rRNA</i>	T1822C	-	-	Homoplasmic
<i>16S rRNA</i>	T2226TA	-	-	Homoplasmic
<i>D-loop</i>	A16316G	-	-	Homoplasmic

*ND*, NADH dehydrogenase subunit of complex I; *CYB*, cytochrome b reductase of complex III; *CO2*, cytochrome oxidase subunits of complex IV; *ATPase 6*, ATP synthase subunit 6 of complex V; *rRNA*, ribosomal RNA.

Table IV. Novel mitochondrial DNA mutations present in the psoriasis patients with type 2 diabetes.

Gene	Nucleotide change	Amino acid change	Type of mutation	Nature of mutation
<i>ND2</i>	T5090C	No change	Synonymous	Homoplasmic
<i>ND4</i>	T11050C	No change	Synonymous	Homoplasmic
<i>ND4L</i>	C10556T	No change	Synonymous	Homoplasmic
<i>ND4L</i>	C10628T	No change	Synonymous	Homoplasmic
<i>ND5</i>	A13419T	No change	Synonymous	Homoplasmic
<i>CYB</i>	C15735T	Ala330Val	Missense	Homoplasmic
<i>ND1</i>	A3720G	No change	Synonymous	Homoplasmic

Ps-T2D, psoriasis with type 2 diabetes mellitus; *ND*, NADH dehydrogenase subunit of complex I; *CYB*, cytochrome *b* reductase of complex III.

#### Known mtDNA sequence variations in patients and controls.

Analysis of whole mitochondrial genomes from Ps, T2D and Ps-T2D patients and controls revealed the presence of numerous known sequence variations in the coding and control regions of mtDNA (Table VI). When the frequency of these variants was compared between patients and controls, significant results ( $P < 0.05$ ) with odd ratios (OR)  $> 1$  were found. Specifically, the G15301A variant in the *CYB* gene was found at a higher frequency in the three groups of patients, and appeared in 32% of the Ps patients (OR, 4.2; 95% CI, 2-9;  $P = 0.0001$ ), 20% of the T2D patients (OR, 2.2; 95% CI, 0.9-5;  $P = 0.04$ ) and 21% of the Ps-T2D patients (OR, 2.4; 95% CI, 1-5.3;  $P = 0.04$ ) compared with the controls (10%). Similarly, the C150T variant in the D-loop was also found at an increased frequency in the

three groups of patients and appeared in 26% of the Ps patients (OR, 3; 95% CI, 1.4-7;  $P = 0.003$ ), 20% of the T2D patients (OR, 2.2; 95% CI, 0.9-5;  $P = 0.04$ ) and 24% of the Ps-T2D patients (OR, 2.8; 95% CI, 1.2-6.3;  $P = 0.008$ ) compared with the controls (10%), whereas the C12705T variant in the *ND5* gene was found at increased frequency in the Ps and Ps-T2D groups: 35% of Ps patients (OR, 3; 95% CI, 1.5-6;  $P = 0.001$ ) and 28% of the Ps-T2D patients (OR, 2.2; 95% CI, 1-4.4;  $P = 0.03$ ) compared with the controls (15%). The variant A1438G in the 12S *rRNA* gene was observed in 100% of Ps patients (OR, 11; 95% CI, 1.3-8.7;  $P = 0.005$ ) and 100% of the Ps-T2D patients (OR, 11; 95% CI, 1.3-8.7;  $P = 0.005$ ) compared with the controls (90%). Some of the identified variants appeared more frequently in specific patient groups compared with the controls (Table VI).

Table V. Known mitochondrial DNA sequence variations present only in the patient groups.

A, Ps group				
Gene	Nucleotide change	Amino acid change	Type of mutation	dbSNP (rs)
<i>ND4</i>	A10819G	No change	Synonymous	rs28358283
<i>ND6</i>	T14212C	No change	Synonymous	rs28357672
<i>CO3</i>	A9377G	No change	Synonymous	rs28380140
<i>ATPase6</i>	A8860G	Thr112Ala	Missense	rs2001031
<i>ATPase8</i>	T8473C	No change	Synonymous	rs386829037
B, T2D group				
Gene	Nucleotide change	Amino acid change	Type of mutation	dbSNP (rs)
<i>ND1</i>	T3396C	No change	Synonymous	rs374875201
<i>ND1</i>	C4025T	Thr240Met	Missense	rs397515509
<i>ND1</i>	T4218C	No change	Synonymous	rs878853061
<i>ND1</i>	A4234G	Thr310Ala	Missense	rs2001030
<i>ND2</i>	C5187T	No change	Synonymous	rs879014605
<i>ND5</i>	G13145A	Ser270Asn	Missense	rs386829175
<i>ND5</i>	T13326C	No change	Synonymous	rs878889334
<i>ND5</i>	T14025C	No change	Synonymous	rs879073899
<i>ND6</i>	T14325C	Asn117Asp	Missense	rs397515505
<i>ND6</i>	T14577C	Ile33Val	Missense	rs386829219
<i>CYB</i>	G14861A	Ala39Thr	Missense	rs2853505
<i>CO1</i>	A6891G	Ser330Gly	Missense	rs879091068
<i>CO1</i>	G7337A	No change	Synonymous	rs386829005
<i>CO2</i>	C7819A	No change	Synonymous	rs878853024
<i>CO2</i>	C7873T	No change	Synonymous	rs879161183
<i>CO3</i>	G9438A	Gly78Ser	Missense	rs267606611
<i>CO3</i>	T9530C	No change	Synonymous	rs879237361
<i>CO3</i>	T9950C	No change	Synonymous	rs3134801
<i>ATP6</i>	C8932T	Pro136Ser	Missense	rs878853013
<i>12S rRNA</i>	G1503A	-	-	rs727503164
<i>tRNA<sup>Ala</sup></i>	C5601T	-	-	rs376884056
<i>tRNA<sup>Thr</sup></i>	CT15939Cdel	-	-	rs878981265
<i>D-loop</i>	T42TC ins	-	-	rs377245343
<i>D-loop</i>	CT151TC	-	-	rs386828863
<i>D-loop</i>	T279C	-	-	rs879199276
<i>D-loop</i>	A512G	-	-	rs1556422458
<i>D-loop</i>	C16167T	-	-	rs371419667
<i>D-loop</i>	T16209C	-	-	rs386829278
C, Ps-T2D group				
Gene	Nucleotide change	Amino acid change	Type of mutation	dbSNP (rs)
<i>ND2</i>	A5390G	No change	Synonymous	rs41333444
<i>ND2</i>	T5426C	No change	Synonymous	rs878866102
<i>ND3</i>	G10143A	Gly29Ser	Missense	rs202131419
<i>ND4</i>	A10876G	No change	Synonymous	rs879036391
<i>ND5</i>	T13020C	No change	Synonymous	rs75577869
<i>ND5</i>	T13879C	Ser515Pro	Missense	rs879087566
<i>CYB</i>	G15734A	Ala330Thr	Missense	rs386829259

Table V. Continued.

C, Ps-T2D group				
Gene	Nucleotide change	Amino acid change	Type of mutation	dbSNP (rs)
<i>COI</i>	C6045T	No change	Synonymous	rs879061193
<i>COI</i>	T6515C	No change	Synonymous	rs878998677
<i>12S rRNA</i>	G1598A	-	-	rs3135027
<i>16S rRNA</i>	T2626C	-	-	rs879158835
<i>tRNA<sup>Thr</sup></i>	A15907G	-	-	rs41383248
<i>D-loop</i>	T125C	-	-	rs144402189
<i>D-loop</i>	C340T	-	-	rs117394573
<i>D-loop</i>	A508G	-	-	rs113683159
<i>D-loop</i>	C16214T	-	-	rs368055283
<i>D-loop</i>	C16290T	-	-	rs386828866
<i>D-loop</i>	C16295T	-	-	rs878874012

Ps, psoriasis; T2D, type 2 diabetes mellitus; Ps-T2D, psoriasis with type 2 diabetes mellitus; *ND*, NADH dehydrogenase subunits of complex I; *CO*, cytochrome oxidase subunits of complex IV; *ATPase*, ATP synthase subunits of complex V; rRNA, ribosomal RNA; tRNA, transfer RNA; dbSNP, Single Nucleotide Polymorphism Database; rs, Reference SNP.

In the Ps group, higher frequencies of variants (OR>1, P<0.05) were observed, namely C10400T and T10873C in the *ND* genes, T14783C in the *CYB* gene, T9540C in the *CO3* gene, and A8701G in the *ATPase 6* gene, as well as C16223T and T16519C in the D-loop control region.

In the T2D group, increased frequencies of variants (OR>1, P<0.05) were found in the coding region, including A4769G, G11914A, C12633A, G13368A, G13590A and G14364A in the *ND* genes, G15148A and A15607G in the *CYB* gene, G15928A in the *tRNA<sup>Thr</sup>* gene, T10463C in the *tRNA<sup>Arg</sup>* gene, and G1719A and G1888A in the *16S rRNA* gene. Variants in the D-loop control region, namely T195C, C16186T, G16274A, C16292T and C16294T were also found.

In the Ps-T2D group, increased frequencies (OR>1, P<0.05) were observed for the T10410C variant in the *tRNA<sup>Arg</sup>* gene and the G16390A variant in the D-loop region.

When these variants' characteristics were analysed (Table VII), the majority of the identified variants were homoplasmic with no amino acid changes. However, the A8701G variant in the *ATPase 6* gene, which was located at a higher frequency in 35% of the Ps patients (OR, 2; 95% CI, 1.1-4; P=0.01) compared with 20% in the controls, was identified as a missense mutation and exhibited a threonine to alanine alteration (Thr59Ala).

## Discussion

To the best of our knowledge, this is the first study to perform a comprehensive analysis of mitochondrial DNA (mtDNA) variants in Kuwaiti subjects with Ps, T2D and Ps-T2D, as well as in healthy controls. The average coverage depth was 24625.2X and the mean read length was 144 bp. However, the average total reads were 3,359,441, the frequency of reads was between 99.4-99.9%, and the coverage of reads was >100%. Whole mitochondrial genome sequencing revealed 36 novel

non-synonymous and synonymous mutations and 51 sequence variations in the patient groups that were not detected in the controls. Additionally, several known sequence variations were seen in both patients and controls.

In general, a synonymous mutation is the substitution of a DNA base pair that does not result in a change in the amino acid sequence; in contrast, a non-synonymous mutation is the substitution of a DNA base pair that results in a single amino acid change in a given polypeptide. Non-synonymous mutations include a missense mutation (a point mutation in which a single nucleotide change results in a codon that codes for a different amino acid), a nonsense mutation (a point mutation in a sequence of DNA that leads to the appearance of a stop codon, resulting in premature termination of translation and the production of a truncated protein), as well as insertion and deletion of one or more DNA base pairs.

Amongst the novel mutations identified in the patient groups, eight non-synonymous mutations resulted in amino acid changes and were detected primarily in the subunit genes of complexes I, III and V. These included missense mutations in the Ps group, primarily found in subunit genes of complex I, including G5262A in the *ND2* gene and A12397G in the *ND5* gene. Moreover, missense mutations were detected in the T2D group. These included the missense mutations C12084T and A11930G in the *ND4* gene and G4959A in the *ND2* gene, as well as the missense mutations C14751T in the *CYB* gene, and T8951C in the *ATP6* gene. Additionally, the C15735T missense mutation in the *CYB* gene was found in the Ps-T2D group. Moreover, 25 synonymous mutations were located in the coding and control regions in patient groups. Known variants previously reported as either missense or synonymous mutations were also identified. The majority of these were located in the coding region, and only a few were found in the control region.

Other known sequence variations were found in the patients groups and controls. Some of these variations were

Table VI. Known mitochondrial DNA sequence variations in patients and controls.

Gene	Nucleotide change	Ps			T2D			Ps-T2D			Controls	
		%	OR, 95% CI	P-value	%	OR, 95% CI	P-value	%	OR, 95% CI	P-value	%	
<i>CYB</i>	G15301A	32	4.2, 2-9	0.0001 <sup>c</sup>	20	2.2, 0.9-5	0.04 <sup>a</sup>	21	2.4, 1-5.3	0.04 <sup>a</sup>	10	
<i>D-loop</i>	C150T	26	3, 1.4-7	0.003 <sup>b</sup>	20	2.2, 0.9-5	0.04 <sup>a</sup>	24	2.8, 1.2-6.3	0.008 <sup>b</sup>	10	
<i>ND5</i>	C12705T	35	3, 1.5-6	0.001 <sup>c</sup>				28	2.2, 1-4.4	0.03 <sup>a</sup>	15	
<i>12S rRNA</i>	A1438G	100	11, 1.3-8.7	0.005 <sup>b</sup>				100	11, 1.3-87	0.005 <sup>b</sup>	90	
<i>ND3</i>	C10400T	18	4, 1.4-11.7	0.003 <sup>b</sup>							5	
<i>ND4</i>	T10873C	35	3, 1.5-6	0.001 <sup>c</sup>							15	
<i>CYB</i>	T14783C	18	4, 1.4-11.7	0.003 <sup>b</sup>							5	
<i>CO3</i>	T9540C	35	3, 1.5-6	0.001 <sup>c</sup>							15	
<i>ATPase 6</i>	A8701G	35	2, 1.1-4	0.01 <sup>b</sup>							20	
<i>D-loop</i>	C16223T	38	2.4, 1.3-4.6	0.005 <sup>b</sup>							20	
<i>D-loop</i>	T16519C	65	1.8, 1-3	0.03 <sup>a</sup>							50	
<i>ND2</i>	A4769G				27	3.3, 1.5-7.3	0.002 <sup>b</sup>				10	
<i>ND4</i>	G11914A				26	3, 1.4-7	0.003 <sup>b</sup>				10	
<i>ND5</i>	C12633A				13	2.8, 0.9-8	0.04 <sup>a</sup>				5	
<i>ND5</i>	G13368A				20	2.2, 0.9-5	0.04 <sup>a</sup>				10	
<i>ND5</i>	G13590A				13	2.2, 0.9-5	0.04 <sup>a</sup>				5	
<i>ND6</i>	G14364A				13	2.2, 0.9-5	0.04 <sup>a</sup>				5	
<i>CYB</i>	G15148A				13	2.8, 0.9-8	0.04 <sup>a</sup>				5	
<i>CYB</i>	A15607G				20	2.2, 0.9-5	0.04 <sup>a</sup>				10	
<i>tRNA<sup>Thr</sup></i>	G15928A				20	2.2, 0.9-5	0.04 <sup>a</sup>				10	
<i>tRNA<sup>Arg</sup></i>	T10463C				20	2.2, 0.9-5	0.04 <sup>a</sup>				10	
<i>16S rRNA</i>	G1719A				13	2.8, 0.9-8	0.04 <sup>a</sup>				5	
<i>16S rRNA</i>	G1888A				20	2.8, 0.9-8	0.04 <sup>a</sup>				10	
<i>D-loop</i>	G16274A				13	2.8, 0.9-8	0.04 <sup>a</sup>				5	
<i>D-loop</i>	C16292T				13	2.8, 0.9-8	0.04 <sup>a</sup>				5	
<i>D-loop</i>	C16294T				27	2, 1-4	0.03 <sup>a</sup>				15	
<i>D-loop</i>	T195C				46	2.5, 1.4-4.6	0.002 <sup>b</sup>				25	
<i>D-loop</i>	C16186T				13	2.8, 0.9-8	0.04 <sup>a</sup>				5	
<i>tRNA<sup>Arg</sup></i>	T10410C							14	3, 1-9	0.02 <sup>a</sup>	5	
<i>D-loop</i>	G16390A							14	3, 1-9	0.02 <sup>a</sup>	5	

<sup>a</sup>P<sub>≤</sub>0.05, <sup>b</sup>P<sub>≤</sub>0.01, <sup>c</sup>P<sub>≤</sub>0.001. Ps, psoriasis; T2D, type 2 diabetes mellitus; Ps-T2D, psoriasis with type 2 diabetes mellitus; OR, odds ratio; CI, confidence interval; ND, NADH dehydrogenase subunits of complex I; CO, cytochrome oxidase subunits of complex IV; ATPase, ATP synthase subunits of complex V; rRNA, ribosomal RNA; tRNA, transfer RNA.



Table VII. Characteristics of mitochondrial DNA sequence variations in patients and controls.

Gene	Nucleotide change	Amino acid change	Type of mutation	Nature of mutation	dbSNP (rs)
<i>CYB</i>	G15301A	No change	Synonymous	Homoplasmic	rs193302991
<i>D-loop</i>	C150T	-	-	Homoplasmic	rs62581312
<i>ND5</i>	C12705T	No change	Synonymous	Homoplasmic	rs193302956
<i>12S rRNA</i>	A1438G	-	-	Homoplasmic	rs2001030
<i>ND3</i>	C10400T	No change	Synonymous	Homoplasmic	rs28358278
<i>ND4</i>	T10873C	No change	Synonymous	Homoplasmic	rs2857284
<i>CYB</i>	T14783C	No change	Synonymous	Homoplasmic	rs193302982
<i>CO3</i>	T9540C	No change	Synonymous	Homoplasmic	rs2248727
<i>ATPase 6</i>	A8701G	p. Thr59Ala	Missense	Homoplasmic	rs2000975
<i>D-loop</i>	C16223T	-	-	Homoplasmic	rs2853513
<i>D-loop</i>	T16519C	-	-	Homoplasmic	rs3937033
<i>ND2</i>	A4769G	No change	Synonymous	Homoplasmic	rs3021086
<i>ND4</i>	G11914A	No change	Synonymous	Homoplasmic	rs2853496
<i>ND5</i>	C12633A	No change	Synonymous	Homoplasmic	rs3926883
<i>ND5</i>	G13368A	No change	Synonymous	Homoplasmic	rs3899498
<i>ND5</i>	G13590A	No change	Synonymous	Homoplasmic	rs28359177
<i>ND6</i>	G14364A	No change	Synonymous	Homoplasmic	rs879086798
<i>CYB</i>	G15148A	No change	Synonymous	Homoplasmic	rs527236206
<i>CYB</i>	A15607G	No change	Synonymous	Homoplasmic	rs193302996
<i>tRNA<sup>Thr</sup></i>	G15928A	-	-	Homoplasmic	rs527236198
<i>tRNA<sup>Arg</sup></i>	T10463C	-	-	Homoplasmic	rs28358279
<i>16S rRNA</i>	G1719A	-	-	Homoplasmic	rs3928305
<i>16S rRNA</i>	G1888A	-	-	Homoplasmic	rs2897260
<i>D-loop</i>	G16274A	-	-	Homoplasmic	rs144095641
<i>D-loop</i>	C16292T	-	-	Homoplasmic	rs144417390
<i>D-loop</i>	C16294T	-	-	Homoplasmic	rs140662392
<i>D-loop</i>	T195C	-	-	Homoplasmic	rs66492218
<i>D-loop</i>	C16186T	-	-	Homoplasmic	rs879166752
<i>D-loop</i>	G16390A	-	-	Homoplasmic	rs41378955
<i>tRNA<sup>Arg</sup></i>	T10410C	-	-	Homoplasmic	rs200478835

*ND*, NADH dehydrogenase subunits of complex I; *CO*, cytochrome oxidase subunits of complex IV; *ATPase*, ATP synthase subunits of complex V; rRNA, ribosomal RNA; tRNA, transfer RNA; dpSNP, Single Nucleotide Polymorphism Database; rs, Reference SNP.

observed more frequently in all patient groups compared with the controls. Specifically, the frequencies of the G15301A variant in the *CYB* gene and the variant C150T in the *D-loop* region were significantly higher in all patient groups compared with the control. In contrast, the C12705T mutation in the *ND5* gene and the A1438G in the *12S rRNA* gene were found at significantly higher frequencies in the Ps and Ps-T2D groups compared with the control group. Moreover, other variants were found at higher frequencies in specific patient groups compared with the control group. Whilst most of these variants were synonymous, the A8701G variant in the *ATPase6* gene that was found at a higher frequency in the Ps patients compared with the control group was identified as a missense mutation and resulted in an amino-acid substitution from threonine to alanine (Thr59Ala).

Mitochondria are the primary intracellular site of energy production, and mutations in the mitochondrial genome can affect mitochondrial function (6). In humans, the mtDNA

encodes 13 protein subunits of the ETC, two rRNAs and 22 tRNAs, all of which are important for normal mitochondrial function (3). Mitochondria are also prone to damage from ROS, and several mutations of the mtDNA-encoded genes can enhance ROS production (37). Indeed, mitochondrial impairment as a result of mtDNA mutations have been observed in somatic tissues during normal aging (7,8), and have also been linked to several diseases, where oxidative stress serves a pivotal role in their development, such as in cancer and neurodegenerative diseases (6,7,9). Moreover, mitochondrial dysfunction serves a role in the pathogenesis of non-alcoholic fatty liver disease (NAFLD), as it affects hepatic lipid homeostasis and promotes ROS production and lipid peroxidation, and NAFLD has been linked to both T2D and psoriasis (38).

In the present study, the identified missense, and insertion mutations in the mtDNA genes were only observed in the patient groups. Although the identified mutations were homoplasmic, they showed changes in the amino acids of

essential polypeptides complexes of the mitochondrial ETC, as well as in rRNAs and tRNAs, which are components of the mitochondrial gene expression system and the non-coding region. A thousand copies of the mitochondrial genome per cell gives rise to an essential feature of mitochondrial genetics: Homoplasmy and heteroplasmy. Homoplasmy is the presence of identical copies of mtDNA that may be normal or mutated. Heteroplasmy is the presence of a mixture of normal and mutated mtDNA. Whereas most deleterious mtDNA mutations are heteroplasmic in nature, not all are pathogenic, as some heteroplasmic mutations in the hypervariable D-loop region may be of little clinical significance (39). Moreover, some homoplasmic mutations have been reported to cause Leigh syndrome, a severe neurological disorder (40), or as secondary mutations that influence the disease severity of Leber's hereditary optic neuropathy (41). Secondary homoplasmic mutations may predispose an individual to specific symptoms of T2D, obesity and Alzheimer's disease from different ethnic groups (31,42).

The present study identified novel mutations that met at least 3 criteria classified as disease-causing mutations (6); they were present in structurally and functionally important regions of the mtDNA, resulted in changes in the amino acids, and were not found in healthy individuals. Therefore, these mutations may have detrimental effects on the structure and function of the ETC complexes. Notably, in the present study, most novel mutations were found in the NADH dehydrogenase subunit genes of complex I, the largest enzyme of the mitochondrial OXPHOS system, and the primary source of ROS in mitochondria (43). Altered complex I activity has been frequently observed in various pathologies such as mitochondrial disorders, cancer, neurodegenerative diseases and T2D (9,44,45).

The results of the present also showed several synonymous mutations in patient groups. Although mutations that do not result in amino changes are considered biologically silent, they have been implicated in human diseases through their direct effect on gene expression and function (46-48).

In addition to mtDNA pathogenic mutations, which are rare in a population, mtDNA polymorphisms have been linked with the susceptibility to or protection from various diseases. In this context, previous population-based studies have found an association between mtDNA variants with the susceptibility and risk of T2D (28-31), whereas a protective effect of mtDNA variants from Ps have also been identified (32).

The current study identified numerous reported mtDNA variations that are already present in the MITOMAP database, which were found more frequently in  $\geq 1$  group of patients compared with the controls. Although most of these were homoplasmic synonymous variants with no amino acid changes, they were reported in several disease conditions. The variants G15301A in the *CYB* gene of complex III and the variant C150T in the hypervariable segment of the D-loop region were found more frequently in all patient groups (Ps, T2D and Ps-T2D) compared with the controls. These variants have not been reported in any of the abovementioned diseases, but were previously reported in other conditions. The G15301A variant was described as a germline homoplasmic mtDNA mutation in 40% of Malaysian females with breast cancer (49), whereas the C150T variant was associated

with the risk of cervical cancer and HPV infection (50). The T10410C variant in the *tRNA<sup>Arg</sup>* gene and the G16390A variant in the D-loop region were found at increased frequencies in Ps-T2D patients compared with the controls. The T10410C variant was previously reported in children with Leigh syndrome (51), and the G16390A variant was found to be weakly associated with T2D in a Tunisian cohort (52). The variant A8701G in the *ATPase 6* gene was found at a higher frequency in Ps patients compared with controls. This homoplasmic variant was previously reported in Japanese patients with T2D (53) and patients with mitochondrial maternally inherited diabetes and deafness (31). Numerous studies have shown a clear association between Ps and T2D, and patients with Ps are at increased risk of developing T2D (19-22). The presence of the G16390A variant in Ps-T2D patients and the A1438G variant in the Ps patients and their previous association with T2D suggest a possible role of these variants to predisposition of Ps and Ps-T2D.

In the present study, the variant A8701G, which occurred at a higher frequency in the Ps patients compared with the controls, was identified as a missense mutation and resulted in amino-acid substitution from threonine to alanine (Thr59Ala) in the ATPase6 subunit of complex V. This variant was previously associated with maternally inherited hypertension and cardiomyopathy in a Chinese pedigree of consanguineous marriage (54). Although the Ps patients triglyceride and total cholesterol levels were normal in the present study, Ps patients are at higher risk of developing cardiovascular diseases (25,26).

The present study has some limitations, including the relatively low number of subjects affecting the statistical power. Additionally, functional analysis should be performed to determine the potential biological significance of these mutations in the context of these diseases, which is lacking from the present study.

In conclusion, the present study is the first study to sequence and analyse the whole mitochondrial genome of Kuwaiti patients with Ps, T2D and Ps-T2D, and compared these with healthy controls. Novel mutations in patients that resulted in a change in the coded amino acid, which may be co-responsible in the determination of these diseases were identified. Additionally, known variants were detected in higher frequencies in the patient group compared with the controls, suggesting their role in predisposing patients to these diseases. These results warrant further functional analysis to determine the role of these variants in T2D, Ps and Ps-T2D.

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## Availability of data and materials

The datasets generated in the present study have been registered in the Sequence Read Archive repository (ref. no. PRJNA699142). The reference BioSample accession nos. are SAMN17766667-SAMN17766764, and the URLs of the datasets have been uploaded as a supplementary file (Table S1).

## Author's contributions

MSA and SA conceived the study; MSA collected the data and performed the experiments; MSA, GAK and MB contributed to data analysis and interpretation, and wrote and edited the manuscript. MB and GAK confirmed the authenticity of all the raw data. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

This study was performed in line with the principle of the Declaration of Helsinki. Approval was granted by the Health Science Center Ethics Committee at Kuwait University and Health and Medical Research Committee in the Ministry of Health and registered on No. 2016/496. Informed consent was obtained from all individual participants included in the study.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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