INTRODUCTION

Mitochondria are present in most eukaryotic cells and deeply involved in various important cell functions, especially energy production in the form of ATP (Adenosine triphosphate) (Wescott, Kao, Lederer, & Boyman, 2019). Mitochondria carry their own circular DNA, which exists in multiple copies per cell and its inheritance is only maternal (Stewart & Larsson, 2014). Several mutations in mitochondrial DNA (mtDNA) have been indicated as cause of human mitochondrial diseases associated with the defects in oxidative production of energy (Sharma & Sampath, 2019).

Previous reports demonstrated that defects in mtDNA function can lead to mitochondrial diabetes (Sarhangi et al., 2017;
Mitochondrial diabetes (MD) is defined as a mitochondrial disease accompanied by impaired secretion of insulin, insulin resistance, or combined defects (Ng, Taylor, & Schaefer, 2017). It is a rare monogenic form of diabetes with a frequency of 1% (Li, Slone, Fei, & Huang, 2019) and classified into type 1 and type 2 (Tabebi et al., 2017). MD is characterized by a strong familial clustering of diabetes with a maternal transmission in conjunction with bilateral hearing impairment in most of the carriers (Mezghani et al., 2013) and also with several other effects such as retinopathy, neuropathy, and nephropathy (Sheetz & King, 2002; Tabebi et al., 2015, 2017).

In many cases, MD was associated with the m.3243A>G mutation in mtDNA, which is located in MT-TL1 (OMIM 590050) gene coding mitochondrial tRNALeu(UUR). MD has also been associated with a range of other point mutations in mitochondrial genes such as mt-tRNA genes, (MT-TI [OMIM 590045], MT-TS1 [OMIM 590080], and MT-TK [OMIM 590060]) and mt-proteins genes (MT-ND1 [OMIM 516000], MT-ND4 [OMIM 516003], MT-COX2 [OMIM 516040], and MT-COX3 [OMIM 516050]) (Maassen, Janssen, & ’t Hart, L.M., 2005; Mezghani et al., 2010; Tabebi et al., 2017; Wilson et al., 2004). In addition, deletion (Mezghani et al., 2013) and depletion (Guillausseau et al., 2001) have also been described in patients with MD.

Here, we investigate the mitochondrial genome anomalies in a family with MD by performing Sanger sequencing for a whole mitochondrial genome screening, a qPCR for mitochondrial copy number analysis, and Long-range PCR for the detection of large deletions.

2 | MATERIAL AND METHODS

2.1 | Patients

In this report, we studied a Tunisian family with clinical features suggestive of mitochondrial diabetes type 2. The main diagnosis criterion was the maternal transmission of diabetes within generations with a bilateral hearing impairment in most of the carriers according to the WHO (World Health Organization) criteria.

The patient (III.1) is an underweight (BMI, 16.4 kg/m²), 38-year-old female suffering from diabetes since the age of 32. The patient suffered from chronic bilateral hearing impairment as well, and the audiogram showed that the defect preferentially affected high-frequency perception. The cardiopulmonary auscultation and the neurological examination were abnormal where the proband presented a psychomotor retardation and global- and axial hypotonia. The brain MRI examination showed a significant cortical atrophy. The electroretinography (ERG) revealed electrical signs of retinopathy. Dilated mitochondrial cardiomyopathy with left ventricular hypertrophy was also found. Laboratory investigations revealed elevated blood lactate level of 2.9 mmol/L (normal value <2 mmol/L) and positive staining for ragged red fibers on muscle biopsy characteristic of mitochondrial cytopathy.

Family history was notable in that her mother had insulin-requiring diabetes while her father was a healthy individual without any family history of diabetes (Figure 1). Her three siblings were all males and healthy. She recalled that her maternal grandmother and one of the maternal aunts had diabetes with deafness, whereas her uncle had only diabetes without deafness.

2.2 | Controls

One hundred Tunisian healthy individuals from the same ethnocultural group were tested as controls. These controls have no personal or family history of diabetes or any other disorder.

All individuals (patient and controls) provided informed consent, in accordance with the ethics committee of Hedi Chaker Hospital (Sfax, Tunisia).
2.3 Methods

2.3.1 DNA extraction

Total DNA was extracted from peripheral blood leukocytes using phenol–chloroform standard procedures (Lewin & Stewart-Haynes, 1992) and from muscular biopsy, only for patient III.1, using «FastPure™ DNA Kit Cat.# 9191» (TAKARA) according to the manufacturer’s recommendations.

2.3.2 Whole mitochondrial DNA screening

PCR amplification and sequencing of the mitochondrial genes

The entire mitochondrial DNA was amplified using 24 overlapping pairs of primers as described elsewhere (Rieder, Taylor, Tobe, & Nickerson, 1998). After the PCR amplification, each PCR product was purified using NucleoSpin (MACHEREN-NAGEL) and sequenced with ABI PRISM 3100-Avant automated DNA sequencer using the BigDye Terminator Cycle Sequencing reaction kit v1.1. The resulting sequences were compared with the updated Cambridge sequence (GenBank accession number: NC_012920). The blast homology searches were performed using the programs available at the National Center for Biotechnology Information website and compared with the wild-type sequence. Areas containing putative novel variations were amplified and sequenced again on both strands to exclude possible PCR artifacts.

Screening of the m.3243A>G mutation in the tRNALeu(UUR) gene using PCR-RFLP and quantification of the heteroplasmic rates

PCR restriction fragment-length polymorphism analysis was used to find the m.3243A>G mutation in blood leukocytes or muscular biopsy. The PCR amplification and the PCR-RFLP analysis with the ApaI restriction enzyme (BioLabs) were performed as described elsewhere (Bannwarth, Procaccio, & Paquis-Flucklinger, 2005; Mkaour-Rebai et al., 2007). The m.3243A>G mutation creates an ApaI restriction site and the digestion results in two fragments of 234 and 294 bp, whereas in the absence of this mutation, a 528 bp fragment corresponding to the PCR product will be observed. For the quantification of the heteroplasmic rates of the m.3243A>G mutation, we analyzed the digested fragments with the Gel DOC™-XR (Bio-RAD).

2.3.3 Long-range PCR amplification in blood leukocytes or muscular biopsy

Long-range PCR was performed to detect mtDNA deletions using Long PCR Enzyme Mix (#K0182) (Fermentas). To amplify the fragment of 10.162 kb, we use a couple of primers corresponding to mitochondrial genome positions 5835–5854 and 15997–15978. The conditions for the PCR reaction and the primer sequences were described previously by Tabebi et al. (2017).

2.3.4 Relative quantification of the mtDNA

For the determination of mtDNA content in the whole blood samples, quantitative PCRs were performed in duplicates in 48-well reaction plates (Applied Biosystems). Each reaction (final volume 10 μl) contained 10-ng DNA, 5 μl of Power SYBR-Green PCR Master Mix (TAKARA), and 0.2 μM each of forward and reverse primers. ND4 (NADH dehydrogenase subunit 4), a mitochondrial-encoded gene, was amplified (ND4-F: CGCAGTATTTACACTCA, ND4-R: GCTAGTCATATTAAGTTGTTG) and GAPDH (glycer-aldehyde-3-phosphate dehydrogenase) (OMIM 138400), a nuclear-encoded gene, was used as normalizing control (GAPDH-F: CCCGTGCAATGAAATTTC, GAPDH-R: CACCCTTTAGGGAGAAAA). The selection of the target gene (ND4, mitochondrial gene) is described in detail in the Supplementary Material.

The PCR condition was 2 min at 50°C and 10 min at 95°C, followed by 40 cycles of denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and primer extension at 72°C for 15 s. The melting curve analysis was provided by the Dissociation Curve software to ensure the assay specificity. The slope of standard curve indicated high real-time PCR efficiency rates, 1.08 for ND4, and 1.19 for GAPDH, in the investigated range from 0.2 to 200 ng DNA input (n = 4) with high linearity (Pearson correlation coefficient r = 0.99).

We then determined the mtDNA copy number relative to nuclear DNA (nDNA) using the method as described by Venegas, Wang, Dimmock, & Wong, (2011). The data are expressed as relative quantification of the mtDNA on nuclear DNA copy number (mtDNA/nDNA). The quantitative differences were statistically analyzed by Student’s t test, where p values lower than .05 were considered significant.

3 RESULTS

3.1 Whole mitochondrial DNA screening

In the present study, we performed a sequence analysis of the whole mtDNA in a Tunisian family presenting three generations of maternally inherited diabetes (Figure 1a). The whole mitochondrial genome screening revealed the presence of several reported substitutions in the D-loop region and the coding genes (Table 1). Among these variations, the
Additional C in the 311–315 track and the m.16519T>C in the D-loop region, m.3480A>G in MT-ND1 and m.14257A>G in MT-ND6 (OMIM 516006) were found in the proband (III.1). They are maternally inherited and associated with diabetes and deafness according to the MITOMAP database (mitochondrial database).

Additionally, the patient (III.1) exclusively presented the heteroplasmic m.3243A>G mutation in the MT-TL1 (Figure 1b). This mutation was absent in the other family members (her mother II.4 and her father II.5) indicating that the mutation is de novo (Figure 1b). This transition creates an Apal restriction site which is absent in the PCR product of wild-type DNA. The PCR-RFLP analysis in leukocyte revealed the absence of the mutation in the patient's parents which is in accordance with the sequencing results (Figure 2a). In addition, the digested fragment profile in the patient quantified with the Gel DOC™-XR (Bio-RAD) analyzer showed that the m.3243A>G mutation was present in the patient with high heteroplasmic loads: 54.7% in muscular tissue and 25% in leukocytes (Figure 2b).

### 3.2 mtDNA deletion and depletion analysis

Patient III.1 presented a severe phenotype comparing to her family members and we speculated MD could be associated with mitochondrial deletions. To test this hypothesis, we performed a Long-range PCR amplification of the 10.162 kb fragment which contains the major arc of mtDNA. The DNA was extracted from the blood leukocytes of studied individuals (II.4, II.5, and III.1) and muscle tissue of the patient (III.1) carrying the m.3243A>G mutation. Amplification results of the proband (III.1) showed the presence of two distinctive fragments, 10.162 kb and ~9.0 kb, in both tissues indicating the presence of heteroplasmic mitochondrial deletion (Figure 3a). These deletions remove several mitochondrial tRNA and protein-coding genes. However, the long-range PCR amplification in the family members (II.4 and II.5) showed only the expected fragment of 10.162 kb indicating the absence of mitochondrial deletions in their blood leukocytes in the mtDNA-tested region (Figure 3a).

The relative quantification of mtDNA copy number using mtDNA/nDNA in the leukocytes of individuals II.4, II.5, or III.1 with qPCR revealed a significant variation in this ratio compared to controls (Figure 3b). The results showed that the proband with the m.3243A>G mutation had low levels of mtDNA compared to controls (mean = 75.07% ± 5.39, t = 4.625; p = .04), which suggests a depletion in the blood leukocytes. However, the mean amount of the relative mtDNA copy number had increased significantly in the leukocytes of the mother (II.4) and of the father (II.5) (p=.04): 112%±2.685 and 121.5%±4.790, respectively.

### 4 DISCUSSION

In the present study, we performed a mutational screening of the whole mitochondrial DNA in a Tunisian family with a history of MD. The mtDNA sequencing showed the presence

| Patients | Locus | Nucleotide change | Homo/heteroplasmic change | AA change | Phenotypic association |
|----------|-------|-------------------|--------------------------|-----------|-----------------------|
| III.1    | MT-HV1| m.16224T>C        | Homoplasmic               |           | LHON                  |
| III.1    | MT-HV1| m.16311T>C        | Homoplasmic               |           | Encephalomyopathy     |
| II.4, III.1 | MT-ND1 | m.3480A>G        | Heteroplasmic             |           | LHON, MIDD            |
| III.1    | MT-ND6| m.14167C>T        | Homoplasmic               | E169E     | LHON                  |
| II.4, II.5, III.1 | MT-ND6 | m.14212T>C   | Homoplasmic               | V154V     | —                     |
| II.4, III.1 | MT-ND1 | m.14257A>G     | Homoplasmic               | P139P     | Diabetes              |
| II.4, II.5, III.1 | MT-HV2 | m.73A>G        | Homoplasmic               |           | —                     |
| II.4, II.5, III.1 | MT-HV2 | m.87A>T        | Heteroplasmic             |           | —                     |
| II.4, II.5, III.1 | MT-HV2 | m.263A>G       | Homoplasmic               |           | —                     |
| II.4, III.1 | MT-HV2 | m.3243A>G     | Heteroplasmic             |           | Diabetes, deafness, MELAS, LHON, MIDD, encephalomyopathy, cardiomyopathy |

Abbreviations: LHON, Leber hereditary optic neuropathy; MELAS, myopathy-encephalopathy-lactic acidosis-stroke-like episodes; MIDD: maternally inherited diabetes deafness.

The bold values are the mitochondrial mutations associated with mitochondrial diabetes.
of several reported substitutions including the m.3243A>G mutation in the \textit{MT-TL1}. This mutation was present only in the patient III.1 at heteroplasmy state in the blood leukocytes and the muscular tissue. This patient also presented \~1.0 kb mtDNA deletion and mitochondrial copy number decrease.

The m.3243A>G mutation in the mitochondrial \textit{tRNA\textsubscript{Leu}(UUR)} was previously reported as the most prevalent mutation in mitochondrial diabetes in several diabetic populations worldwide with a prevalence varying from 0.1% to 10% (Maassen et al., 2004). In Tunisian diabetic population, this mutation was only reported in 1.07% (Bouhaha, Abid Kamoun, Elgaeaied & Ennafaa, 2010 which was similar to the values reported in Japanese (Suzuki et al., 2003), German (Klemm et al., 2001), and French diabetic populations (Guillausseau et al., 2001). In early onset (6.40 years) diabetes, the m.3243A>G mutation was found in 3% of patients while in late-onset (>40 years) diabetes only 0.3% of patients carry this mutation (Ng et al., 2000).
The m.3243A>G has also been associated with various severe mitochondrial diseases and syndromes. Indeed, it has been identified in approximately 80% of MELAS (Myopathy-Encephalopathy-Lactic Acidosis-Stroke-like episodes) patients (Deschauer, Wieser, Neudecker, Lindner, & Zierz, 1999; Mezghani et al., 2011) and in about 38% of them with cardiac involvement (Berardo, Musumeci, & Toscano, 2011). In addition, this mutation has been reported in deafness (Murphy, Turnbull, Walker, & Hattersley, 2008), myopathy (Kärppä et al., 2005), Leigh syndrome (Carelli & Chan, 2014), ophthalmoplegia associated with epilepsy (Mancuso et al., 2014), cardiomyopathy (Alila et al., 2016), and ocular and renal affections (Löwik, Hol, Steenbergen, Wetzels, & Heuvel, 2005; Michaelides et al., 2008).

Several previous cohort studies reported maternal inheritance of the m.3243A>G mutation (Frederiksen et al., 2006) but only rarely at sporadic presentation, in contrast to other mitochondrial mutations (Cree, Samuels, & Chinnery, 2009; de Laat et al., 2016). Furthermore, m.3243A>G at a de novo state was observed in the cases with more severe phenotype, which is in accordance with the presentation of the proband of our studied family (de Laat et al., 2016). This proband carried the de novo m.3243A>G mutation at heteroplasmic state with rate of 25% in the blood leukocytes and much higher 54.7% in the skeletal muscle. As in Table 2, the results are in accordance with a previous description of heteroplasmic rate of the m.3243A>G mutation in blood leukocytes (18%–30%) and in the muscle biopsy (70%–89%) of patients with MD. These heteroplasmic rates were lower than those observed in patients with MELAS syndrome but they both lead to a very severe phenotype in patients presenting mitochondrial diseases including deafness, weakness, myopathy, and so on (Table 2).

The complex phenotype associated with m.3243A>G mutation was explained in several studies by its effect on the oxidative phosphorylation and ATP production. Not surprisingly, the m.3243A>G mutation was described to be responsible for reduced ATP production and impaired oxidative phosphorylation due to the respiratory chain deficiency (Alila et al., 2016). Furthermore, cellular studies investigated that the m.3243A>G mutant revealed deficiencies in RNA processing (Rossmanith & Karwan, 1998), aminoacylation (Park, Davidson, & King, 2003), posttranscriptional tRNA modification (Helm, Florentz, Chomyn, & Attardi, 1999), and translation.

In addition to the m.3243A>G pathogenic mutation, we found four other maternally inherited mitochondrial variations in the proband III.1, which were previously described to be associated with diabetes and deafness. These reported substitutions are in the MT-ND1 (1) and MT-ND6 (1) coding genes or in the noncoding displacement D-loop region (2) (Table 1). The two variations m.3480A>G (p.K58=) and m.14257A>G (p.K6=) found in the MT-ND1 and MT-ND6, respectively, were previously described as potential diabetogenic mtDNA defect markers (Li et al., 2014; Yu, Yu, Liu, Wang & Tang, 2004). Furthermore, the mononucleotide repeat (poly-C) between 303 and 315 nucleotides (D310) in the D-loop has been recently identified as a hotspot mutation and often associated with severe mitochondrial diseases and human neoplasia including breast cancer (Yu et al., 2008). This homopolymeric C stretch is part of the conserved sequence block II within the D-loop and is involved in the formation of the RNA-DNA hybrid leading to the initiation of mtDNA heavy-strand replication (Schwartz, Alazzouzi, & Perucho, 2006). On the other hand, the 16519T>C SNP in the D-loop region was correlated.

### Table 2: An overview of de novo reports of the m.3243A>G mutation

| Phenotype | Sex/age of onset (year) | Heteroplasmic level (%) | Special features | Report |
|-----------|-------------------------|-------------------------|-----------------|--------|
| MELAS     | M/21                    | 89 36  —                | Deafness, weakness | Yamamoto, 1996 |
| MD        | —                       | 79 10  —                | —               |        |
| MELAS     | M/2                     | 70 30  —                | Epilepsy, weakness, psychomotor delay | Campos et al. (1996) |
| MELAS     | M/54                    | 39 6  —                 | Weakness, deafness, diabetes | Deschauer et al. (1999) |
| MD        | F/8                     | — 18 55                | Deafness, hypertension, proteinuria | Maassen, Biberoglu, ’t Hart, Bakker & de Knijff, 2002 |
| MELAS     | M/34                    | 82  — 40               | Encephalopathy, deafness, epilepsy, myopathy | de Laat et al. (2016) |
| MD        | F/32                    | 54.7 25                | Deafness, cardiomyopathy, encephalopathy, retinopathy, psychomotor delay | This study |

Abbreviations: F, female; M, male; MD, mitochondrial diabetes; MELAS, myopathy, encephalopathy, lactic acidosis, stroke-like episodes.
The bold values are our results from this study.
significantly with diabetes mellitus (Navaglia et al., 2006). This variant is thought to alter the transcription levels of mitochondrial proteins involved in oxidative phosphorylation, which, if occurring in the β cells, may lead to β-cell failure (Navaglia et al., 2006; Simmons, Suponitsky-Kroyter, & Selak, 2005). These mitochondrial point mutations together with the pathogenic m.3243A>G mutation could at least partially explain the severe phenotype observed in the proband III.1. Additional mtDNA investigation revealed that this patient also presented a heteroplasmic mtDNA deletion of ~1.0 kb, which was absent in the other family members examined. The mtDNA deletion is a rare anomaly in mitochondrial diabetes, associated with severe phenotypes such as dilated cardiomyopathy and neurological effects (Ballinger et al., 1992; Mezghani et al., 2013; Rigoli, Salpietro, Caruso, Chiarenza, & Barberi, 1999). The severity of phenotypes associated with mtDNA deletions was variable and linked to the loss of function of mitochondrial genes (Chen, 2013). To our knowledge, this is the first report that analyzes the co-occurrence of the mitochondrial deletion with the pathogenic mutation m.3243A>G.

The relative quantification of mtDNA/nDNA showed a significant decrease in the leukocyte mtDNA copy number of patient III.1 presenting the m.3243A>G. Decrease in mtDNA has been often observed in mitochondrial disorders as well as cell lines harboring m.3243A>G mutation, indicating that mtDNA depletion can be a common secondary phenomenon and also that the cause of mtDNA depletion is not always due to mutation of a nuclear gene (Pyle et al., 2007). Interestingly, subjects II.4 and II.5 who do not carry the m.3243A>G mutation presented an increase in the mtDNA content in their leukocytes. This could be explained by the increased expression of the mitochondrial transcription factor A (TFAM, OMIM 600438), which plays a key role in the regulation of mtDNA replication as described recently (Farge & Falkenberg, 2019).

5 | CONCLUSIONS

The whole mitochondrial DNA analysis in a Tunisian family with a history of MD revealed the m.3243A>G mutation (MT-TL1) in a de novo heteroplasmic state in the proband. Together with the high heteroplasmic rate of this mutation, we also found the ~1.0 kb deletion in the mtDNA and the depletion of the mitochondrial content. It is likely that the co-occurrence of these mitochondrial mutations explains the severity of the MD phenotype in the proband compared to other family members, namely diabetes and deafness associated with cardiomyopathy, encephalopathy, retinopathy, and psychomotor delay.

ACKNOWLEDGMENTS

We thank the patient and her family for their cooperation in the present study. This work was supported by The Ministry of Higher Education and Scientific Research in Tunisia. The authors also thank Naomi Yamada for her work in proofreading the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Mouna Tabebi: performed the molecular biology experiment and data mining for the genetic data and wrote the manuscript. Wajdi Safi, Mohamed Abid, and Mouna Mnif: provided clinical samples. Rahma Felhi and Olfa Alila Fersi: performed the molecular biology experiment. Leila Keskes: supplied biological materials. Faiza Fakhfakh: supervised and proofed the manuscript.

DATA AVAILABILITY STATEMENT

Additional supporting information may be found online in the Supporting Information section.

ORCID

Mouna Tabebi https://orcid.org/0000-0002-2873-161X
Leila Keskes https://orcid.org/0000-0002-8420-139X

REFERENCES

Alila, O. F., Rebai, E. M., Tabebi, M., Tej, A., Chamkha, I., Tlili, A., ... Fakhfakh, F. (2016). Whole mitochondrial genome analysis in two families with dilated mitochondrial cardiomyopathy: Detection of mutations in MT-ND2 and MT-TL1 genes. *Mitochondrial DNA Part A*, 27, 2873–2880. https://doi.org/10.3109/19401736.2015.1060417

Ballinger, S. W., Shoffner, J. M., Hedaya, E. V., Trounce, I., Polak, M. A., Koontz, D. A., & Wallace, D. C. (1992). Maternally transmitted diabetes and deafness associated with a 10.4 kb mitochondrial DNA deletion. *Nature Genetics*, 1, 11–15. https://doi.org/10.1038/ng0492-11

Bannwarth, S., Procaccio, V., & Paquis-Flucklinger, V. (2005). Surveyor™ nuclease: A new strategy for a rapid identification of heteroplasmic mitochondrial DNA mutations in patients with respiratory chain defects. *Human Mutation*, 25, 575–582. https://doi.org/10.1002/humu.20177

Berardo, A., Musumeci, O., & Toscano, A. (2011). Cardiological manifestations of mitochondrial respiratory chain disorders. *Acta Myologica: Myopathies and Cardiomyopathies: Official Journal of the Mediterranean Society of Myology*, 30, 9–15.

Boughara, H., Abid Kammoun, K., Elgaaied, A., & Ennaffaa, H. (2010). A3243G mitochondrial DNA mutation in Tunisian diabetic population. *Tunisian medical*, 88, 642–645.

Campos, Y., Martin, MA., Lorenzo, G., Aparicio, M., Cabello, A., & Arenas, J (1996). Sporadic MERRF/MELAS overlap syndrome associated with the 3243 tRNA(Leu(UUR)) mutation of mitochondrial DNA. *Muscle Nerve*, 19, 187–190. https://doi.org/10.1002/smc.19960190203

Carelli, V., & Chan, D. C. (2014). Mitochondrial DNA: Impacting central and peripheral nervous systems. *Neuron*, 84, 1126–1142. https://doi.org/10.1016/j.neuron.2014.11.022

Chen, X. J. (2013). Mechanism of homologous recombination and implications for aging-related deletions in mitochondrial DNA.
Physiology and biochemistry, 110, 186–188. https://doi.org/10.1076/apab.110.3.186.8294.

Maassen, J. A., Janssen, G. M. C., & ’t Hart, L. M. (2005). Molecular mechanisms of mitochondrial diabetes (MIDD). *Annals of Medicine*, 37, 213–221. https://doi.org/10.1080/0785389051007188

Maassen, J. A., ’t Hart, L. M., vanEssen, E., Heine, R. J., Nijpels, G., Jahangir Tafrechi, R. S., … Lemkes, H. (2004). Mitochondrial diabetes: Molecular mechanisms and clinical presentation. *Diabetes, 53*(Suppl 1), S103–109. https://doi.org/10.2337/diabetes.53.2007.S103

Mancuso, M., Orsucci, D., Angelini, C., Bertini, E., Carelli, V., Comi, G. P., … Siciliano, G. (2014). The m.3243A>G mitochondrial DNA mutation and related phenotypes. A matter of gender? *Journal of Neurology*, 261, 504–510. https://doi.org/10.1007/s00415-013-7225-3

Mezghani, N., Mkaouar-Rebai, E., Mnif, M., Charfi, N., Rekik, N., Youssef, S., … Fakhfakh, F. (2010). The heteroplasmic m.14709T>C mutation in the tRNA(Glu) gene in two Tunisian families with mitochondrial diabetes. *Journal of Diabetes and Its Complications*, 24, 270–277. https://doi.org/10.1016/j.jdiacomp.2009.11.002

Mezghani, N., Mnif, M., Kacem, M., Mkaouar-Rebai, E., Hadj Salem, I., Kallel, N., … Fakhfakh, F. (2011). A whole mitochondrial genome screening in a MELAS patient: A novel mitochondrial tRNA(Val) mutation. *Biochemical and Biophysical Research Communications*, 407, 747–752. https://doi.org/10.1016/j.bbrc.2011.03.094

Mezghani, N., Mnif, M., Mkaouar-Rebai, E., Kallel, N., Charfi, N., Abid, M., & Fakhfakh, F. (2013). A maternally inherited diabetes and deafness patient with the 12S tRNA(m.1555A>G) and the ND1 m.3308T>C mutations associated with multiple mitochondrial deletions. *Biochemical and Biophysical Research Communications*, 431, 670–674. https://doi.org/10.1016/j.bbrc.2013.01.063

Michaelides, M., Jenkins, S. A., Bamiou, D.-E., Sweeney, M. G., Davis, M. B., Luxon, L., … Rath, P. P. (2008). Macular dystrophy associated with the A3243G mitochondrial DNA mutation. Distinct retinal and associated features, disease variability, and characterization of asymptomatic family members. *Archives of Ophthalmology, 126*, 320–328. https://doi.org/10.1001/archoph.126.3.320

Mkaouar-Rebai, E., Tiili, A., Masmoudi, S., Belguith, N., Charfeddine, I., Mnif, M., … Fakhfakh, F. (2007). Mutational analysis of the mitochondrial tRNA(Leu(UUR)) gene in Tunisian patients with mitochondrial diseases. *Biochemical and Biophysical Research Communications*, 355, 1031–1037. https://doi.org/10.1016/j.bbrc.2007.02.083

Murphy, R., Turnbull, D. M., Walker, M., & Hattersley, A. T. (2008). Clinical features, diagnosis and management of maternally inherited diabetes and deafness (MIDD) associated with the 3243A>G mitochondrial point mutation. *Diabetic Medicine, 25*, 383–399. https://doi.org/10.1111/j.1464-5491.2008.02359.x

Navaglia, F., Basso, D., Fogar, P., Speri, C., Greco, E., Zambon, C.-F., … Plebani, M. (2006). Mitochondrial DNA D-loop in pancreatic cancer: Somatic mutations are epiphenomena while the germline 16S19 T variant worsens metabolism and outcome. *American Journal of Clinical Pathology, 126*, 593–601. https://doi.org/10.1309/GQFCCJMH5KHNVX73

Ng, M. C., Yeung, V. T., Chow, C. C., Li, J. K., Smith, P. R., Mijovic, C. H., … Chan, J. C. (2000). Mitochondrial DNA A3243G mutation in patients with early- or late-onset type 2 diabetes mellitus in Hong Kong Chinese. *Clinical Endocrinology, 52*, 557–564. https://doi.org/10.1046/j.1365-2265.2000.00898.x
Ng, Y. S., Taylor, R. W., & Schaefer, A. M. (2017). Diabetes mellitus in mitochondrial disease. *Diabetes Associated with Single Gene*, 25, 55–68. https://doi.org/10.1159/000454701

Park, H., Davidson, E., & King, M. P. (2003). The pathogenic A3243G mutation in human mitochondrial tRNA(Leu(UUR)) decreases the efficiency of aminocacylation. *Biochemistry*, 42, 958–964. https://doi.org/10.1021/bi026882r

Pyle, A., Taylor, R. W., Durham, S. E., Deschauer, M., Schaefer, A. M., Samuels, D. C., & Chinnery, P. F. (2007). Depletion of mitochondrial DNA in leukocytes harbouring the 3243A->G mtDNA mutation. *Journal of Medical Genetics*, 44, 69–74. https://doi.org/10.1136/jmg.2006.043109

Rieder, M. J., Taylor, S. L., Tobe, V. O., & Nickerson, D. A. (1998). Automating the identification of DNA variations using quality-based fluorescence re-sequencing: Analysis of the human mitochondrial genome. *Nucleic Acids Research*, 26, 967–973. https://doi.org/10.1093/nar/26.4.967

Rigoli, L., Salti, D. C., Caruso, R. A., Chiarenza, A., & Barberi, I. (1999). Mitochondrial DNA mutation at np 3243 in a family with maternally inherited diabetes mellitus. *Acta Diabetologica*, 36, 163–167. https://doi.org/10.1007/s005920050161

Rossmannith, W., & Karwan, R. M. (1998). Impairment of tRNA processing by point mutations in mitochondrial tRNA(Leu(UUR)) associated with mitochondrial diseases. *FEBS Letters*, 433, 269–274. https://doi.org/10.1016/S0014-5793(98)00928-4

Sarhangi, N., Khatami, F., Kallabi, F., Alila-Fersi, O., Ben Mahmoud, A., Ben Saad, W., ... Fakhfakh, F. (2015). A novel mutation MT-COIII m.9267G>C and MT-COI m.5913G>A mutation in mitochondrial genes in a Tunisian family with maternally inherited diabetes and deafness (MIDD) associated with severe nephropathy. *Biochemical and Biophysical Research Communications*, 459, 353–360. https://doi.org/10.1016/j.bbrc.2015.01.151

Venegas, V., Wang, J., Dimmock, D., & Wong, L.-J. (2011). Real-Time Quantitative PCR Analysis of Mitochondrial DNA Content. *Current Protocols in Human Genetics*, 68, 19.7.1–19.7.12. https://doi.org/10.1002/0471142905.hg190768

Wescott, A. P., Kao, J. P. Y., Lederer, W. J., & Boylan, L. (2019). Voltage-energized calcium-sensitive ATP production by mitochondria. *Nature Metabolism*, 1, 975–984. https://doi.org/10.1038/s4225-019-0126-8

Wilson, F. H., Hariri, A., Farhi, A., Zhao, H., Petersen, K. F., Toka, H. R., ... Lifton, R. P. (2004). A cluster of metabolic defects caused by mutation in a mitochondrial tRNA. *Science*, 306, 1190–1194. https://doi.org/10.1126/science.1102521

Yamamoto, M (1996). Did de novo MELAS common mitochondrial DNA point mutation (mtDNA 3243, A-->G transition) occur in the mother of a proband of a Japanese MELAS pedigree?. *Journal of Biomedical Science*, 15, 535–543. https://doi.org/10.1007/s11377-007-9229-4

Yu, P., Yu, D., Liu, D., Wang, K., & Tang, X. (2004). Relationship between mutations of mitochondrial DNA ND1 gene and type 2 diabetes. *Chinese medical journal (English)*, 117, 985–989.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section.

**How to cite this article:** Tabebi M, Safi W, Felhi R, et al. The first concurrent detection of mitochondrial DNA m.3243A>G mutation, deletion, and depleion in a family with mitochondrial diabetes. *Mol Genet Genomic Med*. 2020;8:e1292. https://doi.org/10.1002/mgg3.1292