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Whole mitochondrial genome screening of a family with maternally inherited diabetes and deafness (MIDD) associated with retinopathy

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Introduction

Mitochondrial diabetes (MD), defined as a mitochondrial disease with chronic hyperglycemia due to inappropriate secretion of insulin, insulin resistance, or combined defects MD (type I or II), is a kind of rare diabetes with a frequency of 1%, characterized by a maternal inheritance and hearing impairment in some cases, giving rise to MIDD (Mitochondrial Inherited Diabetes and Deafness)**.** Individuals with diabetes can present complications that cause their morbidity and mortality. In fact, chronic hyperglycemia is a major initiator of diabetic microvascular complications (eg, retinopathy, neuropathy, and nephropathy). Indeed, MD pathogenesis is caused by mitochondrial dysfunction and rare mitochondrial DNA (mtDNA) mutations.Genetic abnormalities of the mitochondrial diabetes can be caused by mitochondrial DNA .

Patients & Methods

Table 1: Clinical features of the studied family members.

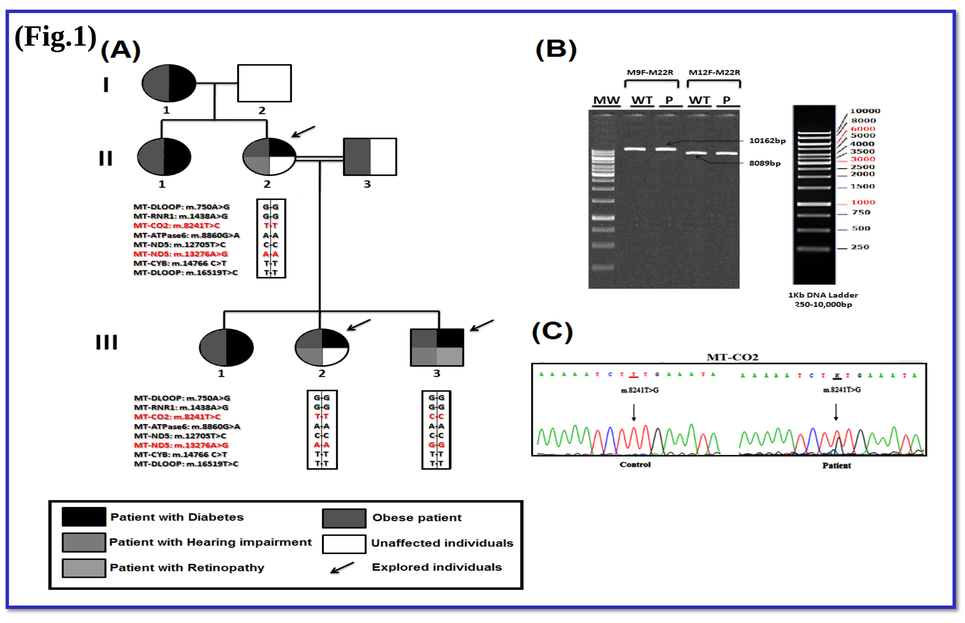
Subjects	Sex	BMI (kg/m²)	Onset diabetes (years)	Clinical features			
				MD	Deafness	Obesity	Diabetic Retinopathy
II.2	F	33.9	18	+	+	+	-
III.2	F	30.11	1	+	+	+	-
III.3	M	30.8	4	+	+	+	+

1- DNA extraction
2- Long-range PCR amplification in blood leucocytes
3- PCR amplification and sequencing of the mitochondrial genes
4- Mitochondrial haplogroup analysis
5- Bioinformatic prediction

Results

1- Long-range PCR amplification of the two fragments of 10.162 kb and 8.089 kb in the DNA extracted from patient's III blood leukocytes. Results showed the expected fragments of 10.162 kb, and 8.089 kb suggesting the absence of mitochondrial deletions in explored regions of mitochondrial DNA in blood leukocytes (Figure 1B).
2- A whole mitochondrial genome screening was performed by sequencing. Results revealed the presence of tA novel heteroplasmic variation *MT-CO2* m.8241T>G in patient III.3. (Figure 1C)
3- The mutational screening, help us to put the item on a specific haplotype composed by "A750G, A1438G, G8860A, T12705, T14766C and T16519C" in the mother and transmitted to her daughter and her son who presented two additional *de novo* variations (m.8241T>G and m.13276G>A) (Figure 1C). Besides, the identification of several variations in polymorphic sites allowed us to classify the studied family under the sub-haplogroup H2a2a1.

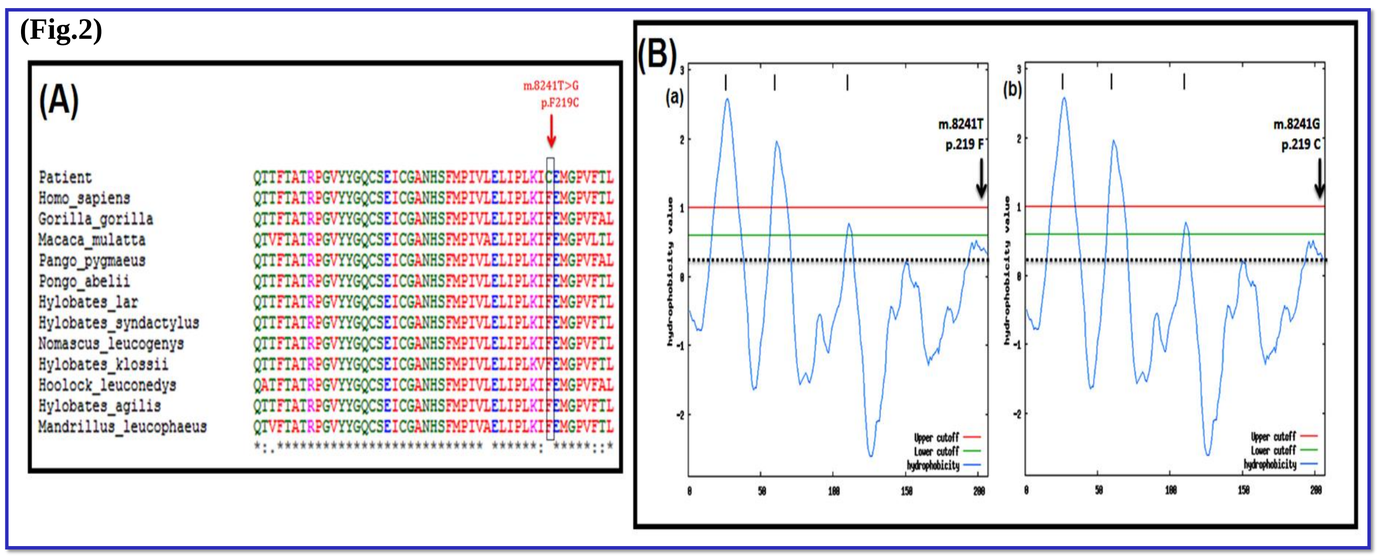
(Fig.1)



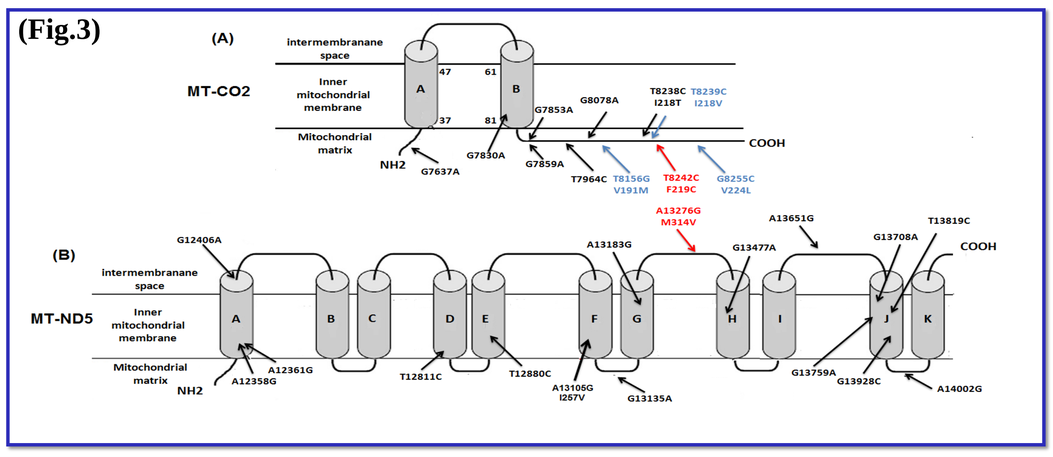
3-The variation m.8241T>G in *MT-CO2* gene substitute the Phenylalanine residue at position 219 to a Cysteine (p.F219C).The analysis of the mitochondrial polypeptide sequences from different species showed that the Phenylalanine (p.F219) residue in MT-CO2 protein was located in an evolutionarily stable domain **(Fig. 2.A)**.
4-A hydropathy plot of the p.219 F>C mutant polypeptide generated with the Kyte–Doolittle algorithm (Toppred program) demonstrated a slight imbalance in its hydrophobicity caused by the m.8241T>G mutation. This substitution substantially reduced the hydrophobicity of the external loop (0.60 to 0.47) **(Fig. 2.B)**.

5-The modeling of the protein secondary structure determined that MT-CO2 is largely a hydrophobic protein and contains just two hydrophilic domains. In addition, the substitution of the Phenylalanine residue with Cysteine located in the C terminal region could be the cause of the slight alteration in the structure of the MT-CO2 protein membrane **(Fig. 3.A)**.

(Fig.2)

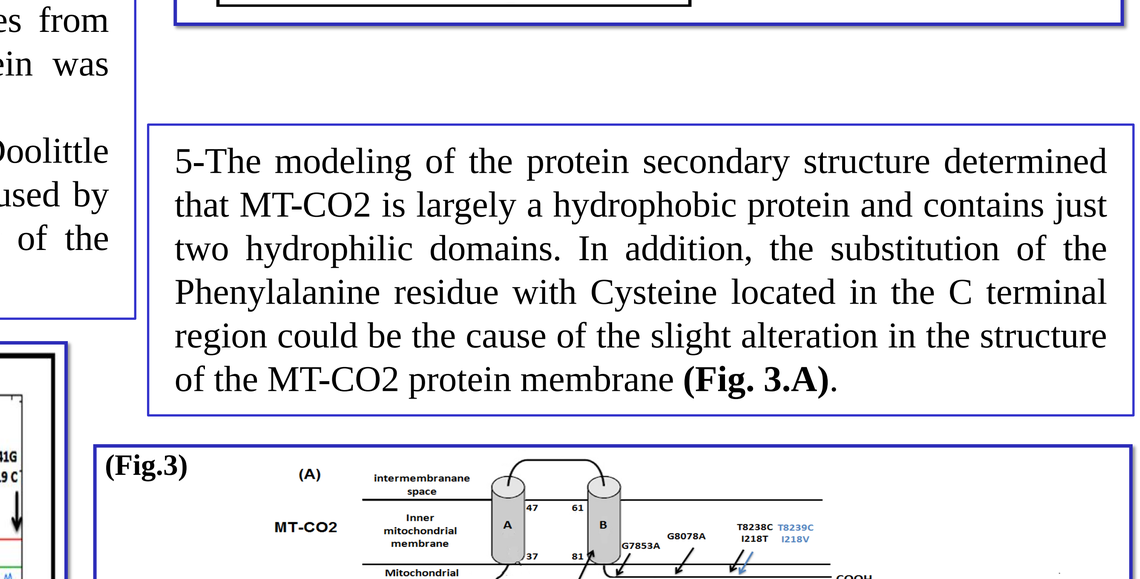


(Fig.3)



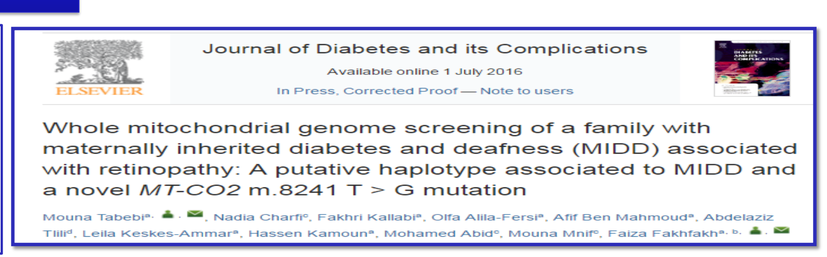
6- Generation of a 3D model of MT-CO2 demonstrated that the presence of amine functional group in the side chain allows to C219 more chance than F residue to share hydrogen bund with I214 and P215 neighbor residues **(Fig. 4.A)**.. Modeling the two adjacent structures (p.V191M and p.I218V) and comparing it to F219C structure showed that this new mutation is the most damaging one **(Fig. 4.B)**..

(Fig.4)



Conclusion

We reported a family with MIDD and presenting a haplotype composed by "A750G, A1438G, G8860A, T12705, T14766C and T16519C"in homoplasmic state. In this family a patient with MIDD2 and retinopathy presented, in addition to this haplotype associated to the MIDD, two *de novo* variations including a novel one m.8241T>G (p.F219C) in MT-CO2 gene and a known one m.13276G>A (p.M314V) in the MT-ND5 gene. The coexistence of these two mutations could explain the retinopathy observed in this patient.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Whole mitochondrial genome screening of a family with maternally inherited diabetes and deafness (MIDD) associated with retinopathy: A putative haplotype associated to MIDD and a novel *MT-CO2* m.8241 T > G mutation

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All authors have no conflict of interest