RESEARCH ARTICLE

POINT MUTATIONS ON MITOCHONDRIAL DNA IN IRANIAN PATIENTS WITH FRIEDREICH'S ATAXIA

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Abstract:

Objective

Mitochondrial DNA (mtDNA) is considered a candidate modifier factor for neuro-degenerative disorders. The most common type of ataxia is Friedreich's ataxia (FA). The aim of this study was to investigate different parts of mtDNA in 20 Iranian FA patients and 80 age-matched controls by polymerase chain reaction (PCR) and automated DNA sequencing methods to find any probable point mutations involved in the pathogenesis of FA.

Materials and Methods

We identified 13 nucleotide substitutions including A3505G, T3335C, G3421A, G8251A, A8563G, A8563G, G8584A, T8614C, T8598C, C8684T, A8701G, G8994A and A9024G.

Results

Twelve of 13 nucleotide substitutions had already been reported as polymorphism. One of the nucleotide substitutions (A9024G) had not been reported before. The A9024G nucleotide substitution does not change its amino acid. The controls were also investigated for this polymorphism which was found in two of them (2.5%).

Conclusion

None of the mutations found in this study can affect the clinical manifestations of FA. This survey also provides evidence that the mtDNA A9024G allele is a new nonpathogenic polymorphism. We suggest follow-up studies for this polymorphism in different populations.

Keywords: Mitochondrial DNA, Friedreich's Ataxia

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Introduction

Mitochondrial genome is exclusively maternally inherited, does exhibit high mutation and mutation fixation rates (1). Modifier genes are defined on the basis of their ability to modulate the clinical phenotype of individuals with monogenic and multigenic disease (2). Mitochondrial DNA (mtDNA) could be considered a candidate modifier factor for neurodegenerative disorders, since mitochondrial oxidative stress is thought to be involved in the pathogenesis of degenerative disorders (3,4). The mutation rate for mtDNA is 10 times higher than that of nuclear genomic DNA (2). Many mtDNA diseases present with neurological symptoms and signs, but the clinical features are often non-specific and diffuse, and mitochondrial diseases have frequently been misdiagnosed when only

clinical abnormalities are considered (5). Friedreich ataxia (FA, OMIM# 229300) is an autosomal recessive ataxia resulting from a mutation of a gene locus on Chromosome 9q13-q21.1 (6). Clinical diagnostic criteria for typical cases basically are Progressive limb and gait ataxia develops before the age of 30 years (7,8). Lower extremity tendon reflexes are absent and evidence of axonal sensory neuropathy is noted (7-9). Dysarthria, areflexia, motor weakness of the lower extremities, extensor plantar responses, and distal loss of joint position and vibration senses are not found in all patients within the first 5 years, but are eventually universal.Footdeformity,scoliosis,diabetesmellitus,and cardiac involvement are other common characteristics. Clinical evidence of ventricular hypertrophy, systolic ejection murmurs, and third or fourth heart sounds may be noted (9-11). Detected in about 96% of FA patients an expanded GAA trinucleotide repeat in intron 1 of the gene X25 that encodes a 210 amino acid protein, Frataxin, which is a nuclear-encoded protein located within mitochondrial inner membrane and crests (12). Frataxin is a component of the human Fe/S cluster (ISC) assembly machinery and it plays role in the maturation of both mitochondrial and cytosolic Fe/S proteins and also in heme biosynthesis (2,13). Recently, it has been shown that frataxin interacts with mitochondrial electron transport chain proteins (2,14). Excess iron in mitochondria of FA patients can cause mitochondrial dysfunction through irreversible oxidative damage. Iron is well-known as catalyst of free radicals (2). Patients with FA have a lower rate of mitochondrial ATP production (15). Following the symptoms, failure in ATP production and presence of free radicals in mitochondria of patients with FA was the reason for us to investigate MT-ATP8 (ATP synthase subunit F0 8), MT-ATP6 (ATP synthase subunit F0 6), and highly mutative genes like; MT-LTI (tRNALeucine1(UUA/G)) , MT-NDI (NADH Dehydrogenase subunit 1), MT-COII (Cytochrome coxidase subunit II), MT-TK (tRNALysine), in 20 Iranian FA patients (7 female and 13 male) from 18 unrelated families, by PCR and automated DNA sequencing to find any probable point mutation that can be involved as an adjunct in the pathogenesis of FA.

Materials and Methods

20 patients were chosen randomly who had clinically and genetically diagnosed to have FA according to the criteria of Harding (16) and Geffroy et al. (17). All individuals included in this study were informed on the aims of the study and gave their informed consents to the genetic analysis. We performed mutations screening of MT-LTI, MT-NDI, MT-COII, MT-TK, MT-ATP8 and MT-ATP6. Peripheral blood samples were obtained and DNA was purified after lyses of white blood cells by use of DNA extraction kit. (Diatom DNA Extraction Kit, Genefanavaran, Tehran). PCR amplification was carried out in a final volume of 50 µl containing 200-300 ng total DNA, 10 pmol each primers, 2.5 mM MgCl2, 200 µM of each dNTP, 5µl l of 10X PCR buffer and 2 Units Taq DNA polymerase. (Roche Applied Science)Two sets of primer used are as follows; Primer ONPF25(8161-8180 nt) 5' -CTA CGG TCA ATG CTC TGA AA -3',ONPR185 (9239-9219 nt) 5-' TAC TAT ATG ATA GGC ATG TGA - 3',These primers amplified a 1078 bp sequence encompassing of MT-COII, MT-NC7, MT-TK(tRNALys), MT-ATP8 and MT-ATP6.Another pair of primers is ; ONPF82 (3187-3206 nt) 5'- CTC AAC TTA GTA TTA TAC CC-3', ONPR164(3550-3530nt) 5' -GAT GGT GAG AGC TAA GGT CG -3' were used to amplify a 363 bp sequence of MT-TL1(tRNALeu1(UUA/G). The PCR reactions for amplification of first fragment were performed in a thermal cycler (MWG-Biotech Primus, Germany) for 35 cycles with denaturation at 94ºC for 1´ (min), primer annealing at 55ºC for 1´ (min) and primer extension at 72ºC for 1´ (min). The amplified fragments were separated by gel electrophoresis in 1.5% agarose and for TL1 fragment were performed for 35 cycles with denaturation at 94ºC for 1´ (min), primer annealing at 60ºC for 1´ (min) and primer extension at 72ºC for 35´´ (sec). The amplified fragments were separated by gel electrophoresis in 1.5% agarose. The nucleotide sequences of the amplicons were directly determined by automated sequencing 3700 ABI machine, using primer ONPF25, ONPF82 (Macrogene Seoul, Korea). The obtained mtDNA sequences were aligned with a multiple sequence alignment interface CLUSTAL_X with comparison to revised Cambridge

reference Sequence (rCRS).

Results

We have identified 13 different point mutations in 40% of our FA patients(15% female, 25% male) on; MT-LTI tRNALeu(UUA/G) , MT-NDI ,MT-COII , MT-TK (tRNALys), MT-ATP8 and MT-ATP6 comparing with human mitochondrial DNA rCRS. Out of this 13 different point mutations: 57.14% of them were observed on MT-ATP6, 21.42% of them were found on MT-NDI, 7.14% of them were occurred on MT-COII and, 14% of them were noted on MT-ATP8. One of the mentioned point mutations (table 1) (18-30) were same in two different patients, G 8251 A and A9024G has been reported for the first time.

Discussion

Iron accumulation in the mitochondria of patients with FA would result in hypersensitivity to oxidative stress as a consequence of fenton reaction (Fe2+ production of hydroxyl radicals),as reactive oxygen species (ROS) are continuously generated by the respiratory chain, they may have cause significant oxidative damage to mtDNA (mtDNA deletions and mutations) if not efficiently eliminated(2). Considering the symptoms of FA including progressive gait and limb ataxia, dysarthria, nystagmus, hypertrophic cardiomyopathy (7,10), FA patients have also a lower rate of ATP production (14). We expected to find point mutations related to pathogenesis of FA in MT-ATP8, MT-ATP6, and highly mutative genes like; MT-LTI, MT-NDI, MT-COII and MT-TK. However 13 different mutations had been noted in 40% of the Iranian FA patients (table1), but our results showed that 12 point mutations out of 13 have been reported before as polymorphisms. Only A9024G mutation was found for the first time in this study. Interpretation of the mtDNA sequence data can be extremely difficult because mtDNA is highly polymorphic and it is often difficult and time consuming to establish whether a mutation is pathogenic or not, particularly if the base change has not been reported before. Of course, several canonical criteria suggest that a novel base change is pathogenic: 1) The base change must affect a site that has been conserved during evolution. 2) The mutation should be absent in normal controls. 3) The mutation must not be a known polymorphism (as described by one of the established sequence data bases). 4) The mutation should be reported in several pedigrees with similar phenotypes. 5) There must be a correlation between the levels of mutated mtDNA and severity of symptoms. 6) Deleterious mutations are usually (but not exclusively) heteroplasmic. This implies that the mutation occurred recently and did not have time to "fix"in the female line, or that there was selection against fixation acting at the level of the organism. 7) There is a correlation between the levels of mutated mtDNA and the occurrence of respiratory chain deficiency in individual muscle fiber segments or in cybrid cell lines. 8) Analysis of cybrid cell lines demonstrates that the mtDNA mutation alone is sufficient to cause a respiratory chain deficiency. These stringent criteria depend upon a good knowledge of the polymorphic sites in the background population. Thus, known polymorphic sites could be useful. In the present investigation, two criteria were applied to determine whether A9024G is pathogenic or not. The mutation was present in 2.5% of normal controls with the same ethnic origin and, according to established sequence data bases; the new base change was not a previously known polymorphism. Our data suggest that A9024G is a new nonpathogenic polymorphism in the Iranian population. To confirm whether this substitution is a polymorphism or a mutation, follow-up studies are needed using other criteria applied to different populations.

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Table 1 : mtDNA point mutations found in Iranian FA patients

Number	Nucleotide position	Locus	Amino acid change	Report	References
1	A3505G	MT-NCI	T-A	Yes	(18)
2	T3335C	MT-NDI	I-T	Yes	(19,20)
3	G3421A	MT-NDI	V-I	Yes	(18)
4	G8251A	MT-CO2	A-T	Yes	Unpublished (20)
5	A8563G	MT- ATP6	Syn	Yes	(21)
6	A8563G	MT- ATP8	Syn	Yes	(21)
7	G8584A	MT- ATP6	A-T	Yes	(22,23,24)
8	T8598C	MT- ATP6	Syn	Yes	Unpublished
9	T8614C	MT-ATP6	Syn	Yes	(25)
10	C8684T	MT- ATP6	T-I	Yes	Unpublished
11	A8701G	MT- ATP6	T-A	Yes	(26,27,28)
12	G8994A	MT- ATP6	Syn	Yes	(29,30)
13	A9024G	MT- ATP6	Syn	No	

References

1. Anderson A, Bankier AT, Barrel BG, de Bruijn MHL, Coulson AR,a.Drouin J, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schrier PH,Smith AJH, Staden R, Young IG. Sequence and organisation of the human mitochondrial genome. Nature 1981; 290, 457–465.
2. Houshmand M, Panahi MS, Nafisi S, Soltanzadeh A, Alkandari FM. Identification and sizing of GAA trinucleotide repeat expansion, investigation for D-loop variations and mitochondrial deletions in Iranian patients with Friedreich's ataxia. Mitochondrion 2006; 6(2):82-8.
3. Orth M, Schapira AH. Mitochondria and degenerative disorders. Am J Med Genet 2001;106, 27–36.
4. Janetzky B, Hauck S, Youdim MBH, Riederer P, Jellinger K, Pantucek F, et al. Unaltered aconitase activity, but decreased complex I activity in substantia nigra pars compacta of patients with Parkinson's disease. Neurosci Lett 1994;169(1-2):126-128.
5. Patrick F Chinnery, Neil Howell, Richard M Andrews, and Douglass M .Turnbull Clinical mitochondrial genetics. J Med Genet , Jun 1999; 36: 425 - 436.
6. Chamberlain S, Koenig M, Richter A. Molecular analysis of the Friedreich's ataxia locus. Adv Neurol 1993; 61: 193-204.
7. Durr A, Cossee M, Agid Y, et al. Clinical and genetic abnormalities in patients with Friedreich's ataxia. N Engl J Med 1996 Oct 17; 335(16): 1169-75.
8. Delatycki MB, Williamson R, Forrest SM: Friedreich ataxia: an overview. J Med Genet 2000 Jan; 37(1): 1-8.
9. Johnson WG. Friedreich ataxia. Clin Neurosci 1995; 3(1): 33-8.
10. Jitpimolmard S, Small J, King RH. The sensory neuropathy of Friedreich's ataxia: an autopsy study of a case with prolonged survival. Acta Neuropathol (Berl) 1993; 86(1): 29-35.
11. Babcock M, de Silva D, Oaks R, Davis-Kaplan S,

Jiralerspong S, Montermini L, Pandolfo M, Kaplan J. Regulation of mitochondrial iron accumulation by Yfh1,a putative homolog of frataxin. Science 1997; 276, 1709–1712.

12. CampuzanoV, Monermini L, Molto MD, Pianese L, Cossee M,Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. Science 1996;271, 1423–1427.
13. Stehling O, Elsasser HP, Bruckel B, Muhlenhoff U, Lill R. Iron– sulfur protein maturation in human cells: evidence for a function of frataxin. Hum. Mol. Genet. 2004; 13 (23), 3007–3015.
14. Gonzalez-Cabo P, Vazquez-Manriue RP, Garcia-Gimeno MA, Sanz P, Palau F. Frataxin interacts functionally with mitochondrial electron transport chain proteins. Hum. Mol. Genet. 2005; 14 (15), 2091– 2098.
15. Bradly JL, Blake JC, Chamberlin S, Thomas PK, Cooper JM, Schapira AH. Clinical, biochemical and molecular genetic correlation in Friedreich's ataxia. Hum. Mol. Genet. 2000; 9, 275–282.
16. Harding AE. Friedreich's ataxia: a clinical and genetic study of 90 families with an analysis of early diagnostic criteria and intrafamilial clustering of clinical features. Brain 1981; 104, 589–620.
17. Geffroy G, Barbeau A, Breton G. Clinical description and roentgenologic evaluation of patients with Friedreich's ataxia. Can. J. Neurol. Sci. 1976 ; 3, 279–286.
18. Jones JB, Song JJ, Hempen PM, Parmigiani G, Hruban RH, Kern SE. "Detection of mitochondrial DNA mutations in pancreatic cancer offers a "mass"-ive advantage over detection of nuclear DNA mutations " Cancer Research 2001; 61 (4): 1299-1304.
19. Kosel S, Grasbon-Frodl EM, Mautsch U, Egensperger R, von Eitzen U, Frishman D, Hofmann S, Gerbitz KD, Mehraein P, Graeber MB. "Novel mutations of mitochondrial complex I in pathologically proven Parkinson disease " Neurogenetics 1998;1 (3): 197-204.
20. Chalmers RM, Robertson N, Kellar-Wood H, Compston DA, Harding AE. "Sequence of the human homologue of a mitochondrially encoded murine transplantation antigen in patients with multiple sclerosis " Journal of Neurology 1995;242 (5): 332-334.
21. Ikebe S, Tanaka M, Ozawa T. "Point mutations of mitochondrial genome in Parkinson's disease" Molecular Brain Research 1995;28 (2): 281-295.
22. Qu J, Li R, Tong Y, Hu Y, Zhou X, Qian Y, Lu F, Guan M. X. "Only male matrilineal relatives with Leber's hereditary optic neuropathy in a large Chinese family carrying the mitochondrial DNA G11778A mutation " Biochemical and Biophysical Research Communications 2005;328 (4): 1139-1145.
23. Silva WA, Jr Bonatto SL, Holanda AJ, Ribeiro-Dos-Santos AK, Paixao BM, Goldman GH, Abe-Sandes K, Rodriguez-Delfin L, Barbosa M, Paco-Larson ML, Petzl-Erler ML, Valente V, Santos SE, Zago MA ."Correction: mitochondrial DNA variation in Amerindians " American Journal of Human Genetics.2003;72 (5): 1346-1348.
24. Yao YG, Macauley V, Kivisild T, Zhang YP, Bandelt HJ ."To trust or not to trust an idiosyncratic mitochondrial data set " American Journal of Human Genetics .2003;72 (5): 1341-1346, 1348-1349.
25. Poetsch M, Wittig H, Krause D, Lignitz E. "The impact of mtDNA analysis between positions nt8306 and nt9021 for forensic casework " Mitochondrion .2003;3 (3): 133-137
26. Abu-Amero KK, Bosley TM. "Mitochondrial abnormalities in patients with LHON-like optic neuropathies" Investigative Ophthalmology and Visual Science 2006; 47 (10): 4211-4220.
27. Li R, Qu J, Zhou X, Tong Y, Hu Y, Qian Y, Lu F, Mo JQ, West CE, Guan MX. "The mitochondrial tRNA(Thr) A15951G mutation may influence the phenotypic expression of the LHON-associated ND4 G11778A mutation in a Chinese family ". Gene 2006; 376 (1): 79-86.
28. Povalko N, Zakharova E, Rudenskaia G, Akita Y, Hirata K, Toyojiro M, Koga Y."A new sequence variant in mitochondrial DNA associated with high penetrance of Russian Leber hereditary optic neuropathy. Mitochondrion 2005; 5 (3): 194-199.
29. Poetsch M, Wittig H, Krause D, Lignitz E. "The impact of mtDNA analysis between positions nt8306 and nt9021 for forensic casework " Mitochondrion. 2003; 3 (3): 133-137.
30. Poulton J, Bindoff LA. "mtDNA: Pathogenic or nonpathogenic sequence changes " American Journal of Human Genetics 1994; 54 (2): 385-386.