Mitochondrial G8292A and C8794T mutations in patients with Niemann-Pick disease type C

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Abstract. Niemann-Pick disease type C (NP-C) is a neurovisceral lipid storage disorder. At the cellular level, the disorder is characterized by accumulation of unesterified cholesterol and glycolipids in the lysosomal/late endosomal system. NP-C is transmitted in an autosomal recessive manner and is caused by mutations in either the *NPC1* (95% of families) or *NPC2* gene. The estimated disease incidence is 1 in 120,000 live births, but this likely represents an underestimate, as the disease may be under-diagnosed due to its highly heterogeneous presentation. Variants of adenosine triphosphatase (ATPase) subunit 6 and ATPase subunit 8 (*ATPase6/8*) in mitochondrial DNA (mtDNA) have been reported in different types of genetic diseases including NP-C. In the present study, the blood samples of 22 Iranian patients with NP-C and 150 healthy subjects as a control group were analyzed. The DNA of the blood samples was extracted by the salting out method and analyzed for *ATPase6/8* mutations using polymerase chain reaction sequencing. Sequence variations in mitochondrial genome samples were determined via the Mitomap database. Analysis of sequencing data confirmed the existence of 11 different single nucleotide polymorphisms (SNPs) in patients with NP-C1. One of the most prevalent polymorphisms was the A8860G variant, which was observed in both affected and non-affected groups and determined to have no significant association with NP-C incidence. Amongst the 11 polymorphisms, only one was identified in the *ATPase8* gene, while 9 including A8860G were observed in the *ATPase6* gene. Furthermore, two SNPs, G8292A and C8792A, located in the non-coding region of mtDNA and the *ATPase6* gene, respectively, exhibited significantly higher prevalence rates in NP-C1 patients compared with the control group (P<0.01). The present study suggests that there may be an association between mitochondrial *ATPase6/8* mutations and the incidence of NP-C disease. In addition, the mitochondrial SNPs identified maybe pathogenic mutations involved in the development and prevalence of NP-C. Furthermore, these results suggest a higher occurrence of mutations in *ATPase6* than in *ATPase8* in NP-C patients.

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Abbreviations: NP-C, Niemann-Pick disease type C; ATP, adenosine triphosphate; ATPase, adenosine triphosphatase; PCR, polymerase chain reaction; SNP, single nucleotide polymorphism; mtDNA, mitochondrial DNA; ASM, acid sphingomyelinase; SMC, Special Medical Center of Tehran, Iran; LHON, Leber's hereditary optic neuropathy; iPSC, induced pluripotent stem cell

Key words: Niemann-Pick disease type C, ATPase6/8, point mutation, mitochondrial DNA, single nucleotide polymorphism, Iran

Introduction

Niemann-Pick disease (NPD) is a rare inherited progressive metabolic disorder, classified into subgroups of lysosomal storage disorders that affect lipid metabolism within cells due to genetic mutations (1). There are four most commonly recognized forms of NPD on the basis of genetic cause and symptoms, namely the classic infantile form or type A (NP-A), the visceral form or type B (NP-B), the subacute or juvenile form or type C (NP-C) and the Nova Scotian variant or type D (NP-D) (2). The various types of NPD have been reported in different populations worldwide, but higher rates

of incidence have been identified in certain populations, for instance, the NP-C variant in the French-Canadian population of Nova Scotia, NP-B in the Maghreb region of North Africa, and Np-C in the Spanish-American population of southern New Mexico and Colorado (2).

NP-A (MIM no. 257200) and NP-B (MIM no. 607616), also known as acid sphingomyelinase deficiencies, are caused by deficiencies of the acid sphingomyelinase (ASM) enzyme due to mutations in the sphingomyelin phosphodiesterase1 acid lysosomal gene (mapped at 11p15.4; MIM no. 607608) (3). By contrast, mutations in either the NPD type C1 (*NPC1*) or NPD type C1 (*NPC2*) gene cause NP-C, which is thus classified as NP-C1 (MIM no. 257220) or NP-C2 (MIM no. 607625), respectively (4,5); both are inherited as an autosomal recessive trait (6,7). The *NPC1* gene (mapped at 18q11; MIM no. 607623) encodes NPC1 protein, which is an integral membrane protein of late endosomes involved in lipid transport; while NPC2 protein, encoded by the *NPC2* gene (mapped at 14q24.3; MIM no. 601015), is a soluble non-enzymatic lysosomal cholesterol-binding protein (8). According to previous reports, more than 380 pathogenic mutations in *NPC1* and more than 20 pathogenic mutations in *NPC2* have been implicated in the development of NP-C (9). Approximately 95% of NP-C patients have mutations in the *NPC1* gene; the remainder, presenting with a rarer form of the disease, exhibit mutations in the *NPC2* gene (9). Thus, NP-C1 is more prevalent than NP-C2 in the general population. The total incidence of NP-C is estimated to be approximately 1 in 150,000 individuals (10).

Generally, patients with NP-C are unable to metabolize cholesterol and other lipids within cells. Consequently, excessive amounts of cholesterol accumulate within the liver and spleen, while other lipids accumulate in the brain (11). Additionally, NP-C causes a secondary reduction in ASM activity (11). NP-C is clinically heterogeneous, and there is a broad spectrum of phenotypes with respect to the age of onset and progression of the disease (Fig. 1) (5). Patients with NP-C usually develop a variety of progressive disabling neurological symptoms including ataxia, vertical supranuclear gaze palsy, gelastic cataplexy, spasticity, dystonia, severe liver disease, hepatosplenomegaly, interstitial lung disease, sleep disturbances, problems with speech and swallowing that worsen over time, impedance with feeding and progressive decline of intellectual function (12). Furthermore, cardiac involvement with cardiomegaly, thickened left ventricular wall and substantial endocardial fibroelastosis has been reported in NP-C patients (13). The detection of NP-C is complicated and initial diagnosis is usually based on learning disability, mild retardation, clumsiness and delayed development of fine motor skills. NP-C is typically fatal and the majority of fatalities occur before the age 20. However, in extremely rare cases, some patients reach age 40 (14). In most cases, neurological symptoms appear between the ages of 4 and 10 years, although the timing of onset may range from perinatal years to adulthood (15). Previous research has focused on low-density lipoprotein cholesterol processing in fibroblasts as the basis for the laboratory diagnosis of NP-C (16). The apparent delay in cholesterol egress clearance from the lysosomal compartment results in intracellular accumulation of cholesterol, which maybe visualized by fluorescence microscopy following filipin staining (17,18). NP-C is classically a neurovisceral disease; however, the involvement of visceral organs (the liver, spleen and sometimes lung) and neurological or psychiatric manifestations arise at different time points, and follow independent courses (5). Liver involvement with varying severity is frequently observed in the first months of life in diagnosed patients, where it constitutes the main feature of the disease (14). Regarding therapy, treatment through bone marrow and/or liver transplantation has been attempted without any effect on the neurological outcome (19,20). Miglustat, with the trade name Zavesca, is the first approved drug for treatment of progressive neurological complications in NP-C disease (21). Additionally, induced pluripotent stem cell (iPSC) lines derived from somatic cells of NP-C patients have been established and maybe used as tools to study the pathogenesis of NP-C and evaluate drug efficacy (22). Soga *et al* (23) produced NP-C iPSCs, and based on their findings, 2-hydroxypropyl-γ-cyclodextrin was suggested as a potential novel drug candidate for the future treatment of NP-C. However, beyond the clinical difficulties associated with NP-C, it has been demonstrated that fibroblasts derived from NP-C1 patients were resistant to Ebola virus due to mutation in the NPC1 protein, which is required for viral escape from the intracellular vesicular compartment (24).

Over the last decade, it has become apparent that cholesterol also accumulates in the mitochondria of NP-C1-deficient neurons, which may affect mitochondrial function (25,26). A possible element associated with oxidative damage in NP-C tissues may be mitochondrial dysfunction (27). Indeed, mitochondrial dysfunction and the concomitant oxidative stress appears to be a key element in many neurodegenerative diseases and pathologies associated with liver and cardiac damage (27,28). In previous studies, oxidative stress has been observed in the liver and brain of NP-C mouse models (25,29). Furthermore, Smith *et al* (30) reported an elevation in the level of oxidative stress markers in the serum of NP-C patients. Previous results also suggest that the mitochondrial dysfunction in NP-C plays an important role in its pathogenesis (27,28). It is well established that the mitochondrial DNA (mtDNA) contains 37 individual genes, of which 13 encode the essential subunits of the four mitochondrial respiratory chain complexes (31). The mtDNA also contains certain genes that serve primordial roles in adenosine triphosphate (ATP) synthesis, including adenosine triphosphatase (*ATPase*)6/8 genes. The *ATPase6/8* genes encode two key subunits of the integral membrane domain F_0 (subunit 'a' of F_0 and 'A6L' of F_0, respectively) in the mitochondrial respiratory chain complex V, which is located on the inner mitochondrial membrane. The mitochondrial complex V coordinates the final step in oxidative phosphorylation and uses the proton gradient, created together with the other four enzymatic complexes across the inner mitochondrial membrane, for ATP production (32).

Based on similarities between symptoms of NP-C and mitochondrial disorders, as well as the reported mitochondrial dysfunction and ATP deficiency in NP-C patients (25,33), the present study hypothesized that NP-C patients may carry mutations in their mitochondrial genome, particularly in the *ATPase6/8* region. Therefore, the present study was undertaken to investigate the correlation between mutations in the mitochondrial *ATPase6/8* genes and NP-C incidence in an Iranian population of patients with NP-C.
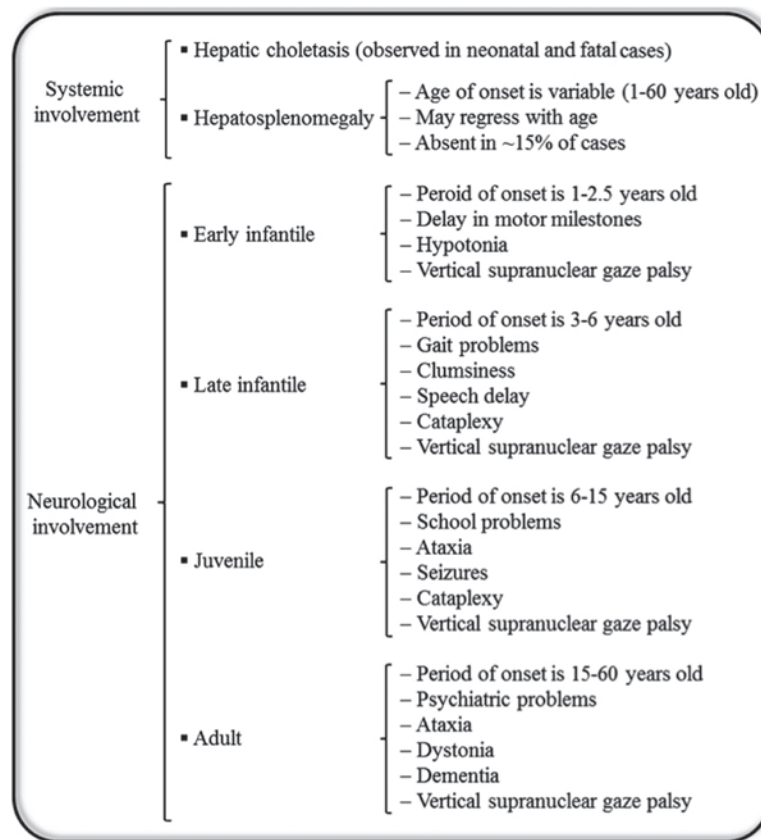
Figure 1. Niemann-Pick disease type C. The diagram represents subtypes and onset ages of visceral and neurological manifestations.

Materials and methods

Patient samples. Peripheral blood samples (10 ml) were collected from 150 healthy donors (75 male and 75 female) as a control group and 22 Iranian NP-C1 patients (16 male and 6 female) with no familiar relation, who were diagnosed based on the criteria of the Special Medical Center (SMC; Tehran, Iran), as described in our previous study (34). The blood samples were collected between March 2009 and March 2012 in Neurology Departments at the SMC. As all subjects in the present study were minor (between 4 months and 12 years old), their legal guardians were informed on the aims of present study, and signed a written consent form prior to blood collection agreeing to subject participation in the genetic analysis study and publication of the related results as anonymized data. This consent form was approved according to a protocol on human and patient rights by the SMC governing Ethics Committee. The study protocol was approved by the SMC board.

DNA sequencing. Total DNA was extracted from the whole blood samples via the salting out method using a Diatom DNA extraction kit (Gen Fanavaran Co., Tehran, Iran), according to the manufacturer's instructions. To analyze the variations in mitochondrial *ATPase6/8* genes, polymerase chain reaction (PCR) sequencing was performed as described previously with some modifications (35). The size of the amplicon was 1,078 bp and the sequences of the oligonucleotides used for amplification of the *ATPase6/8* region are listed in Table I. Sequencing of the PCR products was performed in the forward and reverse directions for confirmation of observed variations using an ABI Prism 3100 automated sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) by Kowsar Company (Tehran, Iran).

Bioinformatics analysis. The sequences were analyzed with the FinchTV software (version 1.5.0; Geospiza, Inc., Seattle, WA, USA) and compared with human reference sequences in the GenBank database (GenBank ID: J01415.2; https://www. ncbi.nlm.nih.gov/genbank/). Finally, observed variations were compared between affected and non-affected groups. Additionally, the Mitomap database (https://www.mitomap. org/MITOMAP) was used to identify sequence variations of the mitochondrial genome in the affected and non-affected groups. The observed non-synonymous single nucleotide polymorphisms (SNPs) were analyzed with the I-Mutant 2.0 (developed by Capriotti *et al* (36); available online: http:// folding.uib.es/i-mutant/i-mutant2.0.html) and the PolyPhen-2 software (developed by Adzhubei *et al* (37); available online: http://genetics.bwh.harvard.edu/pph2/) to predict the possible impacts of amino acid substitutions upon mutations on stability and function of the subjected protein based on comparative physical and evolutionary considerations, respectively. This prediction analysis at the molecular level aimed to identify SNPs affecting actual phenotypes.

Statistical analysis. For statistical analysis, the Fisher's exact test was performed using SPSS software (version 22.0.0.0; IBM Corp., Armonk, NY, USA) to assess associations between observed mutations in NP-C patients and the healthy control

Table I. Sequences of primers used for polymerase chain reaction sequencing of the mitochondrial *ATPase6/8* region.

Primer	Nucleotide position	Sequence, 5'-3'	Ampliconsize, bp
Forward	8161-8180	CTACGGTCAATGCTCTGAAA	1,078
Reverse	9219-9239	TACTATATGATAGGCATGTGA	

Table II. Demographic characteristics of subjects in the affected and non-affected groups.

Group	Sex	No. of individuals	Disease	Age range
Affected	F	6	NP-C1	5-12 years
	M[a]	16	NP-C1	4 months-12 years
Non-affected	F	75	Healthy	5-12 years
	M	75	Healthy	5-12 years

[a]The age of two patients in this group was between 4 months and 5 years old, and the remaining patients were between 5 and 12 years old. M, male; F, female; NP-C1, Niemann-Pick disease type C1.

group. Data are presented as the mean prevalence rate (%) of each observed mutation in the affected and non-affected groups. These data are accompanied with the number of individuals who have carried the point mutation per total examined individuals in each affected and non-affected group. The frequency of each observed mutation in the affected group was compared to the frequency of same mutation in the non-affected group and a value of $P<0.05$ was considered to indicate statistical significance.

Results

Patient characteristics. All cases in the affected group (16 male and 6 female) presented with NP-C1 (Table II).

Gene mutations. Overall, 10 different point mutations in the *ATPase6/8* genes and one point mutation in the non-coding region of mtDNA (MT-C7) were observed in the patient group, which are summarized in Table III. Among all patients, 72.7% (16/22) carried at least one point mutation in the mitochondrial genome. One of the point mutations, the A8860G mutation in *ATPase6*, was commonly observed in the examined individuals. In the investigated population, 72.7% (16/22) of patients as well as 74.7% (112/150) of the healthy subjects carried the SNP A8860G. In addition, the G8292A mutation, which was identified in the non-coding region of mtDNA, was observed in 9.1% (2/22) of the patient group, while none of the healthy subjects carried this mutation; thus it was deemed to be significantly increased in patients ($P<0.01$). Only one of the observed SNPs occurred in the *ATPase8* gene, namely T8473C. This mutation leads to a synonymous amino acid change in the ATPase8 protein, and was observed in 4.6% (1/22) of the patient group while being absent from the control group ($P>0.05$ vs. control group). The remaining eight point mutations were observed in the *ATPase6* gene, of which only one was a synonymous mutation. This mutation, C8574T, was observed in 4.6% (1/22) of patients. The other observed *ATPase6* SNPs were missense

mutations, which overall were detected in 9 individuals of the patient group and 18 individuals of the control group; details of their prevalence in the investigated populations are summarized in Table III. Among these missense mutations, only the C8794T variation had significantly increased frequency compared with controls ($P<0.01$), with a detection rate of 9.1% (2/22) in the examined NP-C1 patients.

Bioinformatics analysis. The results of analysis with I-Mutant 2.0 software indicated that all observed SNPs excluding the synonymous mutations (C8574T and T8473C) and mutation in the non-coding region of mtDNA (G8292A) may decrease the stability of the protein. In addition, PolyPhen-2 software predicted all above mentioned SNPs to decrease protein stability, possibly leading to protein dysfunction, with the exception of SNPs C8684T, C8794T and A8860G, which were predicted to be benign and to not affect protein function. The prediction results of I-Mutant 2.0 and PolyPhen-2 are summarized in Table IV.

Discussion

NP-C is a rare fatal disorder with a lack of effective treatments available. The disease is considered as a neurovisceral lipid storage disorder; however, the underlying pathogenic mechanism that links the accumulation of intracellular cholesterol/lipid with cell death in the central nervous system and liver is currently unknown. According to previous studies, oxidative stress leading to oxidative damage is observed in different tissues of NP-C patients (27,28,30), which suggests its involvement in the pathogenesis of NP-C. Furthermore, previous data suggests that mitochondrial dysfunction is associated with oxidative damage in NP-C patients and serves an important role in NP-C pathogenesis (27). Indeed, it has been reported that mitochondrial dysfunction and the associated oxidative stress appear to be key aspects in many neurodegenerative diseases and disorders associated with liver and cardiac

Table III. Frequency of mitochondrial *ATPase6/8* SNPs in NP-C patients.

Gene	Symbol	Gene location	Variation location	Allele	Type of variation	Mutation status	Prevalence, % (n/total)		P-value	Disease association (ref.)
							Controls	Patients		
MT-NC7	NC7	MT:8270-8294	MT:8292	G/A	SNP	Hm	0.0 (0/150)	9.1 (2/22)	0.007[a]	Myopathy (61), sudden infant death syndrome (62), maternally inherited diabetes and deafness (63)
MT-ATP6	ATPase6	MT:8527-9207	MT:8574	C/T	SNP	Hm	0.0 (0/150)	4.6 (1/22)	0.080	Diabetes (64)
MT-ATP6	ATPase6	MT:8527-9207	MT:8594	T/C	SNP	Hm	0.0 (0/150)	4.6 (1/22)	0.080	Friedreich's ataxia (65)
MT-ATP6	ATPase6	MT:8527-9207	MT:8629	A/G	SNV	Hm	0.0 (0/150)	4.6 (1/22)	0.080	Not reported
MT-ATP6	ATPase6	MT:8527-9207	MT:8684	C/T	SNP	Hm	6.0 (9/150)	9.1 (2/22)	1.000	Multiple sclerosis (66), Huntington's disease (67), autism (35), osteosarcoma (68), thyroid tumor (69)
MT-ATP6	ATPase6	MT:8527-9207	MT:8696	T/C	SNP	Hm	0.0 (0/150)	4.6 (1/22)	0.080	Pancreatic cancer cell line CFPAC (70)
MT-ATP6	ATPase6	MT:8527-9207	MT:8697	G/A	SNP	Hm	6.0 (9/150)	4.6 (1/22)	0.190	LHON (47), thyroid tumor (69)
MT-ATP6	ATPase6	MT:8527-9207	MT:8794	C/T	SNP	Hm	0.0 (0/150)	9.1 (2/22)	0.007[a]	Coronary atherosclerosis risk (56)
MT-ATP6	ATPase6	MT:8527-9207	MT:8860	G/A	SNP	Hm	74.7 (112/150)	72.7 (16/22)	0.000	Colorectal cancer (50), ovarian cancer (51), breast cancer (52), human glioma cells, glioblastoma multiforme cell line (GBM) (71), osteosarcoma (69), LHON (45)
MT-ATP6	ATPase6	MT:8527-9207	MT:8975	T/C	SNP	Hm	0.0 (0/150)	4.6 (1/22)	0.080	Deafness (72)
MT-ATP8	ATPase8	MT:8366-8572	MT:8473	T/C	SNP	Hm	0.0 (0/150)	4.6 (1/22)	0.080	Atypical psychosis (58)

The frequency of each SNP in the affected (22 individuals) and non-affected (150 individuals) groups is presented. Of note, none of the observed mutations in the present study have been reported in NP-C patients to date. Studies that have reported the same mutation in different diseases are listed in the far right column. [a]SNP frequency in the affected group was compared to its frequency in the non-affected group and $P<0.01$ was considered as a significant value. Hm, Homoplasmic; NP-C, Niemann-Pick disease type C; SNP, single nucleotide polymorphism; SNV, single nucleotide variant; MT, mitochondrial; LHON, Leber's hereditary optic neuropathy; ATP, adenosine triphosphate; ATPase, adenosine triphosphatase.

Table IV. Prediction of possible impact of amino acid substitution on protein structure stability and function for point mutations of *ATPase6/8* genes.

Location	SNP	Amino acid substitution	Consequence	I-Mutant (reliability index)	polyphen-2 HumDiv (score)
MT-NC7	G8292A	-	Non-coding	-	-
MT-ATP6	C8574T	G16G	Synonymous	-	-
	T8594C	I23T	Missense	Decrease stability (8)	Possibly damaging (0.620)
	A8629G	K35E	Missense	Decrease stability (5)	Possibly damaging (0.590)
	C8684T	T53I	Missense	Decrease stability (0)	Benign (0.010)
	T8696C	M57T	Missense	Decrease stability (0)	Possibly damaging (0.950)
	G8697A	M57I	Missense	Decrease stability (4)	Possibly damaging (0.890)
	C8794T	H90Y	Missense	Decrease stability (1)	Benign (0.000)
	A8860G	T112A	Missense	Decrease stability (7)	Benign (0.000)
	T8975C	L150P	Missense	Decrease stability (5)	Possibly damaging (0.791)
MT-ATP8	T8473C	P36P	Synonymous	-	-

The prediction of protein structure stability in the presence of SNPs was performed using I-Mutant software; the suggested reliability index of each amino acid substitution is listed. The impact of these amino acid changes on protein function as damaging or benign was predicted using PolyPhen-2 software; the suggested scores for these predictions are listed. MT, mitochondrial; ATP, adenosine triphosphate; SNP, single nucleotide polymorphism.

damage (27,38,39). The role of mitochondrial dysfunction in the pathogenesis of NP-C remains unknown, but studies have identified mitochondrial dysfunction and ATP deficiency in NP-C patients (25,33). In addition, ATP deficiency in parallel with higher cholesterol content has been observed in mitochondria in NP-C mouse models, in which ATP synthesis and mitochondrial function were restored following treatment with the cholesterol chelator cyclodextrin (25). Alterations in ATP production, mitochondrial morphology and input of glutathione to the mitochondrial matrix have been reported in NP-C mouse models, and were correlated with enhanced mitochondrial cholesterol content as well as reduced ATP synthesis, enhanced reactive oxygen species generation, and increased energy demand in NP-C cells (27). These findings support the hypothesis that mitochondrial dysfunction may contribute to oxidative damage in NP-C. In the present study, the potential mutations in mitochondrial *ATPase6/8* genes, which are key elements in cellular energy production, were analyzed in NP-C patients. Different mutations in the *ATPase6/8* genes, which encode subunits of complex V, have been reported in various mitochondrial diseases including neuropathy, ataxia, and retinitis pigmentosa syndrome (40), Leber's hereditary optic neuropathy (LHON) (41), maternally inherited Leigh syndrome (39) and hypertrophic cardiomyopathy (42).

In the present study, 10 SNPs in mitochondrial *ATPase6/8* genes and one variation in the non-coding region of mtDNA were identified in unrelated Iranian NP-C patients. Overall, 73% of examined NP-C patients carried at least one point mutation in their mtDNA. This finding suggests that mitochondria and defects in the respiratory chain may be involved in the pathogenesis of NP-C. An observed mutation was the A8860G variation in the mitochondrial *ATPase6* gene, which was observed in 73% of the NP-C1 patients, and also 75% of unrelated healthy individuals. According to previous reports, this SNP is a common variation and has been identified in

different populations (43). The A8860G SNP has been reported in different diseases including Alzheimer's (44), hypertrophic cardiomyopathy (45), LHON (46,47) and breast cancer (48), and also in the mitochondrial haplogroup R0H2a2a (49). The present findings also support previous data (45) that identified the A8860G SNP in a high proportion of affected and non-affected individuals. Analysis with I-Mutant 2.0 software predicted that A8860G decreases stability of ATPase6 protein. However, based on PolyPhen-2 prediction, it is a benign point mutation and has no impact on protein function. This may be due to the fact, that A8860G is located in a poorly conserved protein region. Thus it is frequently observed in various healthy and patient populations (43,49).

Amongst all observed SNPs, significant differences were observed between NP-C1 patients and healthy subjects regarding the frequencies of the SNPs G8292A and C8794T, which are located in the non-coding region of mtDNA (*NC7*) and in the *ATPase6* gene, respectively. This suggested that the G8292A and C8794T mtDNA SNPs in patients confer genetic susceptibility to NP-C1. The G8292A SNP has been also reported in different cancers including colorectal adenomatous polyps (50), epithelial ovarian cancer (51) and sporadic breast cancer (52), and in the mitochondrial haplogroup R0a1a3 (48). Additionally, it was identified by Cerný *et al* (53) in LHON patients with haplogroup R0a1a1a in Yemen. These patients also carried the minor pathological mutation A3395G. No conservation rate is reported for the G8292A mutation in the Mitomap database (query no. HM185203.1). The other significant SNP, C8794T, is a homoplasmic missense mutation in the *ATPase6* gene, predicted to lead to stability reduction of the protein, but also to be a benign mutation that does not change the function of the ATPase6 enzyme. However, the observed association of C8794T SNP with the NP-C phenotype indicates a potential pathophysiological effect of C8794T, which requires further investigation. The C8794T SNP has been demonstrated to be

involved in different diseases including breast cancer (47), early onset cataracts and focal dystonia (54) and also to be a specific variant of mitochondrial haplogroup A in an East Asian population (55). In addition, Sawabe *et al* (56) reported that Japanese elderly subjects belonging to haplogroup A had a genetic risk of coronary atherosclerosis. Another report by Yu *et al* (57) identified the C8794T mutation in LHON Asian patients belonging to haplogroup A5, who also carried the major pathological mutation G3460A. According to the Mitomap database, the conservation rate of C8794T mutation is 73.33% (query no. GQ999962.1).

The A8629G point mutation, which was identified in the mitochondrial *ATPase6* gene and only in the affected group (1/22), was reported, to the best of our knowledge, for the first time in the present study. The A8629 variant leads to a missense mutation, which was predicted to decrease stability of protein structure without affecting protein function. Although this point mutation was determined to be not significantly associated with NP-C disease, it can be regarded as a novel mitochondrial *ATPase6* single nucleotide variant.

The point mutation T8473C was identified in the mitochondrial *ATPase8* gene and was observed in 5% (1/22) of the affected group. This SNP causes a synonymous mutation in ATPase8 protein and was predicted to cause no functional disturbance. It has been previously detected in patients with a typical psychosis (58) and colorectal cancer (59). As among the 11 different point mutations in NP-C patients reported in the present study, only one occurred in the mitochondrial *ATPase8* gene. It may be assumed that the *ATPase6* gene has a higher susceptibility than the *ATPase8* gene to the occurrence of mutations. Similarly, it has been reported in breast cancer patients that the *ATPase6* gene had greater susceptibility to mutation than *ATPase8* (48). mtDNA point mutations are typically maternally inherited, but can also occur sporadically (60). The reported mtDNA point mutations in the present study were observed only in the examined unrelated NP-C patients, whereas their relatives (especially their mothers) were not analyzed for the presence of these mutations. Therefore, the current study is unable to discriminate whether the reported mutations were sporadic or familial and maternally inherited.

In conclusion, the present study identified that 73% of the Iranian NP-C patients were carrying one or more mitochondrial mutation. Furthermore, the present study, to the best of our knowledge, is the first to report mitochondrial *ATPase6/8* SNPs in NP-C patients. The current findings propose a perspective in the etiology of NP-C by indicating an association between the mitochondrial SNPs G8292A and C8794T and the incidence of NP-C disease. The detection of these SNPs may be clinically important for improving our understanding about the genotypic spectrum of NP-C, which may aid in determining the diagnosis, prognosis and treatment of NP-C patients in the future.

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Availability of data and materials

The datasets used and/or analyzed during the current study included in this published article.

Authors' contributions

AM performed the experimental procedures, data analysis and drafted the manuscript. FS participated in data interpretation and writing the manuscript. LA, SHT, PK, MRA, HS and SM participated in coordination of the study and data analysis. MH conceived the study and aided with the study design. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The blood samples were provided by the Special Medical Center, funded by the Iranian Health Ministry.

Consent for publication

All subjects provided written informed consent to participate in the study and permitting the publication of relevant data on the terms of data anonymization.

Competing interests

All authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this study.

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