Original Article

Mitochondrial Copy Number and D-Loop Variants in Pompe Patients

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Abstract

Objective: Pompe disease is a rare neuromuscular genetic disorder and is classified into two forms of early and late-onset. Over the past two decades, mitochondrial abnormalities have been recognized as an important contributor to an array of neuromuscular diseases. We therefore aimed to compare mitochondrial copy number and mitochondrial displacement-loop sequence variation in infantile and adult Pompe patients.

Materials and Methods: In this retrospective study, the mitochondrial D-loop sequence was analyzed by polymerase chain reaction (PCR) and direct sequencing to detect possible variation in 28 Pompe patients (17 infants and 11 adults). Results were compared with 100 healthy controls and sequences of all individuals were compared with the Cambridge reference sequence. Real-time PCR was used to quantify mitochondrial DNA copy number.

Results: Among 59 variants identified, 37(62.71%) were present in the infant group, 14(23.333%) in the adult group and 8(13.333%) in both groups. Mitochondrial copy number in infant patients was lower than adults (P<0.05). A significant frequency difference was seen between the two groups for 12 single nucleotide polymorphism (SNP). A novel insertion (317-318 ins CCC) was observed in patients and six SNPs were identified as neutral variants in controls. There was an inverse association between mitochondrial copy number and D-loop variant number (r=0.54).

Conclusion: The 317-318 ins CCC was detected as a new mitochondrial variant in Pompe patients.

Keywords: Pompe, Mitochondrial DNA, D-Loop, Copy Number

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Introduction

Pompe disease (PD) or glycogen storage disease type II is a rare neuromuscular genetic disorder (1). Patients have a deficiency or lack of acid alpha-glucosidase (*GAA*) or acid maltase lysosomal enzyme due genetic mutations. It has an autosomal recessive pattern of inheritance (2) with an incidence ranging from 1:33,000 to 1:300,000 in different ethnicities (3). PD is classified by age of onset into two forms of infantile and late-onset (4). Accumulation of glycogen in vital organs such as cardiac, smooth and skeletal muscles leads to a broad spectrum of clinical features (5). The infantile form strongly affects cardiac, respiratory and skeletal muscles and enzyme activity is less than 1% of normal controls. They present hypertrophic cardiomyopathy, respiratory insufficiency, hypotonia and failure to thrive within the first few months of life. Late-onset Pompe is characterized by enzyme activity of about 2 to 40% and a prominent

proximal skeletal involvement (4, 6).

Clinical manifestations are different according to disease severity (7, 8). Several mutations are recognized in the *GAA* gene, but the same mutation in two patients may lead to different manifestations and thus an alternative process seems to affect *GAA* mutations (9). Disease features are probably modified by unknown genetic and environmental factors (10).

Mitochondria generate adenosine thriphosphate (ATP) in cells. There is strong evidence that mitochondrial DNA (mtDNA) variation can enhance oxidative stress. It might also play a secondary role in additive damages (11). Over the past two decades, mitochondrial abnormalities have been recognized as important contributors to an array of neuromuscular diseases (12). Therefore mtDNA variation might be a modifying genetic factor that interacts with *GAA* mutation and may explain lack of strong genotype-phenotype correlation between *GAA* and PD (13). Knowledge about the role of mitochondria in the pathophysiology of PD is limited. Previous studies have shown abnormal mitochondria in Pompe patients, but were not focused on mitochondrial genes (14-17). These studies mentioned mitochondrial structural abnormalities as secondary rather than primary importance. Clinical phenotypes caused by mtDNA mutations are variable. Many phenotypes induced by mitochondrial diseases (cardiomyopathy, hypotonia, developmental delay and skeletal muscle manifestations) are the same as PD. The latter is the most common manifestation of mitochondrial disease. In addition, similar to PD, infantile mitochondrial disease is typically more severe than its adult-onset form. Over the last decade, most of the research on PD has been focused on treatment in spite of an unclear pathophysiology. Understanding the pathogenicity of PD is necessary to therapy. For example, cardiac muscle responds well to therapy in contrast to skeletal muscle. Also, it is not clear why it is such a difficult target for enzyme replacement therapy (ERT). Poor response of skeletal muscle to therapy led us to examine other probable intervening genetics factors.

Human mtDNA comprises a 16569-base pair double strand circular genome, which encodes 13 proteins (from 37 genes) of the respiratory chain, 2 rRNAs and 22 tRNAs (18, 19). D-loop is the regulatory part of mtDNA, which has major role in transcription and replication, and is 1122 bp long (20). D-loop contains two hyper-variable regions (HVR1 at nucleotides 16024-16383 and HVR2 at nucleotides 57-372) (21, 22). There is also a tandem repeat of poly C in D-loop region from 303 to 315 nucleotides. Since D-loop has a regulatory role in mtDNA replication and transcription, mutations in this region might have a significant effect on copy number and gene expression of the mitochondrial genome, thus potentially disturbing mitochondrial function, oxidative phosphorylation (OXPHOS) and ATP production.

If mitochondrial dysfunction contributes to pathogenesis, ameliorating its effects could modify clinical symptoms of patients. In addition, identification of mitochondrial mutations or polymorphisms specific to infantile or adult forms of the disease may be useful as possible biomarkers. Therefore, this study investigated mitochondrial copy number and mitochondrial D-loop region variation in infantile and adult Pompe patients of Iranian origin.

Materials and Methods

Subjects

In this retrospective study, we recruited 28 Pompe patients (15 male and 13 female) from the Department of Neurology of Shariati and Mofid hospitals between December 2013 and February 2015. There were 17 and 11 infant and adult patients, respectively. Moreover, 100 healthy controls were recruited (17 infants and 83 adults). An informed consent was obtained from each participant or parents in the case of infant cases. PD was diagnosed in participants based on clinical findings by two expert neurologists, measurement of *GAA* biochemical activity or mutation detection in the *GAA*. Patients with no family history of mitochondrial or major neuromuscular disorders such as Duchene muscular dystrophy were included. The study was approved by the Ethical Committee of Tehran University of Medical Sciences.

DNA extraction

DNA was extracted from whole blood by standard salting out protocol and using QIAamp DNA Blood Mini Kit according to the manufacturer's instructions (QIAGEN, Germany). Quantity and

quality of DNA were checked by NanoDrop ND-1000 (NanoDrop Technologies, USA) at 260/280 nm and running on agarose gel (1%), respectively.

Polymerase chain reaction-sequencing analysis

Polymerase chain reaction (PCR) was performed with two pairs of primers

F: 5′-GATCACAGGTCTATCACCCT-3′
R: 5′-AGTACACTTACCATGTTACG-3′ and
F: 5′-CTCCTGCTTGCAACTATAGC-3′
R: 5′-GCTCCGGCTCCAGCGTCTGC-3′

to amplify the entire D-loop as described previously (23). PCR master mix included 5 ng of genomic DNA, 0.8 µL of each primer (10 pmol), 0.2 mM of each deoxynucleoside triphosphate (dNTP), 1.5 mM $MgCl_2$ and 1 U of Taq polymerase enzyme (CinnaGen, Inc, Iran). PCR cycling conditions for the first pair of primers were an initial denaturation at 94˚C for 4 minutes, followed by 35 cycles of denaturation at 94˚C for 60 seconds, annealing at 57˚C for 35 seconds and extension at 72˚C for 35 seconds, and a final extension at 72˚ C for 7 minutes. PCR conditions for the second pair of primers were an initial denaturation at 95˚C for 5 minutes, followed by 35 cycles of denaturation at 94˚C for 60 seconds, annealing at 58˚C for 60 seconds and extension at 72˚C for 35 seconds, and a final extension at 72˚C for 5 minutes. PCR-amplified fragments were sequenced by Macrogen (South Korea) using the same PCR primers in two directions and series of overlapping primers to cover all regions of interest for more accurate results. Finch TV 1.4 software (Geospiza, Inc., USA) was used to analyze sequences and were then checked using BLAST. The results were compared with the Cambridge reference sequence (20).

Determination of mitochondrial DNA copy number

The mtDNA copy number was determined using a real-time PCR assay. In brief, to quantify mtDNA content relative to nuclear DNA (nDNA), primers for specific amplification of *ND2* of mtDNA and nDNA-encoded *β-actin* gene were selected as described previously (24).

Forward and reverse primers as follow:
ND2:
F: 5′-CCCTTACCACGCTACTCCTA-3′
R: 5′-GGCGGGAGAAGTAGATTGAA-3′ (279 bp);
β-actin:
F: 5′-ATCATGTTTGAGACCTTCAACA- 3′
R: 5′-CATCTCTTGCTCGAAATCCA-3′ (318 bp).

Real-time PCR was performed on a Corbett 6000 PCR-Real-time Detection System with a total volume of 20 µL reaction mixture containing 1 µL DNA template (5 ng), 10 µL SYBR Green PCR Master Mix (Takara, Japan), 8 µL nuclease- free water and 0.5 µL of each primer (10 pmol). Real time PCR protocol was an initial activation step at 95˚C for 10 seconds followed by 40 cycles including a denaturation step at 95˚C for 12 seconds and an annealing step at 60˚C for 35 seconds. Melting curve analysis was used to validate a PCR product for each primer pair. The copy number of mtDNA D-loop region in each tested specimen was then normalized against that of *β-actin* to calculate relative mtDNA copy number based on the $2^{-\Delta\Delta Ct}$ relative expression formula. Each measurement was repeated in duplicate and a non-template control was included in each experiment.

Statistical methods

Participant characteristics, mitochondrial D-loop variants and copy number were described as mean ± SD by SPSS statistical software for windows (IBM version 16, USA). Fisher's exact and Chi-square tests were used to compare frequency of mitochondrial D-loop variants in infantile and adult Pompe patients, while a linear regression model was used to determine correlation between mtDNA copy number and D-loop variants numbers. $P<0.05$ was considered statistically significant.

Results

Patients' characteristics are shown in Table 1. The mean age of the control group was 23.29 ± 3.12 years. Fisher's exact test showed no statistically significant difference between infant and adult patient groups regarding sex distribution (P=0.687) and family history (P=0.417). Screening of D-loop region in 28 cases of Pompe patients resulted in the identification of 59 variants. The variants absent in MITOMAP (www.mitomap.org) and other databases were checked in controls. A significant frequency difference was seen between the two groups for 12 SNPs ($P<0.05$, Table 2). Six SNPs were found as normal variants in controls compared with patients (Table 2, T152C, 514-515 del CA, A272G, T16352C, G16319A, A73G). From the variants seen in the patient group, 317-318 ins CCC was novel (Fig.1).

Table 1: Baseline clinical characteristics of Pompe patients

Characteristics	Infant	Adult
Gender, (n)		
Male	10	5
Female	7	6
Age of onset (Y, mean ± SD)	0.62 ± 0.43	21.7 ± 12.1
Muscular Pain, (n)		
No	NA	9
Yes	NA	0
Creatine kinase (U/L, mean ± SD)	1316.58 ± 327.11	611.37 ± 211.62
Vital capacity (cm^3, mean ± SD)	NA	56.14 ± 28.08
Walton score (mean ± SD)	NA	2.71 ± 0.49
6 minute waking test (mean ± SD)	NA	369.43 ± 111.58
Family history, n (%)		
Yes	8 (47.06)	4 (37.36)
No	9 (52.94)	7 (63.63)

NA; Denotes values that were not measurable, SD; Denotes standard deviation, and n (%); Denotes number (percent) of patients in each group. Walton score range is 0 to 10, in which 0 and 10 indicate the normal and the worst status, respectively.

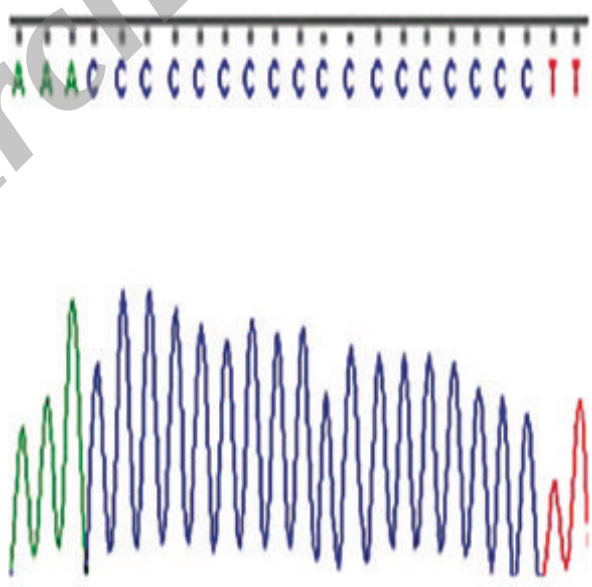

Fig.1: Electropherogram of the 317-318 insertion (CCC) with homoplasy.

Table 2: Mitochondrial D-loop variants in infantile and adult Pompe patients

Infant/Adult	Nucleotide	R/NR[a]	Ho/Ht[b]	Pompe +/- [c]	Control +/- [c]	P value
Adult	A62G	R	Ho	2 / 26	0 / 100	0.012
	A93G	R	Ho	2 / 26	0 / 100	0.012
	C194T	R	Ho	2 /26	0 / 100	0.012
	G207A	R	Ho	1 / 27	0 / 100	0.090
	A235G	R	Ho	1 / 27	1 / 99	0.425
	T310C	R	Ho	1 / 27	0 / 100	0.090
	310 D C7TC6	R	Ho	2 / 26	0 / 100	0.012
	310 D C8TC6	R	Ho	2 / 26	0 / 100	0.012
	317-318 ins CCC	NR	Ho	1 / 27	0 / 100	0.090
	A503G	R	Ho	1 / 27	2 / 98	0.737
	513 ins GCA	R	Ho	1 / 27	0 / 100	0.090
	G16222T	R	Ho	3 / 25	9 / 91	0.786
	C16261T	R	Ho	1 / 27	10 / 90	0.255
	C16266T	R	Ho	2 / 26	0 / 100	0.012
Infant	G103A	R	He	1 / 27	0 / 100	0.090
	C150T	R	Ho	1 / 27	0 / 100	0.090
	C151T	R	Ho	2 / 26	8 / 92	0.882
	T152C	R	Ho	4 / 24	50 / 50	0.002
	A153G	R	Ho	1 / 27	16 / 84	0.052
	G185A	R	Ho	1 / 27	0 / 100	0.090
	G228A	R	Ho	1 / 27	0 / 100	0.090
	C242T	R	Ho	1 / 27	15 / 85	0.07
	A272G	R	Ho	1 / 27	100 / 0	<0.001
	C295T	R	Ho	3 / 25	12 / 88	0.853
	C456T	R	Ho	1 / 27	0 / 100	0.090
	C462T	R	Ho	3 / 25	0 / 100	0.003
	T480C	R	Ho	1 / 27	0 / 100	0.090
	T489C	R	Ho	6 / 22	0 / 100	<0.001
	514-515 del CA	R	Ho	3 / 25	33 / 67	0.028

Table 2: Continued

Infant/Adult	Nucleotide	R/NR[a]	Ho/Ht[b]	Pompe +/- [c]	Control +/- [c]	P value
	C553A	R	Ho	1 / 27	1 / 99	0.425
	G709A	R	Ho	1 / 27	0 / 100	0.090
	A16051G	R	Ho	2 /26	4 / 96	0.493
	G16145A	R	Ho	1/ 27	7 / 93	0.451
	A16163G	R	Ho	1 / 27	4 / 93	0.822
	A16183C	R	Ho	1 / 27	8 / 92	0.372
	T16189C	R	Ho	1 / 27	4 / 96	0.821
	C16248T	R	Ho	1 / 27	1 / 99	0.425
	C16256T	R	Ho	1 / 27	4 / p6	0.493
	C16278T	R	Ho	1 / 27	7 / 93	0.450
	C16294T	R	Ho	1 / 27	1 / 99	0.425
	C16295T	R	Ho	2 / 26	1 / 99	0.068
	T16298C	R	Ho	1 / 27	1 / 99	0.425
	A16300G	R	Ho	1 / 27	1 / 99	0.425
	T16304C	R	Ho	2 / 26	3 / 97	0.326
	A16309G	R	Ho	1 / 27	10 / 90	0.254
	A16318T	R	Ho	1 / 27	0 / 100	0.090
	C16320T	R	Ho	1 / 27	2 / 98	0.737
	T16352C	R	Ho	1 / 27	100 / 0	<0.001
	C16354T	R	Ho	1 / 27	0 / 100	0.090
	T16362C	R	Ho	2 / 26	1 / 99	0.068
	G16390A	R	Ho	2 / 26	7 / 93	0.979
Both	T72C	R	Ho	2 / 26	0 / 100	0.012
	A73G	R	Ho	10 / 18	81 / 19	<0.001
	T146C	R	Ho	6 / 22	0 /100	<0.001
	T195C	R	Ho	7 / 21	2 / 98	<0.001
	A211G	R	Ho	1 / 27	0 / 100	0.090
	A263G	R	Ho	23 / 4	70 / 30	0.214
	A385G	R	Ho	2 / 26	19 / 81	0.146
	T16311C	R	Ho	2 / 26	2 / 98	0.178

[a], R, NR; Denote reported and non-reported variants respectively in previous studies, [b], Ho, Ht; Denote homoplasmy and heteroplasmy, respectively, and [c], + and -; Show positive and negative results for each variant, respectively.

Of the 59 variants, 37 (62.71%) variants were observed in the infant group, 14 (23.333%) in the adult group and 8 (13.333%) in both groups. The mean number of D-loop region variation in each patient was 6.0714. The mean number of variants in infants and adults were 7.058 and 4.54, respectively. All variants were homoplasmic except G103A. Mitochondrial copy number in infantile patients was lower than adult patients (P<0.001, Fig.2), while there was no significant difference between infantile and adult controls (P=0.12).

The range of D-loop variant count among all patients was 2-12 while this was 0-7 in the controls (Fig.3). This study showed that D-loop variant number in infantile patients is higher than adult patients. Chi-square test showed a meaningful difference in the distribution of D-loop variant counts between the infant and adult groups (P=0.038). Some infant patients had 10 variants or more in the D-loop region, while adults had nine variants or less. However, D-loop variant count in healthy adults was more than in healthy infants (P=0.041).

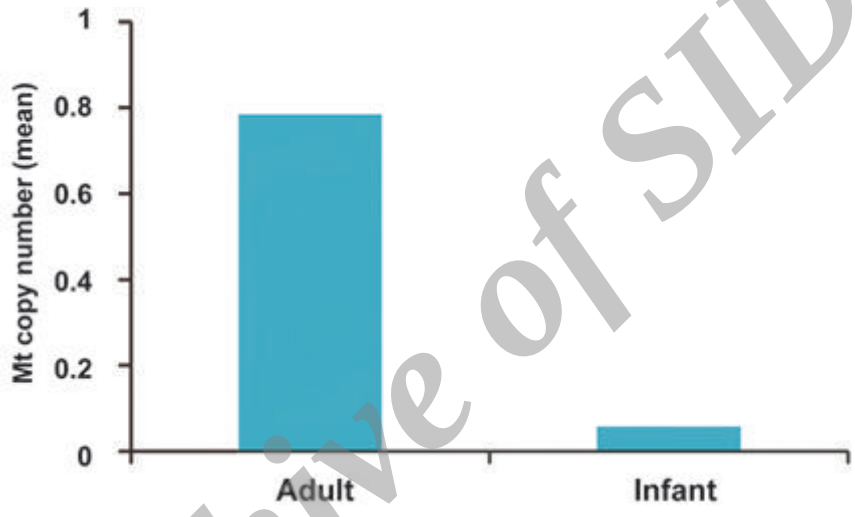

Fig.2: Mitochondrial copy number ratio in infantile and adult Pompe patients versus controls.

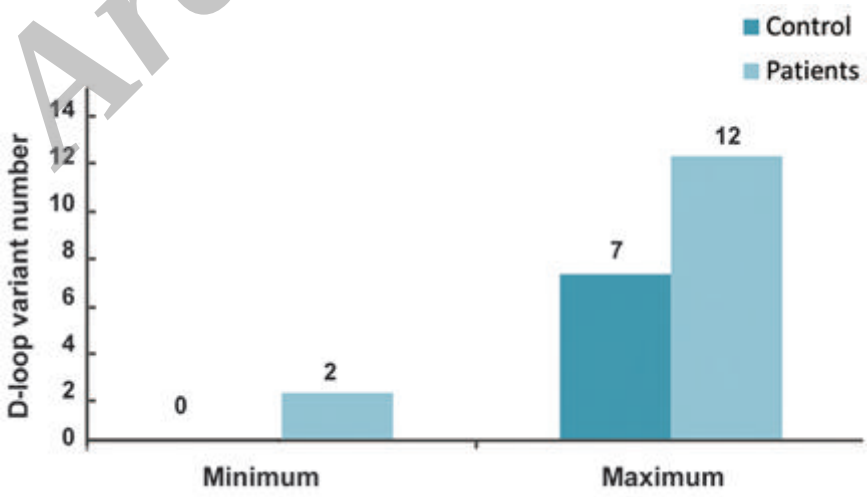

Fig.3: Minimum and maximum D-loop variant number in Pompe patients and controls.

Among the patients in this study, four affected sib pairs were present. They were compared based on their variants. 57.14% of all variants (21 variants) were common in sib pairs (Fig.4).

Linear regression analysis (Fig.5) showed a statistically significant inverse correlation (P=0.003, correlation coefficient R=0.54) between D-loop variant count and mtDNA copy number in patients (Fig.5B). There was no correlation between D-loop variant number and mtDNA copy number in controls (Fig.5A).

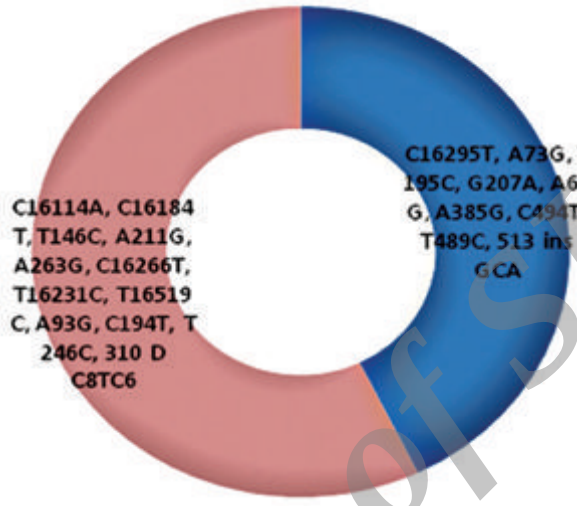

Fig.4: Common/unique variants among four affected sib pairs. Blue area shows common variants and pink area shows unique variants in the sib pair patients.

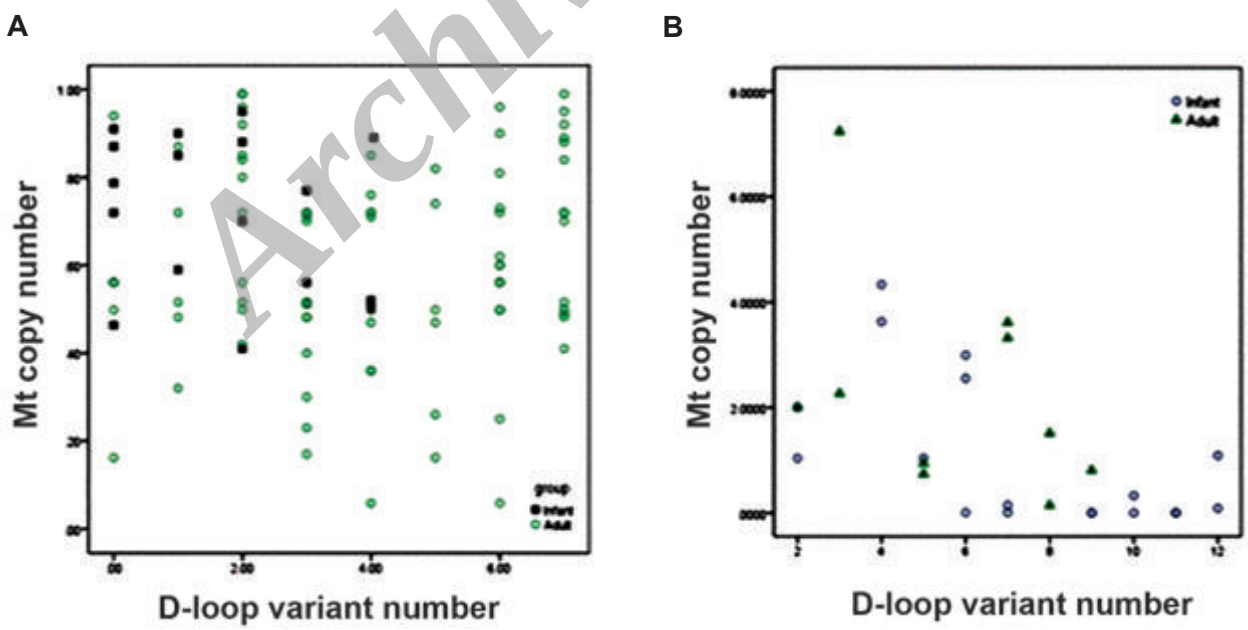

Fig.5: A. Scatter plot of mtDNA copy number and D-loop variant count in controls and **B.** Scatter plot between mtDNA copy number and D-loop variant count in patients. Correlation coefficient R and linear regression line of best of fit should be add.

Discussion

We evaluated mitochondrial copy number and mitochondrial D-loop variants in infantile and adult Pompe patients to investigate the potential role of mitochondria in PD. There are common clinical manifestations (variable expression and variable age of onset) between Pompe and mitochondrial disorders. Some studies have indicated that phenotypic expression of PD has significant variability in affected individuals with identical causal mutations (22).

As mitochondria are controlled by nuclear and mitochondrial genes, mutations in both set of genes may affect mitochondrial function and increase oxidative stress. On the other hand, increasing oxidative stress is a secondary factor that results in mitochondrial dysfunction.

Engel and Dale (25) showed a large number of abnormal mitochondria in skeletal muscle biopsy derived from a patient with adult onset disease. Paracrystalline inclusions were reported in numerous mitochondria among adult patients (15, 26). Hudgson (27) revealed that large subsarcolemmal mitochondrial aggregates exist in an adult patient. Furthermore, Verity reported enlarged "pleomorphic" mitochondria with distorted cristae in muscular tissue of an infant (16). Recently, Huang observed dysfunctional mitochondria with swollen cristae in induced pluripotent stem cells (iPSCs) derived from fibroblasts of two patients with PD and showed that mitochondrial dysfunction is one of the pathophysiological features of PD cells (10, 27). These studies reported abnormalities in mitochondrial structure, but we evaluated mtDNA in Pompe patients.

D-loop part is a major region that acts as the origin of replication of mtDNA and contains essential elements for transcription and replication of mitochondrial genes. D-loop has been shown to be a mutation 'hot spot' in some disorders and is more vulnerable to numerous damages. The rate of mutation in D-loop is much higher than other parts of mtDNA and such alterations perhaps interfere with the sequence of promoters and modify the affinity of binding to the inducer or modulators that is part of the transcription machine (28). In the present study, a large number of point mutations were observed in the D-loop region of mtDNA. Several variants were within nucleotides 110-520. These variants include 146, 150, 152, 153, 203, 228, 263, 295, 456, 489 and 513. These variants were also in cardiomyopathy cases (29). It may thus interfere with mitochondrial replication, transcription and mtDNA copy number. In spite of decreasing mtDNA copy number in the infantile group, D-loop variant count increased. The infantile mean variant count was more than that of the adult group.

We report in this study that almost all D-loop variation in Pompe patients were homoplasmic. The D310 mutations were seen in 17.857% of patients. The D310 region has been recently recognized as a frequent hot spot of insertion mutations in some disorders (30, 31). This polymorphic c-stretch (CCCCCCCTCCCCC) has a role in the formation of persistent RNA-DNA hybrid, which is essential for mtDNA heavy strand replication (31). The T152C variant was also seen in child respiratory morbidity (32) and reported to be present in H, U and K haplogroups. The role of haplogroups is emphasized for several disorders like age-related macular degeneration (33). This variation is associated with haplogroup H and can increase the risk of Parkinson disease (34), while in this study the variant was not significantly overrepresented in the case group. T489C and T146C were observed frequently in the study, both being more frequent in some populations and disorders (35, 36). The T146 variant has been detected in mitochondrial myopathies, hearing loss, mitochondrial encephalomyopathy and ovarian cancer (37, 38). T195C and C462T variants have been associated with repeated pregnancy loss and psychiatric disorders (22). The A73G variant has been reported in Alzheimer's, hearing loss, hypertrophic cardiomyopathy and infantile cardiomyopathy. The A263G variant has been associated with muscle pathology, auditory neuropathy, hypertrophic cardiomyopathy, idiopathic cardiopathy and infantile cardiomyopathy (39). The C16278T has been seen in hearing loss, idiopathic cardiomyopathy, Leigh syndrome, mitochondrial encephalomyopathy and mitochondrial myopathy. These D-loop variants due to mimic Pompe clinical presentations (40).

The T16189C variant has been reported in cardiomyopathy and diabetes and has been suggested to affect mitochondrial DNA replication (39, 41). These variants may change the affinity of transcription factors and other cis acting elements in the D-loop region and affect mitochondrial function. The

role of mitochondrial variants in PD is unknown. This study evaluated mtDNA copy number and the D-loop region variants for the first time in infantile and adult Pompe patients of Iranian origin. It seems that there is a negative correlation between mtDNA variation and mtDNA copy number when analyzing severe PD patients. Other mitochondrial variants within the same haplogroup may have an effect on severity of the disease and may act in synergy with *GAA* mutations.

Conclusion

This is the first study regarding mitochondrial evaluation of infantile and adult Pompe patients of Iranian origin. We identified a novel variant (317-318 ins CCC) in Pompe patients. There was an inverse correlation between mean D-loop variant count and mtDNA copy number. A significant frequency difference was seen between the two groups for nearly a fifth of SNPs.

MtDNA copy number and variant count were different between adult versus infant patients. These differences were large in infants and these results are coordinated with phenotype intensity. It seems that mitochondrial variants may have a secondary role in the pathogenesis of PD. Understanding the role of mitochondria in the pathogenesis of PD could pave the way for the development of new therapeutic strategies.

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References

1. van der Ploeg AT, Clemens PR, Corzo D, Escolar DM, Florence J, Groeneveld GJ, et al. A randomized study of alglucosidase alfa in late-onset pompe's disease. N Engl J Med. 2010; 362(15): 1396-1406.
2. Thurberg B, Carlier P, Kissel J, Schoser B, Pestronk A, Barohn R, et al. A phase 4 prospective study in patients with adult pompe disease treated with alglucosidase alfa. J Neuromuscul Dis. 2015; 2 Suppl 1: S72-73.
3. Lachmann R, Schoser B. The clinical relevance of outcomes used in late-onset Pompe disease: can we do better? Orphanet J Rare Dis. 2013; 8: 160.
4. Dons Sink IJ, Dirckx M, Scoones GP. Anaesthetic management of two patients with pompe disease for caesarean section. Case Rep Anesthesiol. 2014; 2014: 6503104.
5. Lim JA, Li L, Raben NA. Pompe disease: from pathophysiology to therapy and back again. Front Aging Neurosci. 2014; 6: 177.
6. Van der Beek N, Hagemans ML, Reuser AJ, Hop WC, Van der Ploeg AT, Van Doorn PA, et al. Rate of disease progression during long-term follow-up of patients with late-onset Pompe disease. Neuromuscul Disord. 2009; 19(2): 113-177.
7. Yanovitch T, Banugaria S, Proia A, Kishnani P. Clinical and histologic ocular findings in pompe disease. J Pediatr Ophthalmol Strabismus. 2010; 47(1): 34-40.
8. Kishnani PS, Steiner RD, Bali D, Berger K, Byrne BJ, Case LE. Pompe disease diagnosis and management guideline. Genet Med. 2006; 8(5): 267-288.
9. Kroos M, Hoogeveen-Westerveld M, Michelakakis H, Pomponio R, Van der Ploeg A, Halley D, et al. Update of the pompe disease mutation database with 60 novel GAA sequence variants and additional studies on the functional effect of 34 previously reported variants. Hum Mutat. 2012; 33(8): 1161-1165.
10. Huang HP, Chen PH, Hwu WL, Chuang CY, Chien YH, Stone L, et al. Human pompe disease-induced pluripotent stem cells for pathogenesis modeling, drug testing and disease marker identification. Hum Mol Genet. 2011; 20(24): 4851-4864.
11. Ghaffarpour M, Mahdian R, Fereidooni F, Kamalidehghan B, Moazami N, Houshmand M. The mitochondrial ATPase6 gene is more susceptible to mutation than the ATPase8 gene in breast cancer patients. Cancer Cell Int. 2014; 14(1): 21.
12. Tuppen HA, Blakely EL, Turnbull DM, Taylor RW. Mitochondrial DNA mutation and human disease. Biochim Biophys Acta. 2010; 1797(2): 113-128.
13. Schapira AH. Mitochondrial disease. Lancet. 2006; 368(9529): 70-82.
14. Thurberg BL, Lynch Maloney C, Vaccaro C, Afonso K, Tsai AC, Bossen E, et al. Characterization of pre- and post-treatment pathology after enzyme replacement therapy for Pompe disease. Lab Invest. 2006; 86(12): 1208-1220.
15. Lewandowska E, Wierzba Borowicz T, Rola R, Modzelewska J, Stepien T, Lugowska A, et al. Pathology of skeletal muscle cells in adult-onset glycogenosis type II (Pompe disease): ultrastructural study. Folia Neuropathol. 2008; 46(2): 123-133.
16. Verity MA. Infantile pompe's disease, lipid storage, and partial carnitine deficiency. Muscle Nerve. 1991; 14(5): 435-440.
17. Raben N, Wong A, Ralston E, Myerowitz R. Autophagy and mitochondria in pompe disease: nothing is so new as what has long been forgotten. Am J Med Genet C Semin Med Genet. 2012; 160C(1): 13-21.
18. diMauro S, Schon EA. Mitochondrial DNA mutations in human disease. Am J Med Genet. 2001; 106(1): 18-26.
19. Thorburn DR, Dahl HH. Mitochondrial disorders: genetics, counseling, prenatal diagnosis and reproductive options. Am J Med Genet. 2001; 106(1): 102-114.
20. Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, et al. Sequence and organization of the human mitochondrial genome. Nature. 1981; 290(5806): 457-465.
21. Suzuki M, Toyooka S, Miyajima K, Iizasa T, Fujisawa T, Bekele NB, et al. Alterations in the mitochondrial displacement loop in lung cancers. Clin Cancer Res. 2003; 9(15): 5636-5641.
22. Seyedhassani SM, Houshmand M, Kalantar SM, Modabber G, Aflatoonian A. No mitochondrial DNA deletions but more D-loop point mutations in repeated pregnancy loss. J Assist Reprod Genet. 2010; 27(11): 641-648.
23. Houshmand M, Larsson NG, Holme E, Oldfors A, Tulinius

MH, Andersen O. Automatic sequencing of mitochondrial tRNA genes in patients with mitochondrial encephalomyopathy. Biochim Biophys Acta. 1994; 1226(1): 49-55.
24. Shakhssalim N, Houshmand M, Kamalidehghan B, Faraji A, Sarhangnejad R, Dadgar S, et al. The mitochondrial C16069T polymorphism, not mitochondrial D310 (D-loop) mononucleotide sequence variations, is associated with bladder cancer. Cancer Cell Int. 2013; 13(1): 120.
25. Engel AG, Dale AJ. Autophagic glycogenosis of late onset with mitochondrial abnormalities: light and electron microscopic observations. Mayo Clin Proc. 1968; 43(4): 233-279.
26. Fernández R, Fernández JM, Cervera C, Teijeira S, Teijeiro A, Domínguez C, et al. Adult glycogenosis II with paracrystalline mitochondrial inclusions and hirano bodies in skeletal muscle. Neuromuscul Disord. 1999; 9(3): 136-143.
27. Hudgson P. Correlations between histochemical and ultrastructural studies of diseased muscle. Riv Istochim Norm Patol. 1975; 19(1-4): 101-110.
28. Akouchekian M, Houshmand M, Hemati S, Ansaripour M, Shafa M. High rate of mutation in mitochondrial DNA displacement loop region in human colorectal cancer. Dis Colon Rectum. 2009; 52(3): 526-530.
29. Marin Garcia J, Zoubenko O, Goldenthal M. Mutations in the cardiac mitochondrial DNA control region associated with cardiomyopathy and aging. J Card Fail. 2002; 8(2): 93-100.
30. Tang M, Baez S, Pruyas M, Diaz A, Calvo A, Riquelme E, et al. Mitochondrial DNA mutation at the D310 (displacement loop) mononucleotide sequence in the pathogenesis of allbladder carcinoma. Clin Cancer Res. 2004; 10(3): 1041-1046.
31. Mao L. A New marker determining clonal outgrowth. Clin Cancer Res. 2002; 8(7): 2021-2023.
32. Schmuczerova J, Brdicka R, Dostal M, Sram RJ, Topinka J. Genetic variability of HVRII mtDNA in cord blood and respiratory morbidity in children. Mutat Res. 2009; 666(1-2): 1-7.
33. Udar N, Atilano SR, Memarzadeh M, Boyer DS, Chwa M, Lu S, et al. Mitochondrial DNA haplogroups associated with age-related macular degeneration. Invest Ophthalmol Vis Sci. 2009; 50(6): 2966-2974.
34. Khusnutdinova E, Gilyazova I, Ruiz-Pesini E, Derbeneva O, Khusainova R, Khidiyatova I, et al. A mitochondrial etiology of neurodegenerative diseases: evidence from Parkinson's disease. Ann N Y Acad Sci. 2008; 1147(1): 1-20.
35. Dato S, Passarino G, Rose G, Altomare K, Bellizzi D, Mari V, et al. Association of the mitochondrial DNA haplogroup J with longevity is population specific. Eur J Hum Genet. 2004; 12(12): 1080-1082.
36. Kurtz A, Lueth M, Kluwe L, Zhang T, Foster R, Mautner VF, et al. Somatic mitochondrial DNA mutations in neurofibromatosis type 1-associated tumors. Mol Cancer Res. 2004; 2(8): 433-441.
37. Van Trappen PO, Cullup T, Troke R, Swann D, Shepherd JH, Jacobs IJ, et al. Somatic mitochondrial DNA mutations in primary and metastatic ovarian cancer. Gynecol Oncol. 2007; 104(1): 129-133.
38. Filosto M, Mancuso M, Vives-Bauza C, Vilà M, Shanske S, Hirano M, et al. Lack of paternal inheritance of muscle mitochondrial DNA in sporadic mitochondrial myopathies. Ann Neurol. 2003; 54(4): 524-526.
39. Mezghani N, Mnif M, Mkaouar-Rebai E, Kallel N, Haj Salem I, Charfi N, et al. The mitochondrial ND1 m.3337G>A mutation associated to multiple mitochondrial DNA deletions in a patient with Wolfram syndrome and cardiomyopathy. Biochem Biophys Res Commun. 2011; 411(2): 247-252.
40. Chamkha I, Alila-Fersi O, Mkaouar-Rebai E, Aloulou H, Kifagi C, Hachicha M, et al. A novel m.12908T>A mutation in the mitochondrial ND5 gene in patient with infantile-onset Pompe disease. Biochem Biophys Res Commun. 2012; 429(1-2): 31-38.
41. Wallace DC, Chalkia D. Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. Cold Spring Harb Perspect Biol. 2013; 5(11): a021220.