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Ototoxicity and Aminoglycoside Antibiotics Syndromics Presentation of Mitochondrial Disease

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Abstract

Mutations in mitochondrial DNA (mtDNA) have been found to be associated with sensorineural hearing loss. As part of genetic screening program for hearing loss, we studied 40 patients with sensorineural deafness, whose cause might have been after aminoglycoside (ATB-AG), treatment. The affected and control subject's DNA fragments spanning the 12SrRNA gene or tRNASer (UCN) gene, that are associated with both aminoglycoside-induced and non-syndromic hearing loss, were amplified and studied by PCR-RFLP. Three families have the homo plasmic 7444G>A mutation in the tRNAser (UCN) gene, the analysis of the mitochondrial genome in three family's members did not detect any other pathology mutation. The history clinical show one syndromics phenotype for matrilineal family. In the three families the muscle biopsy findings in the proband and their mothers, showed in the electronic microscopy (EM) and in the light microscopy (LM) multiple mitochondrial abnormalities in the striated muscle. These findings have been correlated with the values from Citocromo Oxidase/Citrate Synthase ratio, the indicated poor activity of the Citocromo Oxidase. The matrilineal pedigree clinical feature, and the molecular, biochemical and morphological studies, might indicate that this is a syndromic presentation of the 7444G>A mutation in Córdoba- Argentina. In the fourth family, there port of the clinical, genetic, and molecular characterization in two of their members, revealed the variable phenotype of hearing impairment including audiometric configuration. Mutation an analysis of the mtDNA in these pedigrees showed the presence of nonsyndromichomoplasmic 12 SrRNA A827G mutations. The A827G mutation is located at the A-site of mitochondrial 12SrRNA gene which is highly conserved in mammals. It is possible that the alteration of the tertiary or quaternary structure of this rRNA by the A827G mutation may lead to mitochondrial dysfunction, thereby playing arole in the pathogenesis of hearing loss and aminoglycoside hypersensitivity. Although the 827A>G mutation in the 12SrRNA, is associated with haplo group B, its prevalence ≥2%, does not eliminate its participation and association to Ototoxicity by ATB-AG.

Keywords: Hearing loss; Mitochondrial $tRNA^{ser(UCN)}$; 7444G>A Mutation; Mitochondrial 12S rRNA 827A>G Mutation; Aminoglycosides

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Abbreviations: EM: Electronic Microscopy; LHON: Leber´S Hereditary Optic Neuropathy; NARP: Neuropathy Ataxia and Retinitis Pigmentosa; MERRF: Monoclinic Epilepsy with Ragged Red Fibers; MMC: Maternally Inherited Miopathy and Cardiomyopathy; MELAS: Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-Like Episodes; AG-ATB: Amino Glycoside Antibiotics; PTA: Pure-Tone Audiometry; COX: Cytochrome c Oxidase; CS: Citrate Synthase; H/F: Hematoxilyn-Eosine; SNHL: Severe Sensor Neural Hearing Loss.

Introduction

In 1985, the World Health Assembly estimated that the number of deaf people in the world was 70 million; whereas what was reported in 2018 was of 466 million people. Deafness is defined as the decrease or alteration in the ability to hear, that is of the perception of sounds. It is not compatible with language. While hearing loss, is defined as only the diminution in hearing in a person able to hear. This is compatible with language. Deafness is a sensory disorder that affects: 1 in every 1000 newborns, 4 out of every 1000 people under 45 years old, more than 60% of people over 70 years old. More than half of childhood, originated deafness has a genetic cause. The etiological causes can be classified into: Hereditary, Acquired, and Unknown. Hereditary deafness can be presented as Non-Syndromic in70%ofthe cases. In the rest of the cases, it is part of a more complex syndrome, associated with other signs or symptoms in the 30% of the cases. Also 1 to 2% of Genetic Deafness is presented in the Mitochondrial DNA as Syndromic Deafness or Non-Syndromic Deafness.

An increasing number of disorders associated with mutations in the mitochondrial genome have been reported in recent years. More than 200 pathogenic mtDNA mutations have been reported to date (MITOMAP: A Human Mitochondrial Genome Database, http://www.mitomap.org/MITOMAP). These mutations are located across the 37genes of the mitochondrial genome; however, the vast majority reside in just 5–10% of them tDNA, in the 22 mitochondrial tRNA (mt- tRNA) genes. Mutations in mitochondrial DNA (mtDNA), especially in the 12S rRNA and $tRNA^{ser(UCN)}$ genes, are the most important causes of both aminoglycoside-induced and non syndromic hearing loss [1-6]. These mutations often occur nearly or complete homoplasmically state; specific mutations are consistently related to well-defined clinical entities, suggesting the presence of a relation between molecular and clinical abnormalities. It has been established that a collection of specific mtDNA point mutations are responsible for most of the maternally-inherited syndromes, including Leber´s Hereditary Optic Neuropathy (LHON), Neuropathy Ataxia and Retinitis Pigmentosa (NARP), Monoclinic Epilepsy with Ragged Red Fibers (MERRF), Maternally Inherited Miopathy and Cardiomyopathy (MMC), Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like Episodes (MELAS), Myopathy and Diabetes Mellitus [7-9]. These disorders, with a particular clinical expression affect more over the cochlear function. The tissues that require a high rate of ATP production are more likely to be affected by mutations that affect the mitochondrial function (for example striated muscle, nervous tissues cochlea etc.).The mt DNA dependent dysfunctions have often been found to cause hearing defects in syndromic or non syndromic form. Ototoxicity is a main dose-limiting factor in the clinical application of aminoglycoside antibiotics. Despite long standing research efforts our understanding of the mechanisms underlying aminoglycoside Ototoxicity remains limited. On the other hand, two of the main features of mtDNA-linked deafness, are the specificity of tissue and the variable grade of penetrance [7-10]. In addition, matrilineal relatives of intra-family or inter-family, despite carring the same deafness-associated mtDNA mutation (s), exhibited variable penetrance and expressivity, including the severity, age-of-onset, and progression in hearing loss.

In fact, this shows that the mtDNA mutation (s) itself is not enough to produce the clinical phenotype; it is also necessary the presence either, of modifier genes mutations from nuclear DNA (nDNA), environmental factors like aminoglycoside antibiotics (AG-ATB) and mitochondrial haplogroup/haplotypes [3,11,12]. In the previous studies of mitochondrial genes, tRNA CysT5802C, tRNA CysG5821A, $tRNA^{ser(UCN)}$ G7444A, tRNA Arg, T10454C, ND5T12338C, tRNA GluA14693G, tRNA ThrT15908C, and tRNA ThrG15927A, defined as the secondary mutations, have been reported to possibly influence or worsen the clinical phenotypic manifestation of hearing loss associated with the A1555G mutation in Chinese NSHL pedigrees [13-15].

In this work, were ports the clinical, biochemical, molecular and genetic characterization of three families that had the same mutation in the $tRNA^{ser(UCN)}$ gene and in two have been associated with Ototoxicity after treatment with AG-ATB and of presentation syndromic. We analyzed the 12SrRNA and $tRNA^{ser(UCN)}$ genes in the patient with deafness for Ototoxicity after aminoglycoside antibiotics and her matrilineal and patrilineal family relatives. With the purpose to seek the presence of other mutations in the mitochondrial genome that might to affect the clinical presentation, we analyzed the mtDNA genome complete. The matrilineal pedigree clinical feature, molecular,

biochemical and morphological studies, might indicate that this is a syndromic presentation of the 7444G>A mutation in Córdoba –Argentine.

Subjects and Methods

As part of genetic screening program for hearing loss, we studied 120 patients with sensorineural deafness of which 40 patients might has been cause after Aminoglycoside treatment. The mutations were studied by PCR-RFLP. DNA samples of 220 controls were used, to find the presence of mtDNA mutations. The study protocol and written informed consent, was obtained from all the patients, and it has been approved by the Ethics Committee of the Universidad Nacional de Córdoba and the Ministerio de Salud, Provincia de Córdoba (Resolution Number 296) in accord to Helsinki treaty.

Clinical and Audio Logical Examinations

A comprehensive history and physical examination were performed to identify any syndromic symptoms and signs, to determine the possible use of AG-ATB, and genetic factors related to the hearing impairment in members of this pedigree. These studies were carried out mainly, at the Sanatorio Allende Otorhinolaryngology Service, delaProvinciade Córdoba. An age-appropriate audiological examination was made, and this examination included pure-tone audiometry (PTA) and/or auditory brainstem response (ABR), immittance testing, and Distortion product otoacoustice missions (DPOAE). The PTA was calculated from the sum of the audiometric thresholds at 500, 1000, 2000 and 4000Hz. The severity of hearing impairment was classified into five grades: normal<25Decibels (dB); mild=26–40dB; moderate=41–70 dB; severe = 71–90 dB; and profound, >91dB.

Mutational Analysis of Mitochondrial 12S rRNA and tRNA^{ser} (UCN) Genes

Total DNA was extracted from peripheral blood using standard procedures [16]. Screenings for mutations were carried out using PCR-RFLP. The searched mutations were:

- In the rRNA 12S gene from mtDNA: 1555A>G, 1494T>C and 827A>G [17-19,6].
- In the tRNA$^{ser(UCN)}$ gene from mtDNA: 7443A>G, 7444G>A and 7445A> [20].
- In the tRNA$^{leu(UUR)}$ gene from mtDNA: 3243A>G [21].

DNA fragments completes of the12Sr RNA and tRNA^{ser} genes, from affected patient were amplified by PCR using specific primers PF18-PR18 and PF1-PR1 (all the primer sequences are available from the authors) and each fragment was purified and subsequently submitted for sequencing analysis.

Mutational Analysis of Mitochondrial Genome

The entire mitochondrial genome was PCR-amplified in 19 overlapping fragments by use of sets of the light-strand and the heavy-strand oligonucleotides primers (all the primer sequences are available by the authors, (the primers number 17 was divided in two fragments 17a and 17b) [6]. Each fragment was purified and subsequently submitted for sequence analysis. The resultant sequence data were compared with the updated consensus Cambridge sequence (Gen Bank Accession No.: NC_012920.1) [22].

Mutational Analysis of TRMU Gene

For the role TRMU gene examination, in the manifestation of the phenotype 7444G> A mutation, we genotyping the G28T variant in TRMU gene by PCR-RFLP [12].

Determination of the Enzymes Activities (Cytochrome C Oxidase (COX) and Citrate Synthase (CS)), and COX/CS Ratio

Activity determinations of Cytochrome c Oxidase (COX) and Citrate synthase (CS) was performed in homogenates of quadriceps biopsy [23-25].

Histological and Histochemical Techniques

Histological and histochemical techniques were performed in longitudinal series of the muscle [26,27]. Serial slides were treated with hematoxilyn-eosine (H/E), and modified Gomori trichrome technique to view Ragged Red Fibres (MRRF).

Electronic Microscopy

Tissue was washed in 1 ml of PBS (pH 7.0), and fixed with 2% of glutaraldehyde and 4% formaldehyde in 0.1M CaCO dylate buffer for 2 hour, and then post-fixed with osmium tetroxide at 1% in the same buffer, dehydrated and embedded in Araldite. Thin sections were cut with a diamond knife on a JEOL JUM-7 ultra microtomes and examined in a Zeiss LEO 906 Eelectron microscope [28].

Results

In this population of 120 patients with sensorineural deafness from which 40 might has been the cause after amino glycoside treatment, one family presented the 827A> G mutation in the rRNA12S gene [6]. The others three families do not parent, that we show in this study, has the 7444G>A mutation in the tRNA$^{ser(UCN)}$ from mtDNA.

Clinical Findings

In the family one: The patient (III-5) is from Córdoba, in the centre of Argentine, and she was treated with amino glycosides at birth, due to lung infection. She was considerate to have congenital deafness. Her mother did not have risk factors in her pregnancy. On her matrilineal pedigree were present similar clinical antecedents: Thyroid: Graves Based on disease (I-4; II-5 and III-12); hypothyroidism (II-2, II-8 and III-5) ; the patient III-5 only showed signs since she was 8 years old until 11 years old. The patient II-2 present hypothyroidism, but his children (they have same old than them cousin) no showing any disease. Myopathy: muscle pain and easily fatigued (II-5, 8; III3, 5, 12 and 14) .Rheumatoid arthritis: The patient III-7was diagnosed at the age of nine, she had rheumatoid factor positive (IgM) in serum. Her symptoms were: pain in big articulations, anorexia, and low weight (could not gain weight) and slow growth. She received treatment but at the moment she is not receiving any treatment. Bronchial asthma: (III-6 and 13). Areata alopecia: (III-10 and 12); and Arrhythmia cardiac: (III-11) during fetal stage, until he was six months old. The patients (III-3 and II-5) are pregnant, they have to make rest and both embryos have arrhythmia cardiac too, growing slow intra uterus and them mothers need repose in bed (Figure 1). We observed in this family, that the pathology's, to be present at more early age in the news generations.

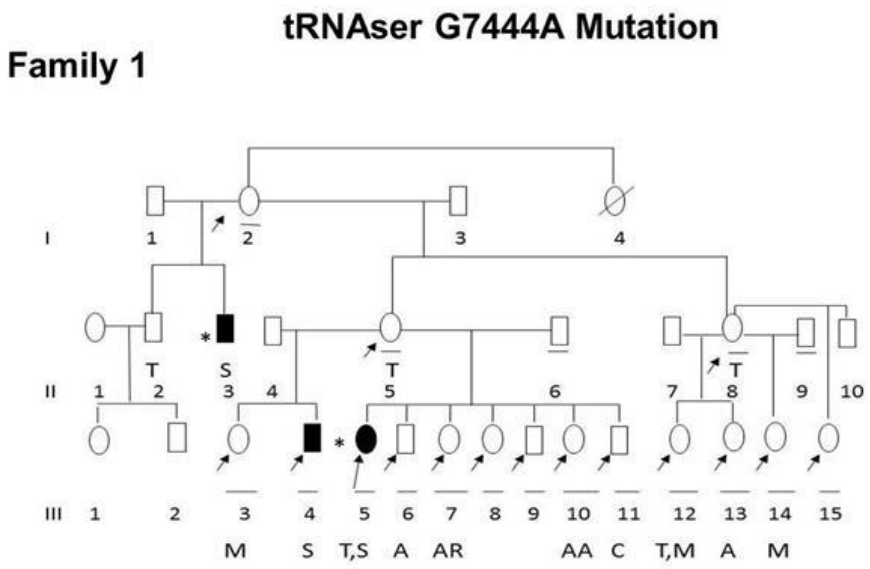

Figure 1: Argentinean pedigrees with aminoglycoside-induced and syndromic hearing-loss.
Filed symbol=deafness; Arrow=probans with G7444A mutation; Asterisks=individual with exposure to aminoglycosides; Underlined= all subjects with molecular studies, AR=rheumatoid arthritic; A=bronchial asthma; C= cardiac arrhythmia; M= miophaty; T= thyroideophaty; AA: alopecia Areate.

As show in Figure 2, the audiogram of pro band III–5, showed severe sensor neural hearing loss (SNHL) . Other audio logical and neurotological examinations, including immittance, ABR and TEOAEs, revealed a cochlear involvement. Otoscopic examination and CT scan of the temporal bones demonstrated normal results. As to be indicated in the Figure1, three members (II-3; III-5 and III-4) in the pedigree exhibit bilaterally symmetric hearing impairment, severe to profound and sensorineural; in two of them, hearing impairment appeared after treatment with ATB-AG (III-5 and II-3). We have not studied the patient II-3; he was treated with ATB-AG when he was 22 years old, and the hearing impairment occurred only 30 days after injection of regular dose of aminoglycosides (gentamycin) . The patient III-5 was treated with gentamicin at birth. In the patient III-4, hearing impairment might have been caused by the excessive use of head phones; the hearing loss was reported to be progressive in serial audiograms. The others family members (I-2, II- 5,6,8, and 9; III-3,6,7,8,9,10,11,13,14, and 15) who did not receive aminoglycoside antibiotics had normal hearing as indicated by audiological and neurological examinations.

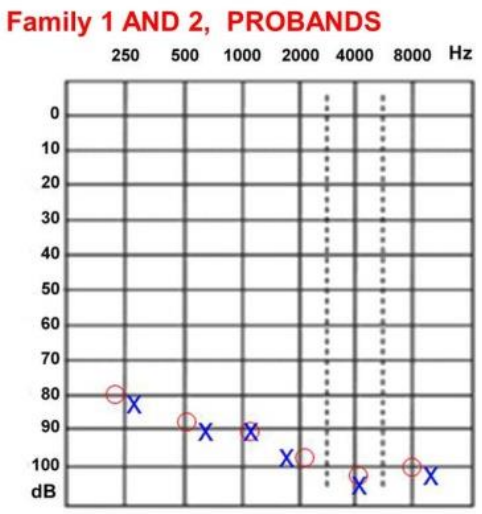

Figure 2: Pure-tone audiometry of patient III-5 with deafness from severe to profound.

In the **family two:** The patient (II-2) is from Córdoba, in the centre of Argentine, and she was treated with aminoglycosides at birth, due to lung infection, and her audiogram of proband II–2 showed severe sensorineural hearing loss (SNHL) . Other audiological and neurotological examinations, including immittance, ABR and TEOAEs, revealed a cochlear involvement. Otoscopic examination and CT scan of the temporal bones demonstrated normal results too. Her mother had thyroid problem, Graves Basedow's disease, Areata alopecia and arrhythmic cardiac. This family is more little (Figure 3).

In the **family three:** The patient (IV-1) is from Córdoba, in the centre of Argentine, she was not treated with aminoglycosides, but she was considerate to have congenital deafness. Her mother did not have risk factors in her pregnancy. On her matrilineal pedigree had got not present similar clinical antecedents how in family 1 and 2 (Figure 3, Table1).

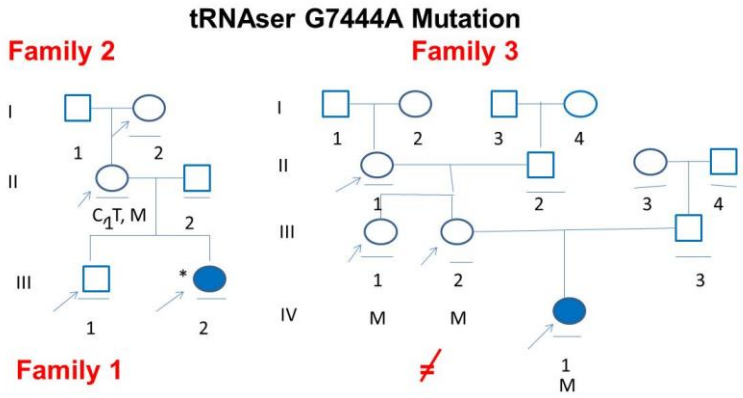

Figure 3: Argentinean pedigrees with/without aminoglycoside-induced and syndromic hearing-loss. Filed symbol=deafness; Arrow=probans with G7444A mutation; Asterisks=individual with exposure to aminoglycosides; Underlined=all subjects with molecular studies, C= cardiac arrhythmia; M= miophaty; T= thyroideophaty.

CL of DFSN			Ototox ATB-AG		S	NS	Birth		New born weight	
Family	Amount of FM	Server to profound	Yes	No			Prem.	A Term.	<2800mg	>2800mg
1	3	X	X		X		X		X	
2	1	X	X		X		X		X	
3	1	X		X	X			X	X	

Diseases						
Family	T	AS	RA	AA	CARD A	MUSC P
1	X	X	X	X	X	X
2	X			X	X	X
3						X

E-spectrophotometry Lab	Morphology

Family	COI(U/g tissue	CS (U/g tissue)	COI/CS	LM	EM	N2M Studied
1	Low	N & L	L & H	RRF, < COX	M A, <COX; >Gly	2
2	Low	N	Low	RRF, < COX	M A, <COX; >Gly	1
3	Low	N	Low	RRF, < COX	M A, <COX; >Gly	2

Table1: Clinical presentation of the mutation G7444A. T: Thyroideophaty; as:asthma; RA:rheumatoid arthritis; AA: Areatealopecia; Card A: Cardiacarrhythmia; MuscleP: miophaty.

Mitochondrial DNA Analysis

Screening of the 12SrRNA and tRNA$^{ser(UCN)}$ genes from mtDNA: The maternal transmission of amino glycoside-induced and syndromic hearing loss in these families (Figures 1 and 3), suggested mitochondrial involvement and this led us to analyze two mitochondrial genes in matrilineal relatives. DNA fragments spanning in the 12SrRNA and tRNA$^{ser(UCN)}$ genes were analyzed, which are the hot spots for deafness-associated mutations and Oto toxicity. The screening by PCRRFLP of A 827A>G, 1494C>T, 1555A>G, 7445A>G, 7444G>A and 7443A>Gmutations were done. We did not detect any of these 1555A>G, 1494C>T, nor 827A>G mutations; but we detected 7445A>G, 7443A>G or 7444G>A mutations (they have the same restriction site), but by sequencing it was detected 7444G>A (Figures 4A and B). We amplified 12SrRNA and tRNA^{ser} genes and each fragment was purified and subsequently submitted for DNA sequencing. We did not detect any mutation in the 12SrRNA gene, and we did not detect the 7510T>C, 7511T>C, 7472 in SC, 7445A>G or7443A>G mutations, in the tRNA^{ser} gene. Interestingly we also failed to find any other nucleotide change, except the mitochondrial tRNA^{ser} 7444G>A mutation, in the homoplasmic form in matrilineal relatives of the Family 1, 2 and 3. Two hundred and twenty Argentinean controls without SNHL did not carry this mutation.

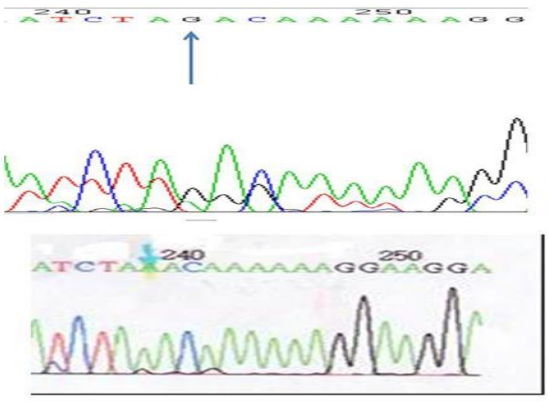

Figure 4: Electrophoregram showed the change of the nucleotide G to A.

With the purpose to look for the presence of other mutations, we analyzed the complete sequence from mitochondrial genome in the patients III-5,II-5 and IV-1. The results positives of the mutations, where analyzed in the remathers.

Analysis mtDNA genome complete in the probans of the three families: The changes observed in the family one were: in the nucleotide (nt) 4580G>A, from the MT–ND2 gene, synonym (syn), in the nt7444G>A from the MT-CO1 gene (nonsyn:term-k) ; in the nt10915T>C, from the MT–ND4 gene (syn.) in the nt12693A>G, from MT–ND5 gene (syn), in the nt14766C>T, from the MT– CYB gene, with the change amino acid (T–I), in the nt14783T>C, from the MT– CYB gene (nonsyn: T-I), in the nt16507C>A, from the region D-Loop (no descript) .

The changes observed in the family two were: in then t263A>G from control region (HV2); in the nt316 Ins. C control region (HV2) not described; in the nt 2831 G>A from MT-RNR2gene; in the nt3107del. N from the MT-RNR2 gene; in the nt4769A>G from the MT-ND2 gene (syn); in the nt6272A>G from CO1 gene (syn); in the nt7444G>A from the MT-CO1 gene (nonsyn: term-k); in the nt7849C>T from MT-CO2 gene (syn); in the nt8383T>C from MT-ATP8 (syn); in the nt 8521A>G from MT-ATP8 (syn); in the nt10915T>C from MT-ND4 (syn) and in the nt 15326 A>G from MT-CYB (non syn: T-A) .

The changes observed in the family three were: in the nt663A>G from MT-RNR1 gene, associated to arteriosclerosis and coronary insufficiency; in the nt750A>G from MT-RNR1gene, to S-Z associated; in the nt3107del. N from the MT-RNR2 gene; in the nt4769A>G from the MT-ND2gene (syn); in the nt6776T>C from MT-CO1 (syn); in the nt7444G>A from the MT-CO1gene (nonsyn:term-k); in the nt7849C>T from MT-CO2gene (syn); in the nt8383T>C from MT-ATP8 (syn); in the nt8860 from MT-ATP6 (nonsyn:T-A); in the nt10915T>C from MT-ND4 (syn) and in the nt 15326 A>G from MT-CYB (non syn: T-A) [29].

In the Family 1 the grandmother had the variant in the nt12152A>G, from the gene MT–TH, that no codify; this variant is not present in the proband (III– 5), nor in her mother (II–5). The resultant sequence data were

compared with the updated consensus Cambridge sequence (Gen Bank Accession No.: NC_012920.1) [19]. We have observed some variants that are repeated in the three unrelated families (Table 2).

F1, 2 and 3						
**7444	MT-CO2	G>A	87	1	No SYN T>A	LHON-DEAF
10915	MT-ND4		52	3		(-)
F 2 and 3						
8383	MY-ATP8	T>C	6	3	SYN T>T	(-)
15326	MT-CYB	A>G	194	1	No SYN T>A	(-)
*4769	MT-ND2	A>G	100	3	SYN M>M	SZ associate (a1 antitripsine

Haplogrupos found in the three families			
Haplogrupos	**FSNS-1**	**FSNS-2**	**FSNS-3**
HV	X		
H1		X	
U1a		X	
HV4a		X	
H1bk		X	X
A K1a1b1a			X

Table 2: Mitochondrial Genome sequencing variants found in the three families. Mutations that are repeated in the three families in the mitochondrial genome.

Mutational analysis of TRMU gene: To examine the role of TRMU gene, over phenotypic manifestation of 7444G>A mutation, we conducted the mutational screening of TRMU gene in the matrilineal members of these families. We failed to detect 28G>T mutation.

Biochemical Analysis

We show the enzyme activities from Cytochromeoxidase (COX) and citrate synthase (CS) in three samples of quadriceps muscle biopsies of the patients in Family 1, 2 and 3. In the Family 1 the patients (III-5andII-5), in Family 2 the patient's mother and in Family 3, the patient's mother and aunt; and controls, were port the cytochromes Coxidase/Citrate Synthase ratio (Table 3).

Enzymes Activities			
Sample	COI (U/g tissue)	CS (U/g tissue)	COI/CS
Patient III	4.4 ± 0.088	32.66 ± 0.65	0.135
Patient II	10.3 ± 2.105	10.9 ± 1.015	0.94
Control	20.6 ± 0.085	33.00 ± 0.30	0.62

Table 3: DFSN1 Enzymes activities of the COX, CS and COX/CS ratio.

The COX/CS ration in III-5 is lower than the control (21.6%) in the patient II-5, the COX and CS are low, therefore the COX/CS ratio is greater than the control; however, the $p<0.05$ this would indicate that the patient is of the range of normal population.

Control values shown are correlated to the ±SD (1.07±0.43) of the samples of Coulbault.

In the **Family 1** the patient III-5 to be show that the activity of the COX (4, 4±0,088U/gtww) is very low respect to control, whereas that the activity of the CS(32,66U/gtww) is near at control. The value of the ratio COX/CS (0.135) is only 21.7% in regard to control. On the other hand, in proband is mother (II-5), activity values of both enzymes are lower, and the relation COX/CS is bigger than registered in control. Because may be her mother had a few amorphous mitochondrial (figure) Table 3. In the **Family 2**, the study in the patient's mother and in the **Family 3**, the patient´s mother and aunt shows that the relationship COX/CS is very low respect to control (Table 4).

Relationship of the specific activities of the COX and CS enzymes	
Sample number	Relationship COX / CS
1	0.72
2	0.44
3	0.54
• 4	0.96
• 5	0.88
• 6	0.51
7	0.67
8	0.75
9	0.74
10	2.9
11	4.8
12	0.58

Table 4: Determination of the relationship of the activities of Cytochrome Oxidase (COX) with respect to Citrate Sintasa (SC), DFSN 2 and 3 (COX/SC).

In red or asterisks the mother of the proband of the DFSN 2 family and the mother. The aunt of the proband of the DFSN 3 family. In green or arrow subjects control. Other patients correspond to another mitochondrial disease: External and Progressive Opthalmoplegia.

Morphological Analysis: The muscle biopsy findings in the Family 1, 2 and 3: The morphological analysis has been realized from the longitudinal quadriceps muscle biopsy from the –5, II–5 and from control, in Family 1, patient II-2, Family 2; and patients III-1 and 2, Family 3. The morphology analyze in muscle biopsy, showed the next results: Family 1: Mather of proband, show sarcomere zig-zag pattern and few amorphous mitochondrial. The Proband, showed amorphous mitochondrial with internal membrane disruption and glycogen increment, plus fibrosis signs. Is important the presentation of, sub sarcolemmal accumulation of abnormal mitochondria in the muscle fiber, which stains red with Gomori trichrome stain (ragged red fibers) (Figures 5A-C and Figure 6).

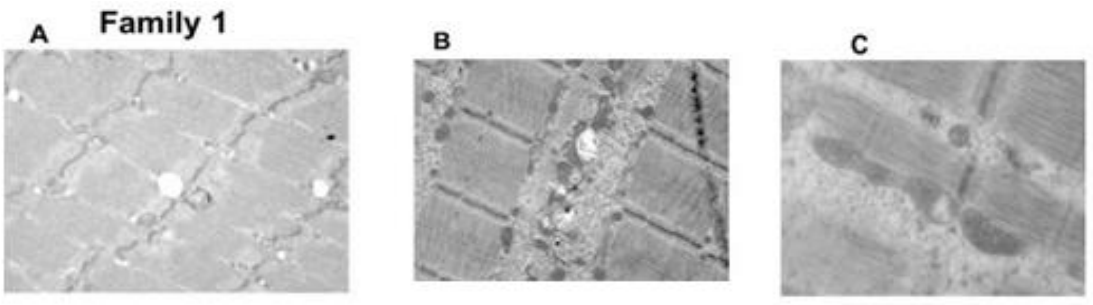

Figure 5: A: Mother of proband: show sarcomere zig-zag pattern and few amorphous mitochondrial.
B: Proband, amorphous mitochondria's and glycogen increment, plus fibrosis signs.
C: Proband: amorphous mitochondrial with internal membrane disruption.

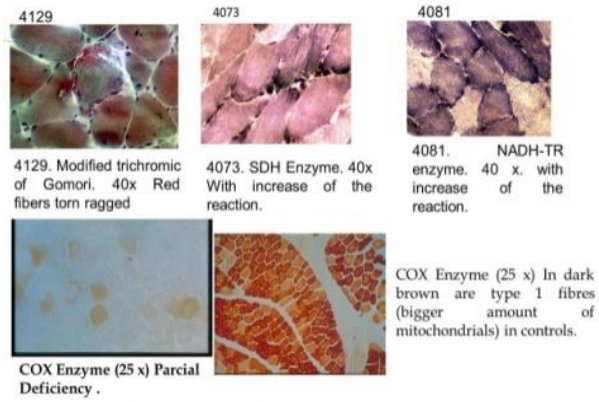

Figure 6: Light microscope. Slides of the function from activities of COX, SDH AND NADH enzymes. $tRNA^{ser}$ G7444A Mutation.

The findings in Family 2 and 3 have similar morphology (Figure7). In the control, the skeletal muscle is conserved, with normal histological characteristics. It is not observed fibrous u other alterations, with H / E color or Gomori technical.

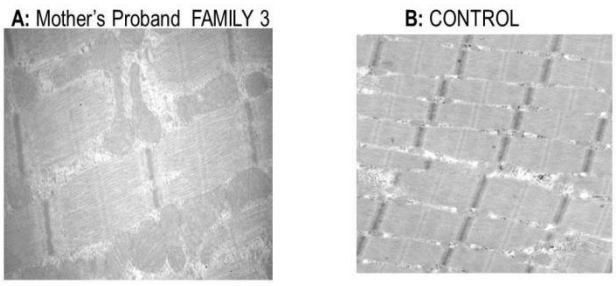

Figure 7: A: Microelectronic photo graph-Very big and deformed mitochondria's.
B: Microelectronic photo graph- Small, Oval, normal mitochondria's. There is one on each side of the Z line (black transversal line).

The cytohistochemical studies show an increase in the activity of the enzymes of complexes 1 and 2 of the oxidative phosphorylation chain, nicotidin- adenine dehydrogenase and the suscinate dehydrogenase, due to the low activity of the Cytochrome Oxidase of the fourth complex (Figure 6).

Haplogroups and Haplotypes Found in the Three Families

In **Family1:** HV; in **Family 2:** HV4a, H1, H1bk; U1a; in **Family 3:** A…K1,a1,b1a (Tabla2).

Discussion

These pathologies are present through matrilineal inheritance; moreover, we have observed that these pathologies are present at an earlier age in each generation in the Family 1. The patient's I-2, II-5 and II-8 were married more than once, and no abnormalities were found through the patrilineal side. In Family 2, cardiac and thyroid diseases as well as alopecia areata are observed. But in the third Family 3 no disease was observed; but, in this Family after the muscle biopsy, by Informed consent, we observed a strange way of walking when the patients got out of the operation theater. And the light and electronic microscope studies, such as spectrophotometry, indicated the syndromic presentation of the mutation (Figures 6-8).

The mutation G7444 A would have a homoplasmic presentation. It has been associated with Ototoxicity sensory neural deafness after AG-ATB in Chinese families. This 7444G>A mutation $tRNA^{ser(UCN)}$ gene has previously been implicated to be associated with both aminoglycoside-ototoxity, and non- syndromic hearing loss in a few genetically unrelated individuals [29]. It has also been associated with the A1555G mutation in the 12srRNA gene [30], and in association to mitochondrial mutation that produces LHON and by itself only producing SN deafness (USA) [31,32,12].

This 7444G>A mutation $tRNA^{ser(UCN)}$ gene has previously been implicated to be associated with both amino glycoside-ototoxity, and non-syndromic hearing loss in a few genetically unrelatedindividuals,indicating that this mutation is involved in the pathogenesis of deafness. It might be that the environment contributes at the syndromics expression of the 7444 mutation, perhaps with other risk factors (the haplotypes mitochondrial or modified and/or associates nuclear genes) that are playing a role and to give one high penetrance.

The more the aminoglycosides are more routinely used, even for relative minor infections. These drugs are highly polarcations, which are not easy to be metabolized [33]. Glomerular filtration rapidly clears aminoglycosides from the majority of tissues and organs. However, these drugs may become concentrated in renal tubular cells and the perilymph and endolymph of the inner ear. The use ofthese drugs can frequently lead to toxicity, which involves the renal auditory and vestibular systems. The renal impairment is usually reversible, but the auditory and vestibular Ototoxicity is usually irreversible. Al through all of aminoglycosides are capable of affecting cochlear and vestibular functions, somehow streptomycin and gentamicin, that produced predominately vestibular damage, while other show neomycin and kanamycin cause mainly cochear damage [33-35]. That is crucial by sub unit association either by RNA-protein or RNA-RNA inter action when the mutation is in the rRNA12S [3,34,36]. In our patients, the damage caused by gentamicin would be cochlear.

The G7444A mutation is adjacent to the site of 3'end endonucleolytic processing of L-strand RNA precursor, spanning $tRNA^{ser(UCN)}$ and ND6 mRNA. Thus, the G7444A mutation, similar to the A7445G mutation, may also cause a defect in the processing of the L-strand RNA precursor, thus causing mitochondrial dysfunctions (Figure 8) [12].

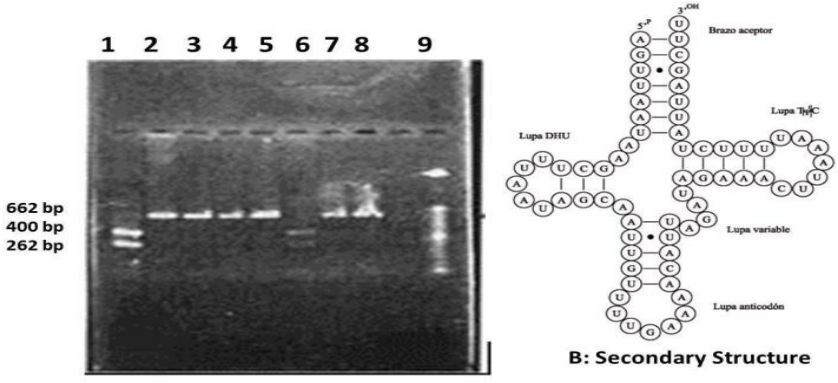

Figure 8: **A:** PCR-RFLP: line 1 and 6 are controls, lines 2 to 5 and 7 to 8 pedigrees with G7444A mutation after the cut with restriction enzyme Xba1; the enzyme did not recognize the site. Line 9 Mq.
B: tRNAser G7444A Secondary structure-mtDNA.

We failed to detect G28T mutation in the gene TRMU, which has been shown to be associated with non syndromicsHearing loss for 12SrRNAand $tRNA^{ser}$ genes. Complete medical histories from matrilineal family of all individuals showed clinical disorders, perhaps with an immunological component, that might constitute a syndromics presentation. Also, the Type A tympanograms and absence of TEOAEs in all patients, and the recordable acoustic reflexes in the patients II-5 and III-2 (Family 1 and 2), to been indicating that the hearing loss is severe to pro fund, which might to be produced after amino glycoside-treated. In the other patients (III-4 and IV-1, Family 1 and 3) without exposition at AG-ATB, his hearing loss might be associated after noise exposition, since that he had a lot of hours used the walkman in Family1, but for other factors in the Family 3. The patients provided strong evidence that the cochlear is the site of lesion.

Respect to the low activity of the COX in III-5 and II-5 patients Family1, II-2 in Family 2 and III-1 and 2 in Family 3, indicated that the muscle function and mitochondria's were affect. With the molecular studies, we have identified a homoplasmic G to A transition at position 7444 in the mitochondrial $tRNA^{ser(UCN)}$ gene. The clinical feature present at ion might be syndromic and the pathologies might be considerate with one component immunology in family 1 and 2. Mitochondrial muscle disease in three families, at the moment (Figures 6-8) .

The following evidence suggests that the 7444G> A mutation present in matrilineal family, should be the major pathogenic mtDNA mutation in these families, and the cause of genetic predisposition to syndromic hearing impairment. In lymphoid cells, the mutation to be presents homoplasmic and is present only in maternal relatives and not in the other members of the family. To accord it is possible that multi systemic mitochondrial syndromes might to be lethal in the homoplasmic state [36]. The phenotypic expression of a mitochondrial molecular defect can be influenced by multiple factors, such as the proportion of the mutation in different cells and tissues (the grade of heteroplasmy), and the nuclear genetic background of a given patient. Additionally mtDNA haplotypes and environmental factors are also known to play a crucial role.

Interesting, in family 1, the disorders to be inherited through the mtDNA, and might to be associated with one younger people presentations

Today, it has not been understood well, which are the factors that share, to that one mutation or variant in the mtDNA, develop one pathology, at one more early age. The variation in the percentage of heteroplasmy in the different tissues, in the news generations, might be one factor probable; but difficult to stablish, to give the problems in the abstention of the samples, from different tissues in the patients. We failed to detect any other mutation in the mitochondrial genome that might give account of the syndromic clinical feature. Moreover, the mutation is not present in 220 Argentinean controls.

It is possible that the 7444G> A mutation in the $tRNA^{ser(UCN)}$ gene in these Argentinean families has a syndromic presentation; as it was observed through the biochemical, molecular, and morphological studies; as well as the phenotype expression. However, it is possible that other mutations in the nDNA, or the haplogroup and haplotype that are present in them tDNA, where is observed the H haplogroup predominantly, as well as environment's risk factors may influence the clinical presentation too. In addition, it is necessary to know more about the mechanism by which ATB-AG induces hearing loss, in the presence of the 7444 G> A mutation in the $tRNA^{ser\ (UNC)}$.

Conclusion

Is possible, that the 7444G> A mutation in the $tRNA^{ser(UCN)}$ gene, in these Argentinean families, have a syndromic presentation; how it was seen, by the molecular, biochemical, and morphological studies, and the phenotype expression. But is possible that other mutations in the nDNA or either haplo group or haplotypes in the mtDNA to be present, and are related with environment's risk factor too.

Regarding the A827 G mutation in the 12 S rRNA genes, mtDNA, already published [6], the site is preserved in different species, nevertheless this mutation is considered a haplotypes of haplogroup B, because it's prevalence in closed populations is over 2%. This could negate the condition of pathological mutation, but, would you dare to prescribe AG-ATB to a patient with this variant, when the sequencing of the mitochondrial genome does not show another pathological mutation associated with AG-ATB?

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