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Analysis of mutations in leu tRNA gene in patients of heart diseases

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ABSTRACT

Cardiovascular diseases (CVD) are the leading cause of death all over the world. Beside general risk factors, there are some genetic factors which lead to cardiovascular diseases. Various nuclear DNA mutation and also mitochondrial DNA mutations have been related with cardiovascular diseases. In the present study, a total of 21 samples were collected from different families residing in district Dir. DNA was extracted from buccal epithelial cells using saliva. The mitochondrial tRNA leu (MT TL1) gene was amplified by PCR and 10 samples of different families were sequenced. The sequence was aligned with revised Cambridge Reference Sequence (rCRS) accession # NC-012920.1. It is concluded that cardiovascular diseases in our subjects are not due to mutation in the mitochondrial leucine tRNA gene. However, a large population of subjects with cardiovascular diseases needs to be studied and whole mitochondrial DNA is needed to be sequenced in the subjects with CVD. This will give an idea about the probable DNA marker which can be used to prevent losses due to these diseases at a very early stages.

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1. Introduction

Heart diseases are main reason of deaths globally. Heart diseases are elevated blood pressure, coronary heart disease, cardiomyopathy, heart failure and stroke. CHD is responsible for 50,200 deaths in USA per year, and above 70,000 deaths in China (Lopez et al., 2006; Zhang et al., 2008). According to global burden of disease study ischemic heart disease is the main reason of global mortality. It is responsible for 1.4 million deaths in low economic country and 5.7 million deaths in high economic countries (Santulli, 2013), as it is third global burdened disease graded by WHO (Murray and Lopez, 1996) and leading deadly disease till 2020 (Murray and Lopez, 1997).

In Middle East and African regions, incidence of CAD is important as per WHO report for South Africa, death rate because of

CAD was 120 per 100,000 populations (Almahmeed et al., 2012). In Tunisia and Egypt the death rates associated with CAD were counts to be 163.8 and 280 per 100,000 populations respectively (Ben et al., 2004). Tajikistan, 17.5% of all deaths were because of CAD (Gaziano et al., 2010). Reports published by Saudi government, deaths in pilgrims in 2006 were associated with heart problems (Sapa, 2006). In Pakistan little data is available about CHD patients having occurrence of CHD from 16.76 to 60% (Ahmad et al., 2002; Sadiq et al., 2001). In Pakistan CVD (12% of all cause mortality) seems on rise because to its aging population and increased urbanization (Ahmad et al., 2002).

In mtDNA mutations A-to-G transition at position 3243 is most frequently observed in tRNA (Leu (UUR)) gene, which shown change in mitochondrial structure, methylation, amino-acylation and codon recognition of tRNA_{Leu} (Goto et al., 1990). This mutation, which gave rise to a wide range of phenotypes including cardiomyopathy deafness, and intolerance to exercise, and diabetes, is predominant in about 80% of patients with MELAS (Uusimaa et al., 2007). Recently described maternally inherited adult-onset syndrome, characterized clinically by variable combination of skeletal and heart muscle failure (MIMyCa, maternally inherited myopathy and cardiomyopathy), and molecularly by presence of heteroplasmic point mutation in mitochondrial DNA (mtDNA) transfer RNA

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(tRNA) Leu (UUR) gene (zevaini et al., 1991). Pathogenicity of mutation, an A → G transition at mtDNA nucleotide position 3260 (Anderson et al., 1981). The present study was conducted to analyze leu tRNA gene for mutation in patients with heart disease and to compare our amplified sequences with the other reported sequences.

2. Materials and methods

2.1. Sample collection

Saliva was collected in 3 to 5 ml sterile cup. The patient's teeth were brushed and were given 5% sugar solution to each patient. Along with this the body weight, age, previous treatment were also recorded from the patient. The samples were collected and brought to the molecular genetics lab of Hazara University Mansehra and were stored at -20 °C.

2.2. Sampling and DNA extraction

Samples were collected from subjects with heart diseases. The DNA was extracted from the buccal cavity epithelial cells of samples by following the procedure of Aidar and Line (2007).

2.3. Technique for making agarose gell

For checking quality of extracted DNA, agarose gel (0.5 g of agarose dissolved in 29.4 ml DDH₂O with 600ul of 50X TAE mixture) 25ul ethidiumbromide was taken, five micro liter of isolated DNA was dissolve with 2ul of loading dye and run for 30 min at 60 V, and was photographed through ultraviolet light using gel documentation system.

2.4. Polymerase chain reaction

Extracted DNA was further used in PCR to amplifying the desired mtDNA Leu tRNA gene.

2.5. Optimal conditions for PCR

There are 3 steps of PCR which run for 40 cycles. First step is denaturation which is for 5 min at 95 °C, 2nd step annealing for 45 sec at 50 °C and 3rd step extension which was done for 5 min at 72 °C. There is also two extra steps one is called Pre PCR denaturation which was done for 5 min at 95 °C. Another extra step was Post PCR extension which is for 5 min at 72 °C.

2.6. Agarose gel electrophoresis of PCR product

Final PCR products were then analyzed on 1% agarose gel. Agarose gel was formerly set through heating 0.5 g agarose mixed with 29.4 ml ddH₂O and 600ul 50X TAE. Then ethidium bromide of 12 μl was added to the mixture. The melted blend used to be stored at 25 °C for cooling. The agarose mixture used to be added into gel plate so that it finally becomes solidified. PCR product of 15 μl of was mixed with 2 μl DNA loading blue dye and loaded into the wells of the agarose gel. Supply 65 voltages for 35 min in electrophoresis approach until DNA fragments move from left to right. Amplified fragments were then checked under UV light and photographed.

2.7. Cleaning of amplified gene piece DNA from gel

The PCR amplification was washed by TIAN gel Midi purification Kit having Cat # DP20902. PCR band including tRNA_{Leu} (UUR) gene

was cut with sterilized surgical blade and kept in labeled Eppendorf tubes.

2.8. Sequencing and data analysis

Eluted DNA of 10 samples were sent to Macrogen, Inc. Korea for sequencing. The resulted sequence data was align with Revised Cambridge Reference Sequence (rCRS) Accession NO NC-012920.1 of whole mitochondrial sequence. The alignment was analyzed for mutation in leu tRNA gene.

2.9. Ethical disclosures

This research was approved from the ethical committee of Hazara University Mansehra.

3. Results

3.1. DNA extraction

All the collected samples were processed for DNA extraction in which we get results of only 10 samples as shown in Fig. 1.

3.2. Amplification of tRNA-Leu gene

PCR was done using the forward and reverse primers specific for a region containing leu tRNA gene. A fragment of 279 bp was amplified as shown in Fig. 2.

3.3. Sequencing analysis

To sequence the cleaned DNA was sent to Macrogen, Inc Korea. We aligned the resulted sequence with mitochondrial genome sequence (Revised Cambridge Reference Sequence (rCRS) (Accession NO NC-012920.1). We sent 10 samples for sequencing and got the sequencing result of 9 samples. The information and analysis of these samples are as follows (see Fig. 3).

3.4. Sample A-1

This is a female subject who was diagnosed with Heart disease (Cardiac valve problem) having age of 90 years. Family history showed that her mother was suffering from heart disease. Her children, brother and sister were also suffering from heart disease. The patient weight was 50 kg and was diagnosed with some other diseases like; skin problem, eye problem and blood pressure. The patient was using canita- D, joutit tablets. No mutation was present in leu-tRNA gene of this subject. Although there is a mismatch of two nucleotides in the alignment, however when we analyzed the chromatogram of this sequencing result we found that it was technical error and not exact miss match (see Fig. 4).

3.5. Sample A-2

This is a female subject who was diagnosed with Heart disease (Cardiac valve problem) having age of 67 years. Family history showed that her mother was suffering from heart disease. Her brother and sister were also suffering from heart disease. The patient weight was 53 kg and was diagnosed with some other diseases like; eye problem and blood pressure. The patient was using canita- D, joutit tablets. No mutation was present in leu-tRNA gene of this subject (see Fig. 5).

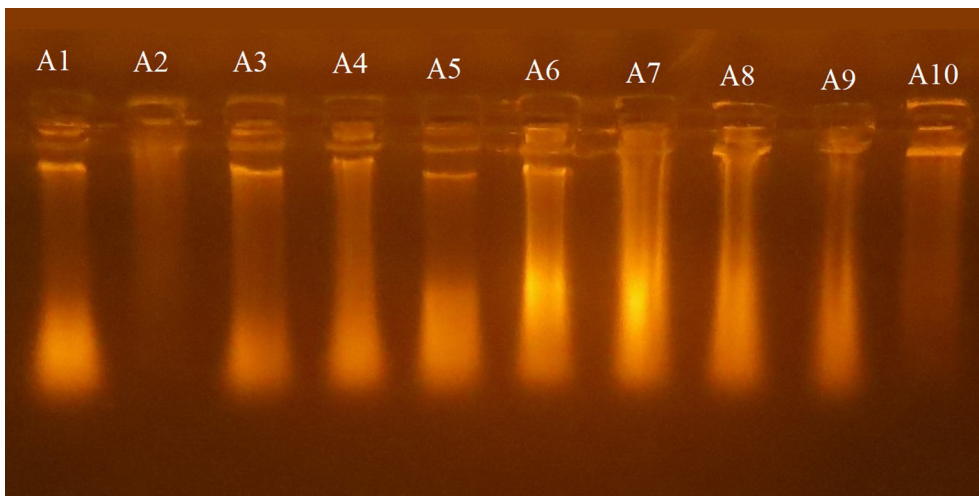


Fig. 1. Total genomic DNA extracted from samples (A1-A10).

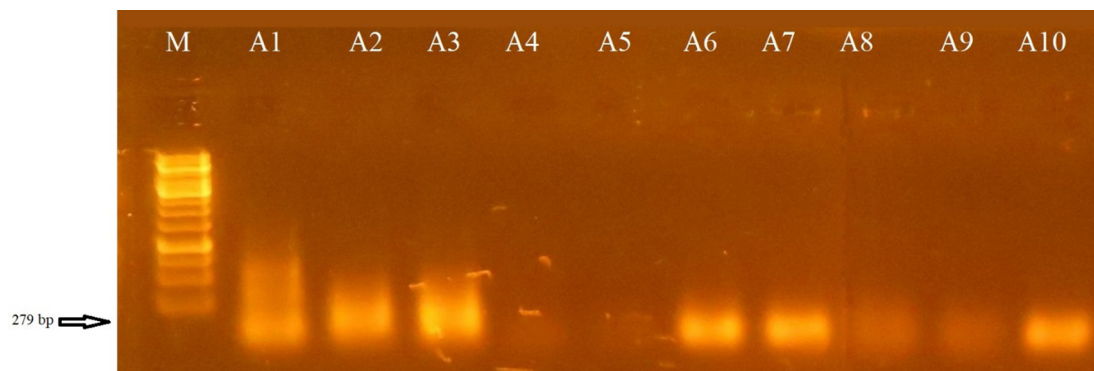


Fig. 2. PCR amplification of leu tRNA gene. A fragment of ca 279 bp was amplified. M = 100 bp DNA Ladder Marker and the remaining are samples.

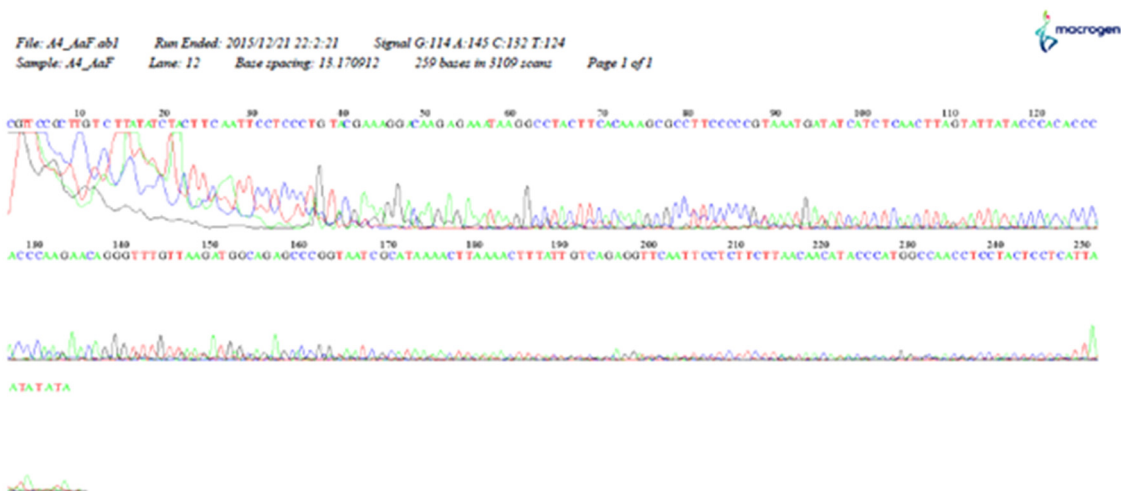


Fig. 3. Chromatogram of sample A-1 as a sequencing result provided by MacroGen Inc. South Korea.

3.6. Sample A-3

This is a female subject who was diagnosed with heart diseases (palpitation) having age of 45 years. Family history showed that her mother was suffering from heart disease. The weight of the

patient was 65 Kg. Hearing loss was also diagnosed in the patient. The patient was using omega (40 mg), andral (10 mg), lopren tablets. No mutation was present in leu-tRNA gene of this subject (see Fig. 6).

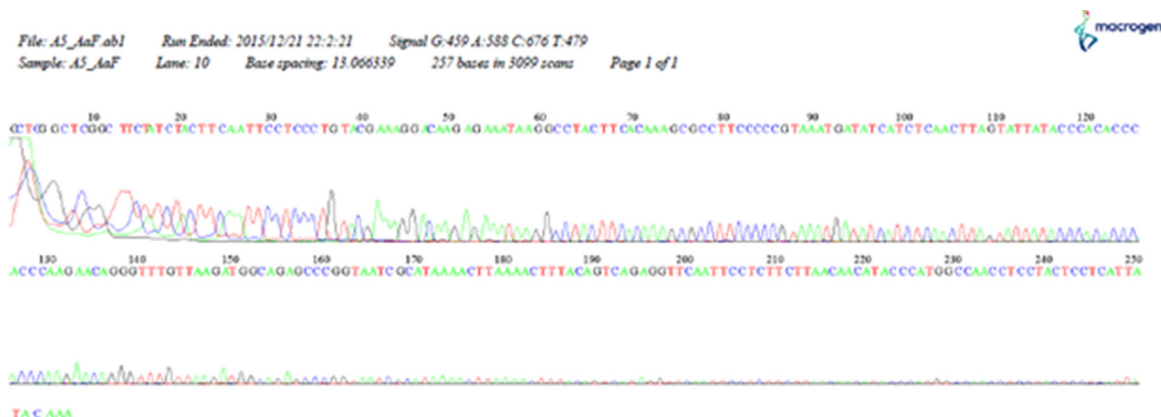


Fig. 4. Chromatogram of sample A-2 as a sequencing result provided by Macrogen Inc. South Korea.

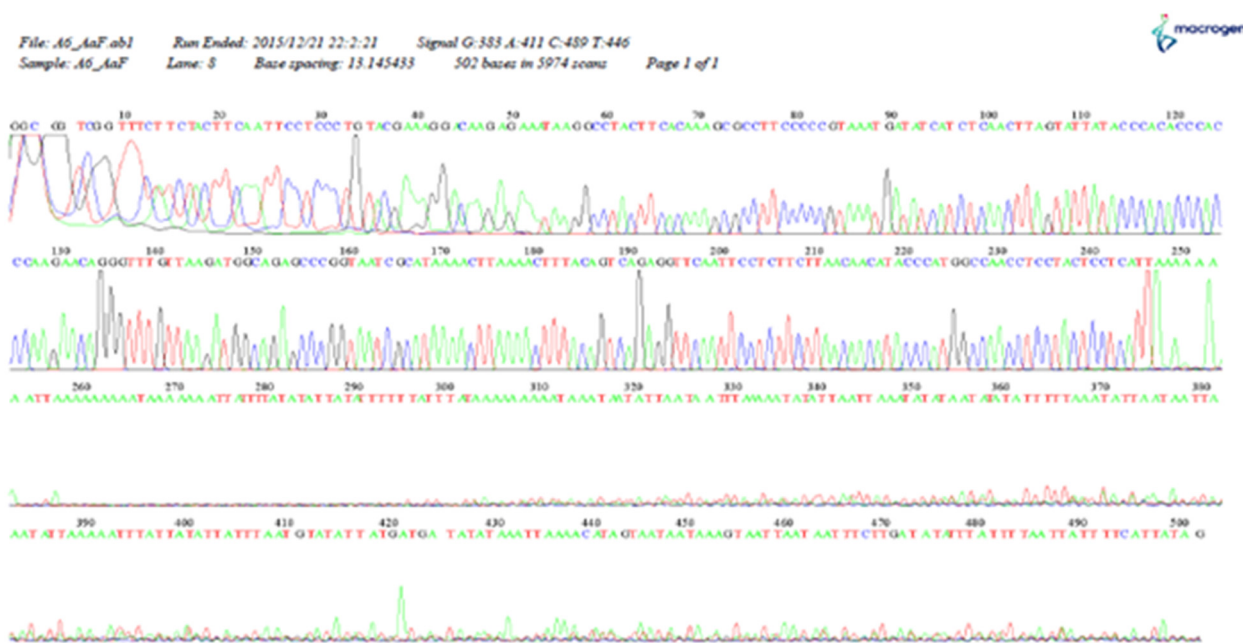


Fig. 5. Chromatogram of sample A-3 as a sequencing result provided by Macrogen Inc. South Korea.

3.7. Sample A-4

This is a male subject who diagnosed with heart disease (MI) having age of 60 years. His mother was suffering from heart problem. Family history showed that his brothers and sisters were also suffering from heart disease. The weight of the patient was 75 Kg and was diagnosed with other diseases like, eye problems and pain. The patient was using toplate (75 mg) tablets. No mutation was present in leu-tRNA gene of this subject (see Fig. 7).

3.8. Sample A-5

This is a female subject who diagnosed with heart disease (arteriosclerosis and palpitation) was having age of 50 years. Her mother was suffering from heart problem. Family history showed that only her one sister has record of heart disease. The weight of the patient was 90 Kg and was diagnosed with joint pain. The patient was using toplate (75 mg) tablets. No mutation was present in leu-tRNA gene of this subject (see Fig. 8).

3.9. Sample A-6

This is a male subject who diagnosed with heart disease (valves problem) was having age of 50 years. His mother were suffering from heart problem. Family history showed that his brothers were suffering from heart disease. The weight of the patient was 70 Kg and was diagnosed with other diseases such as, eye problems, allergy problems and stomach problems. The patient was using bisprol (5 mg), estar (10 mg), loprin tablets. No mutation was present in leu-tRNA gene of this subject (see Fig. 9).

3.10. Sample A-7

This is a male subject who was diagnosed with heart disease (valves problem) having age of 45 years. His mother was suffering from heart problem. Family history showed that his brothers was also suffering from heart disease. The weight of the patient was 75 Kg and was diagnosed with other diseases such as, skin allergy problem, kidney problem and stomach problem. The patient was using low plat (75 mg), rust (20 mg), herbesser (30 mg) tablets.

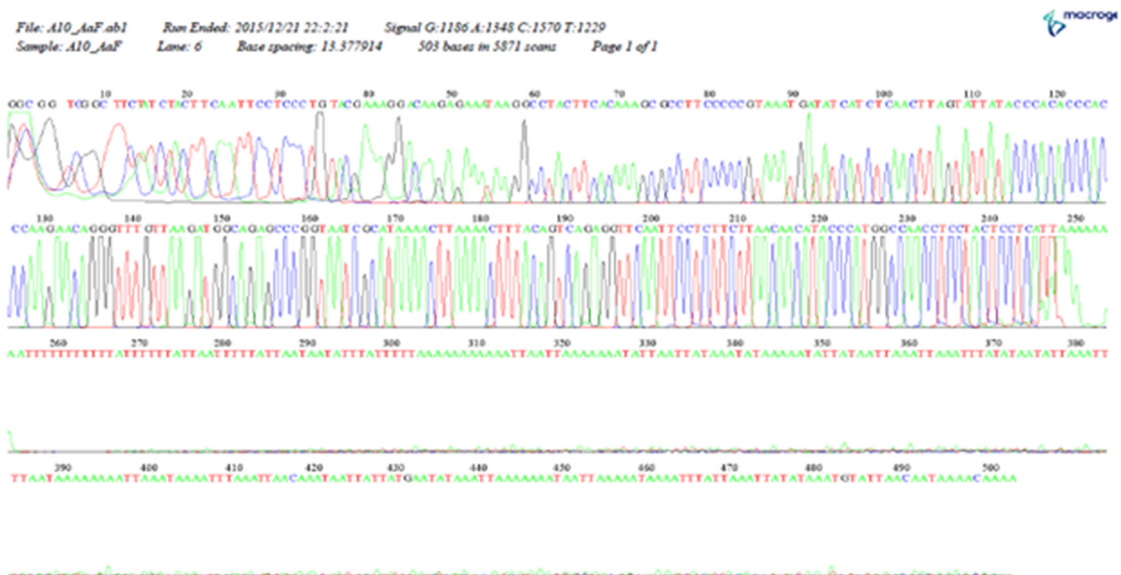


Fig. 6. Chromatogram of sample A-4 as a sequencing result provided by Macrogen Inc. South Korea.

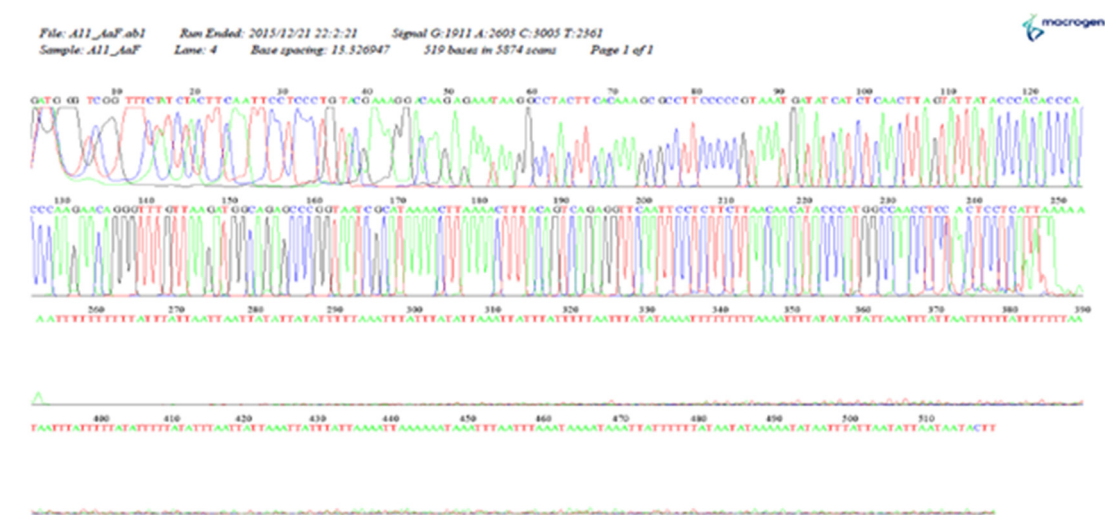


Fig. 7. Chromatogram of sample A-5 as a sequencing result provided by Macrogen Inc. South Korea.



Fig. 8. Chromatogram of sample A-6 as a sequencing result provided by Macrogen Inc. South Korea.

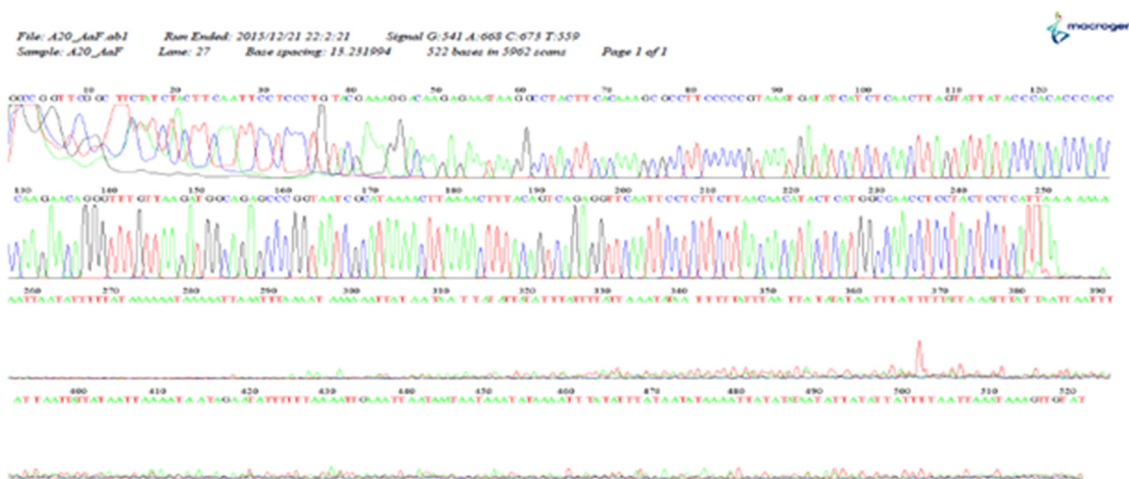


Fig. 11. Chromatogram of sample A-9 as a sequencing result provided by Macrogen Inc. South Korea.

ops before time in A3243G mutation, commonly present in perspective of stroke-like episode or status epileptics. Patients usually are at a highest risk of developing stroke like episodes with the mutation in Leu tRNA (Roger et al., 2015). Family having A3288G mutation in tRNA^{Leu (UUR)} gene have maternally inherited mitochondrial myopathy. They confirmed that tRNA^{Leu (UUR)} is hotspot for mtDNA mutations and frequently associated with respiratory muscle involvement (Hadjigeorgiou and Kim, 1999).

Patient with severe heart failure was analyzed as having a mitochondrial A3243G mutation lies in Leu tRNA. Such patients were reported with cardiomyopathy, either alone or as part of a multi-system disorder (Nan et al., 2002). A to G substitution at nucleotide position 3243 in mtDNA encoded tRNA^{Leu UUR} in a 46 year old man suffering from shortness of breath at rest, chest discomfort, lower limb edema was also reported (Johann and Auer, 2016). Cardiac abnormalities are common and progressive in patients with the A3243G mtDNA mutation and cardiac autonomic regulation is changed. Most frequent causes of death were neuropsychiatric and cardiovascular diseases (Majamaa, 2007).

In our study we attempted to find out relation of mutation in mitochondrial encoded Leu tRNA (MTL1) gene with cardiovascular disease in subjects from District Dir Khyber Pakhtunkhwa. We took a step to uncover the connection of this disease with many other mutations. A total of 21 subjects were included in this study suffering from cardiovascular disease. Amplified Leu tRNA gene of 9 subjects was sequenced. They were in age between 20 and 90 years suffering from other abnormalities like MELAS, kidney and tooth problems as well. However we could not find any mutation in Leu tRNA gene of our subjects related to cardiovascular diseases. This may be due to the small size of the subjects in which we examined the sequence of their Leu tRNA gene or some other genes encoded by mitochondria play its role.

5. Conclusion

It is concluded that in the samples, which we collected from the patients suffering from heart disease, we could not find any mutation in the leu (MT TL1) gene. It is recommended that other genes encoded by mitochondrial genome should be studied in subjects with cardiovascular disease. It is also recommended that a large number of subjects should be included in future studies to reach to some concrete conclusion.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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