MITOCHONDRIAL CYTOCHROME B (MT-CYB) GENE MUTATION CAUSE THE HYPERTROPHIC CARDIOMYOPATHY

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HYPERTROPHIC CARDIOMYOPATHY

Hypertrophic cardiomyopathy (HCM) is a disease in which the heart muscle becomes abnormally thick (hypertrophied). The thickened heart muscle can make it harder for the heart to pump blood.

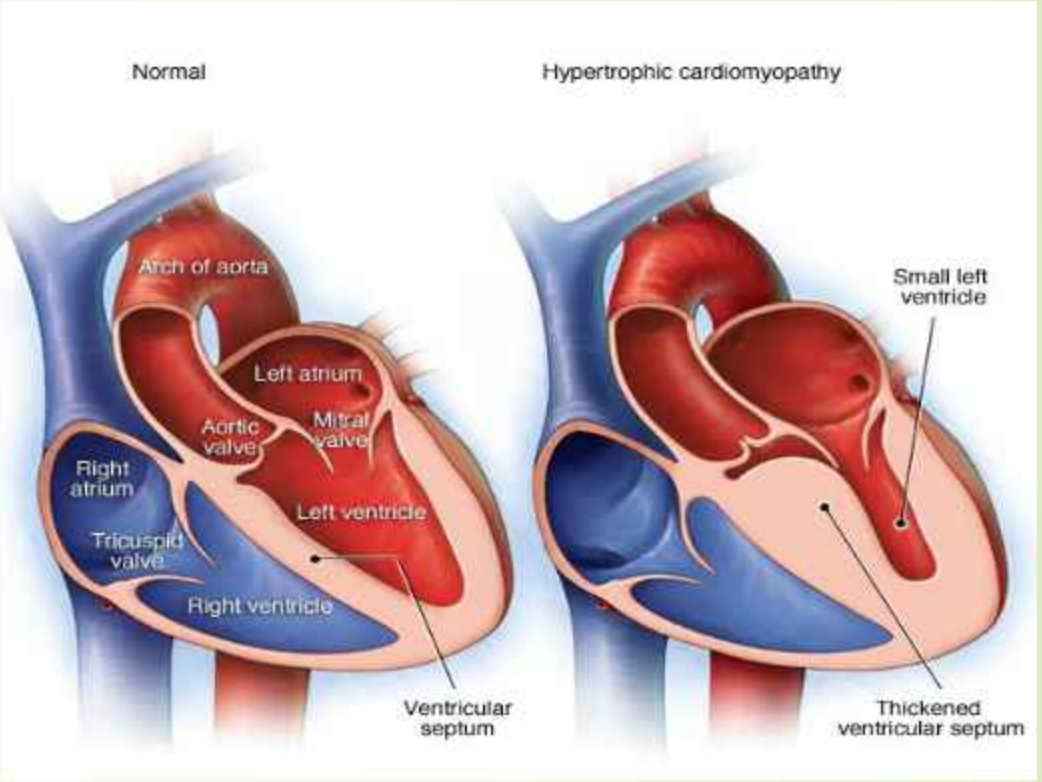


Figure 1. Diagrammatic representation of HCM.

SYMPTOMS

- Chest pain, especially during exercise
- Fainting, especially during or just after exercise or exertion
- Heart murmur, which a doctor might detect while listening to your heart
- Sensation of rapid, fluttering or pounding heartbeats (palpitations)
- Shortness of breath, especially during exercise.

COMPLICATIONS

- Atrial fibrillation.
- Blocked blood flow
- Mitral valve problems
- Dilated cardiomyopathy.
- Heart failure.
- Sudden cardiac death

GENETIC BASIS

- Among the known causal genes, MYH7 and MYBPC3 (myosin-binding protein C) are the 2 most common.
- Mutations TNNT2, TNNI3 (cardiac troponin I), and TPM1 (α-tropomyosin) are relatively uncommon causes of HCM and together are responsible for <10% of cases.
- Mutations in ACTC1 (cardiac α-actin), MYL2 (myosin light chain 2), MYL3 (myosin light chain 3), and CSRP3 (cysteine and glycine-rich protein 3) are also established, albeit uncommon, causes of HCM.

Mitochondrial DNA variations associated with hypertrophic cardiomyopathy

- Mitochondrial DNA (mtDNA) mutations and haplogroups have been found to be associated with several diseases.
- There is a connection between mutations in the mitochondrial DNA and HCM. A phenotype affecting several organs is a common consequence of mutations in mitochondrial DNA, one of which is heart illness

Cytochrome B (Cyt-B)

- The MT-CYB gene provides instructions for making a protein called cytochrome b. This protein plays a key role in structures called mitochondria, which convert the energy from food into a form that cells can use.
- Cytochrome b within both molecular and cell biology, is a protein found in the mitochondria of eukaryotic cells.
- It functions as part of the electron transport chain and is the main subunit of transmembrane cytochrome bc1 and b6f complexes.

CONT…

- Cytochrome b is one of 11 components of a group of proteins called complex III.
- Complex III performs one step of a process known as oxidative phosphorylation, in which ATP is produced.
- Cytochrome b is commonly used as a region of mitochondrial DNA for determining phylogenetic relationships between organisms, due to its sequence variability. It is most useful in determining relationships within families and genera.

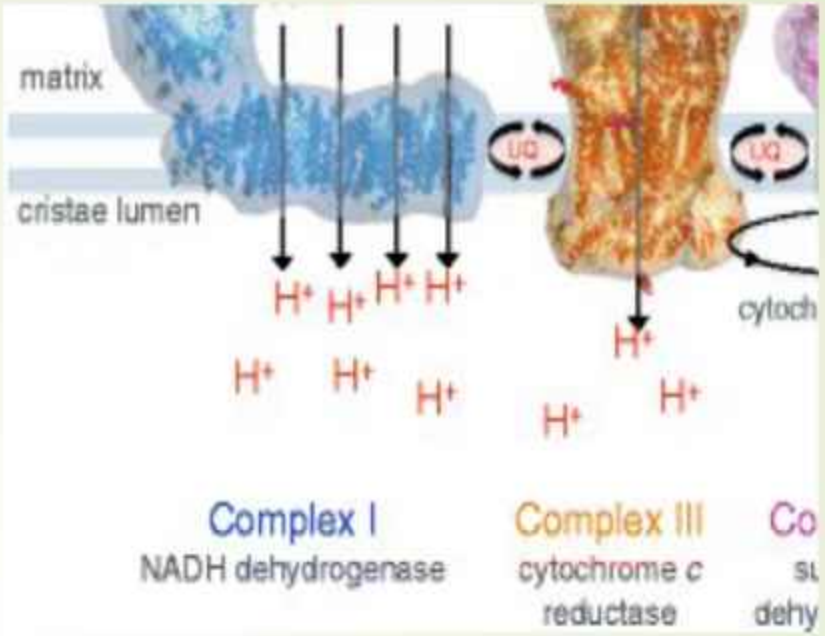


Figure 2. Visual representation of mitochondrial respiratory chain complexes

Position of Cytochrome B (CytB) gene on Mt-genome

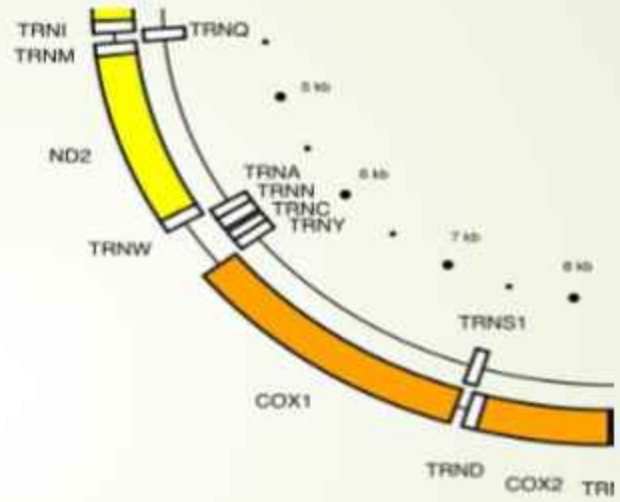


Figure 3. Locus of Cytochrome B (Cyt-B) gene on mitochondrial genome.

STATEMENT OF PROBLEM

- The most common cause of sudden cardiac death (SCD) in children and adolescents is hypertrophic cardiomyopathy (HCM). HCM has been associated to mutations in mitochondrial DNA (mtDNA). Mutations in the mitochondrial DNA have been found to cause isolated hypertrophic cardiomyopathy (HCM), which codes for complex III cytochrome B (CIII) (HCM). A group of people with HCM has been shown to have MT-CYB mutations, which are thought to cause mtDNA leakage. To further understand the role of MT-CYB mutations in HCM, they should be tested in patients.

HYPOTHESIS

MT-CYB mutations might play an important causal or modifying role in hypertrophic cardiomyopathy (HCM).

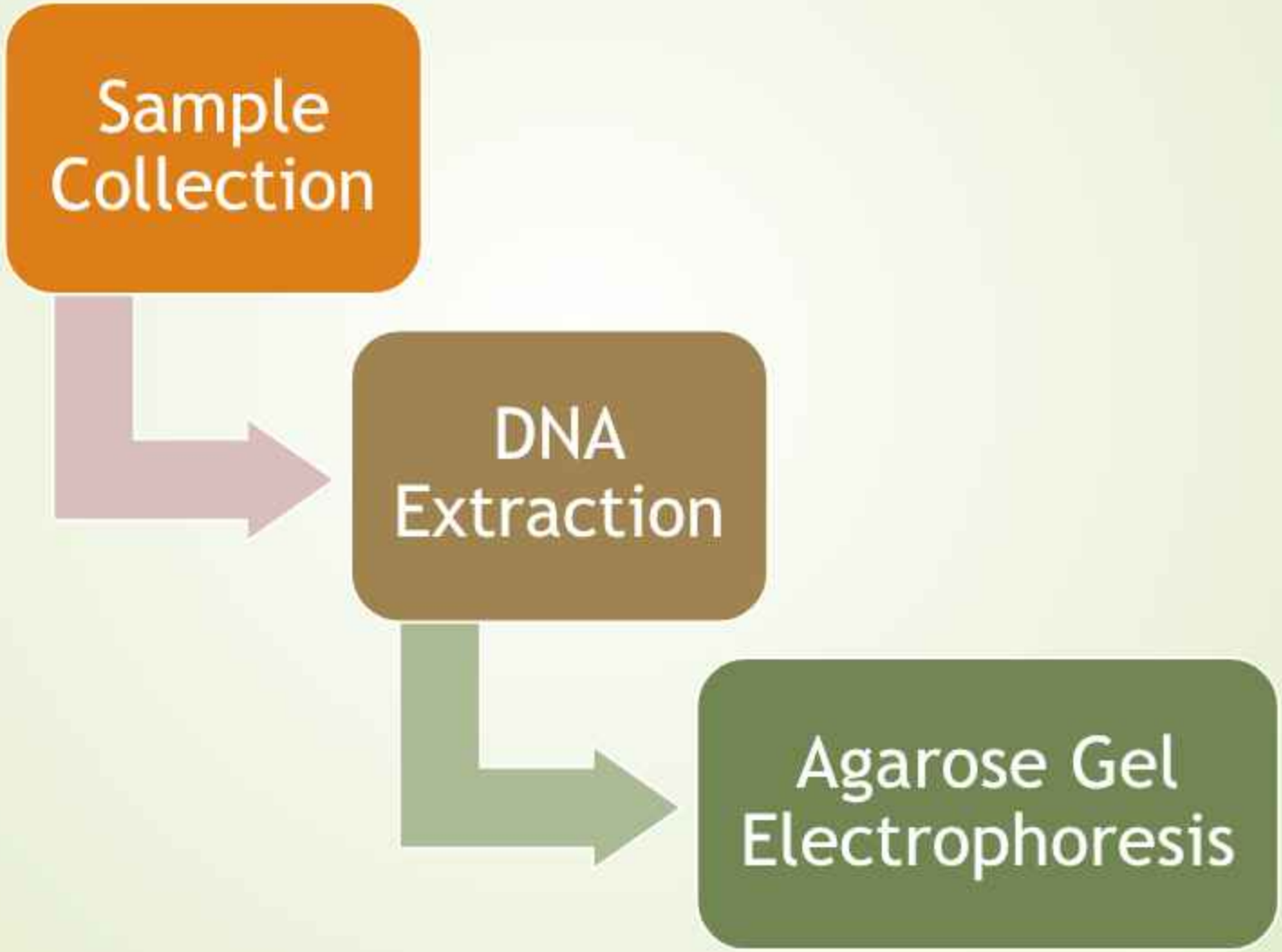
OBJECTIVES

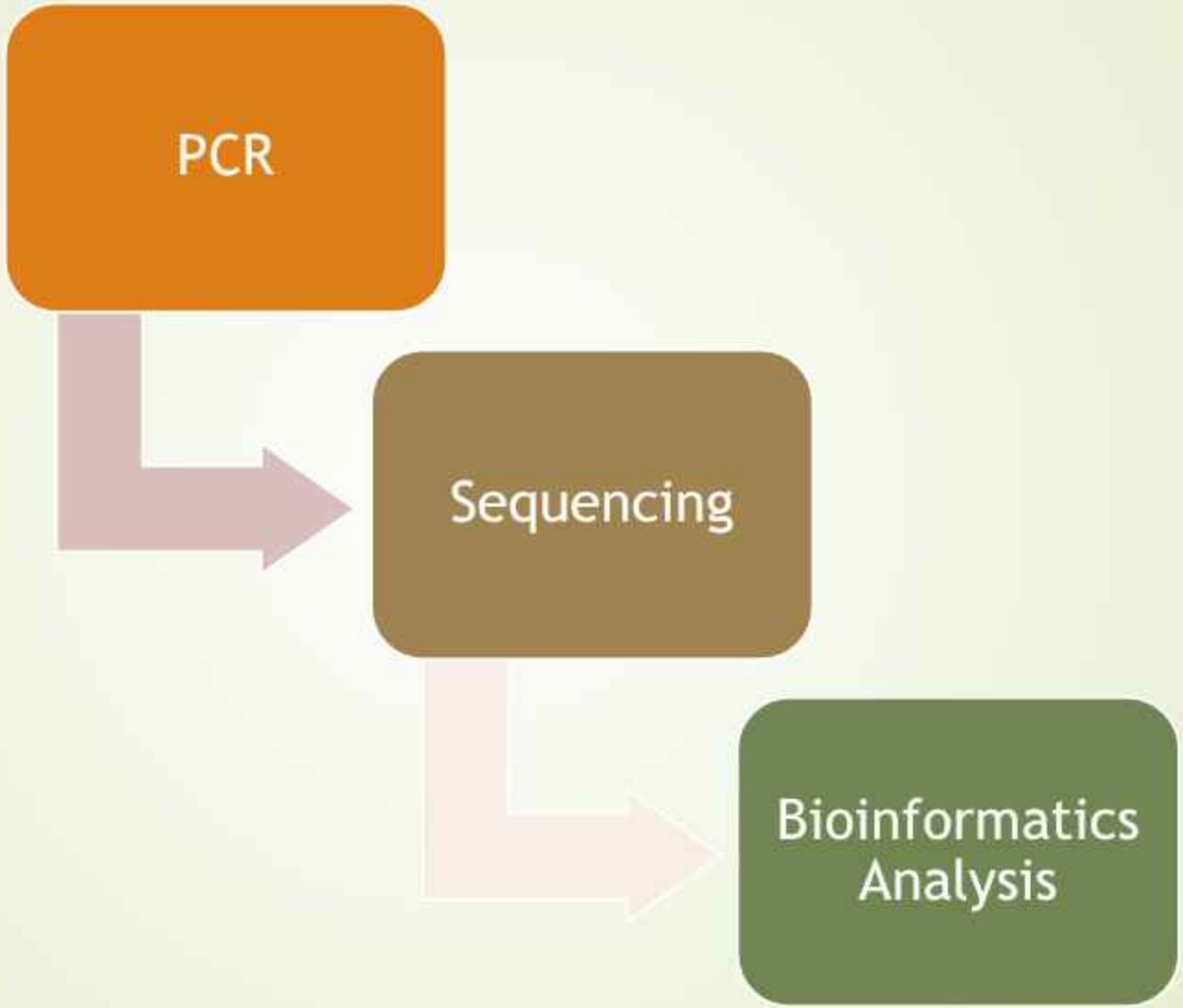
The objectives of the current study are:

1. To screen CYTB gene in CVDs subjects for any variation.

2. To locate the position of variations in CYTB gene across CVD patients.

METHODOLOGY




PCR
Sequencing
Bioinformatics Analysis

RESULTS

➢ Patient's demographics and disease history

Table 1. Representing the details of individuals selected for this study.

03	MA-3	65 years	Fen
04	MA-4	55 years	Fen
05	MA-5	50 years	Fen
06	MA-6	62 years	Fen

➢ Patient's demographics and disease history

12	MA-12	40 years	Fen
13	MA-13	48 years	Fen
14	MA-14	53 years	Fen
15	MA-15	35 years	Fen
16	MA-16	58 years	Fen

➢ DNA Extraction Results

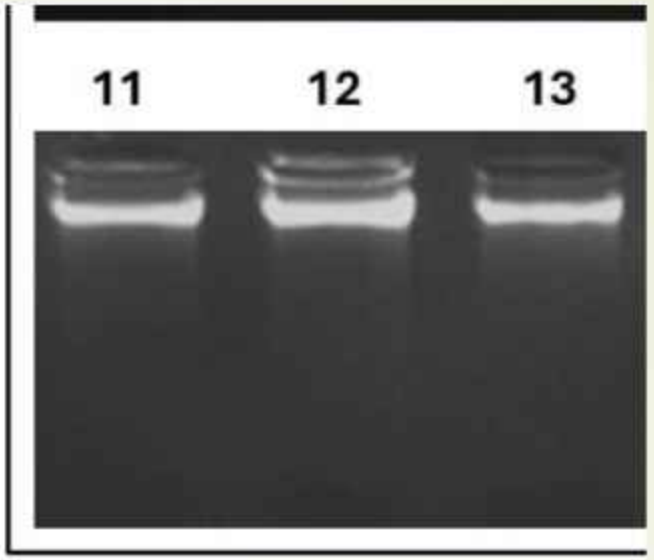


Figure 4. UV-photograph of extracted DNA from 16 patients. 1 to 16 represents the patient ID from MA-1 to MA-16.

➢ PCR Optimization

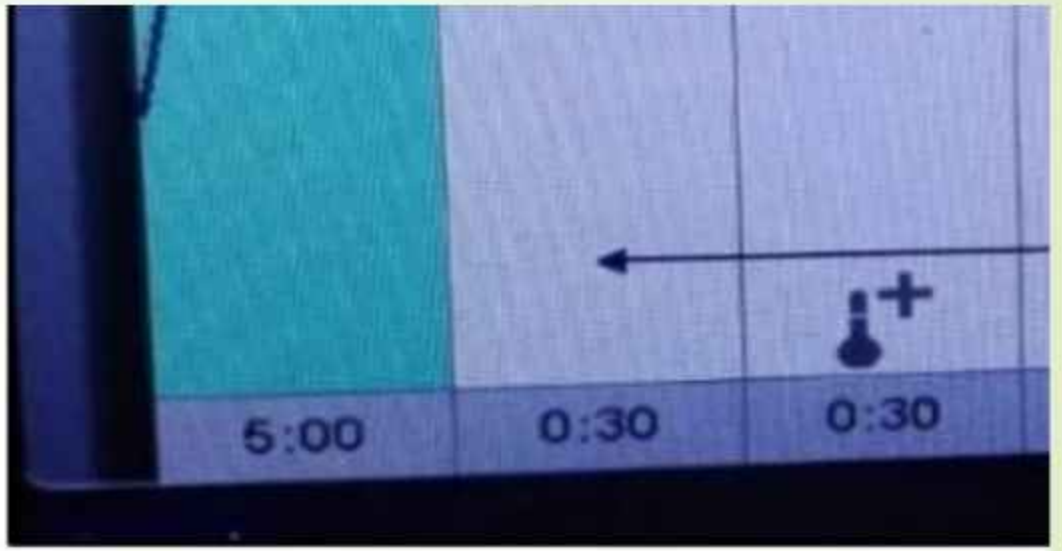


Figure 5. Optimized PCR condition for primer set 1 and primer set 2.

➢ PCR Results

Figure 6. PCR product of MT-CYB gene amplified with primer set 1. 1 to 16 represents the patient ID from MA-1 to MA-16. ‘L’ is the ladder.

➢ PCR Results

Figure 7. PCR product of MT-CYB gene amplified with primer set 2. 1 to 16 represents the patient ID from MA-1 to MA-16.

➢ Sanger Sequencing Analysis

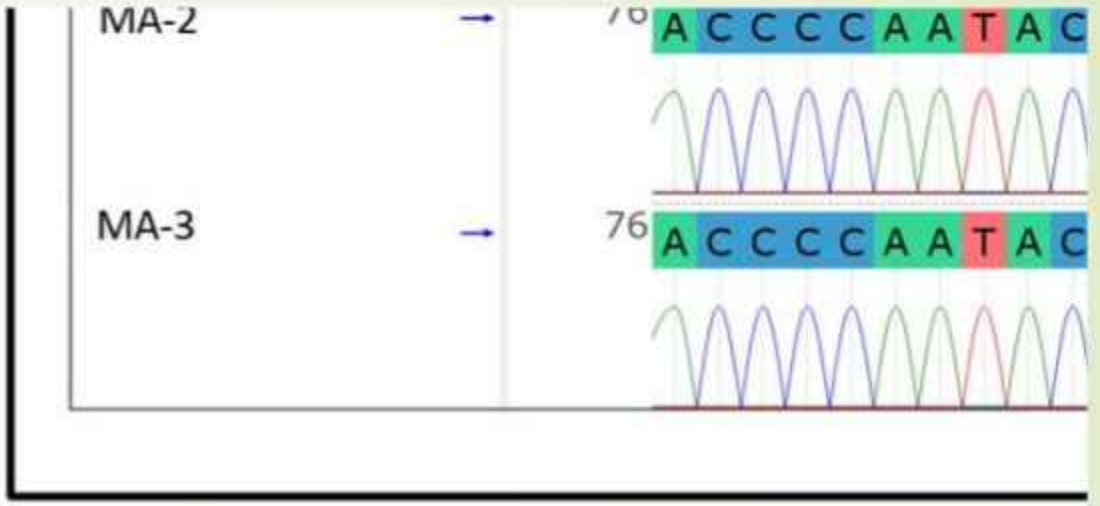


Figure 8. Variation analysis of MT-CYB sequenced with primer set 1 in patient MA-1, MA-2, and MA-3.

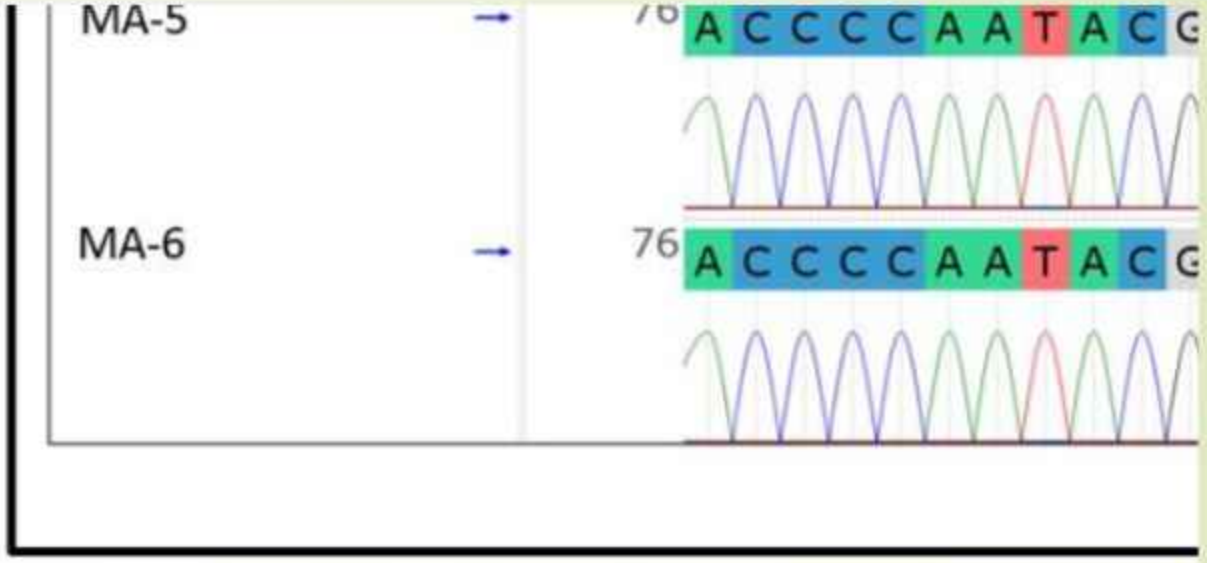


Figure 9. Variation analysis of MT-CYB sequenced with primer set 1 in patient MA-4, MA-5, and MA-6.

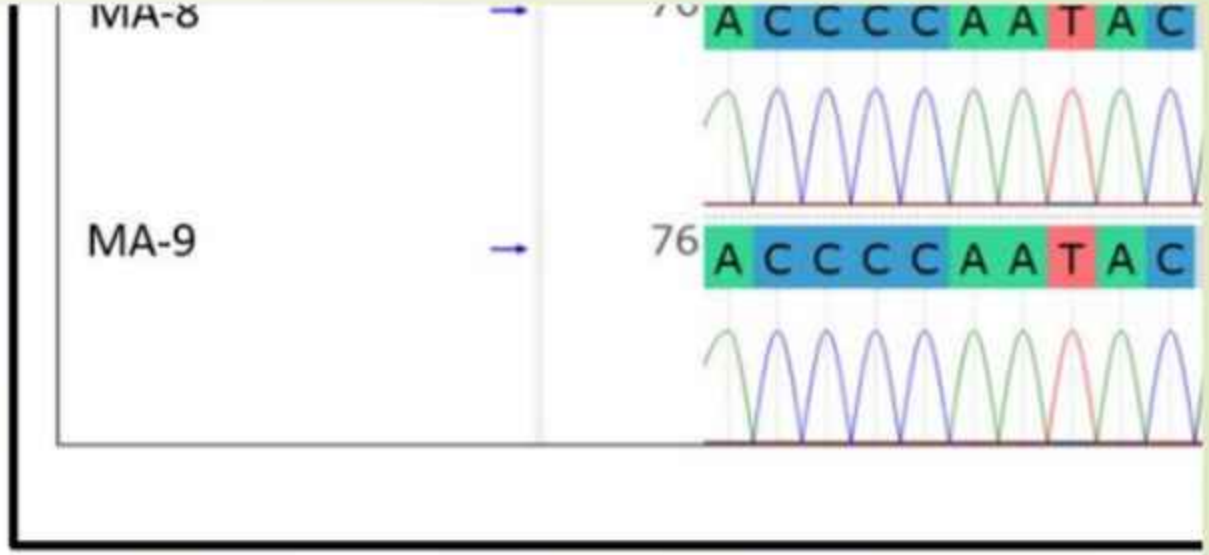


Figure 10. Variation analysis of MT-CYB sequenced with primer set 1 in patient MA-7, MA-8, and MA-9.

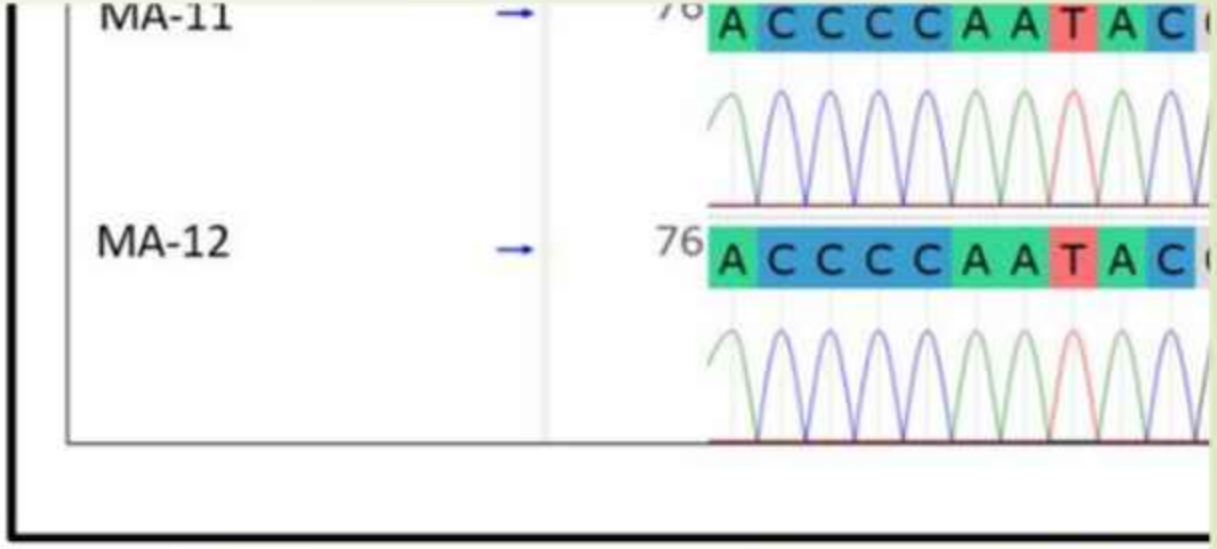


Figure 11. Variation analysis of MT-CYB sequenced with primer set 1 in patient MA-10, MA-11, and MA-12.

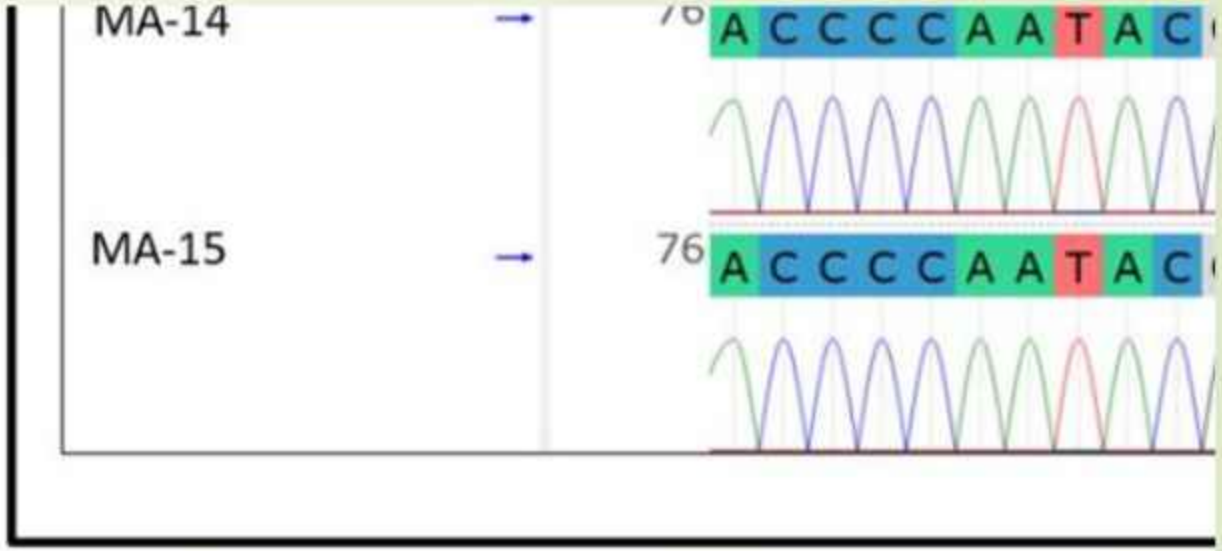


Figure 12. Variation analysis of MT-CYB sequenced with primer set 1 in patient MA-13, MA-14, and MA-15.

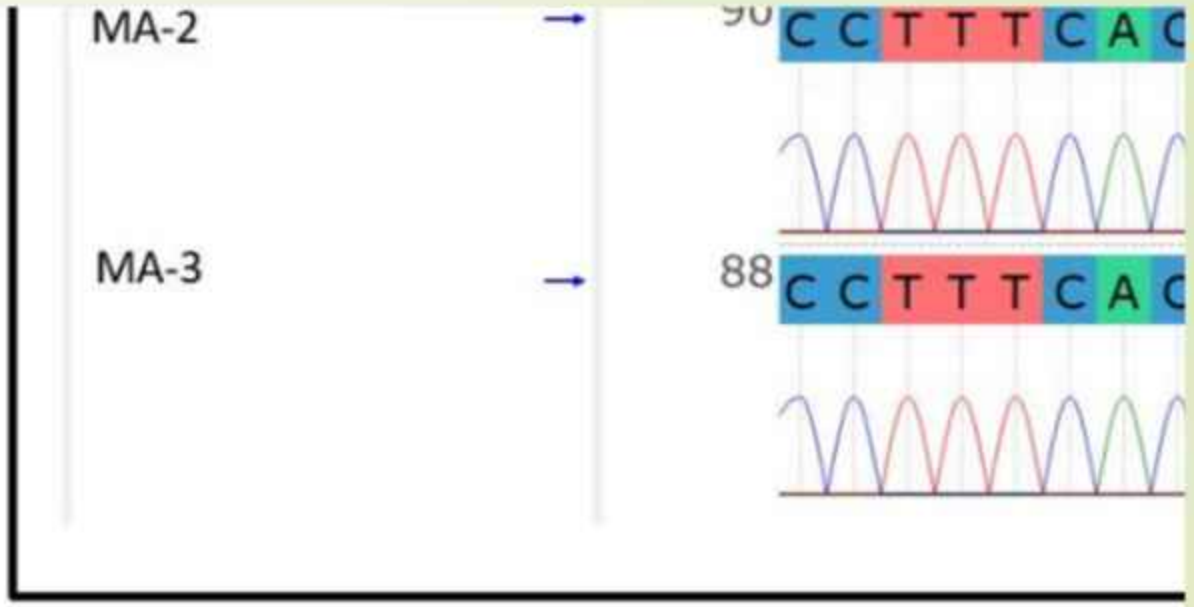


Figure 13. Variation analysis of MT-CYB sequenced with primer set 2 in patient MA-1, MA-2, and MA-3.

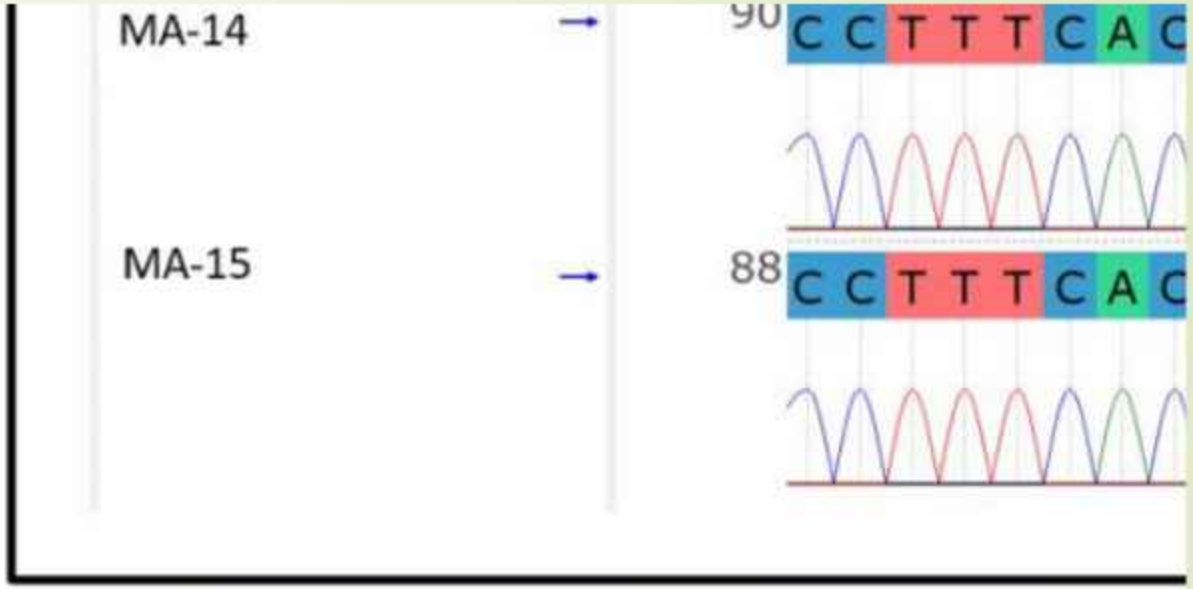


Figure 14. Variation analysis of MT-CYB sequenced with primer set 2 in patient MA-8, MA-14, and MA-15.

CONCLUSION

- This study successfully identified specific mitochondrial cytochrome b (MT-CYB) gene variations in Pakistani patients diagnosed with hypertrophic cardiomyopathy (HCM) through targeted sequencing of 16 patient samples from Abbottabad.
- The study revealed two distinct point variations with varying prevalence patterns among the cohort.
- The C14766T variation demonstrated remarkably high prevalence, appearing in 15 out of 16 patients (93.75%).

- In contrast, the G15301A variation exhibited selective occurrence, present in only 6 patients (37.5%): MA-1, MA-2, MA-3, MA-8, MA-14, and MA-15.
- Both identified variations represent transition substitutions (pyrimidine-to pyrimidine and purine-to-purine changes), which are characteristic of mitochondrial DNA variations.

RECOMMENDATIONS

- The functional validation studies are highly recommended to determine whether C14766T and G15301A directly impair mitochondrial function.
- *In-silico* protein structural modeling should be performed to predict how these amino acid substitutions affect cytochrome b structure, heme binding, and interactions with other Complex III components.
- The small sample size of 16 patients necessitates validation in a larger cohort.
- Critically, this study lacks healthy controls, making it impossible to determine whether C14766T is truly pathogenic or merely a benign regional polymorphism.

- Designing additional primers for complete gene coverage, including the 5' and 3' untranslated regions, would ensure no pathogenic variations are overlooked.
- Comparing variation frequencies across different ethnic groups within Pakistan (Punjabi, Pashtun, Sindhi, Baloch) would also contribute to understanding population specific genetic architecture.
- Long-term goals should include developing genetic counseling protocols for families with identified MT-CYB variations and establishing risk stratification models incorporating MT-CYB genotypes alongside traditional clinical parameters.

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Thank You