Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

https://pakjmcr.com/index.php/1/about

"Mutational Analysis Of Mitochondrial Trna Proline Gene In Myoclonal Epileptic Patients From North Waziristan"

1.Muhammad Riaz Khan
Medical Lab Technologist, UAE.
2. Kaleem Ullah (Corresponding Author)
Senior Lecturer Medical Laboratory Technology Sarhad University Peshawar, Pakistan
3. Tahir Ullah
MS-MLS , Riphah International University, Islamabad.
Lecturer of MLT, Rehman Medical Institute Peshawar
Email: Tahirullah9799@gmail.com
4. Mansoor Badshah
MPHIL in Molecular biology, Islamia College Peshawar.
Email: bmansoor500@gmail.com
5. Noor ul Nisa
Sarhad University of Science and Information Technology, Peshawar
Email: noorulnisa1122n@gmail.com
6. Shaukat Aman
Islamia College Peshawar.
Email: shaukataman1234@gmail.com
7. Anas Bacha Shaheen
Assistant Professor, Rehman Medical Institute (RMI), Peshawar.- Email: Anas.shaheen@rmi.edu.pk
8. Musadiq Khan
Assistant Professor, Rehman Medical Institute (RMI), Peshawar. Email: musadiq.khan@rmi.edu.pk

Author Details

Keywords: COVID-19, Myocarditis, Pericarditis, Vaccination, Males

Received on 25 May 2025
Accepted on 19 June 2025
Published on 28 June 2025

Corresponding E-mails & Authors*:
Kaleem Ullah

Abstract

The term "epilepsy" refers to a group of neurological diseases and syndromes that are characterized by recurring, spontaneous, paroxysmal seizure activity. Juvenile myoclonic epilepsy is a comparatively benign form of idiopathic generalized epilepsy. Genetics appears to have a major role in the genesis of epilepsy. The mitochondrion is the only organelle in the cell that has its own DNA that codes for 13 proteins. The mt-DNA circular genome is 16154bp in length. Mt-DNA mutations cause multisystem mitochondrial disorders having a diverse set of clinical signs and symptoms. More than 20-heteroplasmic point mutations has been linked to Myoclonic epilepsy with ragged red fibers, demonstrating the genetic variability of mitochondrial disorders. In Mt-DNA the most common mutation (m.8344A>G) is observed in tRNA lys (MT-TK) gene, which accounts for 80% of Myoclonic epilepsy ragged red fibers cases. The current study was designed to identify the mutation in Proline (MT-TP) gene of mt-DNA. This cross-sectional study was

conducted in North Waziristan. Non-probability convenient sampling technique was adopted. After taking informed consent questioner was filled and 3mL of blood sample was collected in EDTA tube. A total of 38 samples were collected from 10 families using sample size calculation formula n=p(1-p2) z2/d2. The samples size was calculated by using p(prevalence)=4% (0.04), d (margins of error) =5% (0.05), z; confidence level=95% (1.96) which provided a sample size of (n=38). Thermo Scientific Gene-JET Genomic DNA Purification Kit was used for mt-DNA extraction. DNA quantity was confirmed by nano drop and agarose gel electrophoresis.
Specific primers were designed for 394 bp target of proline gene and the extracted DNA was used as a template for the PCR amplification. The specific amplified region of proline-gene was confirmed through gel electrophoreses. The amplified PCR product bands were carefully removed, purified and cleaned through gene clean process. The amplified product was sequenced in BGI Hongkong for nucleotide. Out of total 14 epilepsy sample population, 10 (71.4%) were males and 4 (28.6%) were females. In the current study, no mutation was found in the (MT-TP) Proline-tRNA gene ranged from 16008-15956 bp of studied specimens. The results of over study suggest that mutation may be found in other region of MT-TP gene or MT-TK gene. The current study also recommends conducting the research on a wider scale to rule out the other mutation in different genes of mitochondrial genome of epileptic families in Pakistani population.

Introduction

The term "epilepsy" refers to a group of neurological diseases and syndromes that are characterized by recurring, spontaneous, paroxysmal seizure activity. The International League Against Epilepsy (ILAE) formed an international committee to develop a common definition of epilepsy in 2013. (Bertram, 2018). Juvenile myoclonic epilepsy is a comparatively benign form of idiopathic generalized epilepsy (Gelisse et al., 2001). Genetics appears to have a major role in the genesis of epilepsy, a frequent episodic neurological illness or condition marked by repeated epileptic seizures. Multiple loci that may have epilepsy susceptibility genes have been identified through early linkage studies, and mutational investigations have identified a number of mutations in both ion channel and non-ion channel genes in individuals with idiopathic epilepsy (Chen et al., 2017).

Seizures can be caused by anything that disrupts the anatomical or functional physiology of the brain, and various diseases might manifest themselves primarily through recurring seizures and hence be labeled as epilepsy. Epilepsy is the most prevalent severe neurological disorder in the world. The number of risk factors for developing epilepsy varies depending on age and region.

Epilepsy is most commonly linked with congenital, developmental, and genetic disorders in infancy, adolescence, and early adulthood. Infections of the central nervous system, head trauma (CNS) and Tumors can develop at any age and can contribute to epilepsy. Infections of the central nervous system (CNS) are a significant cause of epilepsy. Unprovoked seizures are found to occur between 6.8% to 8.3% in population-based cohorts of survivors of CNS illnesses in industrialized nations, and significantly higher in resource-poor countries (Vezzani *et al.*, 2016). Recurrent unprovoked seizures were described as epilepsy i.e. two or more. According to the revised criteria, epilepsy can be diagnosed after a single seizure in those who have other risk factors for a second unprovoked seizure (which is set at 60%). Factors to consider include epilepsy syndrome diagnosis, structural abnormalities such as stroke, CNS infections, intra parenchymal contusions following trauma, and reflex seizures such as photosensitive seizures (Siddiqi and Ali, 2015). Over 70 million individuals worldwide suffer with epilepsy, which is one of the most prevalent brain disorders. Epilepsy affects over 80% of persons living in low- and middle-income nations. Epilepsy is stigmatized in many regions of the globe, and patients may be denied treatment. Over 75% of people with active epilepsy go untreated, resulting in a significant treatment gap, which is mostly concentrated in low- and middle-income nations. Epilepsy should be a top priority for global health, especially now that cost-effective therapies exist that can significantly reduce morbidity, disability, and mortality (Thijs *et al.*, 2019).

The DNA of mitochondria is circular, whereas the genome of nuclear is linear. The mitochondrial genome (comprises 16,569 DNA base pairs) compare to 3.3 billion in the nuclear-genome. The mitochondrion is the only organelle in the cell that has its own DNA (m-tDNA) that codes for 13 proteins. The mitochondrial DNA circular genome is 16,154bp in length with nucleotide composition 28.42% A, 26.53% T, 19.65% G, and 25.40%. (Liu *et al.*, 2020). The mitochondrial genome of human comprises 37 genes, including 13 protein coding genes, two (MT-RNR1 and MT-RNR2) genes that code for the (12S and 16S) section of M-ribosomal RNA and 22-genes that code for various Mt-RNAs (Rovcanin *et al.*, 2020).

Mitochondrial DNA (mt-DNA) mutations cause multisystem mitochondrial disorders having a diverse set of clinical signs and symptoms. Symptoms of myoclonic epilepsy (Myoclonus, generalized epilepsy, cerebellar ataxia, and mitochondrial myopathy with ragged red fibres) are

all with ragged red fibre syndrome. Hearing loss, mental problems, and dysarthria are among the other symptoms. More than 20-heteroplasmic point mutations has been linked to MERRF, demonstrating the genetic variability of mitochondrial disorders. tRNA Lys (MT-TK) gene is m.8344A>G the most common mutation in mitochondrial DNA is, which accounts 80% of MERRF cases. Other MERRF-relevant uncommon mutations, up to 10% patients had no mutations identified of MERRF. Previously, mitochondrial myopathy is linked to the M-tRNA Asn (MT-TN) gene (m.5703G>A) mutation. No link has been found between MERRF syndrome and the m.5703G>A mutation according to our information the current report features a patient with typical MERRF syndrome who has a heteroplastic m.5703G>A mutation, which enhances the clinical spectrum of the m.5703G>A mutation as well as the genotypic spectrum of MERRF (Fu *et al.*, 2019). Five mutation in MT-RNR2 gene (1) m.3197T>C, (2) m.2706A>G, (3) m.2831G>A, (4) m.3010G>A, and (5) m.1811A>G, all of which have a major impact in m-16S- rRNA a secondary framework. All (m.1811A>G, m.2706A>G, m.3010G>A, and m.3197T>C) bring major changes in the 3D structure of m-16S-rRNA. In the MT-TP gene there is a single insertion variant, which codes for mt-RNA for Proline gene (m.15986insG) (Rovcanin *et al.*, 2020). Epilepsy affects between 50 to 70 million individuals globally, accounting for 0.75 percent of the global illness burden. Epilepsy affects 50 out of every 100,000 individuals worldwide, with a prevalence of 700 out of 100,000. Epilepsy affects around 2.4 million individuals every year. Epilepsy claimed roughly 20.6 million disability-adjusted life years in 2012. Asia is home to around 4 billion people (50 percent world's population), with approximately 23 million people suffering from epilepsy (Trinka *et al.*, no date).

About 1.5 million people suffering with epilepsy in Pakistan, accounting for 3% of the global total. Males have a reported yearly incidence of 50.7 per 100,000, while females have a rate of 46.2 per 100,000. Epilepsy is predicted to affect 9.99 individuals out of every 1000 in Pakistan. Persons under the age of 30 have the highest incidence. There is a little decrease in frequency between the ages of 40 and 59. The prevalence of the disease is higher in rural areas. The paediatric population has a higher prevalence of epilepsy aetiology. In 21 to 76 percent of individuals, idiopathic epilepsy was discovered (Nadeem et al., 2018b). To treat both myoclonus and GTCS, broad- spectrum anti-epileptic medications (AEDs) like valproic acid are widely used, as are

adjuvant medicines like clonazepam. The use of a gastrostomy tube for eating might help to prevent aspiration pneumonia. A psychological and clinical examination should be done on a regular basis (Characterization and Epilepsies, 2018). The epilepsy burden has yet to be completely assessed and comprehended. The most prevalent seizure type seen was generalized seizures. Epilepsy and its treatment are both poorly understood. The goal of this study was to look for MT-TP gene alterations in patients with myoclonus epilepsy and their family members. Mutations' structural and pathogenic effects were studied.

MATERIAL AND METHODS

A total of 38 Blood samples were collected from 10 epileptic families. All the participants give informed consents before sample collections. Twenty ml of peripheral whole blood were collected by standard technique. Thermo Scientific Gene-JET Genomic DNA Purification Kit was used for mt-DNA extraction.

PRIMER DESIGNING AND AMPLIFICATION OF TARGET GENE

Specific primer was designed through primer 3. The target region of MT-TP was amplified by using forward primer (TCCTCATTCTAACCTGAATCGGAG) and reverse primer (GGTACCGTACAATATTCATGGTGG). The extracted DNA was used as a template for the PCR amplification. The specific detection of pro-gene was performed by PCR. Thermo Scientific Dream-Taq Green PCR Master Mix (2X) was used for optimization of primers previously designed using gradient PCR (LABNET MULTIGENE™). The PCR reactions were performed with a 20 µl final volume containing 0.6 µl of each primer, 1.5 µl template DNA, 10 µl of Master Mix containing loading dye, and 7.3 µl of sterile distilled-water.

An initial-denaturation at 95 °C for 4 minutes, followed by amplification by 30x cycles of denaturation at 95 °C for 30 seconds, annealing at 62 °C for 30 seconds and elongation at 72 °C for 40 seconds, and a final extension at 72 °C for 6 minutes. PCR amplification products was run on agarose gel and matched with the DNA ladder under UV light and snapped. The amplified PCR product on the gel were safely removed through surgical blades. The DNA bands with a little bit of agarose gel were removed, purified and cleaned through gene clean process.

GEL ELECTROPHORESIS OF AMPLIFIED PRODUCTS

After amplification 12µl -14µl of PCR products was loaded in 1% agarose gel prepared in 1X TBE buffer containing 2.5 µl of Ethidium bromide. 100 base pair DNA ladder (Invitrogen, Merelbeke, Belgium used as a marker). The gel was run for about 60 minutes at 100V in 1X TBE buffer and observed by UV Tran illuminator and photographed by MAJ SCIENCES (USA) Gel Documentation system.

BIOINFORMATICS ANALYSIS

MS excel and SPSS 22 were used for data analysis.

RESULTS AND DISCUSSION

Different studies have shown that epilepsy patients may vary in the prevalence and mutation. Survival and disorder progression can be affected by mutation status in mt-DNA and treatment. In the current study selected families were 10 and epilepsy effected individuals are 14 of different age group, both male and female was selected.

GENDER WISE PREVALENCE OF EPILEPSY

Out of total 14 epilepsy sample population, 10 (71.4%) were males and 4 (28.6%) were females. Male was the dominant gender found throughout the study (Figure 4.1). Pakistan is responsible for 1.5 million people suffering with epilepsy, accounting for 3% of the global total. Males have a reported yearly incidence of 50.7 per 100,000, while females have a rate of 46.2 per 100,000 (Awan et al., 2019a)

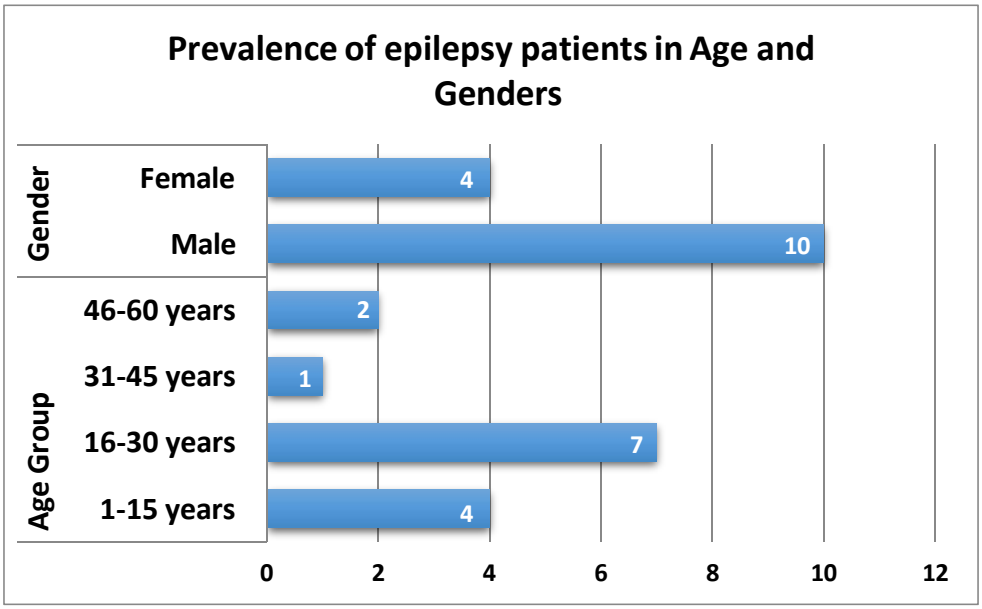

Figure: Prevalence of epilepsy patients in Age and Genders

In the current study 14 patients were epileptic in which 8 (57.14%) patients were taking tab Epival and 6 (42.86 %) were taking both Tab Tegral and Tab Epival medicine while no one was taking Tab Tegral as single medicine.

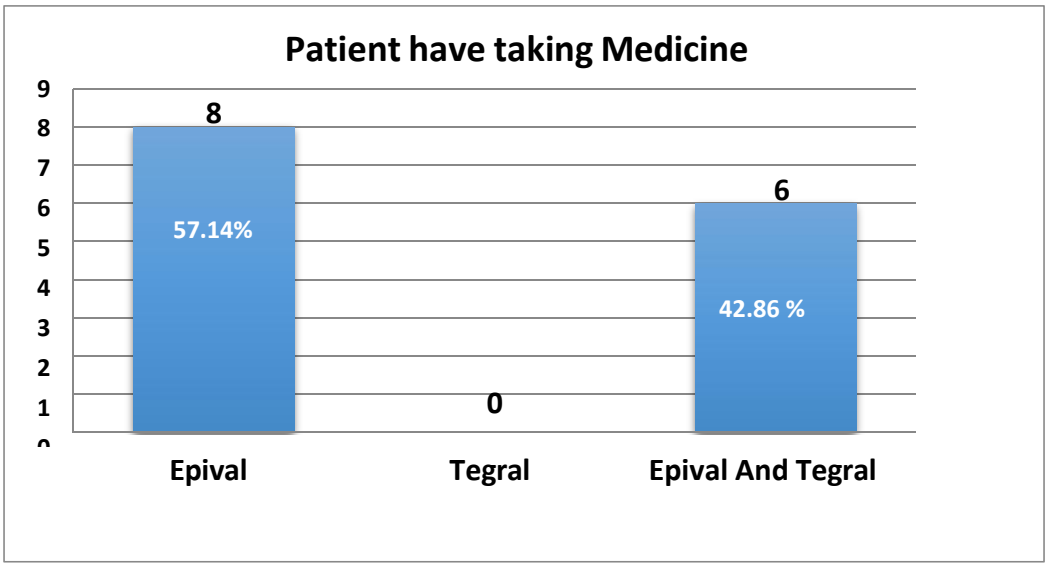

Figure: Patients have taking medicine

PCR AMPLIFICATION OF PROLINE – tRNA GENE

The extracted DNA was then used to amplify the Proline tRNA gene using a PCR equipment. For improved target sequence amplification results, the PCR conditions were adjusted by changing the annealing temperature with time. The optimal PCR condition is graphically represented.

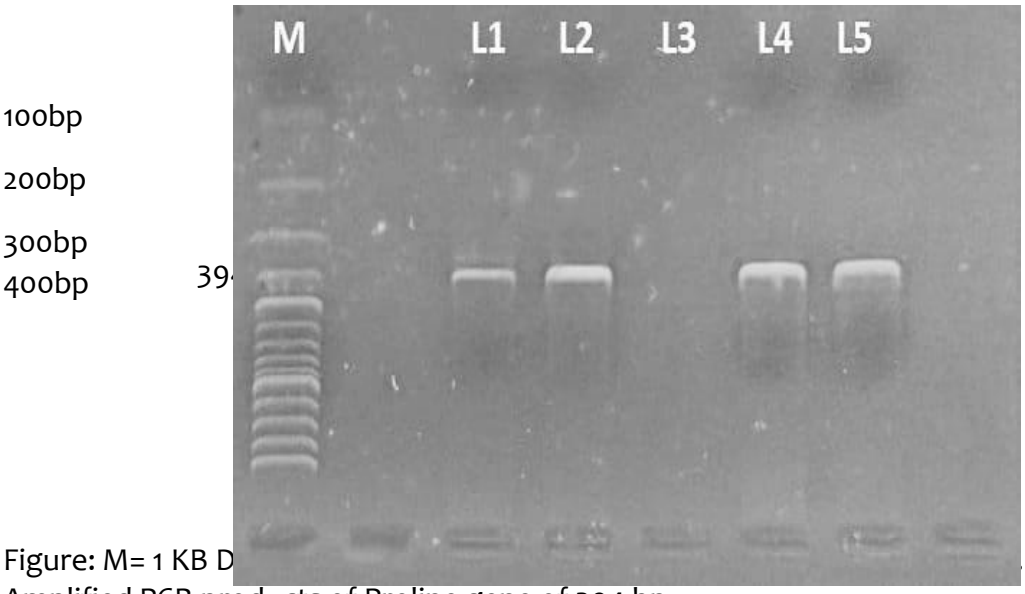

Figure: M= 1 KB D
Amplified PCR products of Proline gene of 394 bp.

NUCLEOTIDE SEQUENCING ANALYSIS

The amplified gene product was then cut and removed from gel and was sent to BGI Hongkong for sequencing of nucleotide. To detect mutations, the relevant sequencing data were aligned with CRS. In the current study, 38 samples were sequenced, with seven samples selected for further investigation based on satisfactory findings and three samples being discarded.

SPECIMEN-02

The patient in this case was a female who was diagnosed with epilepsy at the age of forty-two years. The history of her family revealed that the father was epileptic. The weight of patient was 60 kg and was suffering from other diseases like depression and stress.

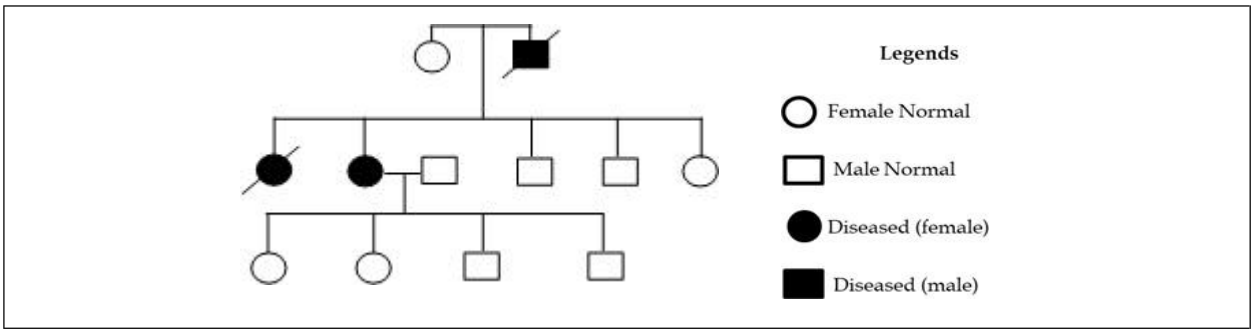

Figure: Pedigree of specimen "06-R" with corresponding legends

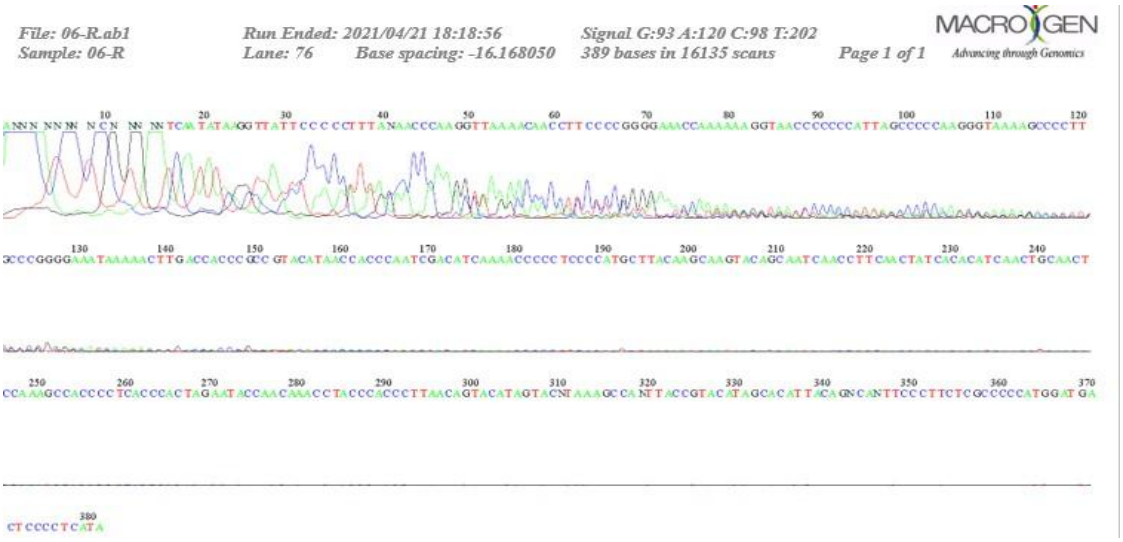

Figure: The chromatography sequence result of specimen-06-R provided by Macrogen Inc. South Korea.

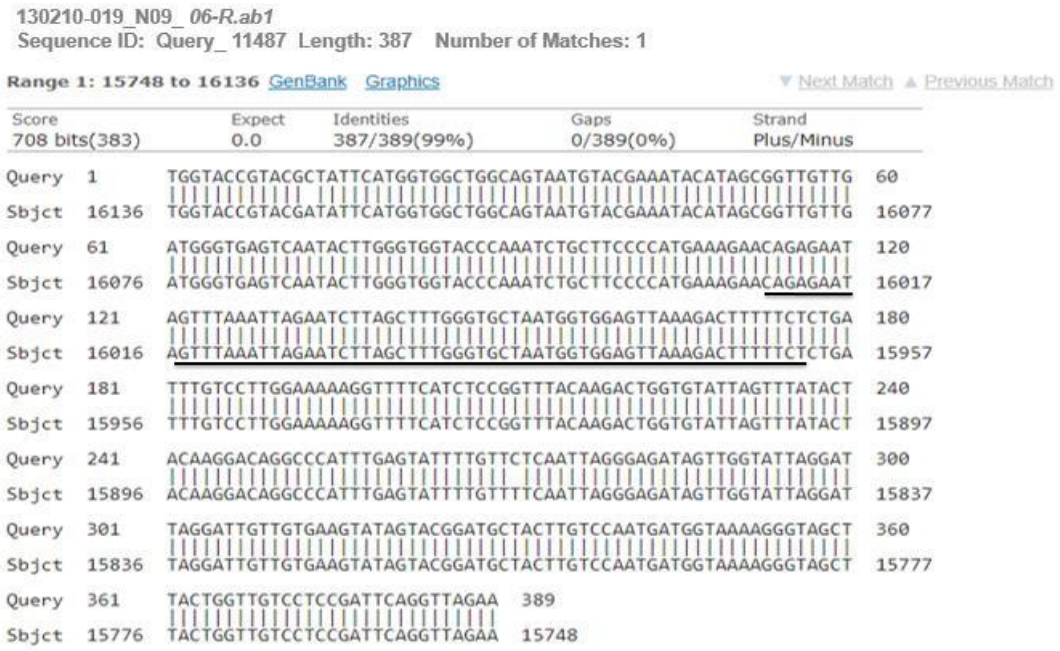

Figure: Sequence alignment of specimen 06-R

Reference Sequence was used to align the sequence of specimen 06-R. The Pro-tRNA gene is highlighted. In the examined 06-R specimens, no mutation was detected in the MT-L1 Pro-tRNA gene, which ranged from 16008 to 15956 bp.

CONCLUSION AND RECOMMENDATIONS

CONCLUSION

This study concludes that epilepsy is common in males as compared to females. The current study also recommends conducting the research on a wider scale to rule out the epilepsy mutation in different ethnicities and geographic distribution in Pakistan. Out of total n=14 epilepsy sample population, 71.4% (n=10) were males and 28.6% (n=04) were females. Males were the dominant gender found throughout the study.

In the current study 14 patients were epileptic in which 57.14% (n=8) patients were taking tab Epival and 42.86 % (n=6) were taking both Tab Tegral and Tab Epival medicine while no one was taking Tab Tegral as single medicine. There is no mutation found in the MT-L1 Pro-tRNA gene ranged from 16008-15956 bp of studied all specimens.

RECOMMENDATIONS

The current study recommended that increases the number of specimens collected in order to obtain better findings and determine the prevalence of the Proline-tRNA gene Not only mitochondrial but also nuclear genes linked to epilepsy should be investigated in the Pakistani population. In epileptic patients, all 37 mitochondrial genes should be examined for any mutations, not just Proline (MT TP). The current study also recommended conducting the research on a wider scale to rule out the Proline-tRNA mutation in different ethnicities and geographic distribution in Pakistan. Further studies with a larger number of epileptic families are needed to assess the causes and treatment of Epilepcy.

REFERENCES

ABRAMOVICI, S. & BAGIC, A. 2016. Epidemiology of epilepsy. *Handb Clin Neurol,* 138, 159- 71.

ADEMUWAGUN, I. A., ROTIMI, S. O., SYRBE, S., AJAMMA, Y. U. & ADEBIYI, E. 2021.
Voltage Gated Sodium Channel Genes in Epilepsy: Mutations, Functional Studies and Treatment Dimensions. *Frontiers in Neurology,* 12, 387.

AUDITEAU, E., CHASSAGNE, F., BOURDY, G., BOUNLU, M., JOST, J., LUNA, J., RATSIMBAZAFY, V., PREUX, P. M. & BOUMEDIENE, F. 2019. Herbal medicine for epilepsy seizures in Asia, Africa and Latin America: A systematic review. *J Ethnopharmacol,* 234, 119-153.

AWAN, S., SIDDIQI, A. I., ASIF, A., AHMED, N., BROHI, H., JALBANI, S. & WASAY, M.
2019a. Spectrum of neurological disorders in neurology outpatients clinics in urban and rural Sindh, Pakistan: a cross sectional study. *BMC neurology,* 19, 1-6.

AWAN, S., SIDDIQI, A. I., ASIF, A., AHMED, N., BROHI, H., JALBANI, S. & WASAY, M.
2019b. Spectrum of neurological disorders in neurology outpatients clinics in urban and rural Sindh, Pakistan: a cross sectional study. *BMC Neurol,* 19, 192.

BOLING, W., MEANS, M. & FLETCHER, A. 2018. Quality of Life and Stigma in Epilepsy, Perspectives from Selected Regions of Asia and Sub-Saharan Africa. *Brain Sciences,* 8, 59.

CARRÉ, F., HERVOCHON, R., FOIREST, C. & TANKÉRÉ, F. 2019. MERRF syndrome
(Myoclonic Epilepsy with Ragged Red Fibres) presenting with cervicothoracic lipomatosis. *European annals of otorhinolaryngology, head and neck diseases,* 136, 113- 114.

CHEN, T., GIRI, M., XIA, Z., SUBEDI, Y. N. & LI, Y. 2017. Genetic and epigenetic mechanisms of epilepsy: a review. *Neuropsychiatric disease and treatment,* 13, 1841.

FU, J., MA, M. M., PANG, M., YANG, L., LI, G., SONG, J. & ZHANG, J. W. 2019. Broadening
the phenotype of m.5703G>A mutation in mitochondrial tRNAAsn gene from mitochondrial myopathy to myoclonic epilepsy with ragged red fibers syndrome. *Chin Med J (Engl),* 132, 865-867.

GELISSE, P., GENTON, P., THOMAS, P., REY, M., SAMUELIAN, J. & DRAVET, C. 2001.
Clinical factors of drug resistance in juvenile myoclonic epilepsy. *Journal of Neurology, Neurosurgery & Psychiatry,* 70, 240-243.

JOBST, B. C. & CASCINO, G. D. 2015. Resective epilepsy surgery for drug-resistant focal epilepsy: a review. *JAMA,* 313, 285-93.

KOVAC, S., PREZA, E., HOULDEN, H., WALKER, M. C. & ABRAMOV, A. Y. 2019. Impaired
Bioenergetics in Mutant Mitochondrial DNA Determines Cell Fate During Seizure-Like Activity. *Mol Neurobiol,* 56, 321-334.

KWAN, P., CABRAL-LIM, L., D'SOUZA, W., JAIN, S., LEE, B. I., LIAO, W., LIM, S. H., OTSUKI, T., TAN, C. T., WANTANABE, M. & FORCE, C. R. T. 2015. Research priorities in epilepsy for the Asia-Oceanian region. *Epilepsia,* 56, 667-73.

LAMPERTI, C. & MANCUSO, M. 2019. Myoclonus Epilepsy with Ragged-Red Fibers (MERRF). *Diagnosis and Management of Mitochondrial Disorders.* Springer.

LIM, A. Z., BLAKELY, E. L., BATY, K., HE, L., HOPTON, S., FALKOUS, G., MCWILLIAM, K., COZENS, A., MCFARLAND, R. & TAYLOR, R. W. 2019. A novel pathogenic m.4412G>A MT-TM mitochondrial DNA variant associated with childhood-onset seizures, myopathy and bilateral basal ganglia changes. *Mitochondrion,* 47, 18-23.

LIU, Z. Q., SHEN, Y. Z., ZHAO, M., WANG, W., CHEN, W., MA, C. Y., ZHANG, F. Y. & MA,
L. B. 2020. Characterization of the complete mitochondrial DNA sequence of the Ariosoma meeki (Anguilliformes, Congridae). *Mitochondrial DNA B Resour,* 5, 2539-2541.

MESRAOUA, B., DELEU, D., AL HAIL, H. J., MELIKYAN, G., ABDALHALIM ALI, M., HADDAD, N.,

MOHAMED ALI, Y. O., ELSHEIKH, L., AYYAD, A., PERKINS, J.,

PALOMO, G. A. & ASADI-POOYA, A. A. 2020. Prevalence and Incidence of Drug- Resistant Temporal Lobe Epilepsy in Qatar. *J Cent Nerv Syst Dis,* 12, 1179573520935031.

MOGAL, Z. & AZIZ, H. 2020. Epilepsy treatment gap and stigma reduction in Pakistan: A tested public awareness model. *Epilepsy Behav,* 102, 106637.

MOREAU, C., RÉBILLARD, R.-M., WOLKING, S., MICHAUD, J., TREMBLAY, F., GIRARD, A., BOUCHARD, J., MINASSIAN, B., LAPRISE, C., COSSETTE, P. & GIRARD, S. 2019.

MYERS, C. T. & MEFFORD, H. C. 2015. Advancing epilepsy genetics in the genomic era.

Genome Med, 7, 91.

MYERS, K. A., JOHNSTONE, D. L. & DYMENT, D. A. 2019. Epilepsy genetics: Current knowledge, applications, and future directions. *Clin Genet,* 95, 95-111.

NADEEM, M. S., AHMAD, H., MOHAMMED, K., MUHAMMAD, K., ULLAH, I., BAOTHMAN, O. A., ALI, N., ANWAR, F., ZAMZAMI, M. A. & SHAKOORI, A. R. 2018a. Identification of variants in the mitochondrial lysine-tRNA (MT-TK) gene in myoclonic epilepsy—pathogenicity evaluation and structural characterization by in silico approach. *Journal of cellular biochemistry,* 119, 6258-6265.

NADEEM, M. S., AHMAD, H., MOHAMMED, K., MUHAMMAD, K., ULLAH, I., BAOTHMAN, O. A. S., ALI, N., ANWAR, F., ZAMZAMI, M. A. & SHAKOORI, A. R. 2018b. Identification of variants in the mitochondrial lysine-tRNA (MT-TK) gene in myoclonic epilepsy-pathogenicity evaluation and structural characterization by in silico approach. *J Cell Biochem,* 119, 6258-6265.

ORSINI, A., ZARA, F. & STRIANO, P. 2018. Recent advances in epilepsy genetics. *Neurosci Lett,* 667, 4-9.

PERUCCA, P. & PERUCCA, E. 2019. Identifying mutations in epilepsy genes: impact on treatment selection. *Epilepsy research,* 152, 18-30.

ROSENOW, F., BAST, T., CZECH, T., FEUCHT, M., HANS, V. H., HELMSTAEDTER, C., HUPPERTZ, H. J., NOACHTAR, S., OLTMANNS, F., POLSTER, T., SEECK, M., TRINKA, E., WAGNER, K. & STRZELCZYK, A. 2016. Revised version of quality guidelines for presurgical epilepsy evaluation and surgical epilepsy therapy issued by the Austrian, German, and Swiss working group on presurgical epilepsy diagnosis and operative epilepsy treatment. *Epilepsia,* 57, 1215-20.

ROVCANIN, B., JANCIC, J., SAMARDZIC, J., ROVCANIN, M., NIKOLIC, B., IVANCEVIC, N., NOVAKOVIC, I. & KOSTIC, V. 2020. In silico model of mtDNA mutations effect on secondary and 3D structure of mitochondrial rRNA and tRNA in Leber's hereditary optic neuropathy. *Exp Eye Res,* 201, 108277.

SCHACHTER, S. C. 2017. Successful health communication in epileptology. *Seizure,* 44, 7-10. TANAKA, A., HATA, J., AKAMATSU, N., MUKAI, N., HIRAKAWA, Y., YOSHIDA, D.,

KISHIMOTO, H., OHARA, T., MIZUNO, T., TSUJI, S., KITAZONO, T. & NINOMIYA, T. 2019. Prevalence of adult epilepsy in a general Japanese population: The Hisayama study. *Epilepsia Open,* 4, 182-186.

TATUM, W. O. T. 2019. Epilepsy & Behavior Reports. *Epilepsy Behav Rep,* 12, 100322.

THIJS, R. D., SURGES, R., O'BRIEN, T. J. & SANDER, J. W. 2019. Epilepsy in adults. *The Lancet,* 393, 689-701.

TRINKA, E., KWAN, P., LEE, B. & DASH, A. 2019. Epilepsy in Asia: Disease burden, management barriers, and challenges. *Epilepsia,* 60 Suppl 1, 7-21.

TSUCHIDA, N., NAKASHIMA, M., KATO, M., HEYMAN, E., INUI, T., HAGINOYA, K., WATANABE, S., CHIYONOBU, T., MORIMOTO, M., OHTA, M., KUMAKURA, A., KUBOTA, M., KUMAGAI, Y., HAMANO, S. I., LOURENCO, C. M., YAHAYA, N. A., CH'NG, G. S., NGU, L. H., FATTAL-VALEVSKI, A., WEISZ HUBSHMAN, M., ORENSTEIN, N., MAROM, D., COHEN, L., GOLDBERG-STERN, H., UCHIYAMA, Y., IMAGAWA, E., MIZUGUCHI, T., TAKATA, A., MIYAKE, N., NAKAJIMA, H., SAITSU, H., MIYATAKE, S. & MATSUMOTO, N. 2018. Detection of copy number variations in epilepsy using exome data. *Clin Genet,* 93, 577-587.

ULLAH, S., ALI, N., KHAN, A., ALI, S. & NAZISH, H. R. 2018. The Epidemiological
Characteristics of Epilepsy in the Province of Khyber Pakhtunkhwa, Pakistan. *Front Neurol,* 9, 845.

VEZZANI, A., FUJINAMI, R. S., WHITE, H. S., PREUX, P. M., BLUMCKE, I., SANDER, J.
W. & LOSCHER, W. 2016. Infections, inflammation and epilepsy. *Acta Neuropathol,* 131, 211-234.

WANG, J., LIN, Z. J., LIU, L., XU, H. Q., SHI, Y. W., YI, Y. H., HE, N. & LIAO, W. P. 2017.
Epilepsy-associated genes. *Seizure,* 44, 11-20.

WANIGASINGHE, J., ARAMBEPOLA, C., MURUGUPILLAI, R. & CHANG, T. 2019. Age,
sex and ethnic differentials in the prevalence and control of epilepsy among Sri Lankan children: a population-based study. *BMJ Paediatr Open,* 3, e000430.