Global Research journal of Natural Science & Technology (GRJNST)

Volume: 04 - Issue 3 (2026), 2083
ISSN P: 2790-7643 ISSN E: 2790-7651
www.grjnst.net
https://doi.org/10.53762/grjnst.04.03.04

Mutational Analysis of TRAPPC9 IN Non-syndromic Intellectual Disability in Selected Patients

Received: 28 March 2026. Accepted: 20 April 2026. Published: 8 May 2026

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GRJNST, Volume: 04 - Issue 3 (2026) / ISSN P: 2790-7643
Article ID: 2083
https://doi.org/10.53762/grjnst.04.03.04

Abstract

Intellectual incapacity (identity) is a neurodevelopmental circumstance characterized through good sized obstacles in cognitive functioning and adaptive behavior, with onset before the age of 18. It is usually diagnosed in early formative years due to developmental delays in motor, cognitive, and speech abilities, and is generally described by way of an IQ under 70. Identity is classed into syndromic and non-syndromic kinds and further divided into mild, mild, extreme, and profound categories based totally on severity. This takes a look at centered on non-syndromic identification patients, regarding medical evaluation and genetic evaluation. Blood samples have been accrued, and DNA became analyzed the usage of PCR with TRAPPC9 gene primers, followed by gel electrophoresis and sequencing. No mutation became detected in exon 14 of the TRAPPC9 gene, suggesting the want for broader genetic investigations to higher understand and help reduce the prevalence of intellectual incapacity.

Keyword: *Mutation, Syndrome, Neurodevelopment, Patients, Cognitive behavior*

INTRODUCTION

Intellectual disability (identity) is a neurodevelopmental sickness characterized through tremendous obstacles in cognitive functioning and adaptive behavior, with onset before 18 years of age, affecting about 1.five–2% of the populace. it is generally identified in childhood because of developmental delays, even though formal analysis is based on an IQ underneath 70. Individuals with id are at a higher danger of intellectual health issues as compared to their generally growing peers. Identification is assessed into syndromic and non-syndromic bureaucracy; syndromic identification includes additional bodily or metabolic abnormalities, while non-syndromic id includes isolated cognitive impairment.

Each genetic and environmental elements make a contribution to id, along with chromosomal abnormalities and monogenic mutations (X-connected and autosomal) (Marangi et al., 2013).

Consanguinity, particularly well-known in countries like Pakistan, drastically will increase the prevalence of autosomal recessive highbrow disability. Neurodevelopmental problems regularly proportion overlapping symptoms and genetic mechanisms, related to pathways consisting of protein synthesis, transcriptional regulation, and synaptic signaling. some of the genes implicated, TRAPPC9 encodes the NIBP protein, which performs a crucial role in neurogenesis, synaptic plasticity, and myelination via NF-κB signaling. Mutations in TRAPPC9 are associated with non-syndromic identification and may present with functions including microcephaly, speech impairment, and brain abnormalities (Abbasi et al., 2017).

Intention was to analyze mutations inside the TRAPPC9 gene in patients with non-syndromic intellectual disability. Basic goals was to perceive the genetic foundation of highbrow impairment throughout age agencies, increase awareness about inherited genetic problems, and explore capability novel gene mutations.

METHODOLOGY

Affected individuals were clinically evaluated with the assistance of qualified psychiatrists. Detailed assessments were conducted to identify cognitive, behavioral, and developmental abnormalities associated with intellectual disability (ID). Clinical history, including developmental milestones, speech development, and behavioral patterns, was recorded. This evaluation helped in confirming the diagnosis of intellectual disability and distinguishing between syndromic and non-syndromic cases.

Families with more than three affected individuals were identified from the district of Mandi Bahauddin, Punjab. Field visits were conducted to collect detailed family histories and demographic information. Pedigree charts were constructed for each family to analyze inheritance patterns and identify possible modes of transmission, particularly focusing on autosomal recessive inheritance due to high consanguinity rates. Only families meeting the inclusion criteria were enrolled in the study.

Prior to sample collection, informed consent was obtained from all participants or their guardians. Approximately 5–10 ml of peripheral blood was collected from each affected individual using sterile techniques. Blood samples were collected in EDTA-containing falcon tubes to prevent coagulation. The samples were properly labeled and transported to the laboratory under controlled conditions. For preservation, the samples were stored at −20°C until further processing.

Genomic DNA was extracted from blood samples using a standard protocol followed at LCWU. The extraction process involved cell lysis to release cellular contents, followed by digestion of proteins using proteinase K. Proteins were precipitated using saturated sodium chloride (NaCl), and DNA was subsequently precipitated using isopropanol. This method ensured the isolation of high-quality DNA suitable for downstream molecular analysis.

Day I: Cell Lysis and Washing

Frozen blood samples were thawed at room temperature using tap water. Tris-EDTA (T.E) buffer was added in two steps to ensure proper mixing and cell suspension. The samples underwent multiple washing steps followed by centrifugation at 3000 rpm for 25–30 minutes at 25°C. After each centrifugation, the supernatant was discarded, and the pellet was resuspended. This washing process was repeated three to four times to remove impurities and obtain a clean cell pellet.

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After the final wash, TNE buffer was added to dissolve the pellet, followed by the addition of 10% SDS and proteinase K to facilitate protein digestion. The samples were incubated overnight at 37°C in a shaking incubator to ensure complete lysis and digestion.

Day 2: Protein Removal and DNA Precipitation

On the second day, samples were checked for complete digestion. Chilled NaCl was added to precipitate proteins, followed by incubation in the freezer. A phenol-chloroform-isoamyl alcohol (PCI) solution was then added, and samples were gently mixed. Centrifugation was performed to separate the layers, and the upper aqueous layer containing DNA was carefully transferred to new tubes.

Equal volumes of isopropanol were added to precipitate DNA, which appeared as visible threads. The DNA was then centrifuged, and the pellet was washed with 70% ethanol to remove impurities. After drying, the DNA pellet was dissolved in low TNE buffer and incubated overnight to ensure complete dissolution.

Day 3: DNA Stabilization and Storage

On the third day, samples were treated to inactivate nucleases by incubating at 65–70°C in a water bath. After cooling, DNA samples were transferred into labeled screw-cap tubes and stored in duplicate at −20°C. DNA concentration and integrity were later assessed using agarose gel electrophoresis.

DNA Quantification

DNA concentration and quality were determined using agarose gel electrophoresis. A 0.8% agarose gel was prepared using TBE buffer, and ethidium bromide was added for DNA visualization under UV light. DNA samples mixed with loading dye were loaded into wells, and electrophoresis was performed for 25–30 minutes. The gel was visualized

using a gel documentation system, and DNA concentration was estimated by comparing band intensity with known DNA standards. This method also allowed assessment of DNA integrity.

Polymerase Chain Reaction (PCR)

PCR was performed to amplify specific regions of the *TRAPPC9* gene prior to sequencing. The reaction mixture was prepared using extracted genomic DNA, gene-specific primers, nucleotides (dNTPs), buffer, and Taq DNA polymerase.

PCR Conditions

PCR amplification was carried out using a thermal cycler with the following conditions:

- Initial denaturation at 94°C for 5 minutes
- Denaturation at 94°C for 30 seconds
- Annealing at 59°C for 30 seconds
- Extension at 72°C for 30 seconds
- Final extension at 72°C for 10 minutes

These steps were repeated for multiple cycles to ensure sufficient amplification of the target DNA region.

Denaturation

During denaturation, the double-stranded DNA was separated into single strands by breaking hydrogen bonds at high temperatures (94–98°C). This step is critical for allowing primers to bind to the DNA template in subsequent steps.

Annealing

In the annealing step, primers specific to the *TRAPPC9* gene bind to complementary sequences on the single-stranded DNA templates. The temperature (59°C) was optimized based on primer characteristics to ensure specificity and efficient binding.

Extension

During extension, Taq DNA polymerase synthesized new DNA strands by adding nucleotides complementary to the template strand at 72°C. This step resulted in the amplification of the target DNA region.

Pre-Sequencing Preparation

Following PCR amplification, the products were verified through gel electrophoresis to confirm successful amplification. The PCR products were then purified and prepared for sequencing to identify potential mutations in the *TRAPPC9* gene.

Reaction mixture for Pre-sequencing PCR

The PCR reaction mixture was prepared in a total volume of 15 µl. It consisted of 2 µl of genomic DNA with a concentration of 50–60 ng/µl. To this, 0.5 µl of forward primer and 0.5 µl of reverse primer (each at 10 nmol concentration) were added. Additionally, 5 µl of master mix was included in the reaction, along with 7 µl of injection water to make up the final volume.

Primer sequence of TRAPPC9 Gene

The primer sequences for exon 14 of the TRAPPC9 gene were designed as follows: the forward primer (F.P) sequence was 5′-GAAGAGGAGCCCCGTACTCT-3′, and the reverse primer (R.P) sequence was 5′-GTGACCCTCGTGCACACTAC-3′. These primers were used to amplify a product with a size of 247 base pairs (bp).

RESULTS

Ethical approval for this study was obtained from the Ethical Committee of Lahore College for Women University (LCWU), Lahore, Pakistan. Intellectually disabled families were identified through field visits in Mandi Bahauddin, where affected individuals were clinically assessed with the assistance of psychologists. Diagnosis was

based on factors including speech disability, motor delay, age, gender, height, weight, prenatal and postnatal history, and IQ level. Behavioral characteristics such as mood, speech patterns, eating habits, sleep, attention, and additional traits like aggression, hyperactivity, impulsivity, and tantrums were also recorded.

Two families with intellectual disability were identified, and one family (PKNSID0091) was selected for molecular analysis. Blood samples were collected with informed consent and stored at −20°C. Pedigree analysis was performed using Cegat software, indicating an autosomal recessive inheritance pattern associated with consanguinity. DNA was extracted, followed by PCR amplification using *TRAPPC9* gene primers. Gel electrophoresis confirmed the amplified products, and DNA sequencing was carried out to detect potential mutations.

The selected family, from Mandi Bahauddin, showed a history of cousin marriages and included three affected individuals across three generations. The pedigree suggested autosomal recessive inheritance, with unaffected parents and affected offspring. All affected members exhibited non-syndromic intellectual disability.

Table 1. History of affected family PKNSID0091

Family ID	PKNSID0091	**Age**	25 years
Gender	Male	**Weight**	70kg
IQ Level	Severe	**No. of affected individuals**	03
At birth time condition	Birth trauma	**Neonatal (1st four weeks of life)**	Fitz after birth

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Table 2. Signs of disorders in the patient with intellectual disability

Signs	Yes	No
Floppy Limbs		✓
Problem in feeding		✓
Cleft Lip		✓
Weak Limbs		✓
Club feet		✓
Lump on back		✓
Lump at navel		✓
Child has serious delays in sitting		✓
Child has serious delays in standing		✓
Child has serious delays in walking		✓
Child has difficulty in seeing in daytime		✓
Child has difficulty in seeing at night	✓	
Child appears to have difficulty in hearing	✓	

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Child has difficulty in understanding	✓	
Child has difficulty in moving arms		✓
Child loses consciousness at sometimes	✓	
Child cannot name objects like toys, books etc		✓
Child is not learning to do things like other children	✓	

Sequencing results were analyzed using Chromas software to visualize and extract DNA sequences obtained from Sanger sequencing. The reference sequence of the *TRAPPC9* gene was retrieved from the Ensemble Genome Browser, including both exons and introns. Sequence alignment was performed using NCBI BLAST by comparing the obtained (query) sequence with the reference (subject) sequence in FASTA format. The query sequence was copied from Chromas and aligned against the reference gene sequence to identify variations.

The analysis of family PKNSID0091 revealed no mutation in exon 14 of the *TRAPPC9* gene. Further analysis is ongoing to investigate potential mutations in other exons of the gene.

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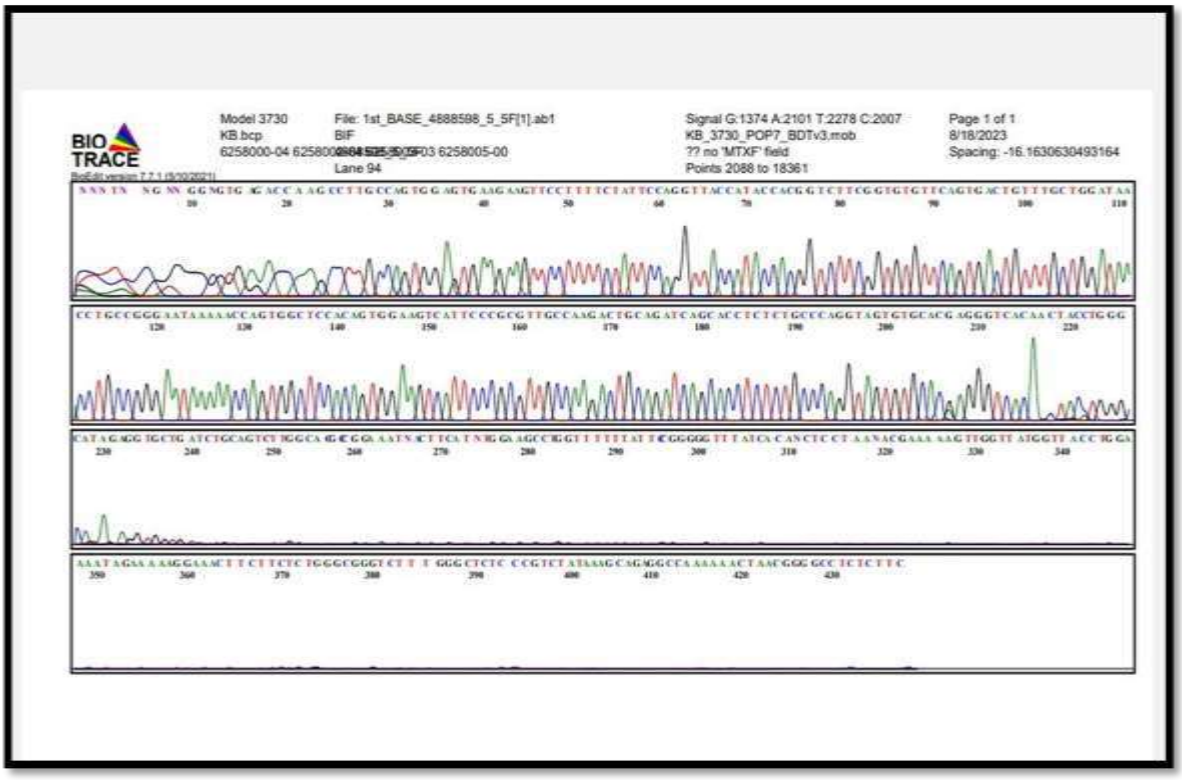

Figure 1. Sequencing chromatogram of exon 14 of TRAPPC9

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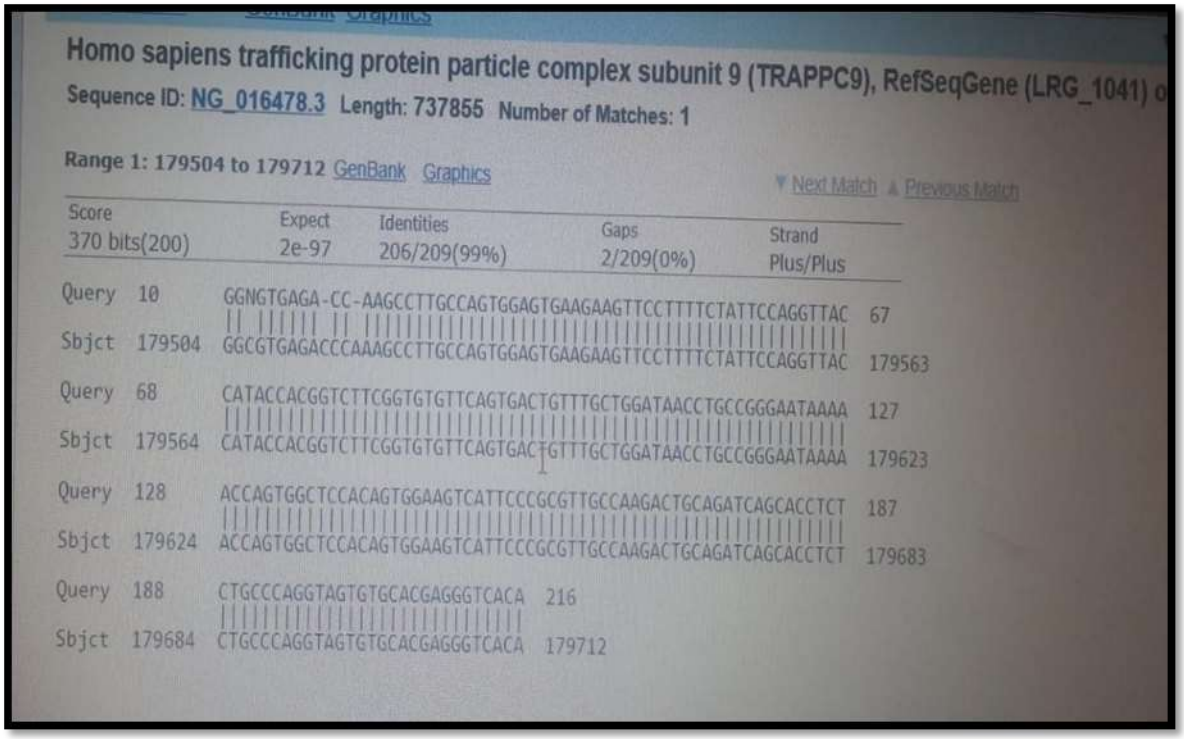

Figure 2. No mutation in exon 14 of family PKNSID0091

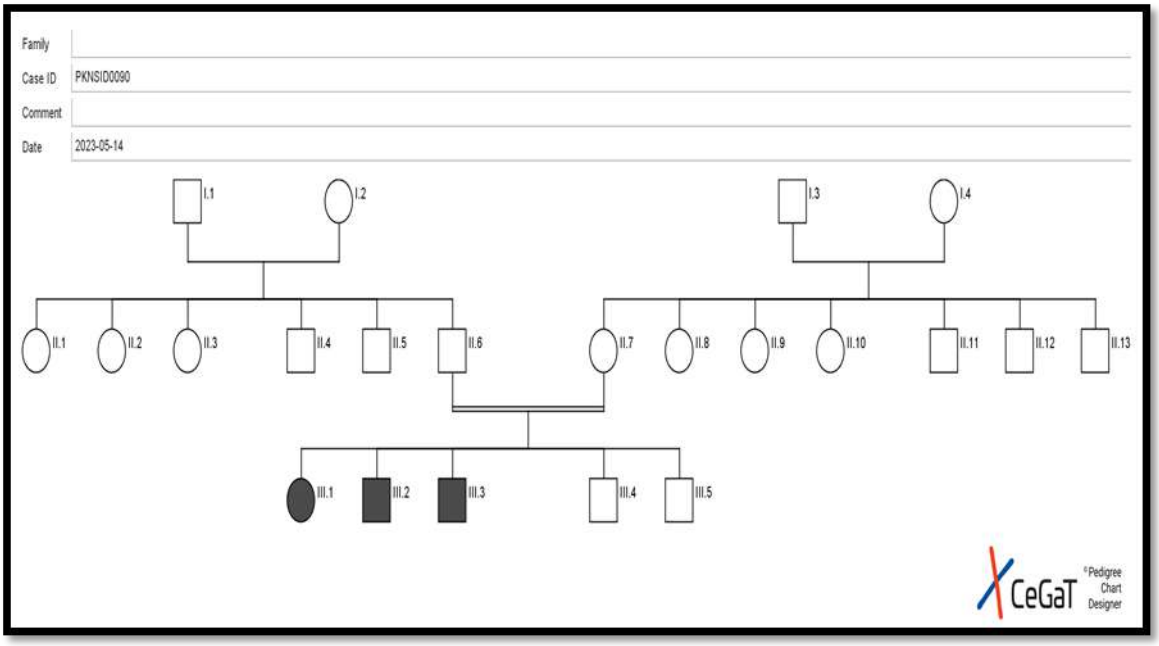

Figure 3. Pedigree of family PKNSID0090

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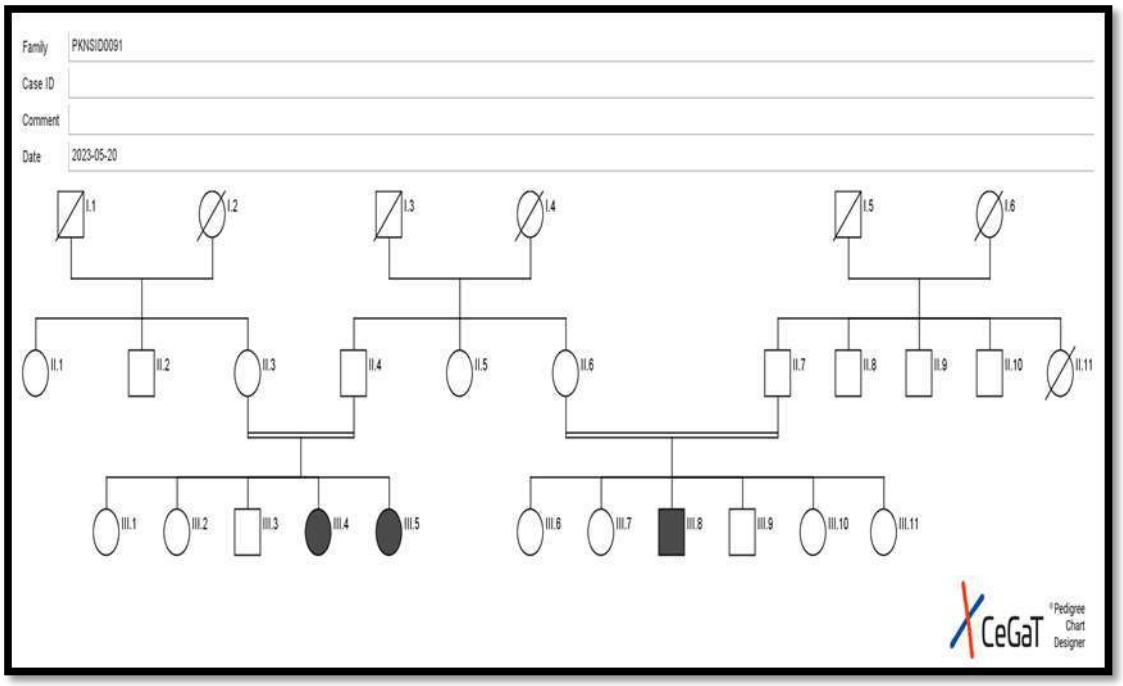

Figure 4. Pedigree of family PKNSID0091

DISCUSSION

Intellectual disability (ID) is a neurodevelopmental disorder characterized by significant limitations in cognitive functioning and adaptive behavior, with onset before 18 years of age. It affects approximately 1.5–2% of populations in Western countries and is usually diagnosed when IQ is below 70, although early identification often occurs due to developmental delays in motor, speech, and cognitive skills. Individuals with ID have a higher risk of mental health disorders compared to typically developing individuals. ID is classified into mild, moderate, severe, and profound categories, and may be syndromic or non-syndromic in nature. (Mefford *et al.,* 2012).

Globally, consanguinity is widely practiced, particularly in South Asia, Africa, and the Middle East, with Pakistan showing a high rate (~65%). This increases the prevalence

of autosomal recessive intellectual disability. In Pakistan, moderate ID prevalence is approximately 65/1000 and severe ID about 19/1000. Increased consanguinity contributes significantly to genetic disorders, including non-syndromic ID (De Ligt *et al.,* 2012).

The present study focused on molecular analysis of families with non-syndromic intellectual disability from Mandi Bahauddin, Punjab. Pedigree analysis revealed an autosomal recessive inheritance pattern. One family (PKNSID0091) with three affected individuals was selected. Blood samples were collected at LCWU, DNA was extracted, and gel electrophoresis confirmed DNA presence. PCR amplification was performed using *TRAPPC9* gene primers (Ibrahim *et al.,* 2023).

The *TRAPPC9* gene, located on chromosome 8, is involved in NF-κB signaling, neuronal development, and intracellular protein trafficking. It plays an important role in neurogenesis and brain development. Although mutations in *TRAPPC9* have been associated with intellectual disability, no pathogenic alteration was identified in this study. Its exact functional role in neuronal impairment remains unclear, particularly whether dysfunction is due to gene mutation or downstream protein effects (Wilton *et al.,* 2020).

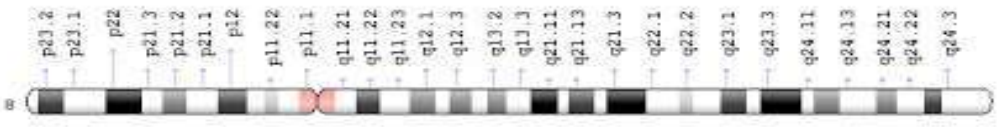

Figure 5. TRAPPC9 Gene on Chromosome 8

https://chromodisorder.org/latest-research-articles/chromosome-8p-abnormalities-studied-in-a-group-of-individuals/

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In Pakistan, cousin marriage is culturally common and is associated with a higher incidence of genetic disorders. Consanguineous families provide a valuable resource for identifying novel genes due to their unique genetic background. In this study, family PKNSID0091 was selected for molecular analysis, and *TRAPPC9* gene primers were used for PCR amplification. However, sequencing results showed no mutation in the analyzed region. This suggests that mutations may be present in other exons of the same gene or that other genes may be responsible for the disorder in this family.

CONCLUSION

This research revealed that there is no mutation detected on the TRAPPC9 gene at exon 14 of ID patient. So, by this research it is concluded that may be some other exon or gene is having mutation for the family PKNSID0091 or we can say that may be some allelic heterogeneity present in them.

REFERENCES

Abbasi, A. A., Blaesius, K., Hu, H., Latif, Z., Picker-Minh, S., Khan, M. N., ... andKaindl, A. M. 2017. Identification of a novel homozygous TRAPPC9 gene mutation causing non-syndromic intellectual disability, speech disorder, and secondary microcephaly. *American Journal of Medical Genetics Part B: Neuropsychiatric Genetics*, **174**(8): 839-845.

Alvarez-Mora, M. I., Corominas, J., Gilissen, C., Sanchez, A., Madrigal, I., and Rodriguez-Revenga, L. 2021. Novel compound heterozygous mutation in TRAPPC9 gene: the relevance of whole genome sequencing. *Genes*, **12**(4): 557.

Amin, M., Vignal, C., Eltaraifee, E., Mohammed, I. N., Hamed, A. A., Elseed, M. A., ... andDorboz, I. 2022. A novel homozygous mutation in TRAPPC9 gene causing

autosomal recessive non-syndromic intellectual disability. *BMC Medical Genomics*, **15**(1): 1-5.

Bessa, C., Lopes, F., andMaciel, P. 2012. Molecular genetics of intellectual disability.

Chelly, J., Khelfaoui, M., Francis, F., Chérif, B., and Bienvenu, T. 2006. Genetics and pathophysiology of mental retardation. *European Journal of Human Genetics*, **14**(6): 701-713.

Chen, C., Chen, D., Xue, H., Liu, X., Zhang, T., Tang, S., ... & Xu, X. (2018). IDGenetics: a comprehensive database for genes and mutations of intellectual disability related disorders. *Neuroscience Letters*, *685*, 96-101.

Eggermann, T., Perez de Nanclares, G., Maher, E. R., Temple, I. K., Tümer, Z., Monk, D., Mackay, D. J., Grønskov, K., Riccio, A., Linglart, A., and Netchine, I. 2015 Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. *Clin Epigenetics* **7**, 123.

Forster, S., Gray, K. M., Taffe, J., Einfeld, S. L., andTonge, B. J. 2011. Behavioural and emotional problems in people with severe and profound intellectual disability. *Journal of Intellectual Disability Research*, **55**(2); 190-198.

Hnoonual, A., Graidist, P., Kritsaneepaiboon, S., andLimprasert, P. 2019. Novel compound heterozygous mutations in the TRAPPC9 gene in two siblings with autism and intellectual disability. *Frontiers in Genetics*, *10*, 61

Ibrahim, N., Naz, S., Mattioli, F., Guex, N., Sharif, S., Iqbal, A., ... and Reymond, A. 2023. A Biallelic Truncating Variant in the TPR Domain of GEMIN5 Associated with Intellectual Disability and Cerebral Atrophy. *Genes*, **14**(3): 707

Kanwal, M., Alyas, S., Afzal, M., Mansoor, A., Abbasi, R., Tassone, F., and Mazhar, K. 2015. Molecular diagnosis of fragile X syndrome in subjects with intellectual

disability of unknown origin: implications of its prevalence in regional Pakistan. *PLoS One*, **10**(4): e0122213.

Karam, S. M., Riegel, M., Segal, S. L., Félix, T. M., Barros, A. J., Santos, I. S., ... abd Black, M. 2015. Genetic causes of intellectual disability in a birth cohort: A population-based study. *American Journal of Medical Genetics Part A*, **167**(6): 1204-1214

Kaufman, L., Ayub, M., and Vincent, J. B. 2010. The genetic basis of non-syndromic intellectual disability: a review. *Journal of neurodevelopmental disorders*, **2**(4): 182-209.

Khan, M. A., Khan, S., Windpassinger, C., Badar, M., Nawaz, Z., and Mohammad, R. M. 2016. The molecular genetics of autosomal recessive nonsyndromic intellectual disability: a mutational continuum and future recommendations. *Annals of Human genetics*, **80**(6): 342-368.

Marangi, G., Leuzzi, V., Manti, F., Lattante, S., Orteschi, D., Pecile, V., ... andZollino, M. 2013. TRAPPC9-related autosomal recessive intellectual disability: report of a new mutation and clinical phenotype. *European Journal of Human Genetics*, **21**(2): 229-232.