Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

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

Online ISSN 3007-2387

Print ISSN 3007-2379

Khan et al., 2026

Association of ABCC1 Exon 21 Genetic Variants with Type 2 Diabetes Mellitus in the Population of District Mardan, Pakistan

Kulsoom Khan*
Department of Biochemistry, Abdul Wali Khan University, Mardan, Pakistan. Corresponding Author Email: Kulsoom.khan9990@gmail.com

Hilal Khan*
Department of Biochemistry, Abdul Wali Khan University, Mardan, Pakistan. Corresponding Author Email: hilalkhanawkum@gmail.com

Maria Manan
Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad, Pakistan.

Mehmood Siddique
School of Biochemistry & Biotechnology, University of the Punjab, Lahore, Pakistan.

Amna Mumtaz
Department of Physiology, Faculty of Life Sciences, Government College University Faisalabad, Pakistan.

Ahmad Rasheed
Department of Microbiology, Faculty of Life Sciences, Government College University, Faisalabad, Pakistan.

Hafiza Sehar Shakoor
Department of Physiology, Faculty of Life Sciences, Government College University, Faisalabad, Pakistan.

Hafiza Mahnoor Baghdadi
The University of Lahore, Pakistan

Usama Anwar
Department of Entomology, University of Agriculture, Faisalabad, Pakistan

Muhammad Ahad Tariq
The University of Lahore, Pakistan

Zeeshan Ali Rajput
Institute of Biochemistry, University of Sindh, Jamshoro, Pakistan.

Syed Muhammad Raza Naqvi
Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad, Pakistan.

Vol. 5 No. 2 (2026)

Pakistan Journal of Medical & Cardiological Review

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

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Ali Hussnain Dahar
Department of Entomology, University of Agriculture, Faisalabad, Pakistan
Sulman Irshad
Department of Entomology, University of Agriculture, Faisalabad, Pakistan
Hamza Hameed
Department of Biochemistry, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan.

Author Details

Received on 20 Jan, 2026

Accepted on 23 March, 2026

Published on 31 March , 2026

Corresponding E-mails & Authors*:
Kulsoom Khan
Kulsoom.khan9990@gmail.com
Hilal Khan
hilalkhanawkum@gmail.com

Abstract

A large class of transporter proteins is called ATP Binding Cassette transporters, or ABC transporters. These proteins transport a wide variety of substrates, metabolites, and xenobiotics by bridging external and intracellular membranes. There are now 49 known ABC transporters in humans. Based on their protein domains and amino acid sequences, ABC transporters are categorized into seven distinct families, numbered A through G. When these transporters are overexpressed, a variety of drugs are exported, which makes treating conditions like cancer, diabetes, and AIDS more difficult. There are twelve full-length transporters in the ABCC transporter family, the first of which is ABCC1. ABCC1 is also known as multidrug resistance protein 1 (MRP1). This transporter has 31 exons and 1531 amino acids, making it a 190 KDa protein. ABCC1 has a role in the cellular efflux of several medications. Insulin production is impacted by mutations in the ABCC1 gene, which is causally associated with Type 2 diabetes and other genetic disorders. The current study looked for mutations in ABCC1 gene Exon 21 in the diabetic population of District Mardan, Pakistan. Patients with diabetes had their genomic DNA taken. Next, using the appropriate PCR primers, exon 21 of the ABCC1 gene was amplified. Single-strand conformation polymorphism analysis was used to identify several genetic variants.

Introduction

A well-known subfamily of transmembrane proteins called ATP-binding cassette (ABC) transporters enables the active transport of solutes across the cell membrane in the face

of concentration gradients (Thomas & Tampé, 2020). All eukaryotes, bacteria, and archaea have been found to contain these ABC transporters, suggesting the evolutionary significance of these polytopic membrane proteins (Köster, 2001). With the possible exception of nuclear membranes, which have pores, transporters in eukaryotic cells transport ions, sugars, amino acids, and other molecules across all cellular and organelle membranes (cell surface, mitochondrial, endoplasmic reticulum, Golgi apparatus, and other vesicles) (Carbó & Rodríguez, 2023).

Osmotic homeostasis, food absorption, bacterial immunity, pathogenesis, lipid transport, cytotoxic drug resistance, cellular immune response, division of antibiotic cells, and cell development response are some of the additional roles for ABC proteins that have been documented thus far (Kotlyarov & Kotlyarova, 2022). In addition to being energy-dependent transporters, ABC proteins are known to alter a drug's pharmacokinetic profile.

In living things, there are various kinds of ABC transporters. Only substrates from the outside can be transported into the cell by importers of Types 1 and 2, as well as transporters with an energy coupling factor (ECF) (importers of Type 3). The particular organisms with ABC importers are called prokaryotes. Although both prokaryotes and eukaryotes have ABC exporters, Type 4 and 5 ABC transporters are known to export ABC (Rice et al., 2014). Type 6 and 7 transporters (mechano-transducers) are unique to bacterial cells. Type 2 diabetes is turning into a chronic health issue that affects people of all ages. in nations that are not industrialized. Diabetes is the seventh leading cause of death. According to the WHO, Pakistan is ranked eleventh (Afzal et al., 2025; Ashraf et al., 2025).

In Pakistan, more than 6.9 million people have diabetes. Diabetes affects 9.2% of men and 11.60% of women in Khyber Pakhtunkhwa (KP). In Pakistan, the prevalence of Type 2 Diabetes mellitus is 10.34% in rural regions and 14.81% in urban areas (Ong et al., 2023). Males are more likely than females to have it, and metropolitan settings are more communal than rural ones. The frequency of Type 2 diabetes and its distribution by age, gender, and level of education in rural and urban areas were examined in the survey. Blood pressure, smoking, family history, WHO and Asian cut-offs for body mass index

(BMI), and blood pressure were measured in a large sample of people in Pakistan who were at least 20 years old (Ghassab-Abdollahi et al., 2023).

Type 2 diabetes accounts for about 85% of all cases. In both industrialized and developing nations, Type 2 diabetes has spread more quickly due to the obesity pandemic and sedentary lifestyle (Shahzadi et al., 2025). Based on current patterns, projections for the next 20 years indicate a 50% growth, particularly in emerging nations (Chen et al., 2012). The prevalence of diabetes is high in Pakistan. based on the International Diabetes Federation's (IDF) estimates. If appropriate steps are not made to control this disease, the number of people with diabetes will rise to 69.2% in 2025 (Adekanmbi et al., 2026).

Multidrug resistance has been linked to mutations in one or more of the ABCC genes. Thus far, no clinically useful inhibitors with high specificity for ABCC1 have been created (He et al., 2011). Clinical trials have only investigated a small number of compounds with inhibitory potential against ABCC1 that are known to block ABCC1 efflux. Exon 21 of the ABCC1 gene was examined in this study in relation to diabetes. According to Errasti-Murugarren and Pastor-Anglada (2010), the study helped identify genetic variants in Exon 21 and later linked them to the Type 2 Diabetic community in Mardan, Pakistan. Aims and objectives of this work: To study Type 2 Diabetes mellitus genetic variations in Exon 21 of *ABCC1* gene. To find out genetic association of Type 2 Diabetic individuals of District Mardan with *ABCC1* gene.

Material and Method

Ethical Approval

The current research was permitted by the ethical committee of Department of Biochemistry, Abdul Wali Khan University Mardan.

Study Area

Blood samples were taken from Khyber Pakhtunkhwa, Pakistan's District Mardan. The current work was carried out at Abdul Wali Khan University Mardan's Molecular Laboratory of the Biochemistry Department.

Inclusion/Exclusion Criteria

Patients with diabetes who were between the ages of 35 and 60 were included in the study; those who had additional conditions, such as cancer or heart issues, were not.

Direct communication based on a structured questionnaire was used to collect all the necessary information. Patients' clinical symptoms, age, gender, and family history of Type 2 diabetes with notable pathological characteristics were also gathered.

Sample Collection

Aseptic vacutainer tubes containing the anticoagulant ethylene diamine tetra acetate (EDTA) were used to collect blood samples from 20 patients with Type 2 diabetes using 5 ml syringes. With the help of numerous lab technicians and hospital administrators, the samples were obtained from various hospitals and private labs. The samples were kept in storage at -20°C.

Genomic DNA Extraction

A conventional Phenol-Chloroform Method procedure was used to extract and purify genomic DNA from human blood in order to amplify Exon 21 of the ABCC1 gene and subsequently test for different mutations (Di Pietro et al., 2011).

Horizontal Gel Electrophoresis

Gel preparation

To verify the isolated DNA, a 1% Agarose gel was made. Agarose gel was made by adding 0.3 grams of agarose and liquefying it in 30 milliliters of 1xTBE buffer. This solution was cooked on a hot plate until it appeared clear. The solution was allowed to cool to 60°C at room temperature. After that, 3 ul of ethidium bromide was added to the mixture to stain the DNA and make it visible. The gel apparatus was assembled, the agarose solution was put into the gel cassette, wells were carefully formed with a comb, and the mixture was allowed to solidify for 30 minutes. The gel was moved to a gel tank with 1X TBE buffer after the comb was removed (Downey, 2003).

Gel running

DNA was loaded into the wells. Gel running was carried out for about 35-40 minutes at 125V (50 mA) in 1 X TBE running buffer.

Gel visualization

Gel documentation system was used to visualize the DNA which was extracted under ultraviolet illuminator (Biometra, Germany) at a wave length of 254 nm.

Polymerase Chain Reaction

Following DNA extraction, PCR was performed on both normal and diabetic DNAs. PCR tubes were utilized for this purpose. The following ingredients were used to make the cocktail in PCR tubes. One ul of DNA (normal/diabetic) was added to each PCR tube after 0.5 ul of the forward and reverse primers were added. After adding 13 ul of green mix, PCR water was used to increase the amount to 25 ul.

Gel Electrophoresis of PCR products

After Polymerase Chain Reaction, the products were run on agarose gel of 3 percent and visualization was carried out on Gel documentation system.

Single Strand Conformation Polymorphism (SSCP) Analysis

PCR results were examined on a 12% polyacrylamide gel. The gel solution was made in a 25 ml beaker and then poured into the center of the 1.5 mm gaps between two glass plates. The comb was then inserted into those areas and allowed to polymerize for an hour at room temperature. After adding 12 ul of loading dye (deionized formamide 9.5 ml, bromophenol blue 25 mg, xylene cynol 2 mg, 0.5M EDTA 400 ul, and water 100 ul) to 8 ul samples, the samples were denatured for five minutes at 95 oC in PCR. After denaturation, samples were then kept at -20°C for roughly ten minutes (to generate specific confirmation). The prepared samples were then put into the wells. between 130 and 180 minutes. SSCP analysis was carried out at 150 volts in a vertical gel tank (Invitrogen X cell). The gel was dyed using an ethidium bromide solution (1 mg/ml). For visualization, a digital camera DC 290 (Kodak, USA) and UV trans illuminator were utilized.

Results

Diabetic Patients Clinical Characteristics

In all, twenty blood samples were used in this investigation. 16 samples were collected from male and female patients with Type 2 diabetes who had been clinically diagnosed. The group consisted of six ladies and ten males. The patients in the study ranged in age from 35 to 60, with a mean age of 47.5. All of the patients had Type 2 diabetes. It was discovered that the blood glucose levels were higher than those of a healthy individual. Four blood samples were obtained from healthy people in addition to those with diabetes. As indicated in Table 1, the primary laboratory and demographic characteristics of both normal individuals and diabetic patients were documented in

accordance with the inclusion criteria. All of the patients have a poor socioeconomic status and live unhealthy, sedate lifestyles.

Table 1: *Demographic Characteristics of samples collected*

S. No	Demographic Characteristics		
1	Total samples		20
2	Number Of Diabetic Patients		16
	Gender		
	a)	No. of Males	10
	b)	No. of Females	6
3	Number of normal individuals		4
	Gender		
	a)	No. of Males	2
	b)	No. of Females	2
4	Socio-Economic Status		Middle Class Family
5	Life Style		Sedentary
6	Age of Patients		
	a)	Lower Limit	35 years
	b)	Upper Limit	60 years
	Mean		47.5 years
7	Level of Glucose		
	Upper Limit		415 mg/Dl
	Lower Limit		124 mg/Dl

Mapping of Genetic Variations in Exon 21 of *ABCC1* in Diabetic Patients

DNA Isolation Results

The phenol-chloroform (organic) approach described in Chapter 3 was used to isolate DNA from blood samples of both normal and diabetic people. 1% DNA was analyzed using an agarose gel. Photographs were shot while the gel was visible in the gel dock.

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Online ISSN 3007-2387
Print ISSN 3007-2379

The gel result of isolated DNA from normal samples is displayed in **Figure 1**. **Figures 2** and **Figure 3** depict DNA isolated from diabetic samples.

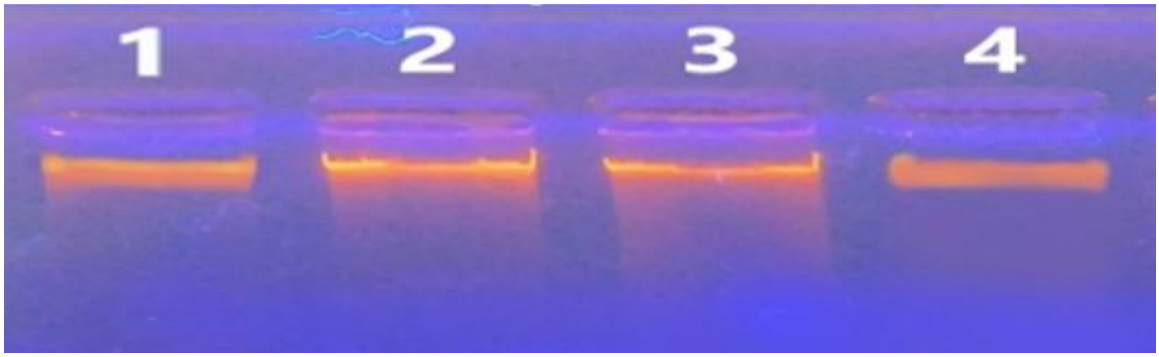

Figure 1: Horizontal gel electrophoresis of normal DNA samples 1-4

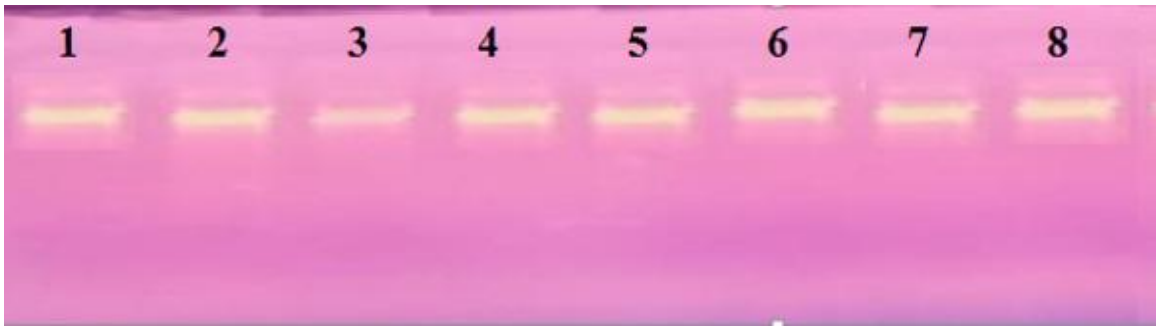

Figure 2: Horizontal gel electrophoresis of Diabetic DNA samples 1-8.

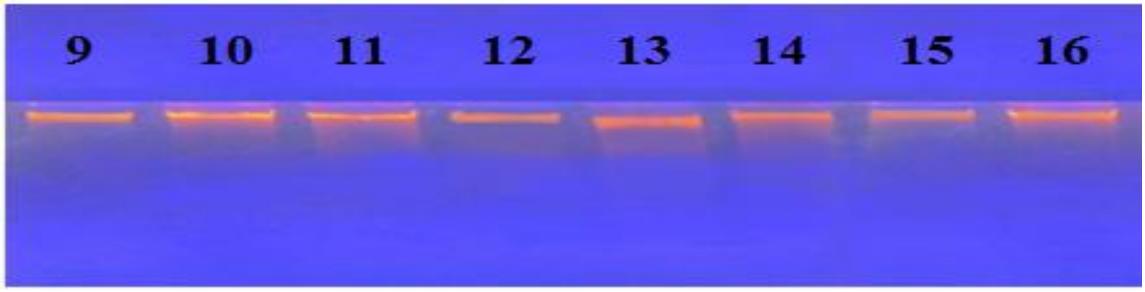

Figure 3: Horizontal gel electrophoresis of Diabetic DNA samples 9-16.

Results of Polymerase chain reaction (PCR) of *ABCC1* Gene

Exon 21 of *ABCC1* gene was amplified by PCR using its respective primers. The products of Polymerase Chain Reaction were run on an agarose gel of 3 percent. The results are depicted in the figures below.

Amplification of Exon 21 from Normal DNA

Exon 21 of the *ABCC1* gene was amplified from normal DNA using a pair of forward and revere primer. The optimal annealing temperature for Exon 21 was 60°C. Length of Exon 21 is 464bp. Result is shown in **Figure 4**.

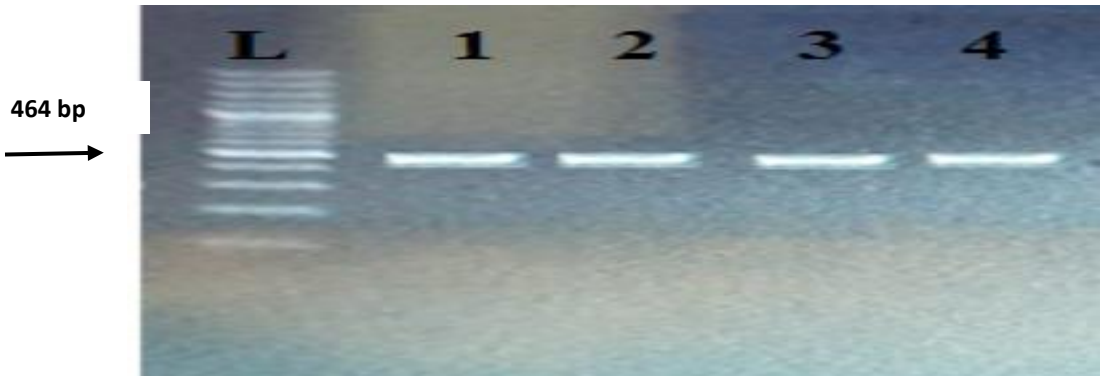

Figure 4: Horizontal gel electrophoresis of PCR products 1-4 of normal samples. 100bp ladder (L) was used.

Amplification of Exon 21 from Diabetic DNA

Exon 21 of the *ABCC1* gene was amplified using a pair of primers that is forward primer and reverse primer. The optimal temperature for annealing was 60°C for Exon 21. **Figure 5** and **Figure 6** shows **16** Diabetic samples of Exon 21.

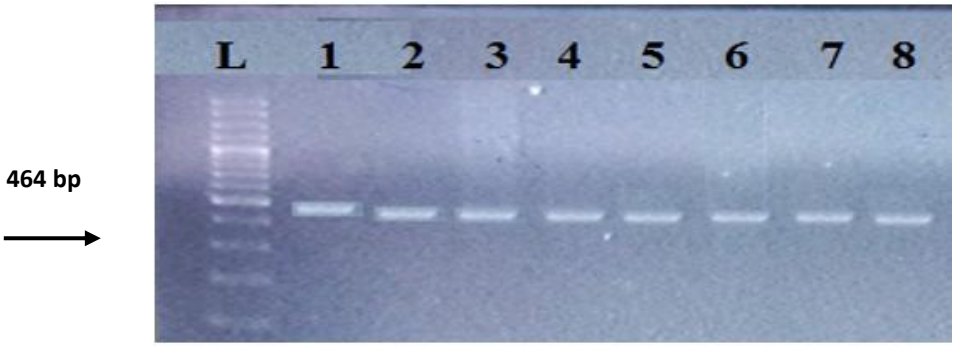

Figure 5: Horizontal gel electrophoresis of PCR product 1-8 of Diabetic samples. 100bp ladder (L) was used.

Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
Online ISSN 3007-2387
Print ISSN 3007-2379

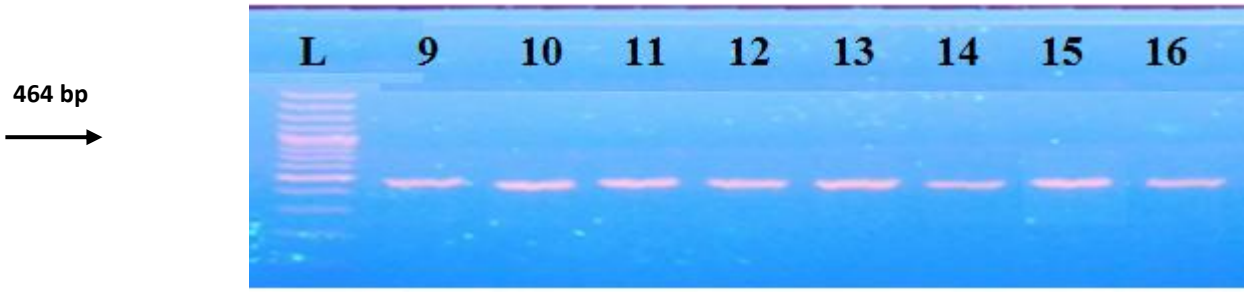

Figure 6: Horizontal gel electrophoresis of PCR product 9-16 of Diabetic samples. 100bp ladder (L) was used.

Polyacrylamide Gel Electrophoresis for Mapping Genetic Variations

Single Strand Conformation Polymorphism (SSCP) analysis

SSCP analysis was done in order to find out variations in Exon 21 of *ABCC1* gene. Therefore, all samples were run on Vertical gel. Results depicted variations in the banding pattern of all 20 samples. Single and double bands were observed based on genetic makeup of each sample. On gel, each conformer has a distinct mobility. However, further sequencing is required to know about mutations in Exon 21.

Figure 7: Electropherogram showing normal samples (1-4) of 12% PAGE.

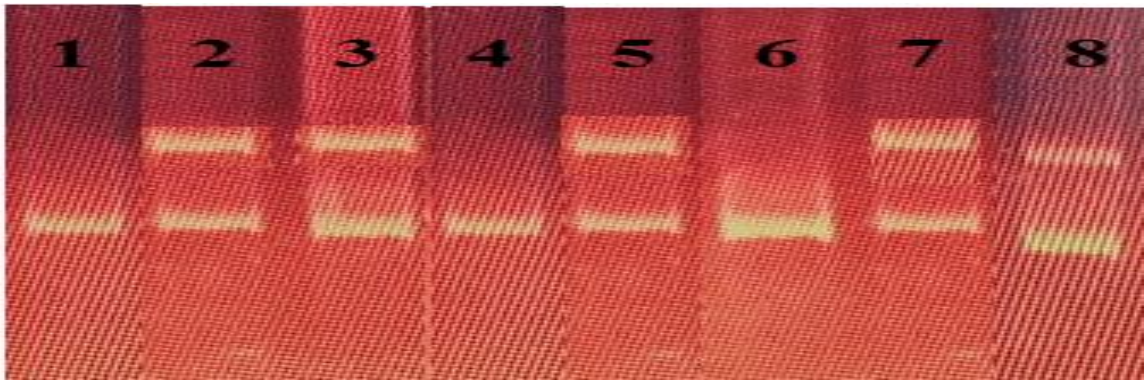

Figure 8: Electropherogram showing Diabetic samples (1-8) of 12% PAGE.

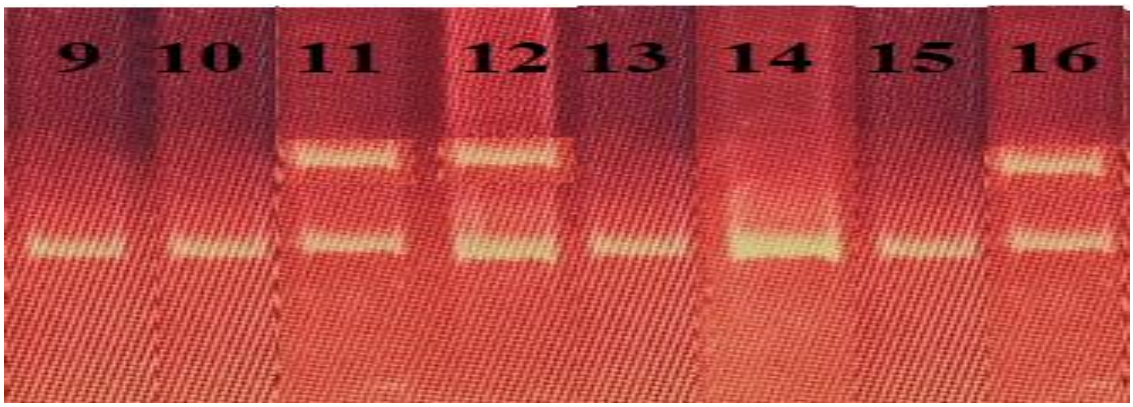

Figure 9: Electropherogram showing Diabetic samples (9-16) of 12% PAGE.

Discussion

Certain hereditary conditions, including cystic fibrosis, diabetes, retinopathies, high-density lipoprotein deficiency, and cardiomyopathies, have been linked to ABC transporters (Afzal et al., 2025). The most substantial multidrug resistance to cells is caused by ABC membrane transporters, particularly ABCC1, which reduces drug accumulation through its energetic efflux. Although the exact role of ABC in multidrug resistance is yet unknown, ABC transporters play a significant role in pancreatic blood membrane blockage. Diabetes mellitus is associated with numerous consequences, including neuropathy and cardiovascular disorders (Dean et al., 2022).

The goal of the current investigation was to identify genetic polymorphisms in the ABCC1 gene's Exon 21. Twenty blood samples in all were taken from clinically identified Patients with diabetes include both men and women. Following DNA extraction, PCR amplification was seen using primers for Exon 21 of the ABCC1 gene. The single strand conformation polymorphism was then used to identify variants or mutations. Every sample had a varied banding pattern, according to the analysis. Our group carried out the first investigation into ABCC1 gene variants in diabetics in District Mardan, Pakistan. Even though Exon 21 has variants based on SSCP, further sequencing is necessary to confirm both known and novel SNPS in the Pakistani population (Ullah et al., 2025).

Numerous mutations in the ABCC1 gene have been found in a number of populations. Four distinct ABCC1 single nucleotide polymorphisms (SNPs), G128C,

G2168A, G1299T, and G2012T, were discovered by researchers during a study in both neuroblastoma samples from the Australian population and normal cord cells. 195 neuroblastoma samples and 158 cord blood samples (Atalay, 2007). There were quite few of the first three polymorphisms. None of the samples had homozygous alternative G1299T alleles, however 13% had heterozygous alternative alleles.

Protein domains contain ABCC1 SNPs, and several mutations have been identified in vitro through mutagenesis (Wang et al., 2011). Researchers changed a very conserved Gly residue at position 671 to Val to create the polymorphic exon 16 variant G2012T. However, no discernible differences between the Gly671Val transmuted and wild type ABCC1 proteins were found. The transportation of several organic anions was significantly decreased in exon 10 when the highly conserved Arg433 was substituted with a Ser residue, while doxorubicin resistance was increased. In summary, the transmutation of exon number 2 G128C reduced the plasma membrane localization in ABCC1 (Yin et al., 2009).

Many polymorphisms have been found in the ABCC1 gene's intronic (non-coding) regions. The human ABCC1 gene has just 14 non-synonymous genetic variants. Different populations have varying frequencies of these single nucleotide polymorphisms. Compared to certain Asian ethnicities, even the allelic frequency of general populations is less than 1%.

Even though certain single nucleotide polymorphisms have been discussed, it has been determined that SNPs are commonly found in the non-coding regions of the ABCC1 gene and that their frequency varies depending on the population. G3140C single nucleotide polymorphisms with a Ser substitution of Cys1047 in CL6 were discovered by researchers in the twenty-third number exon. Compared to the Caucasian population, the American population has a significantly higher frequency of this polymorphism (Conseil et al., 2005). It has been discovered that the ABCC1 promotor gene's 5′ FR/G-260C functional polymorphisms have a significant impact on the production of ABCC1, which includes inflammatory-related activities. Our research revealed a potential link between diabetes and a mutation in the ABCC1 Exon 21 gene. This is corroborated by research showing ABC transporters are implicated in several

illnesses, including as diabetes, cancer, and AIDS. However, the findings requires more investigation on higher populations across the nation.

Conclusion

We found a possible connection between a mutation in the ABCC1 Exon 21 gene and diabetes. Research demonstrating that ABC transporters are linked to a number of diseases, such as diabetes, cancer, and AIDS, supports this. Further research on larger groups across the country is necessary in light of the findings, though.

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