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Evaluation of Single Nucleotide Polymorphism in MDR ABCC1 Gene in Type 2 Diabetic patients from Khyber Pakhtunkhwa Pakistan

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Abstract
ABCC1 is a multidrug resistance ATP-Binding cassette transporter commonly known as multidrug resistance-associated protein 1 (MRP1). It is a full-length transporter and is located on chromosome number 16p13.1. Physiologically, it exports xenobiotics and toxins out of the cell, thereby providing a shielding mechanism for the cell. During stress conditions, *ABCC1* overexpression causes in an efflux of drugs and creating a hurdle in the treatment of various diseases. Several studies have reported the implication of the *ABCC1* genetic polymorphism among cancer patients in different populations. However, studies related to *ABCC1* polymorphism in diabetic patients are rare reported, especially from Pakistan. The present study was therefore conceived to genetically screen the *ABCC1* gene (exon 22 and flanking intron) in diabetic patients (n=100) from Mardan region of Pakistan. The PCR-SSCP analysis followed by DNA sequencing identified variations in 06 subjects followed by sequencing. A total of 10 genetic variations were identified in diabetic subjects. Among these, 9 were novel including 7 non-synonymous, 1 synonymous, and 1 intronic variant. In addition, rs3851716 (A1009G) is identified as a reported variant in dbSNP and the 1000 Genome project. LD analysis revealed a high degree of linkage for region 16111304-16111721 with African populations. Furthermore, the intronic variant 16111592 G/A is reported in association with diabetes in American and African populations. Furthermore, the structural effect of deleterious variants was also analyzed using homology modelling. The variants were identified in TMD2 regions and exhibit drastic differences in interactions with nearby residues when compared to the wild-type protein, which may affect the binding properties of *ABCC1*. This study for the first time reports genetic variations (SNPs) of the *ABCC1* gene and its effect on structural modifications in proteins in diabetic patients from District Mardan, Khyber Pakhtunkhwa, Pakistan.

Keywords: *ABCC1* transporter; polymorphism, SNP; Diabetes; structural analysis

Introduction

There are 49 *ABC* genes in humans, which are classified into seven distinct families (A-G) [1, 2]. Out of these 49 *ABC* transporters, three are multidrug resistance (*MDR*) transporters, such as *ABCB1*, *ABCG2* and *ABCC1 [3]*. *ABCC1* transporter is a 190 kDa protein also called Multidrug Resistance Protein 1 *(MRP1)*, [4] which was discovered by Susan Cole in a human lung carcinoma cell line [5]. *ABCC1* gene is located on chromosome 16p13.1 [6]. It is a full-length transporter, consisting of 31 exons and encoding a protein of 1531 amino acids [7]. Structurally, ABCC1 comprises of 2 NBDs and 3 TMDs [8]. The NBDs are hydrophilic and the TMDs are hydrophobic. TMD1 and TMD2 have six helices each, while TMD0 has 5 membrane-spanning helices that make up the pore in the plasma membrane by which solutes are expelled out of the cell [9]. The NBDs are meant for ATP binding and hydrolysis, which empowers MRP1's trans-membrane solute transport [10].

ABCC1 is approximately found in all major tissues including peripheral blood cell types, tissue-blood barriers, blood-brain barriers with enormous levels in kidney, liver and pancreatic beta cells and played a significant role in drug disposal [11] (Koehn et al., 2008). *ABCC1* is known to transport anti-diabetic drugs like glyburide [12] and drugs that have been chemically conjugated to glutathione or glucuronic acid [13] including glutathione (GSH), oxidized GSH, glucuronide and sulfate-conjugated organic anions actively [14]. MRP1's best physiological substrate is GSH-conjugated and cysteinyl leukotriene C4 (LTC4) [15]. Other physiological substrates are the peptides, the steroid sulfate estrone 3-sulfate (E13SO4) and the glucuronide conjugate 17β-estradiol 17-(β-D-glucuronide) (E217Bg) [16]. *ABCC1* plays a significant role in Absorption, distribution, metabolism, elimination and toxicity (ADMET) properties of drugs. Its overexpression exports a comparatively wide variety of drugs, thereby creating a hurdle in the cure of many diseases, including cancer, AIDS and

diabetes [17]. It has already been reported that genetic variations in ABCC1 might influence its role in drug resistance. *ABCC1* thus may be a possible therapeutic target in the treatment of diabetes.
In different populations, a large number of single nucleotide polymorphisms (SNPs) were detected in the *ABCC1* gene. Saito et al., reported 95 genetic variations in *ABCC1* gene in Japanese individuals [18]. Subsequently, in the same population, they screened the *ABCC1* gene and identified 86 genetic variations, including 31 novel ones [19]. Screening of the *ABCC1* gene in Chinese population showed 32 SNPs [20]. In Caucasian populations, screening of genetic variants in ABCC1 transporter gene identified 61 variants, among them 22 were novel [21]. Comparative study on Chinese, Malay, Indian and Caucasian population showed that apart from population-specific SNPs, only 6 of 71 detected SNPs altered amino acid sequence [22]. Majority of the reported SNPs in *ABCC1* gene are found in intronic region. Studies reported in literature show the importance of ABCC1 polymorphisms in cancer in different populations. Some SNPs alter protein functioning and the effectiveness of cancer chemotherapy. Such cases were reported for neuroblastoma [23], breast cancer [24], ovarian cancer [25], chronic obstructive pulmonary disease (COPD) [26], cystic fibrosis [27] and major depression [28]. However, studies related to diabetes mellitus are very rare, especially in Pakistan. Therefore, the current study was conducted to screen genetic variation of the *ABCC1* gene (exon 22 and flanking intron) in the diabetic population of District Mardan, Pakistan.

2.0 Materials and Methods

2.1 Blood Sampling and human genomic DNA extraction

Blood samples were collected from diabetic and healthy individuals (n=100) of District Mardan Khyber Pakhtunkhwa Pakistan. All the essential data was collected through direct conversation based on a planned questionnaire. Different demographic factors including gender, age, symptoms, complications and family history of diabetes with significant pathological features were collected from the patients. Genomic DNA was extracted from 0.75 ml human blood through a standard protocol (phenol-chloroform method) in an Eppendorf tube [29]. All subjects gave their written informed consent to participate in the study. This study was approved by the ethical committee of Abdul Wali Khan University Mardan, Pakistan.

2.2 PCR-SSCP (Single Strand Conformation Polymorphism) analysis

Exon 22 and flanking intron of the *ABCC1* gene was amplified using forward primer 5´-GTTTACTGCTGACTTTGTTG-3´ and reverse primer 5´-ACTCAAACACCCACTCTACA-3´ to obtain fragment size of 566 bp. Each PCR mixture was prepared with 2 µl of genomic DNA, 1 µl each primer, 26 µl Mater mix and 20 µl PCR water. The following steps amplified exon 22: initial denaturation at 95°C for 3 minutes, final Denaturation at 95°C for 30 seconds, annealing of primer at 57°C for 30 seconds, extension of complementary DNA at 72°C for 1 minute and final extension at 72°C for 5 minutes. SSCP analysis was performed by using modified method of [30, 31]. In principle, if there is no variation, the subjects (healthy/diabetic) must form two conformers. A banding pattern was obtained on 30% polyacrylamide gel electrophoresis, which contained 10X buffer, ammonium persulphate (APS), N, N, N, N-tetramethylethylenediamine (TEMED), acrylamide and distil water. Before loading, PCR products were mixed with SSCP loading dye. SSCP banding pattern was visualized after staining in ethidium bromide.

2.3 DNA Sequencing

The exon 22 and flanking intron region was amplified and subsequently sequenced by using a special set of primers for all subjects. The sequenced data were analyzed using BioEdit software [32] and aligned against the Ensemble Genome Browser genomic reference sequence [33].

2.4 Statistical Analysis

The sequence homology-based webservers, PROVEAN (protein variation effect analyzer) and SNP-nexus were used to predict the damaging effects of 9 variants on protein function. The input format for PROVEAN is the genomic coordinates of the respective variants. The variants were predicted as damaging or neutral/tolerated on the basis of SIFT and PROVEAN prediction scores at cutoff of 2.5 for PROVEAN and 0.05 for SIFT. Linkage disequilibrium (LD pair) analysis was performed using online tool LDpop (https://ldlink.nci.nih.gov/?tab=ldpop). This would explain the non- random association of different alleles in the Mardan, Pakistani population (in the studied exon of *ABCC1*) with all other populations from 1000 Genome Project. This tool uses the rs ID available in dbSNPs and all populations from the 1000 Genome Project. R^2 and D values were calculated to show the correlation with different populations available in 1000 genome project.

2.5 Structural Modelling

The ABCC1 (isoform 1) amino acid sequence was obtained from the UniProt database (identifier: P33527). The MODELLER program was used to construct wild-type and mutant-type ABCC1 structures using three-dimensional modeling techniques [34]. PDB ID: 6YU0 (bovine multidrug resistance protein 1) was used as a template for the construction of wild type. Three mutant models p.Val962Leu, p.Trp995Arg and p.Tyr1019Asn of ABCC1 protein were generated [35]. Structure visualization and image processing were carried out using PYMOL (The PyMOL Molecular Graphics System, Version 1.8 Schrödinger, LLC).

3.0 Results

3.1 Detection of ABCC1 gene (Exon 22) variants in Diabetic population

A total number of 100 subjects, including healthy (n=30) and diabetics (n=70) were amplified using *ABCC1* primers. The demographic characteristics of these subjects are shown in **Table 1.** PCR products were screened for sequence variation through SSCP. According to SSCP analysis, the subjects that do not have any variation showed two bands while those subjects having variation showed multiple banding pattern on vertical gel as shown in **Figure 1**.

PCR- SSCP analysis identified variations in 06 subjects, whereas healthy and most of the diabetic subjects did not show any variation. A total of 10 variants have been identified. Out of these, 8 were in the exonic region and 2 were found in the intronic region, including 16111358 T/G and 16111592 G/A. Exonic variants include G3020C (V962L), G3069T (S978I), T3119A (W995R), G3166T (E1010D), T3191A (Y1019N), C3077A (L981I), C3122A (L996I), and A3162G (E1009R) were identified in the above-mentioned subjects. The most frequent variant, G3069T, existed with a frequency of 3% in studied subjects. Out of these 9 were novel variations, while 1 was already reported in a database (rs943498117, A3162G, Ensemble) but not in the Pakistani population.

The effect of these variants was analyzed using PROVEAN and SNP-nexus servers as shown in **Table 2**. The PROVEAN prediction score cutoff was set at 2.5. The score higher than 2.5 indicated that variant might have damaged or deleterious effects on protein. However, a score of less than 2.5 showed that variants were tolerated in the protein. According to PROVEAN analysis, 3 variants (16,16205244, G, C, 16,16205343, T, A, and 16,16205415, T, A) out of 9 were characterized as deleterious and 3 as neutral including 16,16205293, G, T, 16,16205390, G, T, and 16,16205346, C, A. The corresponding amino acid substitutions for deleterious variants were V962L, W995R and Y1019N. The SIFT algorithm predicted 4 variants damaging (16,16205244,G,C, 16,16205343,T,A, 16,16205415,T,A and 16,16205346,C,A) and 3 variants tolerated at a cutoff score of 0.05. The variants having a SIFT prediction score of less than 0.05 were identified as damaging, and the others having a SIFT score of more than 0.05 were tolerated. A cumulative PROVEAN, and SIFT analyses predicted 3 nsSNPs (16,16205244,G,C, 16,16205343,T,A, and 16,16205415,T,A) as deleterious. SNP-nexus predicted 1 variant as synonymous (chr16:16205387:A/G, Q1009Q) and 1 in the intronic region (chr16:16205215:T/G). These 2 were excluded from further study.

3.2 Linkage Disequilibrium analysis

Linkage Disequilibrium analysis was performed to map geographic correlations of specified alleles with all the populations available in 1000 Genome Project. LD analysis were carried out using the online LDpop tool [36]. The LDpop calculates allele frequencies for each queried population or variants to investigate the LD across a different population of 1000GP. The higher value of D´ and r^2 depicted significant LD among the population of variants. An LD map was generated for globally available variants that are associated with *ABCC1* gene. LD maps were shown in **Figure 2 A-D**. These variants have also been covered our target region SNPs. Five variants that were available in 1000 GP were selected from *ABCC1* intronic/exonic 22 regions. These include rs4238623, rs45607431, rs200753907, which cover the exonic region, while rs3851716 and rs3887893 cover an intronic region as shown in Table 3.

Results revealed a higher degree of linkage (shown in red color) for regions 16111304-16111461 and 16111461-16111521, 16111521-16111592 and 16111592-16111644 with African populations including MSL (Mende in Sierra Leone), LWK (Luhya in Webuya, Kenya), ACB (African Carribbeans in Barbados), ASW (Americans of African Ancestory in SW USA) and YRI (Yoruba in Ibadan, Nigera). Additionally, our intronic variants (16111592-16111644) were strongly linked in American and African populations, including MSL, LWK, ACB, ASW, ESN, GWD, PUB, CLM, and YRI with a high allele frequency of 16111592 G/A. The D values were found to be 1 and the rho square value were less than 1, which showed a strong correlation of variants in the available region with African populations. The four variants identified in the current study (16,16205293, G, T, 16,16205343, T, A, 16,16205415, T, A and 16,16205346, C, A) also lie in the same linked region, indicating their importance in predicting ancestory. Moreover, this will highlight the genetic variations in studied region from other populations.

The amino acids corresponding to the identified variants were also plotted in homology model of human *ABCC1* based on the PDB ID: 6YU0 as a template and highlighted in magenta colour. The model showed that amino acids V962, S978, W995, Y1019, A991, L981, L996 were found in transmembrane domain region of *ABCC1* while remaining residues were found in NBD and extracellular region. All the substituted amino acids were found in different regions of *ABCC1* gene. One change (Val 962 Leu) occurred in NBD1 cytoplasmic region between Walker B and TM12. Two changes (S978I) and (L981I) occurred in TM12. (W995R), (E1010D), (Y1019N), (A991A) and (L996I) were found in extracellular membrane between TM12 and TM13. We also detected one known non-synonymous variant, 3162 A>G (Q1009R), present in extracellular membrane between TM12 and TM13.

3.3 Structural analysis of wild and mutant-type ABCC1

The variants that showed deleterious/damaging effects on proteins according to SIFT and PROVEAN analysis were modeled to identify the structural role of mutated positions by analyzing intramolecular interactions and comparing WT with mutant model. The three-dimensional structures of the WT and mutant *ABCC1* proteins were built using homology modeling technique. WT *ABCC1* (isoform 1) consists of 1531 amino acids (AA) and includes transmembrane domain 1 (red), transmembrane domain 2 (magenta), nucleotide-binding domain 1 (yellow), nucleotide-binding domain 2 (cyan) and linker region (green color) between TMD_0 and TMD_1 **(Figure 3A)**. It is noticed that variants identified in the current study correspond to amino acids, which are located in TMD2 region as shown in **Figure 3A.**

To investigate the potential effect of variations in the transmembrane 2 domain, the structures (203–1531 AA) of WT and mutant *ABCC1* (p. Val962Leu, p. Trp995Arg, p. Tyr1019Asn) were modelled **(Figure 3B)**. The residue Val962 in the alpha

helix is in close proximity to Glu1262 of NBD2 and exhibits a 6.0 Å distance. However, substituting Leu962 for Val962 reduces the distance to 4.0, resulting in a closer interaction of Glu1262 with Leu962. Furthermore, due to the variation, a shortening of the alpha helix can be observed at the local conformation **(Figure 3C)**. Arg1015 formed a hydrophobic contact with neighboring Trp995 (4.7 Å) and Trp999 (3.7 Å) residues in the WT type. The replacement of the hydrophobic side chain (Trp995) with a charged one (Trp995Arg) resulted in a loss of interaction as shown in **Figure 3D**. However, in the case of WT residue Tyr1019, which is found near Phy558 (4.2 Å), Asn590 (2.9 Å), and Leu586 (4.7 Å) of TMD1, the p. Tyr1019Asn interaction increases the distance between neighboring residues, indicating a shift in local conformation **(Figure 3E)**.

Discussion

The present study was conducted to find genetic variations in *ABCC1* exon 22 and flanking intron in a diabetic population of Mardan, Khyber Pakhtunkhwa Pakistan. Results identified a total of 10 genetic variations in diabetic subjects. Among these, 9 were novel including 7 non-synonymous, 1 synonymous, and 1 intronic variant. According to SIFT and PROVEAN Analysis 3 non-synonymous deleterious variations, including 16205244G/C(V962L), 16205343T/A(W995R), and 16205415T/A(Y1019N) were found. However, the other 6 non-synonymous variants were shown as neutral. The deleterious variants were mapped into homology model based on bovine ABCC1 template to see the difference in interactions in mutant model as compared to the wild-type protein. Several studies have already reported the importance of TMD2 residues in transport function of MRP1. Jhonson and Chen reviewed that mutating R1196, W1245, and R1248 results in loss of transport function of the protein [37]. As shown in the homology model, the mutants identified in the current study were found in the vicinity of these residues and showed loss or change of interaction with nearby residues when compared to the wild-type protein. Moreover, bioinformatics analysis also revealed deleterious and damaging effects of these variants on proteins, as shown in **Table 2**. Chen et al., 2021 studied the anti-cancer activity of betulin in the presence and absence of *ABCC1* inhibitor [8]. Their findings show that in the presence of an inhibitor, cells become more sensitive to betulin. Furthermore, the interaction of betulin with *ABCC1* was investigated by docking and simulation studies. It is noticed that botulin binds in the hydrophobic cavity formed by transmembrane domains of *ABCC1* and shares the same binding pocket (partially overlapping) as that of leukotriene C4 (LTC4). The listed residues were L381, F385, F389, Y440, T439, I598, F594, M1092, T2141, Y1242, N1244 and W1245. The deleterious/nonsynonymous variants found in the current study, i.e., V962L, W995R, and Y1019N in diabetic subjects of Mardan, were also found in the same region. These substitutions result in drastic changes in hydrophobic cavity of *ABCC1* as the aromatic tryptophan has been replaced by a positively charged residue and another aromatic residue, tyrosine, has been changed to asparagine. Our homology model of these mutants showed that these replacements W995R result in loss of wild-type interaction with R1015. Furthermore, Y1019N replacement increases the distance between nearby residues Phy558 (4.2 Å), Asn590 (2.9 Å), and Leu586 (4.7 Å), changing the proteins local conformation. There is literature available that shows that the *ABCC1* variant effects the pharmacokinetic properties of drugs. Piatkov et al., 2017 reported the effects of rs212090 (*ABCC1*) and rs1045642 (ABCB1) variants on patients receiving clozapine, an anti-psychotic drug having several side effects, including the new onset of diabetes [38] Their data indicated that patients having these variants shows increase in BMI, BP and CZ serum levels, indicating the effect of ABCB1 and ABCC1 variants in response to drug treatment. In our study, *ABCC1* variants were found in diabetic subjects that might affect the pharmacokinetic and pharmacodynamic properties of drugs. It is already reported in literature that ABC transporters, mainly ABCB1, ABCG2 and members of ABCC family including ABCC1-ABCC6, play a significant role in diabetes type 2 due to their differential expression in the liver that affects the efflux properties of drugs. Additional studies have identified various genes involved in diabetes mellitus, including variations in the *ABCC1* gene [39] (Fukushima-Uesaka et al., 2007), [40-42].

Conclusion

Following major conclusions can be drawn from the present study. ***Firstly***, this study for the very first-time reports 10 variants in *ABCC1* gene in diabetic population of District Mardan, Khyber Pakhtunkhwa Pakistan. ***Secondly***, 3 deleteriuos variations 16205343T/A(W995R) and 16205415T/A(Y1019N) in *ABCC1* gene adversely affects the structural properties of the *ABCC1* transporter especially the TMD2 region. However, in future mechanistic studies are needed to be done to explore the effect of these variants towards treatment of diabetes by doing efflux studies with antidiabetic drugs.

Data Availability Statement

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

Author Contributions

IA and SF performed the experiments. ZP designed the study. AN and HK performed computational analysis and revised the manuscript. AS helped in sequencing and BK helped in scientific discussion. HK and MA helped in submission of sequencing data and its analysis. AK helped in sample collection. ZP, SF, BK and AN helped in finalization of manuscript. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Figure legends

Figure 1. Single nucleotide polymorphism analysis of ABCC1 Exon 22 on 30% PAGE. Figure shows (A) Healthy and diabetic subjects (H= Healthy, D= diabetics (without variation) and (B) diabetic subjects (28,29,35,47,8,25,31,33,34,35,50,55,65 are diabetic subjects in which variation were found). Wild type has two band patterns while the mutated type has single or multiple band patterns.

Figure 2. LD pair analysis for a set of available variants that cover intronic/exonic 22 region. Correlation bar is given at the bottom of each figure with increase in D' value, the red colour intensity increased. At the value of 1 the maximum linkage is shown. In these figures the red colour populations were linked. Figure C shows linkage of intronic variants including 16:16111592 G/A with Americans and African populations.

Figure 3. Membrane topology model of ABCC1. The depicted model display combinations of nucleotide-binding domains (NBDs), which harbor conserved peptide motifs, and transmembrane domains (TMDs), which generally consist of transmembrane helices (TMHs). L_0 linker region between TMD0 and TMD1. Variations reported in this study are mapped with arrow (B). Three-dimensional (3D) structures of the WT ABCC1 predicted using the MODELLER program and visualized with PyMOL software. (C). Representative intermolecular interactions between position at 962 and nearby residues of wild type (upper row) and mutant (lower row) structure. Mutation residues are indicated with bold letter. The residues are shown as stick model. (D). Representative intermolecular interactions between position at 995 and nearby residues of wild type (upper row) and mutant (lower row) structure. The dotted lines display interaction and distance with nearby residues. The distances are shown with Angstrom (Å) (E). Representative intermolecular interactions between position at 1019 and nearby residues of wild type (upper row) and mutant (lower row) structure.

Table 1: Clinical Profiles of diagnosed Type II diabetes mellitus, with Medical and family history of diabetic patients based on demographic and biological variables between male and female of different aged participants.

	Categories	**History of Hypertension**	**History of Obesity**	**Family history of diabetes**	**Glucose Test (HbA1c)**	**Family history of Hypertension**
Age Groups	Below 35	(5.2%)	(5.4%)	(8.5%)	(6.8%-7.3%)	(5.2%)
	35-60s	(31.9%)	(31.2%)	(73.5%)	(6.9%-11.2%)	(38.5%)
	Above 60s	(6.5%)	(3.7%)	(8.2%)	(8.9%-10.3%)	(8.2%)
Gender	Male	(23.8%)	(26.7%)	(14.6%)	(7.3%-9.82%)	(32.5%)
	Female	(32.4%)	(14.2%)	(34.9%)	(7.9%-10.6%)	(19.58%)

Table 2: The PROVEAN analysis of 9 variants characterized 3 variants as deleterious and 3 variants as neutral. The SIFT algorithm predicted 4 variants damaging and 3 variants tolerated. A cumulative PROVEAN, and SIFT analyses predicted 3 nsSNPs (16,16205244,G,C, 16,16205343,T,A, and 16,16205415,T,A) as "deleterious/damaging".

Subject ID.	Variants Input	Server Used	Codon Change	Position	Wild-type Residue	Substituted	Type	PROVEAN Prediction		SIFT Prediction		Frequency
								Score	Prediction (Cutoff=-2.5)	Score	Prediction (Cutoff=0.05)	
33	16,16205244, G,C	PROVEAN	TCC [G/C]TG TAC	962 (3020)	V	L	non-synonymous	-2.65	Deleterious	0.004	Damaging	1%
65, 8, 33	16,16205293, G,T		CTC A[G/T]C ATC	978 (3069)	S	I	non-synonymous	-1.14	Neutral	1	Tolerated	3%
33	16,16205343, T,A		TAT [T/A]GG CTC	995 (3119)	W	R	non-synonymous	-13.38	Deleterious	0	Damaging	1%
33	16,16205390, G,T		CAG GA[G/T] CAC	1010 (3166)	E	D	non-synonymous	-0.62	Neutral	0.567	Tolerated	1%
33	16,16205415, T,A		GTC [T/A]AT GGA	1019 (3191)	Y	N	non-synonymous	-8.53	Deleterious	0	Damaging	1%
25	16,16205346, C,A		TGG [C/A]TC	996	L	I	non-synonymous	-1.78	Neutral	0.031	Damaging	1%

			AGC									
34	chr16:16205307:C/A	SNP-Nexus	C/A	981	L	I	non-synonymous			0.837	Tolerated	1%
55	chr16:16205386:A/G		A/G	1009	Q	R	Non-Synonymous					1%
25	chr16:16205215:T/G		T/G	NA			Intronic					1%

Table 3: LDpop analysis showing r^2 and D values.

Positon ID	dbSNP	R2	D'	Chi-sq	p-value
Chr16:16111304	rs4238623	0.001	1	5.1595	0.0231
Chr16:16111461	rs45607431	0	1	0.006	0.9382
Chr16:16111521	rs200753907	0.0001	1	0.3797	0.5378
Chr16:16111592	rs3851716	0.0529	0.9733	264.6961	0.0001
Chr16:16111644	rs3887893	0.0529	0.9733	264.6961	0.0001

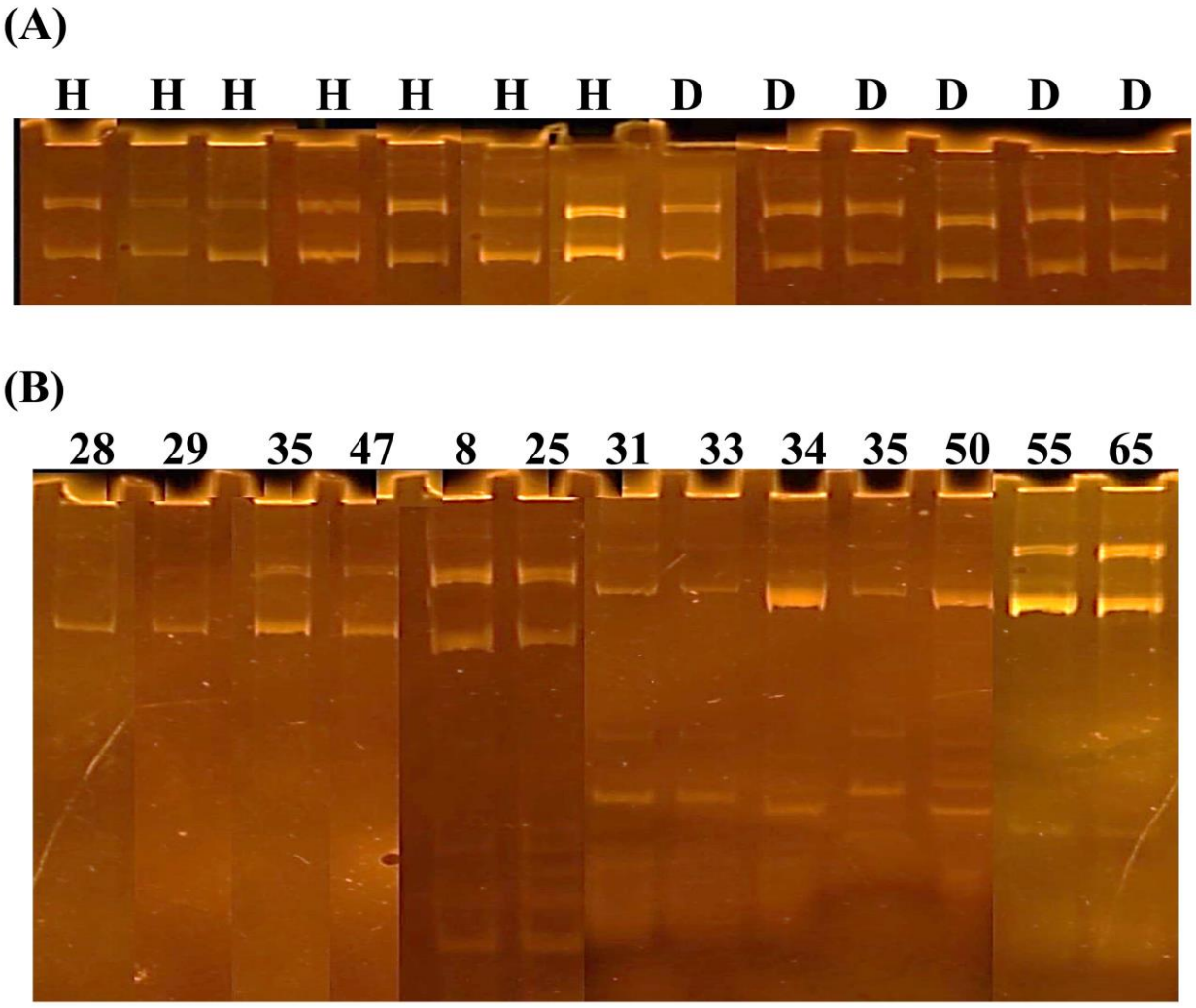

Figure 1. Single nucleotide polymorphism analysis of ABCC1 Exon 22 on 30% PAGE.

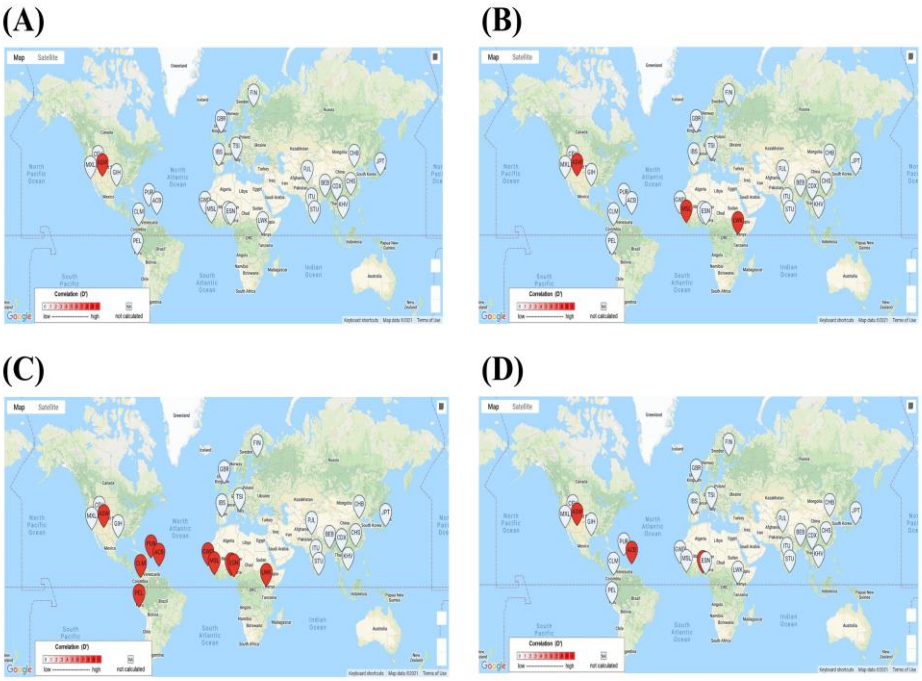

Figure 2. LD pair analysis for a set of available variants that cover intronic/exonic 22 region.

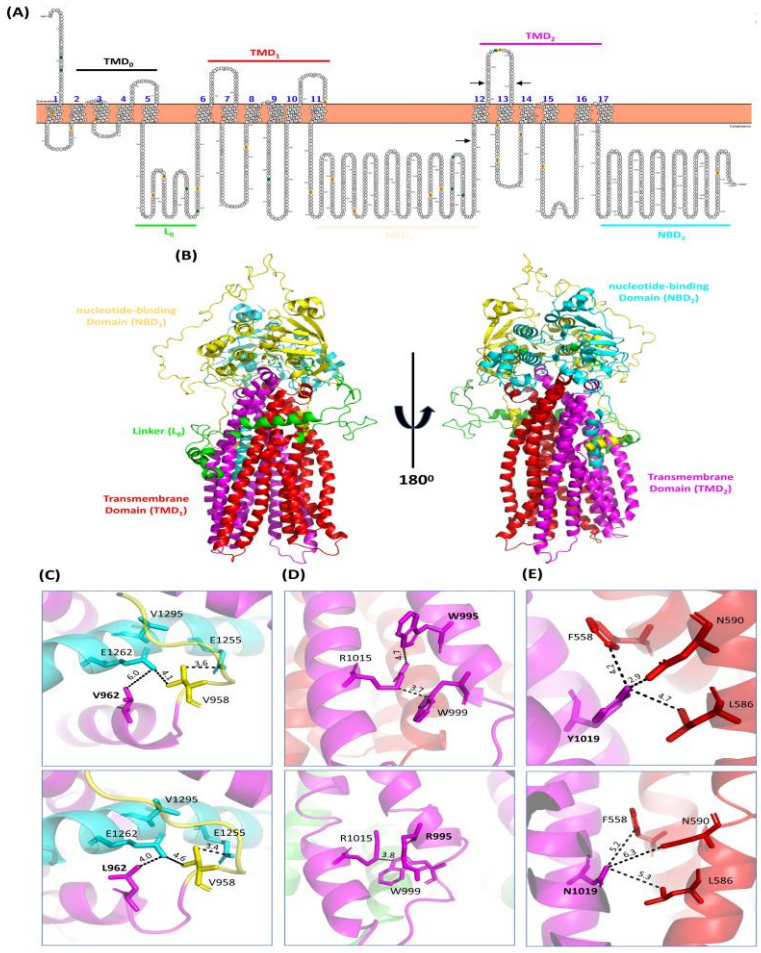

Figure 3. Membrane topology model of ABCC1.

Graphical abstract