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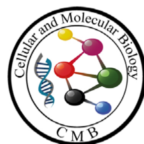

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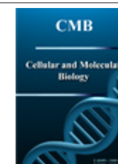

Original Article

Variants of the *ABCG2* gene in Mexican mestizo patients with prostate cancer

Gabriela Monserrat Mimendi-Aguilar[1], Michael Dean[2], Silvia Esperanza Flores-Martínez[3], Alejandra Guadalupe García-Zapién[4], José Sánchez-Corona[3], María Fernanda Romero-Morán[5], Martha Arisbeth Villanueva-Pérez[6], Erick Sierra-Díaz[7], Salvador Sánchez-Benavides[8], Ingrid Patricia Dávalos-Rodríguez[1], Jesús Alejandro Juárez-Osuna[1], Mónica Alejandra Rosales-Reynoso[3], María Cristina Morán-Moguel[9*]

[1] *Doctorado en Genética Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México*
[2] *Laboratory of translational genomics, National Cancer Institute, Gaithesburg, Maryland, United States of America*
[3] *División de Medicina Molecular, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México*
[4] *Departamento de Farmacobiología, Centro Universitario de Ciencias Exactas e Ingenierías, Universidad de Guadalajara, Guadalajara, Jalisco, México*
[5] *Hospital de Especialidades, UMAE, Instituto Mexicano del Seguro Social, Torreón, Coahuila, México*
[6] *Patología y Nefropatología, Centro de Diagnóstico e Investigación, Guadalajara, Jalisco, México*
[7] *División de Epidemiología, UMAE Hospital de Especialidades Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México*
[8] *Hospital General Regional 45, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México*
[9] *Departamento de Biología Molecular y Genómica y Departamento de Disciplinas Filosófico Metodológicas e Instrumentales. Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México*

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Abstract

ABCG2 transporter protein is one of several markers of prostate cancer stem cells (PCSCs). Gene variants of *ABCG2* could affect protein expression, function, or both. The aim of this study was to identify the genetic variability of the *ABCG2* gene in Mexican patients with prostate cancer. Genomic DNA (gDNA) was obtained from peripheral blood samples of 32 Mexican patients with prostate cancer. *ABCG2* gene was sequenced. The electropherograms were analyzed using mutation surveyor DNA mutation analysis software (Softgenetics). The *ABCG2* gene sequence revealed the presence of 22 variants: 19 previously described and three previously undescribed gene variants as part of the *ABCG2* gene variability in the Mexican mestizo population (*R263K G>A, R378K G>A,* and *Q531Q G>A*). No *ABCG2* variant was identified in one patient, but 1 to 12 variants were identified in the remaining 31 patients. The transition *G>A* was the most frequently found substitution. The largest number of *ABCG2* variants was located in exon 9, and at least one of them was present in 28 of the 31 subjects in the Mexican population. The individual genetic variability of *ABCG2* should be analyzed, considering its possible usefulness in personalized medicine in patients with prostate cancer.

Keywords: *ABCG2*, prostate cancer, gene variability, Mexican patients

1. Introduction

Prostate cancer (PCa) is the second most common cancer diagnosed in men and the fifth cause of cancer-related death in men, with 1,414,259 new cases (14.1% of all cancers) reported worldwide each year [1]. The International Agency for Research on Cancer reported that PCa had incidence and mortality rates of 30.7 and 7.7 per 100,000 inhabitants in all age groups worldwide in 2022, 151. 3 and 38.2 per 100,000 inhabitants in men older than 50 years, and 26,742 (13.7%) and 7,457 per 100,000 inhabitants (8.3%) in Mexico in 2020, respectively [2,3].

In cancer cells, overexpression of several ATP-binding cassette (ABC) transporters, which are related to increased effluxes of chemotherapeutic drugs and the development of multidrug resistance, has been observed to be driven by ABC transporters proteins, mostly ABCG2, a protein responsible for conferring the multidrug resistance phenotype [4]. The unfavorable prognosis of patients treated

* Corresponding author.
E-mail address: cristina.moran@academicos.udg.mx (M. Cristina Morán-Moguel).
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with chemotherapy has been related to the *ABCG2* expression in tumor tissue, as some substrates of this transporter are anticancer drugs, including mitoxantrone, doxorubicin, the active metabolite of irinotecan, topotecan, and methotrexate [5].

Tumorigenesis is caused by the dysregulation of key signaling pathways in cancer stem cells (CSCs), which alter the balance of numerous cellular biological processes that include growth, proliferation, and apoptosis [6,7]. CSCs have been identified in most types of liquid and solid cancers, including acute myeloid leukemia, breast, colorectal, glioblastoma, and PCa and are proposed to play roles in tumor growth onset, expansion, and metastasis; in the development of drug-resistant tumors and in cancer recurrence after chemoradiotherapy [8-11]. Moreover, PCa stem cells (PCSCs) are critical in PCa onset and progression. The characteristics of prostate-cancer-stem cells (PCSCs) are not fully understood; however, high *ABCG2* expression levels are found in this cell type, which is thus considered a marker of PCSCs [12-14].

The *ABCG2* gene is located on chromosome 4q22.1 and spans over 141 kilobases (kb), comprising 16 exons and 15 introns. The gene encodes for the ABCG2 protein, which is also called BCRP (breast cancer resistance protein) of 72 kDa. ABCG2 is an N-linked glycoprotein consisting of a polypeptide chain of 655 amino acids. ABCG2 is apically localized in the cell membrane of multiple organs, including the liver, small intestine, kidney, brain, placenta, and prostate, where the *ABCG2* expression has been identified in both the epithelium and endothelium [15-18].

Previous studies have described more than 1,740 *ABCG2* gene mutations [19,20]. The most common genetic polymorphisms in *ABCG2* are single nucleotide polymorphisms (SNPs), to which 90% of the interindividual genetic variability of this gene is attributed. In addition, the gene variability of *ABCG2* is responsible for changes in the expression and function of this transporter, resulting in proteins with functional differences that could affect therapeutic efficacy or induce adverse drug reactions [16].

In patients with PCa of different ethnic groups worldwide at least 180 *ABCG2* gene variants have been described, including some synonymous or missense polymorphisms, and rare variants that lead to nonsense mutations [21,22]. The frequencies of the main *ABCG2* variants have been documented to differ between population groups [16]. Thus, this study aimed to identify the genetic variability of *ABCG2* gene in Mexican mestizo patients with PCa.

2. Material and Methods

A prospective study was conducted at the Molecular Medicine Division of the Centro de Investigación Biomédica de Occidente del Instituto Mexicano del Seguro Social (IMSS), Guadalajara, Jalisco, México. The present study was approved by the IMSS Research Ethics Committee (R-2012-785-105), and the men who consented to voluntary participation signed an informed consent form. The inclusion criteria were as follows: Mexican mestizo men aged ≥18 years who had a confirmed clinical and histological diagnosis of PCa according to the ESMO Clinical Practice Guidelines for diagnosis, treatment, and follow-up [23,24]; no family history of cancer; and without received neither radiotherapy nor chemotherapy treatment before prostate biopsy and/or radical prostatectomy. Information about the sociodemographic, clinical, and pathological features of the patients was obtained from their hospital records, including the tumor staging and grading performed according to the Gleason score.

2.1. DNA collection and sequencing

A total of 5 mL of blood sample was collected by venipuncture in an EDTA tube (BD Diagnostics, Franklin Lakes, NJ). Genomic DNA was isolated using the modified salting-out method [10]. Amplifications were performed using primers previously described complementary to intron sequences up-stream and down-stream of individual 16 exons from the *ABCG2* gene (Table 1) under standard conditions for polymerase chain reaction (PCR) [19]. Briefly, PCR reactions were performed separately in a total of 25 µL containing 12.5 µL of FastStartTM mix (Roche), 5 pmol of each primer (forward and reverse) and

Table 1. Forward and reverse primers used in sequencing.

	Forward primer 5´- 3´	Reverse primer 5´- 3´	Base pair
Exon 1	TgCCCACTCAAAAggTTC	CCAACCCACACTTAACACAC	539
Exon 2	TgTCACCTAgTgTTTgCAATC	gCCAgTTTCTTggAAATAgCC	373
Exon 3	AATCCTgCTTTggTCTCC	TCTCCCATTCTTTTTCCTC	546
Exon 4	AgCATgTgTTggAgggAAAA	ATCAgCCAAAgCACTTAC	266
Exon 5	gCAggCTTTgCAgACATCTA	TgCTgATCATgATgCTTTCA	367
Exon 6	TCTTACAggACTggCACACg	CCCCAAgAATATCTgggACA	312
Exon 7	TCAggCTgAACTAgAgCAAACA	CAAACAgCACTCCTgCAgAC	344
Exon 8	CATgggAAgAAgAgAgAAAg	gTTgACTggTATCAgAAgAC	372
Exon 9	ACTCCTgACCTCgTAATCC	gAAgCAgATgATAACAgAACC	518
Exon 10	TCTAATTgAAACTCTTCCCC	AgTTCgAAgCCAgTCTAgC	479
Exon 11	TgAgTTgACTgCggTgATTT	gTAATCCTCCggATCCCATC	235
Exon 12	gTCTAgCCCTgAggATgTgg	TgCAAAATggACAggTgTTT	399
Exon 13	CAgACACAACATTggAgAC	TAAgggCAAAgAggAAAg	499
Exon 14	CTgCATgAAATTACTCAAgC	CCATCCTCTCATTTACTTC	451
Exon 15	AAACTgTTTACCTTgCCC	gCACCTCACTTCAATCTC	454
Exon 16	gAgTAACATTTgACggATg	CTCTACTCTACCCACAgTTC	826

50 ng of genomic DNA. An initial denaturation of 2 min at 94 ℃, followed by 30 cycles of amplification with denaturation at 94℃ for 15 s, annealing at 58℃ for 30 s and extension at 72℃ for 45s (7 min on the final cycle) were carried out for each of the 16 PCR reactions. The PCR-amplified products were analyzed using electrophoresis on 1.5% agarose gel for 45 min at 100 V.

10 µL de each PCR-amplified fragments were purified with AMPURE XP plates at the Biomek FXP liquid handling automation workstation (Beckman Coulter, Brea, CA) in accordance with the manufacturer instructions. Sequences of purified PCR fragments were obtained by automated DNA sequencing on the 3730 DNA Analyzer (Life Technologies, Carlsbad, CA).

The electropherograms were analyzed for *ABCG2* gene variants using the Mutation Surveyor software (Softgenetics, Pittsburgh, PA).

2.2. Statistical Analysis

Genotype and allele frequencies in Mexican mestizo patients were analyzed by simply counting and comparing them with those described for other populations. Differences were analyzed using χ^2 tests.

3. Results

3.1. Demographic and Clinical Characteristics

Thirty-two Mexican mestizo men with PCa were recruited. Table 2 shows their clinical and pathological characteristics of patients included in the study. Their ages ranged from 50 to 81 years. The prostate-specific antigen (PSA) concentrations ranged from 1.22 to 27 ng/mL (normal concentration, 4 ng/mL) at the time of diagnosis and from 0.01 to 11.2 ng/mL after surgery or treatment. The most prevalent Gleason score was 7 (3+4), and the time (years) from diagnosis to inclusion in the study ranged from 1 to 15 years.

3.2. *ABCG2* variability

We sequenced DNA from the blood samples of 32 Mexican patients with PCa to describe the *ABCG2* gene variability. Sequence analysis allowed for the identification of 22 variants: 15 in the coding regions and seven in the introns. Of the 22 variants, 19 were previously described, and three new *ABCG2* gene variants were identified: *R263K G>A*, *R378K G>A*, and *Q531Q G>A* (Table 3). Figure 1 shows the electropherograms of each of the three new variants identified.

No *ABCG2* polymorphism was identified in one patient, whereas 1 to 12 variants were identified in each of the 31 remaining patients.

Figure 2 shows the number of chromosomes in which the different variants were found and their location along the *ABCG2* gene. In this study included only SNPs, all of which corresponded to base substitutions, both transitions and transversions and were located along the entire gene. Figure 3 shows the ABCG2 transporter structure and amino acids in which the variants were identified in the Mexican population in this study.

3.3. Allelic frequencies

Allelic frequencies described in other populations were compared with some variants found in the Mexican mestizo population in this study (Table 4).

Table 2. Clinicopathological characteristics of the patients with PCa who were included in the study.

Clinical Data	N=32	%
Age (years)		
< 60	4	12.5
> 60	28	87.5
Years since diagnosis		
< 5	25	78
> 5	7	22
PSA at diagnostic (ng/ml)		
<4	5	15.6
>4	27	84.4
PSA after surgery (ng/ml)		
<4	3	9
>4	19	59
ND	10	32
Gleason Score		
6(3+3)	8	25
7(3+4)	9	28
7(4+3)	5	16
8(3+5)	1	3
8(4+4)	2	6
8(5+3)	1	3
9(4+5)	1	3
ND	5	16
Surgery	21	66
Biochemical relapse	12	37.5
Metastasis	2	6
HBP	13	41
DM2	4	12.5
Tobacco consumption	10	31
Alcohol consumption	14	44
Pharmacotherapy		
Goserelin	1	3
Bicalutamide	4	12.5
Goserelin+Bicalutamide	10	31

PSA: Prostate-specific antigen, **ND:** Not determined. **HBP:** High blood pressure, **DM2:** Type 2 *Diabetes Mellitus*

4. Discussion

The stem cell model postulates a hierarchical organization of the tumor in which only a small portion of the cells are responsible for promoting the heterogeneity of prostate tumor cells. It also provides a plausible explanation for therapy resistance and biochemical relapse after initial tumor regression. PCSCs are essential in the onset and progression of PCa, and high *ABCG2* expression level is considered a marker of this cell type [37]. *ABCG2* variability is responsible for changes in the expression or function of this transporter, resulting in proteins with functional differences that can affect therapeutic efficacy or induce adverse drug reactions [16].

In different populations, at least 180 *ABCG2* variants have been identified in patients with PCa. Among the de-

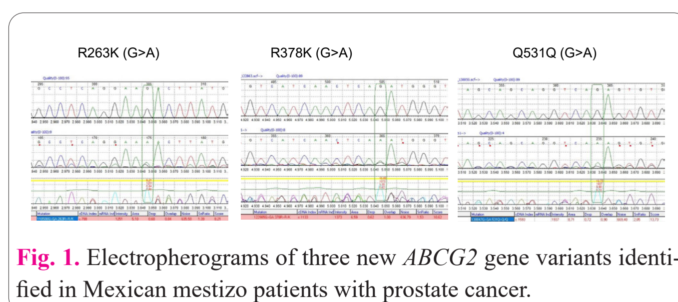

Fig. 1. Electropherograms of three new *ABCG2* gene variants identified in Mexican mestizo patients with prostate cancer.

Table 3. Allelic frequency of variants in *ABCG2* gene found in 32 Mexican mestizo patients with prostate cancer.

Variant or position (nt)	Exon/Intron*	Nucleotide change	rs or mutation ID	Allelic Frequency
78,089	1*	A>G	rs2622605	0.50
V12M	2	G>A	rs2231137	0.09
103,757	3*	A>G	rs2231138	0.11
Q141K	5	C>A	rs2231142	0.09
I206L	6	A>C	rs12721643	0.08
F208F	6	C>T	rs1723993381	0.09
R236Q	7	G>A	rs146672001	0.11
R263K[+]	7	G>A	-	0.11
K357K	9	G>A	rs745374450	0.12
K358K	9	G>A	rs550181756	0.17
K359K	9	G>A	rs745374450	0.17
K360K	9	G>A	COSM3606903	0.27
E366E	9	G>A	rs35622453	0.09
R378K[+]	9	G>A	-	0.03
135,113	11*	C>T	rs2231153	0.69
137,048	12*	C>A,G	rs2231156	0.08
A528T	13	G>A	rs45605536	0.03
Q531Q[+]	13	G>A	-	0.08
138,910	13*	C>T	rs2231162	0.33
140,693	14*	C>T	rs2231164	0.34
N590Y	15	A>T	rs34264773	0.03
141,856	15*	C>T	rs2231165	0.14

[+]Variants not previously described. Nucleotide changes and rs's were verified with dbSNP-NCBI [25].

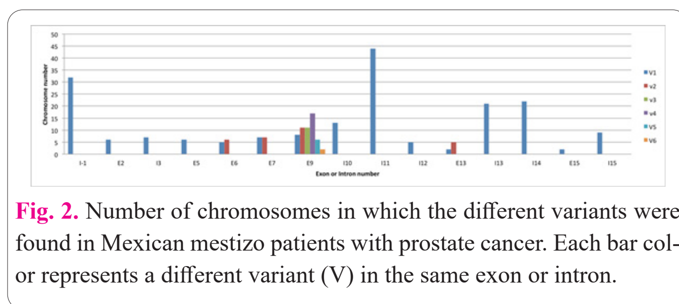

Fig. 2. Number of chromosomes in which the different variants were found in Mexican mestizo patients with prostate cancer. Each bar color represents a different variant (V) in the same exon or intron.

scribed polymorphisms, 99 are located in introns, 47 in promoter regions, and 5 in the 3′UTR, and 29 are located in exons, including 19 non-synonymous variants. In total, 144 SNPs have a frequency >1%, while 36 SNPs show an allele frequency ranging from 0.1% to 0.8% [25,37].

In the Mexican mestizo population in the present study, germline variants were identified by sequencing the 16 exons and the 5′ and 3′ noncoding regions of the *ABCG2* gene. Among the 22 variants, we found five of the 10 most common SNPs in the global population [16]: *135,113*, *78,089*, *140,693*, *138,910*, and *K360K*. Most of the 10 SNPs corresponded to variants whose biological significance is currently uncertain [25]. In patients with PCa, up to 30% of the variants found through germline genetic testing corresponded to variants of unknown significance, which are mostly reclassified as benign and do not change clinical recommendations for carrier patients [38].

The association of *ABCG2* polymorphisms with the risk and susceptibility to different types of cancer has been controversial. In androgen-independent PCa, no significant differences were found in the prevalence of *rs2231142* SNP variants [39]. However, in another study, the time to PCa recurrence after prostatectomy was shorter in the subjects who carried the A allele of this polymorphism [40].

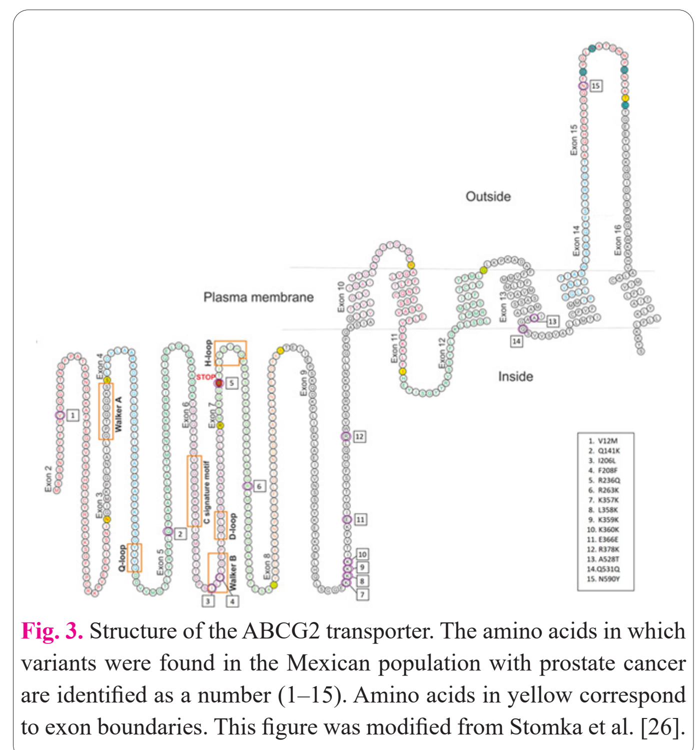

Fig. 3. Structure of the ABCG2 transporter. The amino acids in which variants were found in the Mexican population with prostate cancer are identified as a number (1–15). Amino acids in yellow correspond to exon boundaries. This figure was modified from Stomka et al. [26].

Studies have identified that in cellular models, the minor allele of the variants *rs2231137 G34A* (*V12M*) and *rs2231142 C421A* (*Q141K*) can cause reduced *ABCG2* expression levels and decreased transport of ABCG2 substrates due to the reduced stability of the protein in the endoplasmic reticulum and susceptibility to degradation by ubiquitination. Both amino acid substitutions occur in the nucleotide-binding domain (NBD), which has been

Table 4. *ABCG2* gene allelic frequencies described in prostate cancer patients from other populations and the patients included in this study. Only *p* values with statistical significance are shown.

	Population Frequency					
ABCG2 gene variant	**Mexican mestizo**	**Caucasian**	**Asian**	**African**	**Others**	**References**
***78 089**	0.5	0.5	-	0.5	0.57	[26, 27]
V12M	0.09	0.05-0.10	0.15-0.18	0.04	0.12	[19, 29, 30]
***103 757**	0.11	0.04-0.10	0.07-0.10	-	0.06	[27, 28, 32]
Q141K	0.09	0.11-0.14	0.27-0.35 x^2=4.39 p=0.036	0.05	0.06	[19, 29-31]
I206L	0.08	0.1	-	-	-	[31]
E366E	0.09	-	0.01 x^2=4.48 p=0.034	-	0.02	[32-34]
***135 113**	0.69	0.05-0.18 x^2=27.1 P=0.000	0.16-0.24 x^2=17.2 P=0.000	-	0.22 x^2=15.3 P=0.000	[19, 27, 30,35]
***137 048**	0.08	0.1	0.28-0.33 x^2= 4.52 P=0.003	-	0.13	[29, 32, 36]
***138 910**	0.33	0.04-0.13 x^2=9.95 P=0.000	0.16-0.23	-	0.18-0.22 x^2=19.8 P=0.000	[28, 30, 32, 35]
N590Y	0.03	0.01	-	-	-	[31]
***141 856**	0.14	-	0.12-0.20	-	0.07	[27, 28, 34]

*Variant in Intron.

shown to reduce the expression and function of *ABCG2*, as well as its ability to transport its substrates [16,41]. In the case of *Q141K* (*rs2231142*), the variant allele is known to decrease protein expression levels in in-vitro experiments and alter sensitivity to the topoisomerase I inhibitor metabolite, irinotecan, antineoplastic agent SN 38, mitoxantrone, and topotecan, compared with the wild variant [41]. Substitution of valine for methionine (both nonpolar essential amino acids) at position 12 of the protein (*V12M*) resulted in altered localization of ABCG2 to the apical plasma membrane [41].

The synonymous variants *K357K* and *K359K* are located in a region rich in lysine (K) residues encoded in exon 9, where silent polymorphisms have been previously reported in this location in dbSNP and Cosmic [20,42,43]. The epigenetic effect, if any, of these modifications has not been described, so the possible biological significance of these variants must be analyzed in experiments using protein expression analysis.

In the Mexican patients with PCa, we identified three new variants in the *ABCG2* coding region. Among the nonsense variants, *R263K* is located in exon 7, and *R378K* is located in exon 9, near the first transmembrane domain, and both were located in the intracellular regions of the transporter. The synonymous variant *Q531Q*, is located in exon 13. In this case, the biological meaning may be related only in terms of the availability of the tRNA at the time of translation, as in the genetic code, only two codons for glutamine are involved (Q: CAG, CAA).

In relation to the variants found in the noncoding regions (introns), studies that used the genome-wide association study and other methods to analyze the association of SNPs with various human diseases have allowed the identification of not only coding variants but also a large number of variants located in noncoding regions associated with various traits and diseases, which may mean that these are regulatory regions of genetic expression without being adequately encoded [44]. Therefore, understanding the functional or biological meaning of mutations in this type of region is more complex and could apply to the variants found in introns of *ABCG2*, which makes a greater number of studies necessary to delve into the bioinformatic analysis of nearby sequences in this position and to identify possible regulatory sites or the expression analysis of these variants.

In our study, we found up to 12 variants in the same individual, which could have an additive effect on the interindividual differences in *ABCG2* expression that could alter the pharmacokinetic properties of various substrates or drugs and cause variations in treatment response or adverse effects [16,26]. Thus, a haplotype analysis in a larger number of patients could be appropriate.

The analysis of the mutational spectrum of the *ABCG2* gene allowed us to define the types of mutations present in the Mexican population with PCa. Eight non-synonymous and seven synonymous variants were identified. The replacement that occurred in most variants identified in the Mexican patients was a transition (87%), with *G>A* being the most common followed by *C>T*. Three transversions with equal frequency in the population (33.3%) made up 13% of the variants found.

A limitation of this study is the small sample size, which included only patients with PCa. It is possible that some variants may also be found in Mexican subjects without PCa, which was not determined in this study since a control group of individuals without prostate cancer was

not included. Nevertheless, our results allow us to suggest the inclusion of the *ABCG2* gene in the germline genetic analysis of patients with PCa and to continue to investigate the importance of rare variants and analyze the implications that a single individual can simultaneously carry different *ABCG2* variants.

5. Conclusion

This is the first study in the Mexican population in which gene variability among patients with PCa was analyzed. The *ABCG2* gene variability found in the Mexican patients is similar to that described in other populations. Transversions were the most frequently encountered variants, and most *ABCG2* variants were located in exon 9.

The possible biological significance of the previous and new *ABCG2* variants should be analyzed in experiments using bioinformatic and protein expression analysis, as the literature provides limited information on the topic, which is mainly about the significance of SNPs in introns, untranslated regions, or the *ABCG2* gene promoter. Thus, more research is required in this regard, as this information may be required to establish personalized medicine for carriers of these variants.

Conflict of interest

The authors declare no conflicts of interest.

Consent for publications

All authors have read and approved the final manuscript for publication.

Ethics approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and approved by the Investigation and Ethics Committee of Instituto Mexicano del Seguro Social (Project Registration No. R-2012-785-105).

Informed Consent

Informed consent was obtained from all subjects before they participated in the study.

Availability of data and material

Data generated or analyzed during this study are provided in full within the published article.

Authors' contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by [Gabriela Monserrat Mimendi-Aguilar,], [Silvia Esperanza Flores-Martínez], [Alejandra Guadalupe García-Zapién], [José Sánchez-Corona], [María Fernanda Romero-Morán], [Martha Arisbeth Villanueva-Pérez], [Erick Sierra-Díaz], [Salvador Sánchez-Benavides], [Ingrid Patricia Dávalos-Rodríguez], [Jesús Alejandro Juárez-Osuna], [Mónica Alejandra Rosales-Reynoso] and [María Cristina Morán-Moguel]. The first draft of the manuscript was written by [Gabriela Monserrat Mimendi-Aguilar] and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Abbreviation

ABC ATP-Binding Cassette
BCRP Breast Cancer Resistance Protein
CSCs Cancer Stem Cells
gDNA Genomic DNA
IMSS Instituto Mexicano del Seguro Social
NBD Nucleotide-Binding Domain
PCa Prostate Cancer
PCR Polymerase Chain Reaction
PCSCs Prostate cancer Stem Cells
PSA Prostate-Specific Antigen
SNPs Single Nucleotide Polymorphisms

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