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Germline mutations of B-Raf proto-oncogene and pathological implications in prostate cancer: observational study

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Background: B-Raf proto-oncogene has been found in a variety of neoplasms. BRAF stimulation can promote tumour proliferation through the activation of the MAP/ERK kinase pathway. This study aimed to determine the germline spectra of BRAF and the association with pathological criteria of prostate tumours.

Methods: Fifty blood samples from men treated with prostate cancer were analyzed for BRAF germline mutations and confirmed by Sanger sequencing, in addition, to establishing the frequencies and clinical correlations of frequent mutations in the BRAF gene for both exon 11 and exon 15. The frequency and distribution of high-frequency mutations were analyzed according to the pathological criteria of the patients.

Results: Frameshift mutations: c.1628_1629insA and c.1624_1625insT with a frequency of (46%) and (18%), respectively, Nonsense mutations: c.1181C > A (p.Ser394Ter) was detected in one patient, missense mutations: c.1226A > G (p.Gln409Arg), c.1270T > C (p.Trp424Arg), c.1270_1271delins2 (p.Trp424Leu), with a frequency of (4%) were detected. There was no significant difference between mutation carriers and non-carriers regarding medical and surgical history, but prostate-specific antigen concentration was significantly different between the two groups.

Conclusion: The results of this study elucidate the presence and involvement of germline mutations in prostate cancer, which could serve as a potential indicator for the diagnosis and therapeutic management of prostate cancer in the population studied.

Keywords: BRAF, clinical variant, frequency rate, mutations, Prostate-specific antigen

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HIGHLIGHTS

- Nonsense mutations: c.1181C > A (p.Ser394Ter) was detected in one patient in the population.
- B-Raf germline mutations in prostate cancer, could serve as a potential indicator for the diagnosis and therapeutic management of prostate cancer.
- Prostate-specific antigen concentration was significantly different between the mutation carriers and non-carriers.

Introduction

Prostate cancer is the second most common cancer in the world and the fifth leading cause of death and It is the first cause of death in 2018[1]. In parallel, the frequency of prostate cancer is very variable from one country to another and from one ethnic group to another. In Africa, a closer look reveals that, despite the global situation, prostate cancer has the highest incidence rate, with an estimated 80 971 new cases in 2018, corresponding to 18.1% of whole cancers[2]. It also has the highest death toll on the continent with 42 298 estimated death cases in 2018[3]. In Moroccan population, which presents a profile typical of North African populations, prostate cancer is in principle considered the second most common cancer in men with 3990 new cases in 2018[4,5] Alteration of the BRAF gene in prostate cancer has never been

studied in this population. Among the main risk factors, older age, ethnicity, and family history of prostate cancer are individual risk factors for prostate cancer. Weight, obesity, and height are also risk factors and also genetic factors play a major role in the development of prostate cancer[6]. RAF family proteins are evolutionarily conserved serine/threonine kinases that regulate fundamental cellular processes including growth, differentiation, and survival. The RAF family consists of three members: ARAF, BRAF, and CRAF[7]. The different RAF proteins are all activated by the class A Scavenger Receptor (SRA) and then activate the MEK, thereby triggering the signalling cascade of the MAPK pathway[8]. Constitutive activation of the MAPK pathway caused by oncogenic mutations of RAF genes leads to abnormal proliferation and differentiation, because oestrogen plays a physiological role in prostate development by programming stromal cells and directing early morphogenetic events. However, abnormally high oestrogen exposure at critical periods in the developmental process can lead to permanent changes in prostate branching morphogenesis and cell differentiation, a phenomenon known as impregnation or neonatal impregnation. Developmental oestrogenisation disorders of this type are associated with an increased incidence of prostate damage with age, including hyperplasia, inflammation and dysplasia[8]. The BRAF gene, among the three forms of RAF genes, is most commonly mutated in human cancers[6,9]. According to several studies, no BRAF mutation was detected in white patients[9,10]. A recent study indicates a BRAF mutation rate of 10.2% for cancer of the prostate in Korean patients[11]. The present study aims to investigate the prevalence of different mutations contributing to the development of prostate cancer in Moroccan men and the correlations of these mutations with the characteristics of prostate tumours.

Table 1

Clinical and pathological characteristics of fifty patients treated with prostate cancer engaged in this study

Tumour features	*n* = 50 (%)
Pathological Gleason score	
= 6	16 (32)
> 6	34 (68)
PSA ng / ml	
< 10	11 (22)
≥ 10 < 20	3 (6)
≥ 20	39 (78)
Age at diagnosis	
< 60	15 (30)
≥ 60	35 (70)
Medical background	
Yes	25 (50)
Nope	25 (50)
Surgical history	
Yes	10 (20)
No	40 (80)
Smoking	
Yes	36 (72)
No	9 (18)
Weaned	5 (10)
Alcohol	
Yes	30 (60)
No	10 (20)
Weaned	10 (20)
BMI	
< 20	2 (4)
≥ 20 < 25	30 (60)
≥ 25	18 (36)
Pathological T-stage	
T1	30 (60)
T2 x	2 (4)
T3 x	2 (4)
T4	16 (32)

PSA, prostate-specific antigen.

Materials and methods

Prostate blood samples

A total of 50 blood samples were taken from 50 men being evaluated at the Mohammed V Hospital in the city of Rabat between June 2021 and February 2022. Ethical standards were followed before and during collection, including the patient consent form signed by patients and researchers, and other oral agreements. The necessary ethical validation was carried out by the Moroccan Ethics Committee for Biomedical Research (n°3-/2018/April 30/2018). Histological data had already been collected to identify them as prostate adenomas. The samples were collected according to standard procedures and directly by the clinicians. Medical records including clinical and pathological criteria were recorded for every sample. The clinical and pathological criteria: Age, Gleason score, and Prostate-specific antigen (PSA) concentration of prostate tumours are described in (Table 1). The DNA processing process and genotyping were carried out at the oncology and virology laboratory of the Faculty of Science and Technology of Mohammedia, Morocco.

DNA extraction and genotyping

DNA extraction was performed using the kit (Invitrogen Genomic DNA Mini Extraction Kit, Thermo Scientific) and the concentration and quality of the extracted DNA were measured using the NanoDrop 2000 spectrophotometer (Thermo Scientific) at 260/280 nm. All measurements were carried out following the recommendations of the suppliers. Samples with DNA concentration greater than or equal to 30–60 ng / µl are considered for polymerase chain reaction (PCR) and stored at – 20 °C until further analysis.

Amplification and sequencing of exons 11 and 15 of BRAF

DNA amplified by PCR using The Master Mix Vazyme Green Taq Mix. All these DNA samples were analysed starting with the β-globin gene using specific primers GH20/PCO4 as previously described in[12]. To check the reliability of the assays, a positive control (BRAF gene DNA) was performed at the time of manipulation. PCR analysis for β-globin was performed using the following steps: initial primary denaturation for 10 min at 94°C, 35 cycles of denaturation at 94°C for 45 s, hybridisation at 54°C for 45 s, extension at 72°C for 1 min. For the amplification of the BRAF gene, the following PCR programme was used: 94°C, 35 cycles of denaturation at 94°C for 45 s, annealing at 54°C for 45 s, and extension at 72°C for 1 min, after a final extension, at 72°C for 10 min. Subsequently, all positive β-globin gene PCR products were subjected to another confirmatory PCR for BRAF. The BRAF gene specific to exons 11 and 15 was detected by

polymerase chain reaction based on the specific primers described in[13,14]. Therefore, a PCR directed to the regulatory regions of the BRAF gene was performed with specific primers: The polymerase chain reaction consisting of 50 µl of total volume. PCR reaction which contains genomic DNA (4 ul), 2× Taq PCR master Kit from Qiagen USA mix (25 ul), 4 µl of sense and 4 ul of antisense primers, 12 ul of distilled water. PCR amplification was performed using a Perkin Elmer 2400 thermal cycler. Purification of PCR products was performed with the ExoSAP -IT Express PCR Product Cleanup system to remove primers and nucleotides not involved in the PCR reaction, while that of sequencing products was performed with a Sephadex 50G column (Pharmacia Biotech Co., Ltd). The purified DNA amplicons were sequenced using the BigDye XTer-minator purification kit. Sequencing was performed by 3130 genetic analysers (Applied Biosystems). All sequences were compared to the NCBI RefSeq sequence (NM_001378473.1) for variant determination using Mutation Surveyor software (SoftGenetics LLC., Stage College, Pennsylvania). Purifications and sequencing were performed at the Centre National de la Recherche Scientifique et Technique (CNRST) in Rabat, Morocco.

Mutations identification and registration

The different sequencing results were processed using the MEGA programme and the Nagahama server[15]. Mutation results were evaluated in comparison with Refseq: (NM_001378473.1). The results include missense, nonsense and frameshift mutations, which were analysed in these results. The novel mutations detected by this study were submitted to the ClinVar—NCBI database and were recorded as clinically relevant variants, type Condition ID: MedGen, Condition ID value: C4722327 (hereditary prostate cancer.1) by accession numbers of BRAF exon 15 (SCV002584866–SCV002584907) and accession numbers of BRAF exon 11 (SCV002586285–SCV002586368). The resulting BRAF DNA sequences were successfully submitted to GenBank under the following accession numbers of exon 15 (OP615680–OP615739) and accession numbers of exon 11 (OP580529-OP580586).

Statistical analysis

Statistics were made using *the jamovi* project (V2.2, 2021) https://www.jamovi.org. A T-test was performed to assess continuous variables and Fisher's exact and χ^2 tests were performed for categorical data. The mutation carriers were analyzed against non-carriers regarding every category of clinical and pathological characteristics. For all analyses, a *P* value less than 0.05 was defined as statistically significant.

Results

The clinicopathologic parameters of all the patients are included in (Table 1). Thirty-four patients (68%) had a pathological Gleason score > 6, which implies a high grade. Sixteen patients have a percentage (32%) of those with a score = 6, which indicates that the cancer is probably growing slowly. Thirty-nine patients (78%) have a high prostate antigen (PSA) level in the blood, three patients (6%) have a PSA of (≥ 10 < 20) and eleven patients (22%) have a PSA < 10. Most patients were 60 years or older (70%), and (30%) of patients were under 60 years old. Twenty-five (50%) of patients presented with a medical history and the same percentage was for patients without a medical history. Forty patients (80%) had a history of surgery, while ten patients (20%) had no history of surgery. Regarding patients who smoke, we have 36 patients (72%) and nine (18%) patients who do not smoke, and 5 (10%) patients who have already started smoking. Thirty men (60%) were patients who regularly drink alcohol and ten (20%) were patients who do not drink alcohol and 10 (20%) were patients who had stopped drinking alcohol. We have thirty patients (60%) with a body mass index (BMI) of (≥ 20 < 25), as well as eighteen patients (36%) with a BMI of (≥ 25), and lastly two patients (2%) with BMI (< 20). Thirty (60%) patients were also classified with a stage T1 tumour, which means the tumour affects half of one of the two sides of the prostate or both sides of the prostate, and two patients (4%) with stage T3 reported that the tumour was located either on one side or both sides of the prostate or within the seminal vesicles, lastly, eight patients (16%) have a tumour stage T4 meaning that cancer has reached structures neighbours other than those of the seminal vesicles.

Out of the 50 samples, 35 (70%) carried one or more BRAF exon 15 mutations and 36 (72%) carried multiple BRAF exon 11 mutations. Mutations were identified according to their location in the genome, type of genotype, frequency indicated, and their impact on amino acids. All mutations were located in exon 11 and exon 15 of the BRAF gene. In exon 15, we found the following mutations: deletion mutation: c.1627_1629delTTT (4%) results in the modification of the protein to p.Phe543del. We also found the following mutations: Frameshift mutations of nucleotide number c.1585_0insT with a frequency of (10%) and four mutations: c.1591_1592insG, c.1592delinsGC, c.1607_1608insA, c.1613_1614insT with a frequency of (4%), as well as the frameshift mutation c.1624. _1625insT with a frequency of (18%), another Frameshift mutation of nucleotide number 1626_1627 at the cysteine with a frequency of 10%, we also found a frameshift mutation of nucleotide number 1628_1629 by an adenine resulting in a stop codon, which modifies the structure of the protein at the level of amino acids. This insertion was detected in 23 of the patients with a frequency of 46%, c.1657_1658insT with a frequency of 28%, c.1660_1661insC (8%), c.1663_1664insG (20%), c.1678_1679insG (8%), and c.1684_1685insG (16%). Concerning missense mutations: were detected in five patients the c.1594C > G mutation (10%) resulted in stop codons at the 532 sequence levels of the BRAF protein respectively which alter the structure of the protein at the p.Leu532Val amino acid, the c.1658 G > T mutation was detected in four patients (8%) and alters the structure of the protein at amino acid p.Ser553Ile, the c.1675G > A mutation was observed in seven patients (14%) and alters the structure of the protein at amino acid Glu559Lys, the c.1685 _1686delins2 mutation was detected in three patients (6%) causing a change in the protein at the p.Ser562Trp amino acid, the c.1697T > G mutation was observed in six patients (12%) involving a change in the protein at the p.Leu566Trp amino acid, the c.1703 _1704delins2 mutation was found in three patients (6%), causing a change in the protein at the p.Met568Arg amino acid, and the c.1703T > G mutation was detected in five patients (10%) with a change in the protein at the p. Met568Arg amino acid. For intronic mutations: c.1586-16A > T was found in three patients (6%), c.1586-45delT in four patients (8%), c.1586-46A > C in 10 patients (20%), c.1586-47 _1586-46delins2 found in three patients (6%), c.1586-7A > G found in 9 patients (18%), and the intronic mutation: c.1704 + 9delT was found in 6 patients (12%) (Table 2).

Table 2

BRAF exon 15 high frequent mutations detected in 50 prostate cancer patients arranged by the effect and according to reference genome database: (GRCh38), and human genome variation society (HGVS) nomenclature

Effect	Genome location	CDS change	Protein change	Frequency, *n* (%)
Deletion	Chr7:140753350	c.1627_1629delTTT	p.Phe543del	2 (4)
Frameshift	Chr7:140753393	c.1585_0insT	NA	5 (10)
	Chr7:140753387	c.1591_1592insG	NA	4 (8)
	Chr7:140753387	c.1592delinsGC	NA	4 (8)
	Chr7:140753371	c.1607_1608insA	NA	4 (8)
	Chr7:140753365	c.1613_1614insT	NA	4 (8)
	Chr7:140753354	c.1624_1625insT	NA	9 (18)
	Chr7:140753352	c.1626_1627insC	NA	5 (10)
	Chr7:140753350	c.1628_1629insA	NA	23 (46)
	Chr7:140753321	c.1657_1658insT	NA	14 (28)
	Chr7:140753318	c.1660_1661insC	NA	4 (8)
	Chr7:140753315	c.1663_1664insG	NA	10 (20)
	Chr7:140753300	c.1678_1679insG	NA	4 (8)
	Chr7:140753294	c.1684_1685insG	NA	8 (16)
Missense	Chr7:140753385	c.1594C > G	p.Leu532Val	5 (10)
	Chr7:140753321	c.1658G > T	p.Ser553Ile	4 (8)
	Chr7:140753304	c.1675G > A	p.Glu559Lys	7 (14)
	Chr7:140753293	c.1685_1686delins2	p.Ser562Trp	3 (6)
	Chr7:140753282	c.1697T > G	p.Leu566Trp	6 (12)
	Chr7:140753275	c.1703_1704delins2	p.Met568Arg	3 (6)
	Chr7:140753276	c.1703T > G	p.Met568Arg	5 (10)
Intronic	Chr7:140753409	c.1586-16A > T	NA	3 (6)
	Chr7:140753438	c.1586-45delT	NA	4 (8)
	Chr7:140753439	c.1586-46A > C	NA	10 (20)
	Chr7:140753439	c.1586-47_1586-46delins2	NA	3 (6)
	Chr7:140753400	c.1586-7A > G	NA	9 (18)
	Chr7:140753266	c.1704 + 9delT	NA	6 (12%)

CDS, coding sequences; NA, not applicable..

BRAF contains three highly conserved domains (CR1, CR2, and CR3). CR1 and CR2 represent regulatory regions at the N-terminus of the protein. CR1 contains the RAS-binding domain, which interacts with RAS, the cysteine-rich domain which binds two zinc ions. CR2 is a serine/threonine rich domain. CR3 has a C-terminal kinase domain regulated by phosphorylation (Fig. 1)[9,16,17]. Here are the mutations detected in the BRAF kinase domain (CR3) of BRAF gene in the study. From Dankner *et al.*, 2018, and UniProtKB/TrEMBL A0A2U3TZI2 (Fig. 1).

For exon 11, we found the following mutations: the most frequently encountered mutations are the Frameshift mutations: c.1195_1196insC, c.1197_1198insC, c.1219_1220inC were detected in two patients (4%), and the Frameshift mutation: c.1228delA was found in three patients (6%). For intronic mutations (NA): c.1276 + 13T > G found in five patients with a rate of 10%, the two mutations: c.1276 + 232A > C, c.1276- + 18delT were observed in four patients with a frequency of 8%, c.1276 + 250_1276 + 249delins2 detected in five patients with a rate of 10%, c.1276 + 34C > T observed in 11 patients with a rate of 22%, c.1276 + 50delT was found in ten patients with a rate of 20%, the mutation c.1276 + 91T > A was also observed with a percentage of 12%. And the following two intronic mutations were been recorded: c.1539-10145 T > C, c.1539-10155 T > G with a rate of 8%, as well as c.1539-10161C > T, c.1539-10168G > T with a frequency of 10%, as well as c.1539-10196A > C with a rate of 16% and c.1539-10204C > T with a frequency of 8%. Regarding missense mutations, we identified two mutations with a percentage of 6%: c.1226A > G, c.1270T > C which changes the nature of the protein structure at the amino acid level: p.Gln409Arg, p.Trp424Arg (**Table 3**). Concerning synonymous mutations, three mutations were identified with a rate of 6%: c.1170T > A, c.1203T > C, c.1224A > C. which alter the nature of the protein structure at the amino acid level: p.Gly390 =, p.Pro401 =, p.Gly408 =.

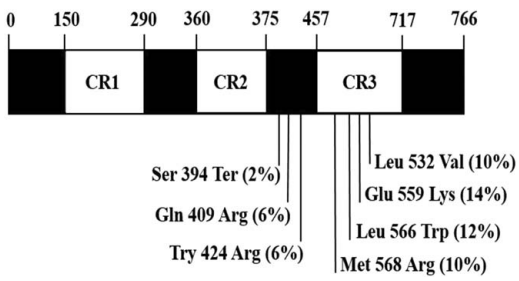

Figure 1. Mutations detected in CR3 (BRAF kinase domain) of the BRAF gene in the study. Based on Dankner *et al.*, 2018, and UniProtKB/TrEMBL A0A2U3TZI2.

Pathological and clinical criteria were compared between carriers and non-carriers of high-frequency BRAF exon 15 mutations in (Table 4). High Gleason scores (> 6) of prostate cancer in carriers were detected in four cases (48%), while in non-carriers, ten cases (20%) [*P* value = 0.722]. In contrast, eleven (22%) cases in carriers of the BRAF mutation were equal to 6, and five cases (10%) in non-carriers. There was no significant difference between carriers and non-carriers for medical and

Table 3

BRAF Exon 11 high frequent mutations detected in 50 prostate cancer patients arranged by the effect and according to reference genome database: (GRCh38), and human genome variation society (HGVS) nomenclature

Effect	Genome location	CDS change	Protein change	Frequency, *n* (%)
Nonsense	Chr7:140781671	c.1181C > A	p.Ser394Ter	1 (2)
Frameshift	Chr7:140781656	c.1195_1196insC	NA	2 (4)
	Chr7:140781654	c.1197_1198insC	NA	2 (4)
	Chr7:140781632	c.1219_1220insC	NA	2 (4)
	Chr7:140781624	c.1228delA	NA	3 (6)
Intronic	Chr7:140781563	c.1276 + 13T > G	NA	5 (10)
	Chr7:140781558	c.1276 + 18delT	NA	4 (8)
	Chr7:140781344	c.1276 + 232A > C	NA	4 (8)
	Chr7:140781326	c.1276 + 250_1276 + 249delins2	NA	5 (10)
	Chr7:140781542	c.1276 + 34C > T	NA	11 (22)
	Chr7:140781526	c.1276 + 50delT	NA	10 (20)
	Chr7:140781485	c.1276 + 91T > A	NA	6 (12)
	Chr7:140764378	c.1539-10145T > C	NA	4 (8)
	Chr7:140764388	c.1539-10155T > G	NA	4 (8)
	Chr7:140764394	c.1539-10161C > T	NA	5 (10)
	Chr7:140764401	c.1539-10168G > T	NA	5 (10)
	Chr7:140764429	c.1539-10196A > C	NA	8 (16)
	Chr7:140764437	c.1539-10204C > T	NA	4 (8)
Missense	Chr7:140781626	c.1226A > G	p.Gln409Arg	3 (6)
	Chr7:140781582	c.1270T > C	p.Trp424Arg	3 (6)
Synonymous	Chr7:140781682	c.1170T > A	p.Gly390 =	3 (6)
	Chr7:140781649	c.1203T > C	p.Pro401 =	3 (6)
	Chr7:140781628	c.1224A > C	p.Gly408 =	3 (6)

CDS, coding sequences; NA, not applicable.

surgical history. The number of cases with elevated PSA levels (PSA > 20 mg/ml) was consistent: three cases (64%) and seven (14%), respectively, [value 0.015]. The majority of carrier mutations were found to be 35 (70%) patients who were diagnosed with prostate cancer at age 60 or older, while no patients were diagnosed at age 60 or younger. While 15 (30%) of the non-carriers were diagnosed at an age below 60 years, [P value = 0.001]. Twenty cases had a medical history of high mutations, while 15 (30%) had no medical history [$P = 0.272$]. Eight cases had a history of surgery with mutations, while 27 (34%) had no history of surgery [$P = 0.296$]. while in carriers, 37 (72%) cases and 8 (16%) cases for smoker patients [value 0.004]. Thirty (60%) cases of men were alcoholics and 28 (36%) were for men who are not carriers of the mutation [$P < 0.001$]. In the cases with a BMI ≥ 25 there are ten (20%) carriers of the mutation and eight (16%) non-carriers with a significant difference of [$P = 0.014$].

Discussion

The good quality of the extracted DNA is strongly linked to the conditions of sample preparation, collection, and storage. Once the DNA extraction phase has been completed, the samples are then subjected to quality and quantity control. To assess the quality of DNA extraction, two methods were used. The first was done by Nanodrop and the second was migration on 1.5% agarose gel. The first method is the Nanodrop assay (nucleic acids absorbed in the UV at a wavelength $\lambda max = 260$ nm). In determining the absorbance value of a nucleic acid solution, therefore the 260/230 ratio is an index of purity and our results are between 2.0 and 2.2 indicating good purity of the nucleic acid. We were able to verify that the PCR process was successful, as the gel results show that the predicted size of the studied exons was obtained, 240 bp for exon 11 and 255 bp for exon 15 compared to a marker of the size of 50 bp. In this study, we exclusively analyzed 50 samples from prostate cancer patients for BRAF mutations. In this population, for the first time for prostate cancer, new mutations have been identified with a very high frequency, never before identified in other populations. Similarly, according to our results, we found the absence of the BRAF V900E germline mutation for prostate cancer in a subgroup of Moroccan men, which also allowed us to determine the most common mutations (Table 4). Indeed, oncogenic mutations of the BRAF gene in prostate cancer tissues have already been indicated[11,18]. Among the studies that have found BRAF mutations in prostate cancer tissue samples, there is one carried out in Korea in 2006, which found 21 cases of BRAF mutations out of 206 cases, which corresponds to a rate of about 10.2%. Among the most frequent mutations detected in codon 600 of the BRAF gene was a GTG→GCG mutation, which corresponds to an amino acid change from valine to alanine, and this mutation was evident in 11 (52.4%) of these 21 mutations. In addition, the second most common mutation is the GTG→ATG (valine to methionine) mutation, which was present in 8 (38.1%) of these 21 mutations. The other 2 mutations were of the GTG→GAG (V600E) type (from valine to glutamic acid)[19]. Other studies show mutations in the BRAF gene but at different frequencies. In China, the study involved 200 samples. Rearrangements of the BRAF gene were demonstrated in five cases (2.5%)[16], Genomic mutations in this gene correlate significantly with high Gleason scores (>7; $P < 0.01$) and generally imply high clinical stage disease. The frequency of copy number increases in the BRAF and RAF1 genes is significant (29 and 15%, respectively). The rate of BRAF copy

Table 4

Correlation between carriers and non-carriers of high frequent mutations of BRAF and the tumour criteria

		BRAF mutation			BRAF mutation		
		Exon 15 ($n=35$)	Exon 15 ($n=15$)		Exon 11 ($n=36$)	Exon 11 ($n=14$)	
Tumour features	**No. Casesn, *n* (%)**	**Carriers, *n* (%)**	**Non-carriers, *n* (%)**	***P***	**Carriers, *n* (%)**	**Non-carriers, *n* (%)**	***P***
Pathological Gleason Score							
=6	16 (32)	11(22)	5 (10)	0.722	12 (24)	4 (8)	0.572
>6	34 (68)	24 (48)	10 (20)		24 (48)	10 (20)	
PSA ng / ml							
<10	8 (16)	2 (4)	6 (12)	0.015	4 (8)	7 (14)	<0.001
≥10 <20	3 (6)	1 (2)	2 (4)		0	3 (6)	
≥20	39 (78)	32 (64)	7 (14)		32 (64)	4 (8)	
Age at diagnosis							
<60	15 (30)	0	15 (30)	0.001	15 (30)	0	0.043
≥60	35 (70)	35 (70)	0		21 (42)	14 (28)	
Medical background							
Yes	25 (50)	20 (40)	5 (10)	0.272	23 (46)	2 (4)	0.037
No	25 (50)	15 (30)	10 (20)		13 (26)	12 (24)	
Surgical history							
Yes	10 (20)	8 (16)	2 (4)	0.296	7 (14)	3 (6)	0.735
No	40 (80)	27 (54)	13 (26)		29 (58)	11 (22)	
Smoking							
Yes	36 (72)	28 (56)	8 (16)	0.004	26 (52)	10 (20)	0.630
No	9 (18)	2 (4)	7 (14)		6 (12)	3 (6)	
Weaned	5 (10)	5 (10)	0		4 (8)	1 (2)	
Alcohol							
Yes	30 (60)	28 (56)	2 (4)	<0.001	26 (52)	4 (8)	0.043
No	10 (20)	2 (4)	8 (16)		4 (8)	6 (12)	
Weaned	10 (20)	5 (10)	5 (10)		6 (12)	4 (8)	
BMI							
<20	2 (4)	0	2 (4)	0.014	0	2 (4)	0.001
≥20 <25	30 (60)	25 (50)	5 (10)		29 (58)	1 (2)	
≥25	18 (36)	10 (20)	8 (16)		7 (14)	11 (22)	
Pathological T-stage							
T1	30 (60)	19 (38)	11 (22)	0.008	22 (44)	8 (16)	0.542
T2 x	2 (4)	0	2 (4)		1 (2)	1 (2)	
T3 x	2 (4)	0	2 (4)		2 (4)	0	
T4	16 (32)	16 (32)	0		11 (22)	5 (10)	

PSA, prostate-specific antigen.

number increase in Chinese cancers was significantly higher than in UK cancers (9.2%) ($P<0.001$) and correlated with a number of clinical parameters[16]. Three studies found BRAF mutations in prostate cancers, and one found no BRAF mutations in the American and German populations. Additionally, 10% of prostate carcinomas have been identified as having an activating BRAF mutation in the Asian population. These differences in the frequency of BRAF mutations have been proven in several different ethnic groups[20]. However, research conducted in 2009 did not find the presence of BRAF mutations in a search of 93 cases of prostate cancer in the United States[21].

The actual effects of these variants on the B-raf proto-oncogene are not fully known and they may alter the kinase function of the protein. These alterations may change the function of the BRAF gene as an oncogene. Further studies and analysis of these frequent mutations are needed before these results can be accepted as valid for other populations. A prostate cancer study with family history data is needed to assess the risk of these variants, as well as a larger study of only cases or individuals with a family history. In addition, a larger study of only cases or individuals with a high risk of prostate cancer would be needed to assess the risk of these variants. The risk of prostate cancer is higher in people with a family history. In addition, data are needed to assess the pathogenicity of the frequent mutations studied in this research.

Conclusions

The main genetic mutations implicated in prostate cancer, and more particularly those of the BRAF gene, have been the subject of discussion for years. The main objectives of this work were to search for the presence of the most frequent BRAF mutations in prostate cancer and to characterize them, in particular the mutations located in exons 11 (240 bp) and 15 (255 bp) in a subset of Moroccan men, this could help explore the hereditary manner of prostate cancer, with consideration that these variants not exclusive for this population and to be confirmed in other populations. Other confirmations to in investigating the pathogenicity of these mutations are needed.

Ethics approval and consent to participate

The study was approved by the Biomedical Research in Morocco code n°3/2018/April 30/2018- Maroc. The study was conducted in accordance with ethical standards and all patients consented to participate in the study.

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Authors contribution

All authors participated in the data analysis, wrote and revised the paper, finally approved the version for publication, and agree to be responsible for all aspects of the work.

Conflicts of interest disclosure

The authors declare no conflict of interest.

Provenance and peer review

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