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Original article

Improving the genetic signature of prostate cancer, the somatic mutations

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Abstract

Background: Somatic mutations have been related to the highest incidence of metastatic disease and different treatment responses. The molecular cause of prostate cancer (PC) is still unclear; however, its progression involves alterations in oncogenes and tumor suppressor genes as well as somatic mutations such as the ones in *PIK3CA* gene. A high percentage of PC is considered sporadic, which means that the damage to the genes occurs by chance after birth (mainly somatic mutations will drive the cancer event). However, little is known about somatic mutations in PC development.

Materials and methods: We evaluated prostate biopsies in the main somatic mutations genes (*PIK3CA*, *TP53*, *EGFR*, *KIT*, *KRAS*, *PTEN*, and *BRAF*) among individuals with PSA values > 4 ng/ml ($n = 125$), including affected and unaffected PC subjects.

Results: Mutations in *KIT* gene are related to aggressive PC: TNM stages II to III, Gleason score ≥ 7 and D'Amico risk ($P = 0.037$, 0.040, and 0.017). However, there are no statistical significant results when more than 3 somatic mutations are presented in the same individual. In relation to environmental factors (smoking, diet, alcohol intake, or workplace exposure) there are no significant differences in the effect of environmental exposure and the somatic mutation presence. The most prevalent mutations among patients with PC are c.1621A > C (rs3822214) in *KIT*, c.38G > C (rs112445441) in *KRAS* and c.733G > A (rs28934575) in *TP53* genes. *KRAS*, *KIT*, and *TP53* genes are the most prevalent ones in patients with PC.

Conclusions: Somatic alterations predisposing to chromosomal rearrangements in PC remain largely undefined. We show that *KIT*, *KRAS*, and *TP53* genes have a higher presence among patients with PC and that mutations in *KIT* gene are related to an aggressive PC. However, we did not find any environmental effect in somatic mutations among PC individuals.

Keywords: Hormonoresistant; *KIT* gene; Prostate cancer; Somatic mutation; Treatment

1. Introduction

In prostate cancer (PC), structural genomic rearrangements, including translocations (e.g., *TMPRSS2-ERG*) and copy number aberrations (e.g., 8q gain, 10q23/*PTEN* loss) are key mechanisms driving tumorigenesis [1]. Somatic events are associated with structural genomic rearrangements but in PC remain largely undefined [2]. Although somatic mutations occur with a low to an intermediate frequency among cancer patients (2–20%), their role in cancer is clearly highlighted, mainly in cancer progression and treatment management [3,4]. Recent large-scale sequencing efforts such as The Cancer Genome Atlas (TCGA) have revealed a complex landscape of somatic mutations in various cancer types [5]. For example, in colorectal cancer, somatic *BRAF* V600E mutation is associated with poor outcomes irrespective of the received

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treatment and regarded as poor prognosis markers [6]. Current data about whole-exome sequencing and whole-genome sequencing have provided a window into the biology, that drives oncogenesis and PC tumors progression, by enabling unbiased exploration of somatic mutations in prostate tumors that span the spectrum of aggressiveness disease [7]. These findings suggest that the genome-wide interplay between somatic single nucleotide variants, indels, and structural variants is important for understanding the repertoire of genomic aberrations that contribute to PC. In spite of these findings, considerable work remains to understand the relationship between somatic genomic alterations and tumor aggressiveness [8].

In PC, somatic mutations rate is in the medium to lower range (0.31 mutations/Mb) in comparison with other tumors like lung squamous cell carcinoma (8.4 mutations/Mb) or malignant melanoma (30 mutations/Mb); even though the rate is also moderate between localized and advanced PC [9]. When talking about somatic mutations in PC there are some candidate genes such as *AR*, *TP53*, *KLF6*, *EPHB2*, *CHEK2*, *ZFHX3* (formerly known as *ATBF1*), *NCOA2*, *PTEN*, *MYC*, *PIK3CA*, *FOXA1*, *KIT*, and various histone-modifying genes [9]. Despite its high incidence, one of the PC main challenges is related to its high heterogeneity, which makes risk stratification and selecting treatments strategies difficult, because tumors classified in the same risk group exhibit different clinical behavior [10]. The inclusion of expression patterns, molecular and genetics biomarkers in PC could create a specific profile classification to assess risk and treatment options [11]. For instance, a recent study has showed an effective prognostic prediction model in relation with several atypical somatic mutations signatures. This model combines the genetic signatures with NICE (National Institute for Health and Care Excellence) factors and improve the prognosis prediction of genetic features or NICE features when they are used alone [10].

The role of environmental factors (like tobacco smoke [including second hand smoke], diet, radiation, and occupational exposures) in cancer development has long been evident from epidemiological studies, and with fundamental implications for primary prevention. There is a clear detailed database of cancer risk molecular effects, and it is well established that environmental factors exert a relevant influence on mutations in all cancers [12]. There are described mutations' signatures related to cancer such as UV damage producing high levels of mutations at Py-Pysequences; tobacco and tobacco smoke exposure with an increase rate of transversional mutations associated with adducts in lung cancer [12]. Moreover in other hormonal cancers, such as breast cancer, the exposure to multiple endogenous and exogenous environmental factors (such as alcohol, smoking, and obesity) are established as risk factors, affecting estrogens metabolism [13].

The term gene and environmental interaction is relevant in cancer cases, and for that reason we make a focus point of the study on the environmental factors effect on somatic mutations and PC risk. Studying the environmental influence includes everything that surrounds us both internally and externally, such as patients with PC and that mutations in *KIT* gene toxicants, hormones, diet, psychosocial behavior, and lifestyle [13].

By September 2013, 125 men with PC clinical symptoms and who had a prostatic biopsy were collected for this study. The main objective of the present work is to obtain a good stratification of patients with high-risk PC giving details in initial steps of the progression of the tumor by the analysis of clinical and environmental exposure data.

2. Materials and methods

2.1. Patient and samples

Men enrolled in this study were selected by urologists of the *Virgen de las Nieves* University Hospital, Granada, Spain. The inclusion criteria were subjects with total PSA/free PSA under 20%, and PSA values above 4 ng/ml. All individuals underwent a systematic 20-core ultrasound guided biopsy to limit the false negative rate. Men with histological confirmed prostatic adenocarcinoma comprised the patient group and negative biopsy individuals were considered as controls. Moreover, patients with positive biopsy were analyzed for T stage, serum PSA, Gleason score and were categorized according to D'Amico risk classification (low, intermediate, and high risk). After primary therapy, PSA was monitored every 3 or 6 months in patients, during 43 months to evaluate the existence of biochemical recurrence (Table 1).

Tissue samples were obtained with 20-core ultrasound guided biopsy as parallel and close cylinders. The anatomopathological analysis classified each biopsy cylinder according to cancer presence/absence. We have analyzed the parallel cylinder to the one previously analyzed by the pathologist. With this methodology we tried to take cells throughout the whole sample, which assures having a deep analysis spectrum and making sure to cover the same sample that the pathologist has analyzed.

Despite the fact that a total of 300 samples were registered, we selected the most representative (samples with no missing data of clinical follow-up, TNM stage Gleason score, and environmental exposure data) 125 tissue samples for somatic mutation analysis. Several samples were discard because of samples' quality, so the total final samples that fulfill all the criteria were reduced to $n = 119$ (PC [$n = 60$] and no patients with PC [$n = 59$]). Moreover, we have quantified the samples with Qubit 4 Fluorometer and NanoDrop 2000c (Thermofisher Scientific, MA). These systems provide quantification and purity assessments for DNA; those samples that were under 1.8 in the 260/280 rate in fluorometry measurements; or those with a difference value over 3 between the fluorescence and

Table 1
Summary of clinical variables

	Patients, $n = 60$		Controls, $n = 59$	
	n	%	*n*	%
Initial PSA (ng/ml)				
>4 < 10	32	53.3	42	71.2
>10 < 20	7	11.7	15	25.4
>20	21	35.0	2	3.4
Gleason				
<7	30	50.0	n.a.	
>7	30	50.0	n.a.	
T Stage				
I	36	60.0	n.a.	
II	19	31.7	n.a.	
III	5	8.3	n.a	
D'Amico Risk Group				
Low	18	30.0	n.a.	
Medium	19	31.7	n.a.	
High	23	38.3	n.a.	
Age				
Median, y	69.02		68.27	
Range, y	47–87		49–84	
Observational period				
Median, mo	36.15		n.a.	
Range, mo	5–43		n.a.	
Missing	6		n.a.	

n = total numbers of samples); n.a. = not applicable.

absorbance measurements were also discarded from the analysis.

For a specific somatic mutation analysis, samples' selection was done in all biopsy cylinders with clear cancer and cancer-free areas, to avoid collecting parts of the tissue that could interfere in final results.

Fresh tissue biopsy samples colleted were stored at −80° C until genetic analysis processing. And peripheral EDTA coated tubes blood samples were extracted and stored at −20°C until genomic DNA extraction.

All subjects provided a written informed consent to be enrolled in the study, which was previously approved by the Research Ethics Committee of Granada Center (CEI-Granada) following Helsinki ethical declaration.

2.2. Genetic analysis

DNA extraction was carried out according to the method detailed in Ref. [14] a nonorganic (proteinase K and salting out) protocol with some modifications described [15] and quantified by Qubit 4 Fluorometer and NanoDrop 2000c systems (Thermo Scientific, MA, USA).

Mutations were selected from a comprehensive somatic mutation database (COSMIC) and by a peer-reviewed scientific literature based on their clinical or functional relevance and frequency of occurrence [16]. The somatic mutation analysis was performed by the Custom qBiomarker Somatic Mutation PCR Array Human (Applied Biosystems, CA, USA) that carry information about the most 86 common somatic mutations in *BRAF*, *PIK3CA*, *TP53*, *EGFR*, *KIT*, *KRAS*, and *PTEN* genes (more details in Supplementary Table 1). These arrays contain panels of bench-verified hydrolysis probe-based real-time PCR assays that detect as low as 0.01% mutant DNA in a wild-type background. Allele-specific amplification is achieved by Amplification Refractory Mutation System (ARMS) technology. These arrays were analyzed by 7900 HT Fast Real-Time PCR System software. A 10% of the samples were confirmed by amplification and sequencing using Kit Big Dye v3.1 (Applied Biosystems, CA, USA) with specific designed primers.

The sensivity of this array (i.e., the detection of low-percentage mutations) and assay specificity (i.e. the detection of only the mutant allele), has been verified on average with a detection window of 13.6 cycles (allowing a very sensitive detection of low-percentage mutations). The calculated average assay sensitivity is 0.008%, and the calculated median assay sensitivity is 0.024% [17].

To confirm the specificity of somatic mutations results we have performed a blood sample extraction in a 10% of the samples, to eliminate possible germline mutations from the data.

2.3. Questionnaire analysis

All participants have been interviewed face to face in the hospital to obtain details about environmental exposure and lifestyle patterns using a structured questionnaire by trained researchers and dieticians. The questionnaire included information about socio-demographic factors (age, residence, and occupation); exposure to smoke; powder and toxic products; and leisure time physical activity. Exposure to environmental factors and practice of sport were evaluated by yes/no questionnaire.

2.4. Statistical analysis

Software package SPSS v.22 was used to execute statistical analyses (IBM Corporation, USA). The analyses including chi-square and Fisher's exact tests. They were considered statistically significant with a $P < 0.05$.

2.5. In silico analysis

We use several computational analyses (COSMIC, PolyPhen-2, UniProtKB, ClinVar, and MutationTaster) to evaluate the possible effect of somatic mutations by the comparison of proteins structure and function.

COSMIC (Catalog of Somatic Mutations in Cancer) (http://cancer.sanger.ac.uk/cosmic) is a database which accumulates the described somatic mutations in all human cancers [16]. PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) is an on-line software, which uses structural and comparative evolutionary approaches to predicts the impact of amino acid substitutions on the structure and

function of human proteins [18]. UniProtKB (http://www.uniprot.org/) it is a database with a large resource of protein sequences and associated detailed annotations. Offering information about phenotypic and functional consequences of sequence variations [19]. ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) is an on-line archive of the National Center for Biotechnology Information (NCBI) used for interpretations of variants clinical significance [20]. Finally, MutationTaster (http://www.mutationtaster.org/) is a web tool which works on DNA level and informs about single base exchange, insertions, and deletions [21].

3. Results

3.1. Somatic mutations in cases-controls and cases-cases analysis

The presence of the specific number of somatic mutations has not been reported with significant differences ($P > 0.05$) among patients and controls in *BRAF*, *PIK3CA*, *TP53*, *EGFR*, *KIT*, *KRAS*, and *PTEN* genes, data shown in Table 2.

Among the 95 analyzed somatic mutation we have found that some were exclusive in patients such as c.1637A > G (rs397517201) in *PIK3CA*; c.586C > T (rs397516435), c.844C > T (rs28934574); and in certain combinations including c.733G > A, c.586C > T, and c.733G > A, c.844C > T in TP53; c.2573T > G (rs121434568) in *EGFR*; the combination c.1621A > C (rs3822214), c.2447A > T (rs121913507), and c.2446G > T (rs121913506) in *KIT*; c.35G > T/C (rs121913529), c.37G > T (rs121913535), c.38G > T (rs112445441), and the combination of c.34G > C, c.38G > C and c.34G > T, c.182A > G (rs121913240) in *KRAS*. The most prevalent mutations among patients with PC were c.1621A > C (rs3822214) in *KIT*, c.733G > A (rs28934575) in *TP53* and c.38G > C (rs112445441) in *KRAS* genes.

3.2. Somatic mutations and clinical status

We have performed different analysis including clinical parameters and somatic mutations implications. In relation to Gleason score, a 6.3% of the patients had a value ≥7 vs. 26.7% <7 ($P = 0.040$) in *KIT* gene. When comparing D'Amico risk data, patients with *KIT* mutation had a statistical significant data vs. those with any mutation ($P = 0.017$), with a 70% of the patients in an intermediate–high risk classification. Furthermore, *KIT* mutations were also related with higher T stages (II and III), $P = 0.037$.

In relation to aggressiveness in clinical parameters (Gleason, D'Amico risk and TNM), although there were no statistical significant results when we cluster 3 somatic mutations, a 54.1% of the patients with at least 3 somatic mutations were clustered in Gleason score above 7 (data not shown). Moreover, a follow-up of the patients during 43 months of observation were performed; however, there were not statistical significant values with this comparison (data not shown).

3.3. Somatic mutations and environmental exposition

There were no significant differences between the presence of the mutation and environmental factors, such as tobacco, diet, alcohol intake, or workplace exposure; (data not shown). Just in the residence area effect combined with the presence of *TP53* mutation, it was nearly to be significant $P = 0.052$; including higher rates (16.7%) of patients in areas <100,000 habitants contrasted to a 97.2% with the absence of the mutation in residences >100,000 habitants. *PIK3CA* was also presented in all patients that combine more than one environmental exposure factor ($P = 0.080$), contrasting to those without any exposition.

3.4. In silico analysis in somatic mutations

3.4.1. Patients cohort

In silico analysis also reported several relevant data in some of the most frequent mutations in patients cohort such as c.34G > A and c.38G > A/C (*KRAS*), which was classified as pathogenic in several databases (ClinVar, COSMIC and MutationTaster). *TP53* variants c.733G > A and c.844C > T were included as pathogenic by ClinVar, COSMIC, PolyPhen-2, MutationTaster and UniProtKB; c.1799T > C (*BRAF*) was included as a pathogenic somatic mutation with high rates in PC [21].

3.4.2. Controls cohort

In relation to controls, c.35G > A and c.182A > T (*KRAS*) were classified as pathogenic variants (COSMIC, ClinVar and MutationTaster). c.388C > G and c.952_955delCTTA (*PTEN*) were considered as pathogenic and disease causing (UniProtKB and MutationTaster); c.3129G > T and 3139C > T (*PIK3CA*) both also included as pathogenic variants. More details in Supplementary Table 2.

4. Discussion

The important role of somatic mutations has been proven in cancers such as colorectal, non–small-cell lung and breast. In the case of non–small-cell lung, some studies have confirmed *EGFR* mutations as predictive treatment response to tyrosine kinase inhibitors, gefitinib and erlotinib [22]. Similar results have been obtained in *KRAS* mutations in colorectal cancer patients. It is proven that *KRAS* mutations confers resistance to treatment with *EGFR* antibodies and only patients with wild-type *KRAS* tumors obtain benefit from these agents [23]. Nevertheless, at the

Table 2
Clinical data and environmental analysis

Gene	Mutations	PC	Controls	Total	T stage[a]	Gleason[b]	D'Amico risk	Population[c]	Environmental exposure
PIK3CA	c.1637A > G	1 (50%)	0 (0%)	1 (12.5%)	$P = 0.218$	$P = 1$	$P = 0.447$	$P = 0.420$	$P = 0.080$
	c.3129G > T	0 (0%)	2 (33.3%)	2 (25%)					
$P^{d} = 0.157$	c.3139C > T	1 (50%)	4 (66.7%)	5 (62.5%)					
	Total	2 (100%)	6 (100%)	8 (100%)					
	c.586C > T	1 (10%)	0 (0%)	1 (6.7%)	$P = 1$	$P = 1$	$P = 0.863$	$P = 0.052$	$P = 1$
	c.659A > G	0 (0%)	2 (40%)	2 (13.3%)					
	c.733G > A	4 (40%)	2 (40%)	6 (40%)					
TP53	c.733G > A, c.586C > T	1 (10%)	0 (0%)	1 (6.7%)					
$P^{d} = 0.317$	c.733G > A, c.844C > T	1 (10%)	0 (0%)	1 (6.7%)					
	c.818G > A	1 (10%)	1 (20%)	1 (13.3%)					
	c.844C > T	2 (20%)	0 (0%)	2 (13.3%)					
	Total	10 (100%)	5 (100%)	15 (100%)					
	c.2235_2249del15	0 (0%)	1 (11.1%)	1 (7.7%)	$P = 1$	$P = 0.613$	$P = 0.883$	$P = 0.448$	$P = 0.474$
	2235, 2236, c.2155G > T	0 (0%)	1 (11.1%)	1 (7.7%)					
EGFR $P^{d} = 0.204$	c.2236_2250del15	3 (75%)	4 (44.4%)	7 (53.8%)					
	c.2237_2251del15	0 (0%)	1 (11.1%)	1 (7.7%)					
	c.2239_2247del9	0 (0%)	2 (22.2%)	2 (15.4%)					
	c.2573T > G	1 (25%)	0 (0%)	1 (7.7%)					
	Total	4 (100%)	9 (100%)	13 (100%)					
	c.1621A > C	8 (80%)	8 (100%)	16 (88.9%)	$P = 0.037$	$P = 0.040$	$P = 0.017$	$P = 0.533$	$P = 0.472$
KIT	c.1621A > C, c.2447A > T	1 (10%)	0 (0%)	1 (5.60%)					
$P^{d} = 0.887$	c.2446G > T	1 (10%)	0 (0%)	1 (5.60%)					
	Total	10 (100%)	8 (100%)	18 (100%)					
	c.182A > T	0 (0%)	2 (20%)	2 (7.4%)	$P = 1$	$P = 1$	$P = 0.209$	$P = 0.759$	$P = 0.544$
	c.34G > A	2 (11.8%)	2 (20%)	4 (14.8%)					
	c.34G > A, c.38G > T	0 (0%)	1 (10%)	1 (3.7%)					
	c.34G > C	1 (5.9%)	0 (0%)	1 (3.7%)					
	c.34G > C, c.38G > C	1 (5.9%)	0 (0%)	1 (3.7%)					
KRAS	c.34G > T, c.182A > G	1 (5.9%)	0 (0%)	1 (3.7%)					
	c.35G > A	1 (5.9%)	2 (20%)	3 (11.1%)					
$P^{d} = 0.244$	c.35G > C	1 (5.9%)	0 (0%)	1 (3.7%)					
	c.35G > T	1 (5.9%)	0 (0%)	1 (3.7%)					
	c.37G>C	1 (5.9%)	1 (10%)	2 (7.4%)					
	c.37G > T	1 (5.9%)	0 (0%)	1 (3.7%)					
	c.38G > A	2 (11.8%)	1 (10%)	3 (11.1%)					
	c.38G > C	4 (23.5%)	1 (10%)	5 (18.5%)					
	c.38G > T	1 (5.9%)	0 (0%)	1 (3.7%)					
	Total	17 (100%)	10 (100%)	27 (100%)					
PTEN	c.388C > G	1 (50%)	4 (66.7%)	5 (62.5%)	$P = 0.218$	$P = 0.492$	$P = 0.107$	$P = 1$	$P = 1$
$P^{d} = 0.157$	c.952_955delCTTA	1 (50%)	2 (33.3%)	3 (37.5%)					
	Total	2 (100%)	6 (100%)	8 (100%)					
BRAF $P^{d} = 1$	c.1799T > C	3 (100%)	2 (100%)	5 (100%)	$P = 0.597$	$P = 1$	$P = 0.983$	$P = 1$	$P = 0.286$
	c.3934G > T	1 (25%)	0 (0%)	1 (25%)	$P = 0.597$	$P = 0.346$	$P = 0.121$	$P = 0.550$	$P = 0.556$
APC	c.4132C > T	2 (50%)	0 (0%)	2 (50%)					
$P^{d} = 0.119$	c.4285C > T	1 (25%)	0 (0%)	1 (25%)					
	Total	4 (100%)	0 (0%)	4 (100%)					
SKT11 $P^{d} = 1$	c.1062C > G	1 (100%)	0 (0%)	1 (100%)	$P = 1$	$P = 1$	$P = 0.334$	$P = 1$	$P = 0.380$

[a]T stage: *P* value comparing cases with PC stage I or II to III.
[b]Gleason: *P* value comparing cases with Gleason values <7 and ≥ 7.
[c]Population: *P* value comparing living environment with populations under or over 100,000 people.
[d]*P* value under the name of each gene: comparing the number of mutations between PC and controls.

moment there are not many relevant data in PC. Recent published data, have reinforce the role of *SPOP* somatic mutations, as relevant cancer predisposition of noncoding variants that lead to allele-specific transactivation of central tumoral factors programs [24].

With the present study we have proven a high presence level of certain somatic mutations (*KIT*, *PIK3CA*, *KRAS*, and *EGFR*) among patients with PC, mutations with a no described relevant role in others cancers (breast and lung cancer mainly). As it has previously exposed in Table 2, there are several somatic mutations with presence in controls and non in patients, as well as others with a higher rate in controls cohort. These data seem to be controversial; however similar data has been previously reported in pancreatic intraepithelial neoplasms [25]. Indicating that more than 99% of the earliest-stage, lowest-grade, pancreatic intraepithelial neoplasm-1 lesions contain mutations in *KRAS*, *p16/CDKN2A*, *GNAS*, or *BRAF* [25]. Furthermore, recent publications have proven that somatic mutational burden in normal human tissues can vary by several orders of magnitude, depending on biological and environmental factors. These data proved that normal colonic epithelium from 11 individuals showed prevalence's mutation that significantly increased with age [26]. So the presence of somatic mutations exposed in our control population can be explained by other biological factors, and the event of the old age of the population analyzed. It is known that PC is rare in young men (<40 years), and present data confirm that aging is associated with the accumulation of somatic mutations, and strongly suggest that the level of genome instability is the main risk factor for cancer [27].

Moreover, the overall median age among this cohort is 68.65 years old, which is an old age population and DNA repair machinery has a poor efficiency in elder ages, explaining the high rates of somatic mutations. Several data reported in stem cells suggested that a mutation spectra in normal tissues is explained because a substantial fraction of the mutations found in cancers occur in normal stem cells and not only attributed to cancer-specific processes [26].

Some authors indicate that several somatic mutations (like *KRAS*-mutant) could exert an autocrine and paracrine influence affecting to the developmental genes, senescence stress-inducing signals; and induce metaplastic features in mutant cells [25]. Here we have just reported a significant data between *KIT* and clinical variables (TNM, Gleason and D'Amico risk). It has previously been confirmed that KIT protein overexpression or mutations are involved in growth and development of a variety of cancers. An abnormal activations of *KIT* can be caused by KIT overexpression, gene amplification, or mutations in a variety of cancers, such as gastrointestinal stromal tumors, melanoma, and breast cancer [28]. But this is the first study that reported *KIT* mutations associated with PC aggressiveness.

Moreover, it is well known that different rates of somatic mutations are due to differences reflecting exposures to unidentified commonly encountered mutagens, or tissue-specific processes such as DNA repair. Here we have reported that there are not many effects of the analyzed environmental factors (place of residence, occupational status, lifestyle habits, tobacco, and alcohol intakes) in somatic mutations. Just incipient data are exposed with resident area and *TP53* mutations, which is similarly to recent publications where differences in demography between populations' areas have an effect on somatic mutations rates. Thus, it seems that differences in the proportion of rare or private, somatic variants are driven by differences in the rate of recent population growth rather than differences in mutation rate [29].

Furthermore, in silico analysis point out that some of the described mutations in patients' cohort are classified as pathogenic in several databases. c.34G > A (*KRAS*) classified as pathogenic, produces protein features affected and splice site changes. c.38G > A/C (*KRAS*) is also classified as pathogenic and with a known adverse effect on colorectal and lung cancers [20]. c.1799T > C (*BRAF*) is included as a pathogenic somatic mutation with high rates in PC, producing amino acid sequence changes, protein features affected and splice site changes [21]. c.4132C > T (*APC*) is a nonsense substitution with a pathogenic described effect in colorectal cancer (COSMIC, ClinVar and MutationTaster). In relation to controls, c.388C > G and c.952_955delCTTA (*PTEN*) are considered as pathogenic and disease causing, for example in c.388C > G there is a described loss of phosphatase activity and an affection of splice site changes (UniProtKB and Mutation Taster). c.3139C > T (*PIK3CA*) is considered as one of the main three *PIK3CA* hotspots (with E542K and E545K) participating in cellular transformation and tumorigenesis induced by oncogenic receptor tyrosine kinases and *HRAS/KRAS* [19].

5. Conclusion

To conclude, the role of somatic mutations in PC remains unclear. Here we reported for the first time an incipient relation between mutations in *KIT* gene; and an aggressive PC (TNM > II, D'Amico risk and Gleason ≥ 7). However, not many influences of environmental effect has been demonstrated between PC and somatic mutations in this study, just limited data in *TP53*. It is known that the neoplastic development is a fitness selection process in where environmental, epigenetic, and physiological events are relevant for a selection process. As suggested with this study, KIT somatic mutations seems to be a disease-specific potential gene which enable other genetic cascade events that classify high-risk patients PC. Further analysis in a larger cohort and also exploring the role of germline events will probably supports data about the specific role of somatic mutations in PC.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.urolonc.2018.03.012.

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