PTEN Mutations Mapped in BPH and PCa in a Selected Egyptian Population; A Pilot for New Diagnostic Markers

Maha A. Bassuoni[1], Randa M. Talaat[2], Ahmed A. sonbol[1], Ayman A. Diab[3],Ahmed A.sadik[4]

[1]Clinical pathology department, Faculty of Medicine, Menoufiya University,
[2] Microbiology and Immunology Department, Genetic Engineering and Biotechnology Research Institute, El Sadat University, Cairo, Egypt,
[3]Dean of Faculty of Biotechnology, Modern science and Arts University of 6th October, Egypt,
[4]BSC of Pharmacy, Faculty of Pharmacy, Cairo university
ahmed_sonbolmmm@yahoo.com

Abstract: *PTEN* is one of the tumor suppressor genes involved in secondary cancer progression prevention. In Egypt, Prostate Adenocarcinoma (PCa) recorded the highest incidence and mortality among cancers of the genitourinary system. **Aim:** This was a pilot study that aimed to detect the presence or absence of mutations along exons 5 & 7 in the tumor suppressor PTEN gene in BPH and PCa patients and correlate between PTEN and the pathogenesis of both PCa and Benign Prostate Hyperplasia (BPH). **Patients and Methods:** Twenty benign and malignant patients were recruited, as well as seven apparently normal subject served as controls. Whole blood samples were collected from patients previously diagnosed using TRUS-Biopsies. Control subjects were free of chronic disorders and tumors, yet some had family history of benign and malignant tumors. Studied groups had age range between 54 and 77yrs that averaged 64 ± 7.5 and average PSA level of 2.3, 19.9 and 35ng/ml for Control, BPH and PCa groups respectively. PCR were performed, using intronic primers specific for exons 5 and 7, to map possible mutations (for positive PCR reactions sequencing done using both forward and reverse primers). **Results:** Analyzing mutations mapped for the selected studied groups proved the likeliness of correlating between both BPH and PCa patients. PTEN association in PCa patient was correlated with their pathology findings and clinical picture. One patient out of 10 showing complete deletion had the highest Gleason score (9) and worst clinical picture, two patients out of 10 showed partial deletion (one losing the phosphatase function and the other losing the C2 domain of the direct protein binding). Upon thorough analysis high occurring genotypes were defined. Ten mutations were mapped across the exons and their near end site introns both upstream and downstream revealing 5 potentially significant mutations. All 5 potentially significant mutations (g.74482dupA, g.74818dupG, g.74845dupC and g.99506C>G, g.99582dupA) shared the presence of the highly occurring genotypes except g.99506C>G of exon 7 benign patient (7b6). **Conclusion:** Mutations mapped have revealed some potentially significant mutant variants of PTEN pointing towards its role in both BPH and PCa Pathology and the possibility of a bridging correlation between both benign and malignant prostate tumors.

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1. Introduction

In Egypt the overall contribution of cancer mortalities is approximately 8.5% of all causes of death (**GBD, 2004**), 2.2% of which is contributed to PCa. According to the Egyptian Ministry of Health (MOH) 95% of the total incidences of cancers of the male genital organs are attributed to PCa (**MOHP, 2010**). *PTEN* also known as *MMAC1* is the most known gene on 10q that has been regarded as one of the main causes of PCa, due to the large span of abnormalities that have been recorded for this gene (**Sirintrapun and Parwani, 2008**), it was also found to be the 2nd most mutated gene after *P53* (**Arnoldussen *et al* 2009**) in most tumors. *PTEN* is found at the 10q23.1-3 locus of approximately 111 Kb in size, comprising 9 exons that give a protein of 403 amino acids.

The ability to suppress tumorogenesis is attributed to its dephosphorylating ability of Phosphatidylinositol 3,4,5-trisphosphate (PIP3) to Phosphatidylinositol-4,5-bisphosphate (PIP2). This dephosphorylation process inhibits the activity of the PI3K pathway which mediates tumorigenic activities within the cell (**Maehama and Dixon, 1998**). *PTEN* has 5 functional domains mapped and more definitively identified PIP2 binding domain 1-15, phosphatase domain 22-185, C2 regulatory domain 190-351, two consecutive PEST homology Domains 350-375 379-396, and PDZ binding domain 401-403 (**Bonifant *et al* 2007**).

Mutations of *PTEN* are one of the main reasons for its inactivation in addition to Loss of Heterozygosity (LOH) (**Dong *et al* 1998; 2001**). The occurrence of mutations leading to the loss of the phosphatase function are numerous, abrogating the ability of PTEN to show tumor suppressive activity through down regulation of cell growth rate (**Koul *et al* 2002**). Other mutations occurring in the C2 domain were markedly significant in their impact on the tumor suppressor ability of PTEN, where, point mutations of the central C2 domain have been demonstrated to impact proper PTEN localization (**Trotman *et al* 2007**).

2. Patients and Methods

Patients Groups

Blood samples used in this study were collected from patients of Kasr Al Aini Teaching Hospital, Cairo University, Cairo, Egypt. The age range of the patients had an average of 64 ± 7.5. Schistosomiasis, was one of the major exclusion criteria considered. BPH and PCa Patients had TRUS-Biopsies and were examined following standard Hematoxy Eosin staining. Gleason Scores, Capsular infiltration and metastasis were recorded for the PCa group. The malignant samples were only considered, when the Gleason score was not less than 6.

A number of 7 normal blood samples were gathered, the individuals from whom the samples were collected from were healthy non-diabetic, non-hypertensive, free of chronic atherosclerotic disorders and above the age of 45 years, some patient history included, family history of benign and/or malignant tumors.

Statistical Analysis

The malignant patient profiles were subject to Pearson Correlation between PSA and Gleason score, a value of $p<0.01$ was considered significant. Independent-T test and the Mann Whitney U test, to evaluate PSA and Gleason score association significance in addition to the significance of their association with Capsular Infiltration and Metastasis, a value of $p<0.05$ was considered significant. Data analyses were performed by the SPSS software (Statistical Package for the Social Sciences, version 19.0, SPSS Inc, USA).

DNA Extraction

The DNA extraction was conducted using QIAmp Mini Blood DNA extraction kit. The extracted DNA was measured on the nanometer after gel electrophoresis and the concentration of all samples was adjusted to 100ng/µl.

PCR

PCR was conducted at the Molecular Biology Lab, Modern University of Science and Technology. Primers used were those described by **Feilotter *et al* (1998)** and were purchased from Invitrogen® GmBH. The PCR was conducted using the Hybaid PCR sprint thermal cycler. The reaction mixes were denatured at 94°C for 4 min followed by 30 cycles of 94°C for 1 min, 56°C for 30 s and 72°C for 30 s followed by 1 cycle of 72°C for 10 min. A volume of 10 µl of each PCR product was loaded on previously prepared 1.5% agarose gel and electrophoresed at 65 Volts for approximately 90 min.

DNA Sequencing

Samples of the benign group (b1-b10), seven samples from the malignant group (m1-4, m6, m8 and m10) and tow samples from the normal group having family history of benign or malignant tumors (n3 and n6), were all subject to sequencing. Samples selected for sequencing were sent to Macrogen® (908 world meridian venture center, #60-24, Gasan-dong, Geumchun-gu, Seoul 153-781, Korea). All PCR tubes were firmly packed and were delivered to Lab-Technology® (Macrogen agent in Egypt) on crushed ice. Sequencing was performed using the ABI PRISM BigDyeTM Terminator Cycle Sequencing Kits, ABI PRISM 3730XL Analyzer (96 capillary type) sequencer (Applied Biosystems), MJ Research PTC-225 Peltier Thermal Cycler, DNA polymerase (FS enzyme) (Applied Biosystems).

Sequencing Analyses

The DNA sequences were aligned using the DNA Star Software Package – Lasergene (Expert Sequence Analysis Software, USA). DNA sequences were translated to protein using the EditSeq program and then alignment using the MegaAlign program was done. Four reference sequences (AF067844.1, NG_0074466.1, NT_030059.13 and NW_001838005.2) were used in the comparative analysis of the sequenced fragments. Further blasting using tblastx (**Johnson *et al* 2008**) was conducted alongside with Sequence analysis using Human Splice Finder (**Desmet *et al* 2009**). The reference sequences NG_007466.1 was chosen for the pairwise alignment that was performed prior to the mutation analysis and splice variation evaluation conducted using HSF.

3. Results

Patient groups

The correlation between PSA and Gleason scores showed the significance of high association of Gleason score and high PSA values (**Table, 1**). A marked significance between the association of either PSA and Gleason scores with both Metastasis and Capsular Infiltration was recorded (**Table, 2**). The only result that didn't show a significant value during the analysis was that of the T-test result of PSA and Capsular infiltration. This result although non-significant, yet the significant relation still holds,

attributed to the small sample size used, the non-parametric nature of the Capsular Infiltration values and the significance of the U-test for the same parameters studied.

PCR amplification of Exons 5 and 7 from the DNA of collected patients

Results showed that all normal and benign group samples showed PCR-positive reactions when tested for detection of exons 5 & 7. On the other hand, 7 out of 10 malignant group samples showed PCR-positive reactions when tested for detection of exons 5 & 7 (**Figures, 1 & 2**). The two exons were not amplified from the DNA of the malignant sample m5. Exon 5 was not detected in the DNA of the malignant samples m9, while exon 7 was not detected in the DNA of the malignant sample m7, therefore, the malignant samples 5, 7 and 9 were not selected for further studies.

DNA Sequencing

Results showed PCR products of exon 5 of the studied group samples, revealing different lengths ranging from 369 to 385 nt. These nucleotides showed a number of translated amino acids from 123 to 129 aa. In case of exon 7, the PCR products of the selected group samples had variable lengths from 253 to 268 nt and their respective translated amino acid sequences from 85 to 90 aa.

Sequencing Analyses

Comparison of the three groups for exons 5 and 7

The collective exon 5 data set (5n, 5b and 5m selected samples) showed that samples 5m2, 3, 4, 6 and 10; 5b1, 2, 3, 5, 6, 9 and 10; 5n3 were of highest similarity (**Table, 3**) ranging from 86 to 99%. Slight variances from the exon 7 data sets were shown for the samples with highest similarity, 7m4, 6, 8 and 10; 7b1, 3, 5, 9 and 10; 7n3 and 6 (**Table, 4**). The findings of both exon data sets when compared indicate that the High Probability Occurring Genotypes (**HPOG**) will be those of the samples b1, 3, 5, 9 and 10; m2, 4, 6, 8 and 10; n3 and n6. These results came consistent with those obtained when using the different reference sequences.

The pairwise alignment showed a wide set of mutant variants (**Table, 5**). These mutant variants were all checked with tblastx and HSF online software to identify which of these mutations were significant, in addition to identifying possible splice variations (**Table, 6**). The categorization of these mutations is shown in **Figure (3)**.

Table (1): Pearson correlation between PSA and Gleason scoring, showing a significant relation between increased Gleason scores and PSA.

PSA	Gleason score		**Parameter**
.896**	1	Pearson Correlation	Gleason score
.000		Sig. (2-tailed)	
10	10	N	
1	.896**	Pearson Correlation	PSA
	.000	Sig. (2-tailed)	
10	10	N	

**. Correlation is significant at the 0.01 level.

Table (2): Significance of both PSA and Gleason score association with Metastasis and Capsular infiltration using Mann Whitney U test and the Independent T test. The results show a strong correlation with deteriorating patient clinical pictures.

T test						**U test**		**Parameter**
p-value	**t-value**	**SE**		**Mean**		***p*- value**	**z-value**	
		Prs	**Abs**	**Prs**	**Abs**			
PSA ng/ml								
0.001*	4.87	20.67	2.52	79.33	16.07	0.017*	2.40	**Metastasis**
0.059	2.20	18.45	3.21	55.60	14.50	0.032*	2.20	**Capsular Infiltration**
Gleason score								
0.003*	4.18	0.33	0.22	8.67	7.00	0.017*	2.45	**Metastasis**
0.010*	3.30	0.37	0.20	8.20	6.80	0.032*	2.36	**Capsular Infiltration**

*Significance is achieved at $p<0.05$.

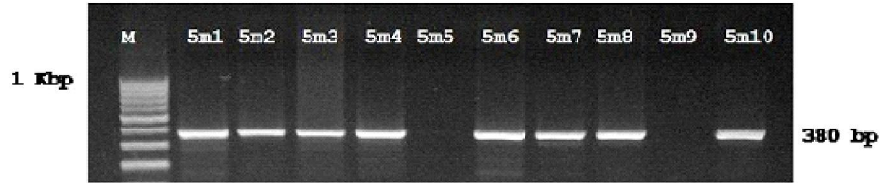

Figure (1): Agarose gel (1.5%) stained with ethidium bromide showing PCR amplification of exon 5 of *PTEN* gene from DNA extracts of 10 malignant group patients. Both 5m5 and 5m9 gave a negative PCR indicating the absence of the fragment of study. A PCR product of about 380 bp was amplified. M: 100 bp DNA marker.

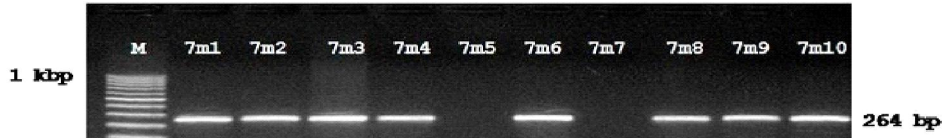

Figure (2): Agarose gel (1.5%) stained with ethidium bromide show PCR amplification of exon 7 of *PTEN* gene from DNA extracts of 10 malignant group patients. Both 7m5 and 7m7 gave a negative PCR indicating the absence of the fragment of study. A PCR product of about 264 bp was amplified. M: 100 bp DNA marker.

Table (3): Similarities between nucleotide sequences of exon 5 amplified from DNA extracts of 10 benign and 7 malignant group patients and 2 normal group subjects using Clustar V Method.

	5n6	5n3	5m8	5m6	5m4	5m3	5m2	5m10	5m1	5b9	5b8	5b7	5b6	5b5	5b4	5b3	5b2	5b10	
5b1	26	29.5	25.4	36.4	98.1	36.5	98.4	29.5	29.6	41.4	32.2	25.4	25.4	98.4	34.6	36.5	30	93.5	***
5b10	26.8	30	24.9	34.2	96	33.3	94.5	30	28.5	35.1	31.3	24.9	24.9	94.2	41	33.3	30.3	***	
5b2	29	98.7	29.7	35.6	29.6	35.3	30.5	98.7	27.1	33.7	26.1	31.2	29.7	29.5	29.7	35.3	***		
5b3	24.9	34.7	28.6	99.5	35.5	100	35.7	34.7	25.5	95.3	24.4	30.2	28.6	35.2	30.4	***			
5b4	27.4	29.5	25.7	30.7	36.6	30.4	35.5	29.5	30.2	30.4	32.6	27	25.7	35.6	***				
5b5	26	29.5	25.1	35	99.2	35.2	96.9	29.5	29.6	40.1	31.7	25.1	25.1	***					
5b6	35	30.3	100	28.6	25.1	28.6	25.1	30.3	28.6	27.8	29.5	32.7	***						
5b7	27.9	30.4	32.7	30.2	25.5	30.2	25.4	30.4	27	29.4	26.2	***							
5b8	28.5	25.8	29.5	24.8	31.2	24.4	32.6	25.8	87.3	25.9	***								
5b9	24.9	33.2	27.8	96.8	37.9	95.3	40.3	33.2	25.7	***									
5m1	26	27.1	28.6	25.6	29.3	25.5	29.5	27.1	***										
5m10	28.5	100	30.3	35	29.6	34.7	30	***											
5m2	25.7	30	25.1	36.7	97.8	35.7	***												
5m3	24.9	34.7	28.6	99.5	35.5	***													
5m4	26.3	29.6	25.1	34.8	***														
5m6	24.9	35	28.6	***															
5m8	35	30.3	***																
5n3	28.5	***																	
5n6	***																		

Table (4): Similarities between nucleotide sequences of exon 7 amplified from DNA extracts of 10 benign and 7 malignant group patients and 2 normal group subjects using Clustar V Method.

	7n6	7n3	7m8	7m6	7m4.	7m3.	7m2	7m10	7m1	7b9	7b8	7b7	7b6	7b5	7b4	7b3	7b2	7b10	7b1
7b1	28.8	29.2	28.8	28.9	28.8	29.5	92	28.8	28.4	23.3	27.7	28.8	28.8	28.8	96.6	100	100	28.8	***
7b10	100	97.4	100	100	100	38.5	37	99.6	26.4	24.1	91.7	100	99.6	100	32.2	28.8	29.1	***	
7b2	28.8	28.9	29.1	28.9	29.1	29.7	91.7	28.6	28.2	23.3	27.8	29.1	29.1	29.1	96.6	100	***		
7b3	28.8	29.2	28.8	28.9	28.8	29.5	92	28.8	28.4	23.3	27.7	28.8	28.8	28.8	96.6	***			
7b4.	32.2	32.6	32.2	32.3	32.2	33	88.6	32.2	27.7	22.5	31.1	32.2	32.2	32.2	***				
7b5	100	97.4	100	100	100	38.5	37	99.6	26.4	24.1	91.7	100	99.6	***					
7b6	99.6	97	99.6	99.6	99.6	38.5	37	99.2	26.4	24.5	91.3	99.6	***						
7b7	100	97.4	100	100	100	38.5	37	99.6	26.4	24.1	91.7	***							
7b8	92	94	91.7	92	91.7	47	28.6	91.4	27.8	24.5	***								
7b9	24.1	24.1	24.1	24.1	24.1	26.5	22.9	24.1	28.5	***									
7m1	26.5	27.2	26.4	26.6	26.4	27.1	26.7	27	***										
7m10	100	96.6	99.6	100	99.6	38.3	36.5	***											
7m2	36.7	34.2	37	36.5	37	30.5	***												
7m3	38.6	41	38.5	38.4	38.5	***													
7m4	100	97.4	100	100	***														
7m6	100	97.7	100	***															
7m8	100	97.4	***																
7n3	97.7	***																	
7n6	***																		

Table (5): Analysis of mutations spanning exons 5 and 7 and the near end sites of their introns after pairwise alignment against NG_007466.1. Mutations were described according to the Human Genome Variation Society (HGVS) (den Dunnen and Antonarakis, 2000).

Predicted changes	Mutation types	Nucleotide changes	Samples	Exons	No.*
L101X**	Insertion	g.74482dupA	5b1, 5b4, 5m1, 5m2, 5m10 & 5n3	**5**	1
Non-significant	In/Del	g.74482_74486delinsAAAG	5b10		2
Non-significant	Substitution	g.74504G>C	5b5		3
Spl. Mut.	Insertion	g.74818dupG	5b9 and 5b10		4
Spl. Mut.	Insertion	g.74845dupC	5b4 and 5m2		5
Non-significant	Insertion	g.99386dupA	7m3	**7**	6
Non-significant	Deletion	g.99363delC	7b4		7
E241D***	Substitution	g.99506C>G	7b6		8
Spl. Mut.	Insertion	g.99582dupA	7b8 and 7m2		9
Non-significant	Insertion	g.99605dupA	7n3		10

*Genbank sequences accessions JQ037769-JQ037778 (in the same order).
**Lysine has been replaced at position 101 with a stop codon.
***Glutamic acid is replaced by Aspartic acid at position 241.

Table (6): HSF splice analysis of mutations g.74818dupG, g.74845dupC, g.99582dupA showing possible splice variations at different acceptor and donor splice sites.

Donor splice sites		Acceptor splice sites		Mutation	Spanning Exon
New sequences	motifs	New sequences	motifs		
-	-	TAAggttat	taaggttat	g.74818dupG	**5**
-	-	ttcctccttcctGA	tttcctccttcctgg	g.74845dupC	**5**
CTTgtgaaa	CCTGTGAAA	-	-	g.99582dupA	**7**

4. Discussion

Mutation detection was conducted through the isolation of exons 5 and 7 through direct PCR as previously described. The choice of exons 5 and 7 was made in accordance to their location in the functional domains of the PTEN protein, where exon 5 is located in the phosphatase domain and exon 7 is located in the C2 domain. Another reason for the choice of these two exons in particular was the high incidence of mutations reported in these exons in prostate cancer as described in previous studies (**Cairns *et al.*, 1997**; **Dong *et al* 1998; Suzuki *et al* 1998; Bonneau and Longy, 2000**), therefore their presence or absence would indicate the presence of either partial or complete deletion of PTEN.

The m5 patient manifested complete loss of PTEN which came in agreement with the clinical picture of the patient, which was aggressively deteriorating owed to the high Gleason score (9), PSA >100ng/ml and the presence of both capsular infiltration and metastasis. The m9 patient lost the phosphatase domain and thus had a highly deteriorating clinical picture with Gleason score of 8, capsular infiltration and metastasis. The exon 7 partial deletion of the m7 patient, losing the C2 domain functions and suggestively indicating the loss of the PDZ domain, didn't show an aggressively deteriorating clinical picture suggesting that loss of phosphatase activity was more significant in manifesting a more progressive and aggressive cancer. It is noteworthy, that the findings of **Gajewski *et al* (2007)** showed that both domains were equally important. Although, this was supportive of the findings of **Vazquez *et al* (2000), Liu *et al* (2007) and Trotman *et al* (2007)** proving the importance of the tyrosine residues at the STT on the C2 domain that play an important role in PTEN activation, still, our findings directed us towards believing the higher importance of the phosphatase domain.

The deteriorating clinical picture of PCa patients m5, m7 and m9 (high Gleason score, elevated PSA, capsular infiltration and metastasis) was consistent with their genetic findings as proved before by **McMenamin *et al* (1999)** among others. The use of PTEN in combination with Gleason scoring, PSA, capsular infiltration and metastasis, was thus considered to provide a useful tool in the tumor prediction, staging and evaluation of PCa progression as suggested by **Koksal *et al* (2004)**, which was contradicted by the study conducted by **Crawford (2003)** that suggested that PSA cannot be used alone or in combination because it is not specific to PCa.

The normal subject samples gave +ve PCR for both exons and both had a family history of neurofibromatoma and breast cancer respectively. The choice of the normal samples was highly consistent will all previous studies indicating the high mutation rate of PTEN in multiple advanced cancers

as those conducted by **Perren *et al* (1999), Podsypanina *et al* (1999), Wappenschmidt *et al* (2004) and Chang et al. (2006)**.

All sequenced samples and their translations were aligned against the reference sequences translations. The discrepancy appearing in terms of protein sequence alignment was very high and suggestive of a more thorough pairwise alignment of each individual sequence against the reference sequence, NG_007466.1.

The results of these alignments showed several mutant variants of PTEN along both exons but not in all samples. Samples that were free of mutant variants from both exons were n6; b2, b3 and b7; m4, m6 and m8. The probability of occurrence of these variants was highest for samples n6, b3, m4, m6 and m8, covering about 38% of the 12 highly occurring genotypes as concluded from the previous alignment and phylogenic data sets analyzed. The b2 and b7 samples although not included in the highest probably occurring samples, yet they share an intermediary evolutionary relationship with b3 of about 40%, and thus their occurrence was also possible but at lower expectations.

The lack of mutations mapped on these fragments has led to many assumptions. Most importantly, the absence of a significant correlation based on the genetic findings between normal, benign and malignant studied groups as supported by clinical evidence of PSA and Gleason scores elevation, which was challenged by **Ørsted *et al* (2011)**, yet consistent with the findings **Kopp *et al* (2011)**, who conducted a study on the association of BPH and PCa by comparing the clinical picture and chronic disease history of the same 3 million Danish patient cohort done by **Ørsted *et al* (2011)** finding no clear significant relation between BPH and PCa progression pointing out to the fact that although diabetes, hypertension, dyslipidemia and atherosclerotic disorders provide a clear understanding of their role in evolving either BPH or PCa, yet, PSA among other clinical parameters cannot be used in defining a relation between them. In another study, **De-Nunzio *et al* (2011)** showed that inflammation, should also be included in the process of defining a correlation between BPH and PCa prognosis.

Another very important assumption was, the inability to correlate between the clinical manifestations of malignancy that these patients suffered and PTEN mutation at exons 5 and 7 and thus directs us towards the believe of presence of LOH (**Li *et al* 1997; Dong *et al* 1998; Dong *et al* 2001; Pourmand *et al* 2007**), microsatellite genetic instability (Latini, 2001) and/or other unmapped mutations on different exons of PTEN (**Dong *et al* 1998; Bonneau and Langy, 2000; Dong *et al* 2001; Pourmand *et al* 2007**) to be the cause of the clinical picture the patients had in addition to other factors involved in the pathogenesis, not including PTEN.

The aggressively deteriorating clinical picture comprising a high Gleason score (9), and a PSA above 100 ng/ml, capsular infiltration and metastasis of patient m4, was suggestive for the presence of the previous reasons as contributing causes of the patients clinical pictures in addition to the assumption of the presence of other genetic factors that were out of the scope of this study, that needed further investigations. The possible use of PTEN as a genetic marker in association with Gleason scores and PSA values to determine the progression of PCa in cancer patients (**Koksal *et al.*2004**) is undermined by these findings as suggested by **Crawford (2003)**.

The probability of occurrence of the mutant variant groups of PTEN represents approximately 62% of the high probability occurring genotypes (n3; b1, b5, b9 and b10; m2, m3 and m10). The rest of the samples which manifested mutations but were not from the high probability occurring genotypes had intermediary occurring possibilities except for 5m1 and 5b8. Sample m1 which was the least likely to occur variant shared an evolutionary relation with m2 and also happens to share a mutation with m2 that has the highest recorded mutation of incidence among all mutant variants, hence justifying the presence as a mutant variant. Samples b4 and b6 shared an intermediary evolutionary relation with b10 and b3 (HPOG), respectively, hence justifying the manifestation of a PTEN mutant variant. Despite being grouped among the least occurring genotypes for both exons, sample b8 exhibited mutations in both exons. The 7b mutant variant was considered, due to the presence of the same mutation in the highly occurring genotype of sample m2.

The evidence from the findings reached so far, provided for the understanding towards PTEN high association with the pathogenesis of BPH and PCa. Although supporting evidence of lack of PTEN mutations point at the association of other PTEN unrelated factors to be of higher significance, owed to the absence of PTEN mutations in 38% of the HPOG which was supported in other studies (**Wang *et al* 1998 and 2003**), but the mutation presence in 62% of the HPOG support the opposite as inferred by **Gray *et al* (1995); Cairns *et al* (1997); Suzuki *et al* (1998) and Haiman *et al* (2006)**. Not only PTEN association can be inferred from that, but also the presence of a relation between BPH and PCa from the mutations found in the 62% mutant variants of the highly occurring genotype, with mutations repeated among both benign and malignant studied groups, genotypically supporting the clinical associative

findings of **Ørsted *et al* (2011)** shedding light on the role of genetic factors towards BPH and PCa association as inferred from correlating clinical pictures and their different parameters.

All 5 potentially significant mutations (g.74482dupA, g.74818dupG, g.74845dupC and g.99506C>G, g.99582dupA) shared the presence of HPOG except for the 7b6, g.99506C>G, substitution that caused the missense of Glutamic acid to be replaced by Aspartic acid (E241D), which might not have a great influence on the pathogenesis caused by PTEN, thus its presence in a LPOG was well justified, yet further 3D protein modeling should be made followed by a protein – protein docking to one of the PTEN counter-partners in order to fully evaluate this mutation.

The g.74482dupA mutation was found among 6 different samples from the 3 different groups, 4 of these samples were among HPOG (n3, b1, m2 and m10). The importance of this mutation was derived from being positioned in the upstream near end site of exon 5 that contains the splice site of exon 5. The frame shift this mutation causes leads to the formation of a stop codon at position 101 replacing Lysine (L101X) causing the formation of a truncated protein. This provided for the importance of this mutation in the progression of PCa and of the correlation between benign and malignant patients, since the mutation was present in 4 patient samples belonging to the 3 different group samples that were found in the highly occurring genotypes.

The g.74818dupG and g.74845dupC mutations that lie downstream near the end site of exon 5 and affect the splicing sites of exon 5, need more experimental evidence to support their effect on the splicing of PTEN and the effect of the alternative splicing on the protein function. The exon 7, g.99582dupA, at the final nucleotide of the exon is believed to cause an in-frame shift of the splicing site downstream that leads to the formation of a new donor splicing site at the end of exon 7 that will lead eventually to the formation of a new protein resulting from the alternative splice, which might be missing a codon at the end of exon 7. This mutation happens to appear in two samples, one of which was sample m2 which was found among the highest occurring genotypes and has a Gleason score of 8 accompanied by capsular infiltration, thus directing us towards the importance of this mutation's occurrence.

Not only do these findings support our believe of the correlation between BPH and PCa pathogenesis and the presence of a genetic predisposition as shown through analyzing the g.74482dupA mutation, but also are further supported through other different findings involving both genetic and clinical associations as shown in previouse studies (**Cairns et al., 1997; Bonneau and Langy, 2000; Haiman et al., 2006; Ørsted et al., 2011**) conducted to identify the significance of clinical correlations that show BPH and PCa association in addition to mutations and the role of PTEN in the pathogenesis of BPH and/or PCa, not to mention much less support to those of **Cooney et al. (1999) and Kopp et al. (2011)**. It is noteworthy, that studies conducted by **Lefebvre (2010)** suggested that PTEN affected the expression of three different peptides that were found in serum, all of which were suggested to undergo further investigations as possible serum markers for the better diagnosis of prostate cancer.

The suggestive introduction of PTEN regulated markers **(Lefebvre, 2010)** also supports this study, however, the use of PTEN in association with Gleason scoring and PSA should also be introduced as a suitable follow up measure and diagnostic protocol for PCa patients as suggested by **Koksal *et al* (2004)**. Further recommendations of increasing the size of the population studied to better correlate BPH and PCa and further identify the importance of the exon 5 g.74482dupA and exon 7 g.99582dupA mutations as early diagnostic markers is thought out for.

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