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Research Square

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Development of a Custom NGS Panel for the Determination of Bladder Cancer Risk

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Research Article

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Abstract

Backgound

Bladder cancer (BCa) is a heterogeneous disease caused by the interaction between environmental and genetic risk factors. The objective of this study was to design a panel that evaluates the role of some selected variants in BCa susceptibility. We are also interested in studying the interaction between environmental and genetic risk factors.

Methods

The case/controls cohort was composed with 249 BCa cases and 255 controls. The designed Bladder cancer hereditary panel (BCHP) is composed of 139 selected variants. These variants were genotyped by an amplification-based targeted Next-Generation Sequencing (NGS) on the Ion Torrent Proton sequencer (Life Technologies, Ion Torrent technology).

Results

We have found that rs162555, rs2228000, rs10936599, rs710521, rs3752645, rs804276, rs4639, rs4881400 and rs288980 were significantly associated with decreased risk of bladder cancer. However the homozygous genotypes for *VPS37C* (rs7104333, A/A), MPG (rs1013358, C/C) genes or the heterozygous genotype for ARNT gene (rs1889740, rs2228099, rs2256355, rs2864873), GSTA4 (rs17614751) and APOBR/IL27 (rs17855750) were significantly associated with increased risk of bladder cancer development compared to reference group (OR=2.53, 2.34, 1.99, 2.00, 2.00, 1.47, 1.96 and 2.27 respectively). We have also found that non–smokers patients harboring heterozygous genotypes for ARNT/rs2864873 (A>G), ARNT/ rs1889740 (C>T) or GSTA4/rs17614751 (G to A) were respectively at 2.775, 3.069 and 6.608 –folds increased risk of Bca development compared to non-smokers controls with wild genotypes. Moreover the *ARNT* CT (rs1889740), *ARNT* CG (rs2228099), *ARNT* TC (rs2864873) and *GSS* GA genotypes were associated with an increased risk of BCa even in absence of professional risk factors. Finally the decision-tree analysis produced a three major BCa class. These three classes were essentially characterized by an intensity of tobacco use more than 20 pack years (PY) and the CYP1A2 (rs762551) genotype.

Conclusions

The determined association between genetic variations in BCa and environmental factors, as well as the effect of studied pathway SNPs in comparison with environmental exposition may provide urologists additional genetic information that may help for clinical assessment and treatment decisions. Nevertheless, the underlying mechanisms through which these genes or SNPs affect the clinical behavior of BCas require further studies.

Highlight

In this study we determined the effect of studied pathway SNPs in comparison with environmental exposition using Next-Generation Sequencing (NGS). We have found that homozygous minor genotype or heterozygous genotype for rs1889740 C/T, rs2228099 C/G, rs2256355 T/C, rs2864873 A/G in ARNT gene (Aryl hydrocarbon receptor nuclear translocator) were significantly associated to increased risk of bladder cancer even in the absence of tobacco risk factors, or professional risk factors. Finally we noticed the importance of tobacco status and the CYP1A2 (rs762551) genotype in the stratification of Tunisian BCa patients.

Background

Bladder cancer (BCa) is a common malignancy of the urinary tract and the ninth most frequent cancer in the world, affecting nearly 3.4 million people with 430 000 new cases diagnosed in 2012 and 165 000 deaths per year (2% of all the cases)[1]. In the few last years, the incidence of BCa was higher in developed countries and some African countries, with an increase in the aggressiveness of the disease and the mortality rate [2]. According to the Cancer Registry of the north of Tunisia (1999–2003), BCa is the most frequent urological cancer in Tunisia in men and in 2018 it became the second malignant tumor with an age-standardized incidence rates of 17.7 and 2.0 per 100 000 in men in women respectively[3]. In 2018, the number of new BCa cases was 1323 [4]. The mean age at diagnosis is 65.9 years, without sex significant difference. At the time of diagnosis, non-muscle-invasive BCa (NMIBC) represents the majority of BCa whereas muscle-invasive BCa (MIBC) represents around 25% of bladder tumors with a 5-year survival rate ranging from 35–70%. NMIBC is usually associated with a favorable prognosis but it is characterized by a variable rate of recurrence and progression depending on tumor characteristics. MIBC is, however, initially aggressive and given the associated high risk of developing a metastatic disease requires a radical treatment when still localized[5].

BCa is a heterogeneous disease associated with many risk factors. The most known risk factor is tobacco smoking, which accounts for the occurrence of up 50% of BCa [6]. Occupational exposure to chemicals, genetic factors, and other environmental factors such as dietary factors, lifestyle, medical condition, fluid intake, also contribute to BCa carcinogenesis, although the evidence of the role of some of these risk factors is still inconclusive [6, 7]. Most carcinogens undergo activation by Phase I enzymes, mostly through oxidation reactions, and detoxification by Phase II enzymes, which is the case for aromatic and heterocyclic amines, well-known BCa carcinogens present in smoking and occupational exposure [8]. Therefore, early studies evaluated whether candidate genetic variants in such metabolic pathways could modify the risk of BCa induced by the above-mentioned carcinogens. For example, the deletion of xenobiotic metabolism gene "*GSTM1* and/or *GSTT1*" characterized as *GSTM1*- and *GSTT1-null* genotypes have been shown to be BCa susceptibility loci in several studies [9, 10]. Moreover, N-acetyltransferase 2 (*NAT2*) and Glutathione-s-transferase M1 (GSTM1) variants were the first described genes showing a gene-smoking interaction associated with increased risk of BCa[11]. Hypothetically the deficiency in xenobiotic metabolizing pathway could result in the accumulation of somatic mutations which should be corrected by DNA-repair pathways. There are five major important DNA-repair pathways consisting of

more than 130 genes: nucleotide excision repair (NER), base excision repair (BER), mismatch repair (MMR), double-strand break repair (DSBR) and transcription-coupled repair (TCR) [12–14]. Among these pathways, NER is the most important DNA repair mechanism responsible for various types of DNA damage consisting of oxidative DNA damage, bulky adducts cross-links, alkylating damage, and thymidine dimmers[15]. Deficient DNA repair capacity is known to be a cancer-predisposing factor. Indeed the presence of some single nucleotide polymorphisms (SNP) in DNA repair genes can impair the function of the repair enzymes, thus reducing DNA Repair Capacity (DRC) and inducing genetic instability [16]. In addition others studies have reported a significant association between variations in gene encoded for cell cycle and/or inflammatory response proteins and bladder development and/or prognosis[17].

All of this reported information highlights the complexity of the mechanisms implicated in the etiology of BCa which warrants further investigation. The goal of this case-control study was to evaluate the implication a selected SNP panel in BCa development of the Tunisian population. We developed an amplification-based targeted Next-Generation Sequencing (NGS) assay to simultaneously genotype 139 polymorphisms in 97 genes, selected according to their eventual implications in the etiology and the prognosis BCa such as those located in genes encoded for enzymes implicated in xenobiotic metabolism, DNA repair, treatment response, and inflammatory reaction process. The design of the panel was done, after consulting published data and available databases (dbSNP, UCSC Genome Browser, and GWAS).

Methods

Study population

A case-control study was conducted in the Urology department of Charles Nicolle Hospital in Tunis (Tunisia) and the Laboratory of Protein Engineering and Bioactive Molecules (LIP-MB 11ES24) of the National Institute of Applied Sciences and Technology of Tunis (INSAT), in collaboration with the Genetic Cancer Susceptibility group (GCS group) of the International Agency for Research on Cancer in Lyon (IARC/WHO). Recruitment was carried out after the agreement of the ethics committee of the Charles Nicolle Hospital and approved by an Ethics Committee (IEC Project No. 17–35) and an MTA (MATERIAL TRANSFER AGREEMENT MTA/ 2017 / IMP / GCS)/ 0356) from IARC.

This study was performed on a cohort of 504 cases (249 BCa patients and 255 controls). A total of 255 control cases, randomly selected from healthy volunteer cases enrolled in the Biochemistry Department, were matched to those of the BCa patients group according to the age range (64.28 ± 11.54), sex, and geographic origin. Epidemiological, clinical and anatomopathological data of BCa patients were collected from the medical records of the urology department and the histological reports from the pathology department of the Charles Nicolle Hospital in Tunis (Table 1). Epidemiological data (age, sex, smoking status) and pathological data (grade, stage, histological type, type, and follow-up of treatment) were recorded and made available for the study (Table 1). Histological reviews of the tumors were performed confirmed by a trained pathologist in urological oncology. The BCa tumors of the 249 patients were classified as 45 Specimens of High-Grade (HG) NMIBC, 119 specimens of Low-Grade (LG) NMIBC, and 66 specimens of MIBC.

Table 1: Clinical and Epidemiological characteristics of bladder cancer patients

Clinical and epidemiological parameters	Bladder cancer patients	Controls
Samples sizes	249	255
Male	224 (89.96%)	196 (76.86%)
Female	25 (10.04%)	59 (23.14%)
Mean Age at diagnosis (years)	68.18 ± 12.80	64.28 ± 11.54
Smoking status		
Smokers	199 (79.91%)	138 (54.12%)
Non smokers	27 (10.84%)	117 (45.88%)
ND	23 (9.25%)	0 (00.00%)
Number of pack/years		
< 20 PY	31 (15.58%)	70 (50.72%)
≥ 20 PY	168 (84.42%)	68 (49.28%)
Exposure to professional risk factors (farmer, painter, building, chemical factory…)		
Not exposed	119 (47.80%)	225 (88.24%)
Exposed	83 (33.33%)	30 (11.76%)
ND	47 (18.87%)	0 (00.00%)
TNM classification		
LG NMIBC	119 (47.80%)	-
HG NMIBC	45 (18.07%)	-
MIBC	66 (27.50%)	-
ND	19 (07.63%)	

ND: Not Determined; PY: Packet per Year; LG: Low Grade; HG: Haut Grade; MIBC: Muscle Invasive Bladder Cancer; NMIBC: Non-Muscle Invasive Bladder Cancer

Biological Samples And Dna Extraction

Peripheral blood samples were collected into tubes with ethylene diamine tetra-acetic acid EDTA (PH 8). Genomic DNA was extracted from leukocytes using a phenol /chloroform procedure. First, the integrity of the genomic DNA was visualized by electrophoresis on a 1% agarose gel stained with ethidium bromide. In a second step, DNA samples were quantified by Nanodrop and Qubit (High Sensitivity HS / Broad Rang BR) and were dried with the speedvac (Eppendorf™ Vacufuge™ Concentrator) to be normalized into 6*96-well plates.

Panel Design

We have selected a panel of 139 polymorphisms from 97 genes. These SNP were selected according to their eventual implications in poor-prognosis BCa such as those affecting the xenobiotic metabolism, DNA repair, treatment response, and inflammatory reaction. The design of the panel was done, after consulting published data and available databases (dbSNP, UCSC Genome Browser, and GWAS).

In a second step, the implication of the SNPs selected in our SNP panel in BCa susceptibility was confirmed by the following tools: Kyoto Encyclopedia of Genes and Genomes (KEGG) available on the website https://www.genome.jp/kegg/pathway.html [18, 19], Panther classification system available on the website (

http://pantherdb.org/tools/gxIdsList.do?list=upload_1&organism=Homo%20sapiens)[20], STRING database available on the website https://string-db.org/ ,AmiGO2[21], Reactome Pathway Database[22] and GWAS [23].In addition, we constructed candidate pathways based on our defined panel consisting of 97 genes. The pathways were defined according to the STRING protein–protein interaction networks. A complete list of the studied variants was summarized in Table 2. Pathways and protein-protein interaction, in which the studied genes were implicated, were presented and described in Fig. 2.

Table 2
A comprehensive list of all regions targeted by the Tunisian bladder cancer hereditary panel and Allelic Association (BCHP panel)

CHR	SNP	GENES	VARIATION	Reference variant	Mutated variant	Major allele /Minor allele	Major.allele.freq	Allelic A
								p-value.lo additive
1	rs2020902	CASP9	splice_region_variant,intron_variant	A	G	A/G	82,5	0,873
1	rs2647396	BCL10	intron_variant	C	T	T/C	65,3	0,685
1	rs560018	GSTM4	intron_variant	T	C	T/C	82,8	0,938
1	rs11101992	GSTM5	intron_variant,non_coding_transcript_variant	A	C	A/C	65,9	0,596
1	rs4970774	GSTM5	intron_variant,non_coding_transcript_variant	A	C	C/A	50,1	0,754
1	rs4970776	GSTM5	intron_variant,non_coding_transcript_variant	T	A	T/A	53,3	0,87
1	rs1571858	GSTM3	intron_variant	C	T	C/T	75,9	0,888
1	rs15864	EPS8L3	3_prime_UTR_variant	G	C	G/C	81,6	0,947
1	rs3136701	CD2	downstream_gene_variant	G	C	C/G	52,8	0,79
1	rs1889740	ARNT	intron_variant	C	T	C/T	57,8	0,207
1	rs2228099	ARNT	synonymous_variant	C	G	C/G	57,4	0,203
1	rs1027699	ARNT	intron_variant	T	C	T/C	64,8	0,937
1	rs2256355	ARNT	intron_variant	T	C	T/C	56,7	0,181
1	rs2864873	ARNT	intron_variant	A	G	A/G	64,1	0,78
1	rs763110	FASLG	upstream_gene_variant	C	T	T/C	58,6	0,998
1	rs228001	ASTN1	intron_variant,non_coding_transcript_variant	C	A	C/A	96	0,937
1	rs1800890	IL10	upstream_gene_variant	A	T	A/T	74,9	0,886
1	rs2854461	EPHX1	intron_variant	C	A	C/A	73,8	0,223
1	rs1051740	EPHX1	missense_variant	T	C	T/C	72,5	0,66
1	rs2260863	EPHX1	intron_variant	G	C	C/G	64,9	0,89
1	rs1805410	PARP1	intron_variant	T	C	T/C	82,7	0,432
2	rs391835	AC012593.1/CCR2	intron_variant,non_coding_transcript_variant	G	A	A/G	53,5	0,93
2	rs1056836	CYP1B1/RMDN2	downstream_gene_variant	G	C	C/G	56,1	0,618
2	rs162555	CYP1B1	upstream_gene_variant	T	C	T/C	76,1	0,017
2	rs3771171	IL18R1	intron_variant	T	C	T/C	80	0,165

Chr: Chromosome; SNP: Single Nucleotide Polymorphism ; P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval

CHR	SNP	GENES	VARIATION	Reference variant	Mutated variant	Major allele /Minor allele	Major.allele.freq	Allelic A
2	rs11892031	UGT1A10	intron_variant	A	C	A/C	90,6	0,213
3	rs2228000	XPC	missense_variant	G	A	G/A	81,5	0,024
3	rs1050450	RHOA	downstream_gene_variant	G	A	G/A	74,6	0,953
3	rs10936599	ACTRT3	upstream_gene_variant	C	T	C/T	85,2	0,004
3	rs710459	MASP1	downstream_gene_variant	G	A	G/A	71,6	0,968
3	rs710521	TP63	regulatory_region_variant	T	C	T/C	76,7	0,035
4	rs798766	TACC3	intron_variant	T	C	C/T	69,7	0,836
4	rs4073	IL8	upstream_gene_variant	A	T	A/T	55,3	0,83
4	rs2227306	IL8	intron_variant	C	T	C/T	68,5	0,428
4	rs3804099	TLR2	synonymous_variant	T	C	T/C	53	0,259
4	rs3087455	CASP3	intron_variant	T	G	T/G	65	0,4
5	rs34847072	AHRR	3_prime_UTR_variant	G	C	G/C	82,9	0,106
5	rs401681	TERT/CLPTM1L	intron_variant	C	T	T/C	53,8	0,415
5	rs2070874	IL4	5_prime_UTR_variant	C	T	C/T	80,9	0,455
6	rs4510656	CDKAL1	intron_variant	C	A	C/A	61,9	0,103
6	rs451774	GPX5	3_prime_UTR_variant	A	G	A/G	60,5	0,377
6	rs8193036	IL17A	upstream_gene_variant	C	T	T/C	74,3	0,9
6	rs2275913	IL17A	upstream_gene_variant	G	A	G/A	79,8	0,207
6	rs2180314	GSTA2	missense_variant	C	G	G/C	54,9	0,398
6	rs2144698	GSTA2	intron_variant	T	G	G/T	79,5	0,679
6	rs17614751	GSTA4	downstream_gene_variant	G	A	G/A	95,4	0,029
6	rs367836	GSTA4	downstream_gene_variant	G	T	T/G	59,7	0,764
6	rs150126	MAP3K7	intron_variant	C	T	T/C	74,9	0,999
6	rs4880	SOD2	missense_variant	A	G	A/G	53	0,406

Chr: Chromosome; SNP: Single Nucleotide Polymorphism ; P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval

CHR	SNP	GENES	VARIATION	Reference variant	Mutated variant	Major allele /Minor allele	Major.allele.freq	Allelic A
7	rs1062492	SNX8	downstream_gene_variant	C	T	C/T	79,8	0,234
7	rs2017000	EGFR	intron_variant	A	G	A/G	74,5	0,139
7	rs1140475	EGFR	synonymous_variant	T	C	C/T	92,3	0,59
7	rs2293347	EGFR	synonymous_variant	C	T	C/T	96	0,698
7	rs1045642	ABCB1	synonymous_variant	A	G	G/A	69,1	0,12
7	rs1858923	ABCB1	intron_variant	A	G	A/G	61,8	0,611
7	rs2740574	CYP3A4	upstream_gene_variant	C	T	T/C	79,4	0,129
7	rs11773597	CYP3A4	upstream_gene_variant	G	C	G/C	92,3	0,247
7	rs3752645	PRKAR2B	intron_variant	G	A	G/A	90,9	0,023
8	rs804276	NEIL2	upstream_gene_variant	G	A	G/A	67,4	0,03
8	rs8191604	NEIL2	intron_variant	T	G	T/G	69,3	0,409
8	rs1874546	NEIL2	intron_variant	C	G	C/G	85,2	0,769
8	rs4639	NEIL2	3_prime_UTR_variant	A	G	A/G	65,4	0,009
8	rs1961456	NAT2	intron_variant	A	G	A/G	76,8	0,952
8	rs1799929	NAT2	synonymous_variant	C	T	C/T	54,3	0,374
8	rs1799930	NAT2	missense_variant	G	A	G/A	70,9	0,17
8	rs1799931	NAT2	missense_variant	G	A	G/A	97,3	0,814
8	rs12674710	NAT2	downstream_gene_variant	A	C	A/C	89,8	0,538
8	rs1495741	NAT2	regulatory_region_variant	G	A	A/G	79,6	0,509
8	rs13278062	TNFRSF10A	upstream_gene_variant	G	T	G/T	55,7	0,633
8	rs1126452	EPHX2	synonymous_variant	A	C	A/C	69,9	0,325
8	rs3136717	POLB	intron_variant	C	T	T/C	71,6	0,439
8	rs17226566	LY96	intron_variant	T	C	T/C	84,3	0,611
8	rs9642880	CASC11	intron_variant,non_coding_transcript_variant	G	T	T/G	50,3	0,405
8	rs2294008	PSCA	5_prime_UTR_variant	C	T	T/C	54,4	0,426
10	rs1937845	AKR1C3	5_prime_UTR_variant	A	G	G/A	52,8	0,886
10	rs3763676	AKR1C3	5_prime_UTR_variant	A	G	A/G	72	0,289

Chr: Chromosome; SNP: Single Nucleotide Polymorphism ; P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval

CHR	SNP	GENES	VARIATION	Reference variant	Mutated variant	Major allele /Minor allele	Major.allele.freq	Allelic A
10	rs12529	**AKR1C3**	missense_variant	C	G	**G/C**	52,8	0,886
10	rs1937843	**AKR1C3**	intron_variant	A	G	A/G	72	0,289
10	rs4881400	**AKR1C3**	intron_variant	T	G	T/G	76,5	0,029
10	rs12775701	**AKR1C3**	intron_variant	A	G	**G/A**	59,3	0,665
10	rs2475377	**CYP2C9**	intergenic_variant	C	T	C/T	87,3	0,283
10	rs9332197	**CYP2C9**	intron_variant	T	C	T/C	98,2	0,132
10	rs12357751	**BLNK**	intron_variant	C	T	C/T	73,5	0,66
10	rs3789928	**BLNK**	intron_variant	G	C	G/C	52,8	0,057
10	rs2031920	**CYP2E1**	upstream_gene_variant	C	T	C/T	97,9	0,249
10	rs915908	**CYP2E1**	intron_variant	G	A	G/A	84,5	0,869
11	rs7104333	**VPS37C**	downstream_gene_variant	G	A	G/A	52,5	0
11	rs625978	**GSTP1**	regulatory_region_variant	C	T	C/T	61,5	0,73
11	rs1695	**GSTP1**	missense_variant	A	G	A/G	62,5	0,376
12	rs3213427	**CD4**	3_prime_UTR_variant	T	C	T/C	64,2	0,761
12	rs11046349	**AICDA**	3_prime_UTR_variant	T	G/A	T/G	73,6	0,283
12	rs10878176	**TBK1**	intron_variant	G	C	G/C	71,9	0,807
12	rs1866074	**TDG**	intron_variant	A	G	A/G	59,4	0,247
12	rs3890995	**UNG**	upstream_gene_variant	T	C	T/C	81,4	0,796
12	rs4765621	**SCARB1**	intron_variant	C	T	C/T	51,6	0,155
13	rs1050112	**PARP4**	missense_variant	G	T	G/T	50,6	0,164
13	rs13428	**PARP4**	missense_variant	C	G	C/G	58,8	0,131
13	rs17655	**ERCC5**	missense_variant	G	C	G/C	67,7	0,887
14	rs2228026	**TEP1**	synonymous_variant	A	G	A/G	94,4	0,475
14	rs1130409	**OSGEP**	upstream_gene_variant	T	G	T/G	53,8	0,978
14	rs696	**NFKBIA**	3_prime_UTR_variant	C	T	C/T	52,5	0,236
14	rs10133290	**CHURC1**	downstream_gene_variant	A	C	A/C	72,7	0,797
14	rs7101	**FOS**	5_prime_UTR_variant	C	T	**T/C**	81,8	0,698

Chr: Chromosome; SNP: Single Nucleotide Polymorphism ; P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval

CHR	SNP	GENES	VARIATION	Reference variant	Mutated variant	Major allele /Minor allele	Major.allele.freq	Allelic A
14	rs861539	**KLC1**	intron_variant	G	A	G/A	60,8	0,985
15	rs1048943	**CYP1A1**	missense_variant	T	C	T/C	96,7	0,587
15	rs2472299	**CYP1A1**	intergenic_variant	A	G	**G/A**	58,6	0,601
15	rs762551	**CYP1A2**	intron_variant	C	A	**A/C**	57,6	0,901
15	rs976072	**POLG**	downstream_gene_variant	A	G	A/G	71,1	0,393
16	rs1013358	**MPG**	intron_variant	C	T	**T/C**	68,1	0,01
16	rs1799801	**ERCC4**	synonymous_variant	T	C	T/C	67,7	0,044
16	rs17855750	**APOBR/IL27**	downstream_gene_variant	A	C	A/C	96,1	0,021
16	rs153109	**IL27**	upstream_gene_variant	T	C	T/C	75,6	0,632
16	rs1800566	**NQO1**	missense_variant	G	A	G/A	73,8	0,507
16	rs689452	**NQO1**	intron_variant	C	G	**G/C**	86,1	0,61
16	rs899729	**IL17C**	upstream_gene_variant	C	A	C/A	67,3	0,598
17	rs4791489	**MAP2K4**	downstream_gene_variant	C	T	C/T	74,7	0,251
17	rs4796030	**LIG3**	3_prime_UTR_variant	A	C	**C/A**	52,6	0,525
17	rs2333227	**MPO**	upstream_gene_variant	C	T	C/T	68,7	0,275
17	rs7209435	**MAP3K3**	intron_variant	T	C	T/C	64,5	0,09
17	rs11655650	**BIRC5**	intron_variant	C	T	C/T	56,9	0,485
18	rs288980	**ROCK1**	intron_variant	T	C	**C/T**	71,4	0,03
18	rs10775480	**SLC14A1**	intron_variant	T	C	**C/T**	61	0,363
18	rs10853535	**SLC14A1**	intron_variant	C	T	**T/C**	61,3	0,479
18	rs723279	**SOCS6**	intron_variant	G	A	G/A	69,1	0,963
19	rs3746162	**SBNO2**	intron_variant	C	T	C/T	77,5	0,392
19	rs5498	**ICAM5**	upstream_gene_variant	A	G	A/G	62,8	0,664
19	rs25487	**XRCC1**	missense_variant	T	C	**C/T**	56,7	0,969
19	rs1799782	**XRCC1**	missense_variant	G	A	G/A	94,3	0,765
19	rs13181	**ERCC2**	downstream_gene_variant	T	G	T/G	65,9	0,092
20	rs7265992	**GSS**	intron_variant	G	A	G/A	90,4	0,148

Chr: Chromosome; SNP: Single Nucleotide Polymorphism ; P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval

CHR	SNP	GENES	VARIATION	Reference variant	Mutated variant	Major allele /Minor allele	Major.allele.freq	Allelic A
20	rs6088662	**GSS**	upstream_gene_variant	T	G	T/G	69,9	0,091
20	rs7260770	**GSS**	intergenic_variant	G	A	G/A	88,6	0,046
21	rs2173962	**AP000253.1**	downstream_gene_variant	T	C	T/C	96,6	0,943
21	rs2839488	**TFF1**	intron_variant	G	C	G/C	50,4	0,904
22	rs4633	**COMT**	synonymous_variant,NMD_transcript_variant	C	T	C/T	57,9	0,152
22	rs4680	**COMT**	missense_variant,NMD_transcript_variant	G	A	G/A	58	0,081
22	rs1006771	**DDTL**	3_prime_UTR_variant	G	T	T/G	74,3	0,177
22	rs1014971	**GSTT2**	regulatory_region_variant	C	T	**T/C**	69,1	0,526
22	rs5751222	**CYP2D6**	downstream_gene_variant	T	A	T/A	87,7	0,823

Chr: Chromosome; SNP: Single Nucleotide Polymorphism ; P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval

Snp Genotyping

SNP genotyping was performed by NGS on the Ion Torrent Proton sequencer (Life Technologies, Ion Torrent technology). The GeneRead DNAseq Panel PCR Kit V2 (Qiagen) was used for the amplification of the targeted SNPs. The Gene Readprimers provided in different three pools. The samples were all re-quantified by Picogreen (using Fluoroscan) and normalized to 5ng/ul and 20 ng of each sample was plated into 3 daughter plates for the analysis of each group of primers. A validated in-house protocol was used to perform multiplex PCRs in 10 µL reaction volumes, containing 20 ng DNA, 60 nM of each primer pool and 0.73 µL of HotStarTaq enzyme. Amplification was carried out in a 96-well format plates using the standard conditions recommended by Qiagen: 15 min at 95°C and 22 cycles of 15 s at 95°C and 4 min at 60°C and 10 min at 72°C. Amplification products from the same sample were pooled purified using paramagnetic Serapure beads at 1.8X and quantified with the Qubit DNA high-sensitivity assay kit (Invitrogen Corporation). Barcoded libraries were prepared from 10ul of purified products and as previously described [24]. For template preparation, the barcoded libraries were pooled in equimolar concentrations and 7 µL of 100 pM was subsequently used for emulsion PCR (emPCR) using the Ion Torrent Template Ion PI Hi-Q OT2 200 kit on the Ion OneTouch2 Instrument (Thermo Fisher Scientific). The sequencing reaction was performed on an Ion Proton System using Life Technologies' Ion PI™ Chip Kit v3 and Ion PI™ Hi-Q™ Sequencing 200 Kit (Thermoscience Fisher Scientific) **(**Fig. 3**).**

Bioinformatics Analyses

Primary analyses were done using the Torrent_Suite 5.6.0 and included signal processing, base calling, reads alignment to the human genome reference 19 (Genome Reference Consortium GRCh37), quality control of mapping and coverage analysis, and variant calling. Subsequently, a list of detected sequence variants, including SNPs and small insertions/deletions, was imported into (Thermo Fisher Scientific) for annotation. The GeneRead data analysis workflow runs the GATK Variant Annotator program using the TVC output to populate the INFO field in the VCF file with the parameters necessary for downstream filtering. The INFO field of the GATK Unified Genotyper output already contains all the necessary parameters. Alignments were visually verified with the Integrative Genomics Viewer (IGV) v2.3. A combination of SnpEff v4.3, SnpSift v4.3 were used for annotation and filtration, and PLINK and in-house algorithms (R packages …) were used for analysis. QC filtered variants were then merged, annotated, and filtered using various databases including, ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), HGMD (http://www.hgmd.cf.ac.uk/ac/index.php), and dbSNP (https://www.ncbi.nlm.nih.gov/snp/).

Results

All assays were performed using DNA samples from a total of 544 cases and controls and duplicated samples for quality control. Only 40 samples (40/544; 7.35%) failed the library preparation and were excluded from the analysis. In this series, two runs led to 75% and 88% ISP loading and to the generation of total bases of 4.33 and 11.4 Gigabases for total bases of 33, 329, 31 and 84,548,378 respectively, 99% of which aligned to the reference genome (Hg19) **(**Fig. 4**).** This analysis provided high analytical sensitivity and allowed detecting SNPs. Using this approach, we have generated molecular profiles of a large number of individuals and identified specific BCa variations.

Among the 139 retained SNP (Table 2) nine minor alleles were significantly inversely associated with bladder cancer development [**rs162555** (CYP1B1*C), **rs2228000** (XPC*A), **rs10936599** (ACTRT3*T), **rs710521** (TP63*C), **rs3752645** (PRKAR2B*A), **rs804276** and **rs4639** (NEIL2*A and NEIL2*G), **rs4881400** (AKR1C3*G) and **rs288980** (ROCK1*T)]. When we used the co-dominant model (the reference group is composed of subjects with homozygous genotype for major allele) we also found a statistically significant inverse association for heterozygous genotypes of *ACTRT3* (rs10936599), *TP63* (rs710521), *CYP3A4* (**rs2740574**), *BLNK* (**rs3789928**) and *COMT* (**rs4680**) (OR1 = 0.51, 0.62, 0.61, 0.55, and 0.66 respectively) or homozygous genotypes of *CYP1B1* (rs162555), *XPC* (**rs2228000**), *NEIL2* (**rs4639**), *NAT2* (**rs1799930**), *AKR1C3* (**rs4881400**) and *ROCK1* (**rs288980**) (OR2 = 0.36, 0.12, 0.49, 0.38, 0.4 and 0.42 respectively) (Table 3**).** However six minor alleles in six genes were significantly associated with increased risk of bladder cancer development: *MPG* **(rs1013358 T > C)**, *GSTA4* (**rs17614751 G > A**); *GSS* **(rs7260770 G > A); *VPS37C/***CD5 **(rs7104333 G > A)**; *APOBR/ IL27* **(rs17855750 A > C)**; and *ERCC4* **(rs1799801 T > C)**. These aggravating effects were attributed to homozygous genotypes for *VPS37C* (**rs7104333**, A/A) or MPG (**rs1013358**, C/C) genes (OR2 = 2.53 and 2.34 respectively) or to the heterozygous genotype for ARNT gene (rs1889740, rs2228099, rs2256355, rs2864873), GSTA4 (rs17614751) and APOBR/IL27 (rs17855750) with OR1 respectively estimated at 1.99, 2.00, 2.00, 1.47, 1.96 and 2.27 compared to reference group. When comparing the distribution of these unfavorable genotypes according to exposition to environmental risk factors (Tables 4 and 5), we have found that non–smokers patients harboring heterozygous genotypes for ARNT/rs2864873 A > G, ARNT/ rs1889740 (C > T) or GSTA4/rs17614751 (G to A) were respectively at 2.775, 3.069 and 6.608 – folds increased risk of Bca development compared to non-smokers controls with wild genotypes. When we analyzed genotypic distribution in heavy smokers (BCa and Controls smoking more than 20PY) we found that smoker's patients harboring *ARNT* CT (rs1889740), *ARNT* CG (rs2228099), *ARNT* TC (rs2864873) or *ROCK1* TC genotypes were respectively associated with 2.690, 2.864, 2.779 and 3.00 fold-increased risk of BCa. In the other hand we have found that *ARNT* CT (rs1889740), *ARNT* CG (rs2228099), *ARNT* TC (rs2864873) and *GSS* GA genotypes were associated with an increased risk of BCa even in absence of professional risk factors (comparison of Non-exposed patients to Non-exposed controls). However when we compare genotypic distribution of unfavorable genotypes in exposed patients and controls according to professional risk factor, we observed that *ARNT* TT (rs1889740), *ARNT* GG (rs2228099), *ARNT* CC (rs2864873), *ROCK1* CC/CT genotypes were respectively associated with 12.000, 12.666, 12.666, 3.976, 3.960 fold-increased risk of BCa development.

Table 3
Comparison of genotypic distribution between case and control groups

					Dominant model (DM)				Recessive model (RM)				Co-dominant mode			
Gene	SNP	Raisonnement/	Alleles	MjAF	p-value	OR	(95% CI)		p-value	OR	(95% CI)		p-value ¥	OR_{CD1}	(95% CI)*	
ARNT	rs1889740	T	C > T	C	0,00	1,7	1,17	2,48	0,26	0,77	0,49	1,21	0,00	1,99	1,34	2,
ARNT	rs2228099	G	C > G	C	0,00	1,72	1,18	2,5	0,26	0,77	0,49	1,21	0,00	2	1,34	2,
ARNT	rs2256355	C	T > C	T	0,00	1,73	1,19	2,53	0,31	0,8	0,51	1,24	0,00	2	1,34	2,
ARNT	rs2864873	G	A > G	A	0,16	1,29	0,9	1,84	0,14	0,68	0,41	1,14	0,05	1,47	1,01	2,
CYP1B1	rs162555	C	T > C	T	0,06	0,72	0,5	1,02	0,02	0,39	0,17	0,9	0,03	0,79	0,55	1,
XPC	rs2228000	A	G > A	G	0,19	0,78	0,54	1,13	0,00	0,12	0,03	0,53	0,00	0,92	0,62	1,
ACTRT3	rs10936599	T	C > T	C	0,00	0,52	0,35	0,79	0,62	0,76	0,26	2,23	0,01	0,51	0,34	0,
TP63	rs710521	C	T > C	T	0,01	0,64	0,44	0,91	0,89	0,95	0,44	2,06	0,04	0,62	0,42	0,
GSTA4	rs17614751	A	G > A	G	0,02	2,11	1,1	4,04	0,24	NA	0	NA	0,03	1,96	1,02	3,
CYP3A4	rs2740574	C	**C > T**	**T/C**	0,03	0,67	0,46	0,96	0,35	1,51	0,63	3,59	0,02	0,61	0,42	0,
NEIL2	rs804276	A	G > A	G	0,03	0,67	0,47	0,96	0,25	0,73	0,42	1,26	0,08	0,69	0,48	1
NEIL2	rs4639	G	A > G	A	0,03	0,68	0,48	0,97	0,03	0,56	0,33	0,94	0,03	0,76	0,52	1,
NAT2	rs1799930	A	G > A	G	0,86	0,97	0,68	1,37	0,00	0,36	0,18	0,74	0,01	1,14	0,79	1,
AKR1C3	rs4881400	G	T > G	T	0,11	0,75	0,52	1,07	0,02	0,42	0,2	0,91	0,05	0,84	0,58	1,
BLNK	rs3789928	C	G > C	G	0,01	0,58	0,39	0,86	0,78	0,94	0,62	1,43	0,02	0,55	0,36	0,
VPS37C	rs7104333	A	G > A	G	0,01	1,73	1,17	2,55	0,00	2,03	1,34	3,09	0,00	1,42	0,94	2,
MPG	rs1013358	C	**C > T**	**T/C**	0,09	1,36	0,95	1,92	0,00	2,2	1,26	3,84	0,01	1,15	0,79	1,
ERCC4	rs1799801	C	T > C	T	0,15	1,3	0,91	1,84	0,05	1,8	1	3,22	0,09	1,18	0,81	1,
APOBR	rs17855750	C	A > C	A	0,01	2,36	1,16	4,79	0,49	NA	0	NA	0,02	2,27	1,11	4,
ROCK1	rs288980	T	**T > C**	**C /T**	0,21	0,8	0,56	1,13	0,01	0,43	0,23	0,8	0,02	0,94	0,65	1,
GSS	rs7260770	A	G > A	G	0,03	1,62	1,05	2,5	0,97	1,02	0,25	4,14	0,08	1,67	1,07	2,
COMT	rs4633	T	C > T	C	0,03	0,67	0,46	0,96	0,99	1	0,64	1,57	0,07	0,63	0,42	0,
COMT	rs4680	A	G > A	G	0,01	0,63	0,44	0,92	0,83	0,95	0,61	1,49	0,04	0,6	0,41	0,

P: P value; OR: Odds ratio; 95% CI: Confidence Interval, OR_{CD1}: Heterozygous genotype VS Homozygous Wild genotype; OR_{CD2}: Mutant homozygous genotype genotype; p-value ¥: P value for co-dominant model

Table 4
Comparison of genotypes distribution of significant unfavorable SNP from "Tunisian BCHP" panel in bladder cancer p

	Genotypes	Controls				Bladder cancer				Bladder cancer (NE) Vs Controls (NE)		Bladder cancer (E) Vs Controls (E)		Bladd Contr
Gene/Variant														
		NE	< 20PY	≥ 20PY	E	NE	< 20PY	≥ 20PY	E	p	OR (95% CI)	p	OR (95% CI)	P
ARNT rs1889740 C > T														
	CC	44	24	34	58	5	8	50	58	,	1*	,	1*	,
	CT	43	35	23	58	15	19	91	110	0.044	3.069 (1.026–9.184)	0.009	1.896(1.169–3.075)	0.013
	TT	30	11	11	22	7	4	27	31	0.254	2.053(0.595–7.080)	0.305	1.409(0.730–2.716)	0.453
ARNT rs2228099 C > G														
	CC	43	23	34	57	5	8	48	56	,	1*	,	1*	,
	CG	44	36	23	59	15	19	93	112	0.054	2.931(0.979–8.771)	0.007	1.932(1.189–3.138)	0.013
	GG	30	11	11	22	7	4	27	31	0.270	2.006(0.581–6.925)	0.283	1.434(0.741–2.772)	0.478
ARNT rs2256355 T > C														
	TT	30	22	33	55	5	8	46	54	,	1*	,	1*	,
	TC	44	36	24	60	15	19	93	112	0.207	2.045(0.671–6.228)	0.010	1.901(1.165-3.100)	0.230
	CC	43	12	11	23	7	4	29	33	0.970	0.976(0.283–3.371)	0.253	1.461(0.761–2.803)	0.008
ARNT rs2864873 A > G														
	AA	51	26	37	63	7	10	71	81	,	1*	,	1*	,
	AG	42	36	23	59	16	17	79	96	0.040	2.775(1.044–7.377)	0.317	1.265(0.797–2.008)	0.157
	GG	24	8	8	16	4	4	18	22	0.773	1.214(0.324–4.549)	0.855	1.069(0.5189 to 2.204)	0.111
CYP1B1 rs162555 (T to C)														
	TT	63	38	36	74	18	16	106	122	,	1*	,	1*	,
	TC	47	24	27	51	7	12	60	72	0.179	0.521 (50.201–1.349)	0.509	0.856 (0.540–1.357)	0.335
	CC	7	8	5	13	2	3	2	5	1.000	1,000 (0.190–5.240)	0.007	0.233 (0.079–0.680)	0.099
XPC rs2228000 (G to A)														
	GG	72	43	42	85	22	20	114	134	,	1*	,	1*	,
	GA	37	19	20	39	5	11	52	63	0.127	0.442 (0.154–1.262)	0.921	1.024 (0.632–1.661)	0.725

P: P value; OR: Odds ratio; 95% CI: Confidence Interval; NE: Non Smoker; E: Smoker

	Genotypes	Controls				Bladder cancer				Bladder cancer (NE) Vs Controls (NE)		Bladder cancer (E) Vs Controls (E)		Bladd Contr
Gene/Variant														
	AA	2	8	6	14	0	0	2	2	0.779	0.644 (0.029–13.924)	0.001	0.090 (0.020–0.408)	0.538
TP63, P3H2 rs710521 T/C														
	TT	55	46	35	81	16	23	107	130	,	1*	,	1*	,
	TC	56	21	28	49	9	7	53	60	0.195	0.552 (0.225–1.355)	0.257	0.763 (0.477–1.218)	0.001
	CC	6	3	5	8	2	1	8	9	0.874	1.145 (0.210–6.237)	0.482	0.701 (0.259–1.890)	0.409
ACTRT3/MYNN rs10936599 (C to T)														
	CC	76	51	44	95	18	24	135	159	,	1*	,	1*	,
	CT	39	18	19	37	9	5	29	34	0.9543	0.974 (0.400-2.369)	0.026	0.549 (0.323–0.933)	0.001
	TT	2	1	5	6	0	2	4	6	0.903	0.827 (0.038–17.969)	0.384	0.597 (0.187–1.905)	0.663
GSTA4 rs17614751(G to A)														
	GG	114	63	63	126	23	31	168	199	,	1*	,	1*	,
	GA	3	7	5	12	4	1	17	18	0.017	6.608 (1.385–31.530)	0.894	0.949 (0.442–2.038)	0.051
	AA	0	0	0	0	0	0	2	2	0.431	4.872 (0.094-251.797)	0.457	3.170 (0.151–66.582)	0.497
CYP3A4 rs2740574 C > T														
	CC	5	3	1	4	3	3	5	8	,	1*	,	1*	,
	CT	46	23	28	51	2	8	50	58	0.010	0.072(0.009–0.542)	0.379	0.568(0.161-2.000)	0.693
	TT	66	44	39	83	22	20	113	133	0.445	0.555(0.122–2.516)	0.724	0.801(0.233–2.744)	0.695
PRKAR2B rs3752645 (G to A)														
	GG	88	57	58	115	22	25	149	174	,	1*	,	1*	,
	GA	26	10	10	20	5	6	19	25	0.629	0.769 (0.265–2.231)	0.554	0.826 (0.438–1.556)	0.019
	AA	2	3	0	3	0	0	0	0	0.878	0.786 (0.036–16.971)	0.119	0.094 (0.004–1.847)	0.141
NEIL2 rs804276 G/A														
	GG	39	37	30	67	18	17	82	99	,	1*	,	1*	,
	GA	59	25	31	56	7	12	67	79	0.005	0.257 (0.098–0.672)	0.844	0.954 (0.601–1.515)	0.012

P: P value; OR: Odds ratio; 95% CI: Confidence Interval; NE: Non Smoker; E: Smoker

	Genotypes	Controls				Bladder cancer				Bladder cancer (NE) Vs Controls (NE)		Bladder cancer (E) Vs Controls (E)		Bladd Contr
Gene/Variant														
	AA	19	8	7	15	2	2	19	21	0.063	0.228 (0.047– 1.085)	0.885	0.947 (0.455– 1.969)	0.024
NEIL2 rs4639 A/G														
	AA	37	36	29	65	16	15	82	97	,	1*	,	1*	,
	AG	58	23	28	51	8	13	68	81	0.017	0.319 (0.124– 0.819)	0.795	1.064 (0.664– 1.703)	0.014
	GG	22	11	11	22	3	3	18	21	0.091	0.315 (0.082– 1.205)	0.194	0.639 (0.325– 1.256)	0.005
NAT2 rs1799930 G > A														
	GG	56	38	32	70	11	19	83	102	,	1*	,	1*	,
	GA	46	26	28	54	14	10	78	88	0.329	1.549(0.642– 3.738)	0.630	1.118(0.709– 1.763)	0.842
	AA	15	6	8	14	2	2	7	9	0.637	0.678(0.135– 3.398)	0.071	0.441(0.181– 1.075)	0.014
AKR1C3 rs4881400 T > G														
	TT	63	37	43	80	18	22	101	123	,	1*	,	1*	,
	TG	41	28	20	48	7	9	61	70	0.291	0.597(0.229– 1.556)	0.822	0.948(0.597– 1.506)	0.592
	GG	13	5	5	10	2	0	6	6	0.442	0.538(0.111– 2.609)	0.079	0.390(0.136– 1.115)	0.005
BLNK rs3789928 G/C														
	GG	24	18	16	34	8	11	55	66	,	1*	,	1*	,
	GC	59	37	42	79	17	13	70	83	0.767	0.864 (0.329– 2.269)	0.019	0.541 (0.323– 0.906)	0.022
	CC	36	15	10	25	2	7	43	50	0.031	0.166 (0.032– 0.853)	0.926	1.030 (0.546– 1.941)	0.034
VPS37C/CD5 rs7104333 G > A														
	GG	18	14	14	28	10	11	53	64	,	1*	,	1*	,
	GA	53	32	35	67	11	14	76	90	0.055	0.373(0.136– 1.025)	0.056	0.587(0.340– 1.013)	0.020
	AA	46	24	19	43	6	6	49	45	0.013	0.234(0.074– 0.741)	0.012	0.457(0.248– 0.842)	0.000
MPG rs1013358 C/T														
	CC	10	5	6	11	5	4	25	29	,	1*	,	1*	,
	CT	39	34	29	62	8	13	68	81	0.184	0.410(0.110– 1.529)	0.073	0.495(0.229– 1.069)	0.421
	TT	68	31	34	65	14	14	75	89	0.153	0.411(0.121– 1.391)	0.092	0.519(0.241– 1.115)	0.047
ERCC4 rs1799801 T/C														

P: P value; OR: Odds ratio; 95% CI: Confidence Interval; NE: Non Smoker; E: Smoker

	Genotypes	Controls				Bladder cancer				Bladder cancer (NE) Vs Controls (NE)		Bladder cancer (E) Vs Controls (E)		Blad Cont
Gene/Variant														
	TT	58	33	34	67	10	15	70	85	,	1*	,	1*	,
	TC	52	29	29	58	13	16	71	87	0.421	1.450 (0.586–3.585)	0.4768	1.182 (0.745–1.875)	0.587
	CC	7	8	5	13	4	0	27	27	0.093	3.314 (0.817–13.438)	0.188	1.637 (0.784–3.414)	0.034
APOBR rs17855750 A/C														
	AA	111	67	65	132	26	27	151	178	,	1*	,	1*	,
	AC	6	3	3	6	1	4	16	20	0.757	0.711(0.082–6.167)	0.059	2.471 (0.965–6.326)	0.128
	CC	0	0	0	0	0	0	1	1	0.475	4.207 (0.081-216.956)	0.624	2.226 (0.090–55.100)	0.701
ROCK1 rs288980 T/C														
	TT	13	8	14	22	0	1	14	15	,	1*	,	1*	,
	TC	42	28	22	50	12	10	66	76	0.160	7.941(0.440-143.219)	0.035	2.229(1.056–4.705)	0.289
	CC	62	34	32	66	15	20	88	108	0.195	6.696(0.377-118.925)	0.017	2.400(1.163–4.951)	0.316
GSS rs7260770 G/A														
	GG	94	61	56	117	17	24	129	153	,	1*	,	1*	,
	GA	20	9	11	20	9	7	36	43	0.057	2.488 (0.970–6.377)	0.094	1.644 (0.918–2.943)	0.354
	AA	3	0	1	1	1	0	3	3	0.605	1.843 (0.180-18.781)	0.474	2.294 (0.235–22.338)	0.555
COMT rs4633 C > T														
	CC	38	19	19	38	8	9	75	84	,	1*	,	1*	,
	CT	60	35	35	70	14	15	62	77	0.833	1.108(0.424–2.891)	0.006	0.497(0.301–0.821)	0.036
	TT	18	16	14	30	5	7	31	38	0.663	1.319(0.377–4.606)	0.075	0.573(0.310–1.057)	0.894
COMT rs4680 G > A														
	GG	38	20	18	38	9	10	75	85	,	1*	,	1*	,
	GA	62	33	35	68	14	13	63	76	0.919	0.953(0.376–2.415)	0.006	0.499(0.302–0.826)	0.020
	AA	17	17	15	32	4	8	30	38	0.992	0.993(0.268–3.679)	0.040	0.530(0.289–0.973)	0.998

P: P value; OR: Odds ratio; 95% CI: Confidence Interval; NE: Non Smoker; E: Smoker

Table 5
Comparison of genotypes distribution of significant unfavorable SNP from "Tunisian BCHP" panel in bladder cancer patients according to professional risk factors

		Controls		Bladder cancer		Bladder cancer (NE) Vs Controls (NE)		Bladder cancer (E) Vs Controls (E)		Bladder cancer (E) Vs Controls (NE)	
Gene/Variant	Genotypes	Non Exposed (NE)	Exposed (E)	Non Exposed (NE)	Exposed (E)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)
ARNT rs1889740 C > T											
	CC	90	12	36	19	-	1*	-	1*	-	1*
	CT	84	17	65	45	0.010	1.934(1.168–3.203)	0.269	1.671(0.670–4.166)	0.002	2.537(1.374-4.685)
	TT	51	1	18	19	0.710	0.882(0.455–1.710)	0.022	12.000(1.416-101.672)	0.123	1.764(0.856–3.635)
ARNT rs2228099 C > G											
	CC	88	12	35	18	-	1*	-	1*	-	1*
	CG	86	17	66	46	0.010	1.929(1.163–3.201)	0.207	1.803(0.720–4.518)	0.002	2.615(1.405-4.864)
	GG	51	1	18	19	0.724	0.887(0.456–1.725)	0.020	12.666(1.491-107.602)	0.108	1.821(0.876-3.783)
ARNT rs2256355 T > C											
	TT	86	12	34	18	-	1*	-	1*	-	1*
	TC	87	17	65	46	0.014	1.889(1.133–3.149)	0.207	1.803(0.720–4.518)	0.003	2.526(1.357-4.701)
	CC	52	1	20	19	0.933	0.972(0.507–1.865)	0.020	12.666(1.491-107.602)	0.135	1.745(0.840-3.625)
ARNT rs2864873 A > G											
	AA	101	13	43	33	-	1*	-	1*	-	1*
	AG	85	16	62	39	0.029	1.713(1.055–2.780)	0.926	0.960(0.403–2.283)	0.222	1.404(0.813-2.424)
	GG	39	1	14	11	0.636	0.843(0.415–1.710)	0.180	4.333(0.507–37.030)	0.710	0.863(0.397-1.875)
CYP1B1 rs162555 (T to C)											
	TT	120	17	72	58	-	1*	-	1*	-	1*
	TC	89	9	42	25	0.315	0.786 (0.4920-.257)	0.666	0.814 (0.3199–2.072)	0.050	0.581 (0.337 1.000)
	CC	16	4	5	0	0.221	0.183–1.482	0.024	0.033 (0.0017–0.648)	0.054	0.062 (0.003–1.058)
XPC rs2228000 (G to A)											
	GG	146	17	79	58	-	1*	-	1*	-	1*
	GA	68	8	39	25	0.811	1.059 (0.6562–1.712)	0.858	0.915 (0.349–2.397)	0.782	0.925 (0.533–1.604)
	AA	11	5	1	0	0.090	0.168 (0.021–1.325	0.016	0.027 (0.001–0.516)	0.126	0.108 (0.0063–1.877)

P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval; NE: Non Exposed; E: Exposed

		Controls		Bladder cancer		Bladder cancer (NE) Vs Controls (NE)		Bladder cancer (E) Vs Controls (E)		Bladder cancer (E) Vs Controls (NE)	
TP63, P3H2 rs710521 T/C											
	TT	124	12	78	53	,	1*	,	1*	-	1*
	TC	89	16	36	26	0.071	0.643 (0.3980–1.038)	0.026	0.367 (0.152–0.890)	0.169	0.683 (0.397–1.175)
	CC	12	2	5	4	0.455	0.662 (0.2247–1.952)	0.390	0.452 (0.074–2.765)	0.678	0.779 (0.2405–2.529)
ACTRT3/MYNN rs10936599 (C to T)											
	CC	150	21	95	65	,	1*	,	1*	-	1*
	CT	70	6	21	16	0.007	0.473 (0.2730–0.822)	0.782	0.861 (0.298–2.485)	0.041	0.527 (0.284–0.976)
	TT	5	3	3	2	0.941	0.947 (0.2213–4.056)	0.104	0.215 (0.033–1.377)	0.925	0.923 (0.174–4.881)
GSTA4 rs17614751(G to A)											
	GG	213	27	107	77	,	1*	,	1*	-	1*
	GA	12	3	11	6	0.165	1.824 (0.779–4.271)	0.632	0.701 (0.163-3.000)	0.530	1.383 (0.501–3.813)
	AA	0	0	1	0	0.275	5.958 (0.240-147.496)	0.606	0.354 (0.006–18.319)	0.613	2.754 (0.054 140.045)
CYP3A4 rs2740574 C > T											
	CC	8	1	5	3	-	1*	-	1*	-	1*
	CT	85	12	29	21	0.320	0.545(0.165–1.802)	0.656	0.583(0.054–6.251)	0.561	0.658(0.160 2.699)
	TT	132	17	85	59	0.959	1.030(0.326–3.254)	0.902	1.156(0.112–11.850)	0.800	1.191(0.305 4.653)
PRKAR2B rs3752645 (G to A)											
	GG	178	25	101	75	,	1*	,	1*	-	1*
	GA	43	4	18	8	0.322	0.737 (0.4041–1.346)	0.535	0.666 (0.184–2.404)	0.045	0.441 (0.198–0.984)
	AA	4	1	0	0	0.275	0.195 (0.010–3.666)	0.185	0.112 (0.004–2.851)	0.371	0.262 (0.014–4.940)
NEIL2 rs804276 G/A											
	GG	93	13	64	39	,	1*	,	1*	-	1*
	GA	100	15	45	37	0.079	0.653 (0.406–1.050)	0.658	0.822 (0.345–1.959)	0.644	0.882 (0.518 1.500)

P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval; NE: Non Exposed; E: Exposed

		Controls		Bladder cancer		Bladder cancer (NE) Vs Controls (NE)		Bladder cancer (E) Vs Controls (E)		Bladder cancer (E) Vs Controls (NE)	
	AA	32	2	10	7	0.046	0.454 (0.208–0.988)	0.858	1.166 (0.214–6.336)	0.156	0.521 (0.212–1.282)
NEIL2 rs4639 A/G											
	AA	90	12	60	42	,	1*	,	1*	-	1*
	AG	94	15	48	34	0.273	0.766 (0.475–1.234)	0.335	0.647 (0.267–1.566)	0.352	0.775 (0.453–1.325)
	GG	41	3	11	7	0.016	0.402 (0.191–0.844)	0.595	0.666 (0.149–2.978)	0.025	0.365 (0.151–0.883)
NAT2 rs1799930 G > A											
	GG	111	15	56	42	-	1*	-	1*	-	1*
	GA	89	11	57	39	0.311	1.269(0.799–2.015)	0.603	1.2662(0.519–3.089)	0.578	1.158(0.690· 1.943)
	AA	25	4	6	2	0.124	0.475(0.184–1.226)	0.060	0.178(0.029–1.076)	0.040	0.211(0.048· 0.932)
AKR1C3 rs4881400 T > G											
	TT	121	22	68	58	-	1*	-	1*	-	1*
	TG	85	4	45	23	0.802	0.942(0.590–1.503)	0.191	2.181(0.677–7.025)	0.044	0.564(0.323· 0.985)
	GG	19	4	6	2	0.241	0.561(0.214–1.474)	0.065	0.189(0.032–1.110)	0.046	0.219(0.049· 0.974)
BLNK rs3789928 G/C											
	GG	50	8	39	30	,	1*	,	1*	-	1*
	GC	119	19	56	32	0.059	0.603 (0.356–1.020)	0.103	0.449 (0.171–1.178)	0.008	0.448 (0.246–0.814)
	CC	56	3	24	21	0.064	0.549 (0.291–1.037)	0.395	1.866 (0.442–7.873)	0.172	0.625 (0.318–1.228)
VPS37C/CD5 rs7104333 G > A											
	GG	40	6	41	21	-	1*	-	1*	-	1*
	GA	105	15	48	45	0.004	0.446(0.256–0.775)	0.779	0.857(0.291–2.522)	0.529	0.816(0.433· 1.537)
	AA	80	9	30	17	0.001	0.365(0.199–0.669)	0.319	0.539(0.160–1.818)	0.017	0.404(0.192· 0.851)
MPG rs1013358 C/T											
	CC	17	4	19	14	,	1*	,	1*	-	1*
	CT	84	17	41	36	0.031	0.436(0.205–0.927)	0.431	0.605(0.173–2.116)	0.113	0.520(0.232· 1.167)
	TT	124	9	59	33	0.020	0.425(0.206–0.878)	0.945	1.047(0.276–3.974)	0.005	0.323(0.144· 0.722)
ERCC4 rs1799801 T/C											
	TT	109	16	51	34	,	1*	,	1*	-	1*

P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval; NE: Non Exposed; E: Exposed

		Controls		Bladder cancer		Bladder cancer (NE) Vs Controls (NE)		Bladder cancer (E) Vs Controls (E)		Bladder cancer (E) Vs Controls (NE)	
	TC	97	13	55	35	0.422	1.211 (0.7581 to 1.937)	0.594	1.267 (0.5304 to 3.026)	0.600	1.156 (0.670–1.996)
	CC	19	1	13	14	0.339	1.462 (0.670–3.189)	0.080	6.588 (0.795–54.560)	0.033	2.362 (1.071–5.207)
APOBR rs17855750 A/C											
	AA	214	29	105	76	,	1*	,	1*	-	1*
	AC	11	1	14	6	0.023	2.593 (1.138–5.910)	0.452	2.289(0.264–19.849)	0.413	1.535(0.549-4.296)
	CC	0	0	0	1	0.723	2.033 (0.040-103.184)	0.929	1.156(0.045–29.208)	0.193	8.411(0.339-208.705)
ROCK1 rs288980 T/C											
	TT	28	7	9	4	,	1*	,	1*	-	1*
	TC	88	4	43	36	0.325	1.520(0.659–3.503)	0.020	3.976 (1.239–12.756)	0.064	2.863(0.937-8.751)
	CC	109	19	67	43	0.116	1.912(0.850–4.300)	0.044	3.960 (1.035–15.154)	0.071	2.761(0.914-8.341)
GSS rs7260770 G/A											
	GG	186	25	89	60	,	1*	,	1*	,	1*
	GA	35	5	28	23	0.070	1.671 (0.957–2.919)	0.235	1.916 (0.654–5.609)	0.020	2.037 (1.116–3.716)
	AA	4	0	2	0	0.960	1.044 (0.187–5.812)	0.667	0.421 (0.008–21.829)	0.474	0.342 (0.018–6.454)
COMT rs4633 C > T											
	CC	71	6	43	35	-	1*	-	1*	-	1*
	CT	109	21	52	31	0.352	0.787(0.476–1.302)	0.008	0.253(0.090–0.707)	0.057	0.576(0.326-1.018)
	TT	45	3	24	17	0.689	0.880(0.472–1.642)	0.969	0.971(0.216–4.363)	0.449	0.766(0.384-1.526)
COMT rs4680 G > A											
	GG	69	7	45	35	-	1*	-	1*	-	1*
	GA	111	19	50	31	0.1489	0.690(0.417–1.141)	0.027	0.326(0.121–0.880)	0.039	0.550(0.311-0.972)
	AA	45	4	24	17	0.525	0.817(0.439–1.522)	0.814	0.850(0.218–3.306)	0.402	0.744(0.373-1.485)

P: P value for Fisher Test; OR: Odds ratio; 95% CI: Confidence Interval; NE: Non Exposed; E: Exposed

Finally the decision-tree analysis produced a three major BCa class. All of these classes were characterized by an intensity of tobacco use ≥ 20 pack years (PY) and subdivided according to the genotype of the CYP1A2 C > A variation (rs762551). The first class (58/249) is defined by the presence of CYP1A2 CC or CYP1A2 CA genotype and also defined by others 8 variations: ARNT C > G (rs2228099), CYP1B1 T > C (rs162555), SOD1 T > C (rs2173962), ROCK1 T > C (rs288980), IL10 A > T (rs1800890), LY96 T > C (rs17226566), AICDA T > G (rs11046349) and MAP2K4 C > T (rs4791489). The second class of BCa group (31/249) is only defined by the intensity of tobacco use ≥ 20PY and the inheritance of the **homozygous genotype for** rs762551 in CYP1A2 (CYPA2 A/A), XPC GG or AG genotype (rs2228000, G > A), and MAP2K4 CC or CT genotype (rs4791489, C > T). The third class (25/249) of BCa patients was defined by rs762551 (CYP1A2 C/A or C/C genotype) and 6 other variables (ARNT rs2228099 C > G, MPO rs2333227 C > T, LY96 rs17226566 T > C, CCR2 rs391835 G > A; APOBEC3 rs1014971 C > T and CASP3 rs3087455 T > G) **(**Fig. 5**).**

Discussion

As all complex diseases, BCa isn't one SNP/gene disorder. Rather, many SNPs with small effects may result in the impairment of key pathways involved in their pathophysiology. The identification of such SNP-signatures represents an analytical challenge requiring the appliance of novel comprehensive statistical approaches. To our knowledge, this is the first study on BCa in Tunisian population analyzing a large number of SNPs with NGS technique. Indeed, in this study, we report the development and validation of the targeted amplification-based NGS panel analyzed on the Ion Torrent Proton Platform. This panel is named BCHP (BCa Heredity Panel) and used for detecting clinically useful genetic variations associated with the development of BCa in a total of 249 patients and 255 controls. After filtration the BCHP was composed of 139 SNPs in 97 genes. These SNPs are located in many different regions: introns sites (*BCL10, GSTM4, GSTM3*...), regulatory region (*TP63, NAT2, GSTP1, GSTP2*), up and downstream regulation genes sequences (*GSTA4, NAT2 APOBR/IL27, CYP2D6*...), splicing sites (*CASP9)*.... The encoded enzymes modulate biological processes (xenobiotic metabolic process, cellular response to xenobiotic stimulus, Base-excision repair, immunology response...) and/or affect molecular function (Damaged DNA binding, Enzyme model we found statistically significant association between some genotypes and risk of bladder cancer development. These associations were reported for gene encoding for enzymes implicated in xenobioitic and drug metabolisms [*CYP1B1* (**rs162555**), *NAT2* (**rs1799930)**, *CYP3A4* (**rs2740574)**, *COMT* (**rs4680)**, *AKR1C3* (**rs4881400)**, *PRKAR2B* (**rs3752645**), *ARNT* (**rs1889740, rs2228099, rs2864873**), GSS **(rs7260770)**, *GSTA4* (rs17614751)], or in DNA repair pathway [*NEIL2* (**rs4639)**, *XPC* (**rs2228000)**, *MPG* **(rs1013358)**, *ERCC4/XPF* **(rs1799801)]** or in other molecular functions such as cell proliferation, immunology response.

Xenobiotic metabolism pathway

In this study, we have found that *CYP1B1* C/C (**rs162555**), NAT2 A/A (**rs1799930**), *AKR1C3* G/G (**rs4881400)** genotypes and heterozygous genotypes for *CYP3A4* (**rs2740574)**, *COMT* (**rs4680)** and *PRKAR2B* (**rs3752645**) were associated with a decreased risk of BCa development compared to reference group. *CYP1B1* encodes for the CYP1B1 enzyme which is implicated in the NADPH-dependent electron transport and xenobiotic oxidation [25]. Nevertheless, no previous study has presented conclusive results for an association between *CYP1B1* rs162555 and BCa development. The CYP3A4 (Cytochrome P450 3A4) enzyme encoded by the *CYP3A4* gene is expressed in adult human liver and it is responsible for the oxidative metabolism of many clinically used drugs. The *CYP3A4*1B* variant (rs2740574 C to T) is associated with the reduction of the expression of the activity of CYP3A4 enzyme which could explain its effect on the development of BCa [26]. These results were supported by others studies which reported a significant protective effect of homozygous wild genotype in other cancer types such as prostate cancer, breast cancer, leukemia, gastric cancer, colorectal cancer, and ovarian cancer [27].

The N-acetyltransferase 2 (*NAT2*) gene encodes for an important phase II xenobiotic-metabolizing enzyme frequently present in liver and intestinal mucosa. It catalyzes the reaction of aromatic and heterocyclic amine carcinogens via O-acetylation and N-acetylation [28–30]. In our case–control study we have found an inverse association between *NAT2 A/A* genotype (rs1799930) and BCa development. This result concord with those of Lei Quan who reported that *NAT2 A/A* carriers (rs1799930) were at 50% decreased risk of bladder cancer development compared to rapid acetylator[31]. Catechol-O-methyltransferase (*COMT*) encodes a phase II enzyme mainly liable for the degradation of catecholamines, like dopamine and noradrenalinev [32]. It catalyzes the O-methylation of 2-hydroxyestradiol to yield 2-ME2 [32]. Additionally, it is involved within the inactivation of potential carcinogenic compounds that produce inflammation and catechol estrogens and thus might protect DNA from oxidative damage. Rs4680 (Ex4-12 G > A, or val158met) is the most studied *COMT* single nucleotide polymorphism (SNP). In our cohort we explain the significant inverse association of heterozygous genotype against BCa development by the modification of enzyme activity associated to this genotype. Indeed it has been reported that valine (val) variant enzyme is 3–4 times more active than the methionine (met) variant [33, 34]. Moreover we observed that *PRKAR2B* A/A genotype (rs3752645) is inversely correlated with BCa risk. The rs3752645 G/A SNP lies within the *PRKAR2B* gene, which encodes a regulatory subunit for cyclic adenosine 3', 5'-monophosphate kinase. Our result confirms previous findings of a GWAS study where the authors showed that the rs3752645 SNP had a strong inverse association with BCa risk [35].

In contrast we have found that homozygous minor genotype or heterozygous genotype for rs1889740 C/T, rs2228099 C/G, rs2256355 T/C, rs2864873 A/G in ARNT gene (Aryl hydrocarbon receptor nuclear translocator) were significantly associated to increased risk of bladder cancer. These SNPs are described for the first time in association with increased risk of BCa development. *ARNT gene* encodes a protein that binds to ligand-bound aryl hydrocarbon receptor and promotes the expression of genes involved in xenobiotic metabolism [36]. We also found that the inheritance of rare homozygous genotype of *GSS* **(rs7260770)** is associated with an increased risk of BCa which confirms findings reported in other studies [37]. These findings may be explained by the functional impact of this variant on the enzyme activity. Indeed it has been reported that GSS deficiency resulted in decreased GSH levels and caused dramatic metabolic consequences. However, the role of *GSS* in cancers has not been studied in details. Finally we showed that *GSTA4* G/A genotype (rs17614751) was associated with an increased risk of BCa in our population. This result could be explained by the perturbation of enzyme activity or expression (the rs17614751 SNP is a downstream gene variant). Moreover, it has been reported that the deregulation of GSTA4 enzyme activity was implicated in cell proliferation in nasopharyngeal carcinoma, breast cancer, and liver cancer cells [38].

DNA repair pathway

When considering the DNA repair pathway we have found that the minor alleles of the **rs4639 and rs2228000** SNPs in *NEIL*2 and *XPC* genes respectively were associated with a decreased risk of BCa development compared to reference groups carrying major alleles. However the minor alleles observed in the studied population for the **rs1013358 and rs1799801 SNPs** in respectively *MPG* and *ERCC4/ XPF* genes increased the risk of bladder cancer.

The *NEIL*2 (Nei-like DNA glycosylase 2) gene encodes for an enzyme implicated in the first step of the base excision repair (BER) mechanism which consists of cleaving oxidatively damaged bases and introducing a DNA strand break via the associated lyase reaction [39]. The rs4639, is located on 3'UTR of *NEIL2*[40]. In our study, we found that *NEIL2 G/G* genotype for rs4639 was associated with 0, 49-fold decreased risk of BCa. This inverse association could be explained by the fact that the minor allele interacts with some specific miRNA, which activates the BER pathway [41]. Moreover, we have found that rs4639 and rs804276 in *NEIL2* gene were in linkage disequilibrium (*p-value* = 0.03). Beside *NEIL2*, we found that *XPC* AA genotype (rs2228000) was associated with a decreased risk of BCa compared to reference group. The *XPC* gene is located on 3p25 and encodes for an enzyme involved in global genome repair. This

enzyme represents the earliest damage detector by initiating the NER pathway and eliminating the DNA damages induced by chemical and environmental exposures such as aromatic amines and UV light [42]. Our result confirms the study of Zhu Y. *et al*. which demonstrates a significant association between the presence of *XPC* rs2228000 AA genotype and a decreased risk of BCa [43]. However, the recent study of Dai Y. *et al*. suggests that *XPC* AA genotype may be linked to an increased risk of bladder and breast cancer [44]. These conflicting results and differences in risk associations may be explained by the different etiology and mechanisms of BCa in study populations with different ethnic backgrounds [15].

On the other hand, ERCC4/XPF and MPG enzymes represent the headmaster in DNA metabolic process, DNA repair, and cellular response to DNA damage stimulus. *ERCC4* (16p13.12) is a NER gene that plays a key role in DNA repair that protects against genetic instability and carcinogenesis [45]. The *ERCC4*-rs1799801 is a synonymous variation (Ser835Ser). A significant association between the *ERCC4*-rs1799801 polymorphisms and increased risk of Bca in Tunisian population could be explained by the effect of this variation on the enzyme activity. Indeed a previously genotype-phenotype correlation analysis indicated that the *ERCC4*-rs1799801 rare homozygous C/C genotype carriers had an increased trend of *ERCC4* expression levels [46]. In contradiction with our results, the meta-analysis of Shi T.Y. *et al*. also did not provide statistical evidence for an association between *ERCC4* gene and the overall risk of several human cancers, they also report that stratification analysis showed an inverse correlation with cancer in Caucasians [47]. These conflicting results could be explained by a difference in both ethnic origins of patients and exposure to environmental risk factors. MPG, a BER gene, also plays an important role in DNA repair.

Others cellular pathway

In addition to xenobiotic metabolic pathway and DNA repair process we are interested in this study to investigate the impact of others variations in gene implicated in cell proliferation pathway and immunology response in the etiology of BCa. Among the analyzed variations in this panel we have found that *APOBR/IL27* A/C genotype **(rs17855750)** was associated with 2.27 fold-increased risk of BCa. The APOBR (Apolipoprotein B Receptor)/ *IL27* (Interleukin 27) encodes for a protein that has been recognized as a pleiotropic cytokine with both pro- and anti-inflammatory properties [48]. This significant association could be explained by the implication of encoded enzyme in the inflammatory process. In the same context, Zhou B. *et al*. showed a significant association between *IL-27* gene polymorphisms and *IL-27*plasma level and reported that rs17855750 GG (CC) genotype was associated with increased risk for muscle invasive bladder carcinoma. Moreover we found that rs7104333 in *CD5/VPS37C* gene. The Vacuolar protein sorting-associated protein 37C (VPS37C*)* is a Component of the ESCRT-I complex, and a regulator of vesicular trafficking process. This protein may be involved in cell growth and differentiation and it is known as a negative regulator of T- and B-cell receptor signaling. Its expression has been shown to be implicated in T lymphocytes tolerance toward tumor cells [49]. In contrast we have found that genetic variations in *BLNK* (**rs3789928**), *TP63 (***rs710521**), *ROCK1* (**rs288980**) and *ACTRT3/MYNN* (**rs10936599)** were associated with a decreased risk of bladder cancer. *BLNK* gene encodes for a cytoplasmic linker or adaptor protein that plays a critical role in B cell development [50]. It plays an important role in the pro-B cell to pre-B cell transition and B-cell apoptosis via BCR signaling pathway and reported to have a tumor-suppressive function in various hematologic malignancies [51]. Moreover the Human Protein Atlas consortium showed that high expression of BLNK had a favorable prognosis value in urothelial cancer [52]. *TP63* (3q27-28) has a homolog sequence to *TP53* (tumor suppressor) and *TP73*[53]. This gene encodes for an enzyme implicated in the control of cell cycle and plays an important role in apoptosis. Abnormal expression of TP63 is associated with a loss of urothelial differentiation [54]. In our study the TP63 T/C genotype (rs710521) was reported to be inversely associated with BCa (*p-value =* 0.035 and an OR = 0.62; CI95% 0.42–0.89). Our results seem to agree with those of Kimeney L. *et al.* and Lehmann M.-L *et al.* who confirmed that this variant plays a role in decreasing the risk of BCa in European patients [55, 56]. We have also found that *ROCK1* TT genotype (rs288980) was associated with 0.75 fold-decreased risk of BCa. *ROCK1* enzyme is a necessary effector kinase downstream of Rho GTPases, a very important pathway involved in cell migration and has been identified as a possible therapeutic target [57]. The Cancer Genome Project identified three non-synonymous mutations within the *ROCK1* gene [58] but the effect of these variations in protein activity or expression was not more elucidated. Finally *ACTRT3/ MYNN* (Actin Related Protein T3; 3q26.2) gene is poorly described in the literature. The minor T allele of rs10936599 in *ACTRT3/ MYNN* has been described in the Genome-wide association study conducted by Figueroa J. *et al*. in 2014, to be highly associated with decreased risk of BCa [59], which was confirmed by Wang M. *et al*. [60]. However, the effect of rs10936599 polymorphism on the *MYNN* activity is not well known and more biological functional studies are needed to draw more concise conclusions regarding the underlying molecular mechanism of this variation.

Interaction between genetic and environmental risk factors

When we compare the distribution of unfavorable genotypes [*GSS G/A* (rs7260770), ARNT A/G (rs2864873), ARNT C/T (rs1889740) ARNT C/G (rs2228099), ARNT T/C (rs2256355), GSTA4 G/A (rs17614751), *ERCC4* C/C (rs1799801), MPG C/C (rs1013358), *VPS37C* A/A (rs7104333), APOBR/IL27 A/C (rs17855750)] between exposed bladder cancer cases and controls according to environmental risk factors we found that *ARNT* C/T (rs1889740), *ARNT* A/G *(*rs2864873) and *GSTA4* G/A (rs17614751*)* genotypes were associated with an increased risk of BCa even in the absence of tobacco risk factors, or professional risk factors. Moreover when we compared exposed cases to exposed controls, we have not found a significant additive effect between the majority of unfavorable genotypes and exposure to environmental risk factors (smokers' ≥ 20PY or exposed to professional risk factors). The absence of significant additive effect between the majority of unfavorable genotypes and BCa risk could be explained by the fact that the targeted SNPs encoding enzymes were not directly implicated in the metabolism of xenobiotics. For example, the proinflammatory role of APOBR/IL27 enzyme could explain the role of rs17855750 in BCa development independently to exposition to environmental risk factors. However this additive effect reached more than 12 fold-increased risk of BCa development for *ARNT* rs1889740 T/T, *ARNT* rs2228099 G/G and *ARNT* rs2256355 C/C genotypes in subjects exposed to professional risk factors. This association may be explained by the fact that *ARNT gene* encodes a protein thats binds to ligand-bound aryl hydrocarbon receptor and promotes the expression of genes involved in xenobiotic metabolism [36]. Whether this association is caused by specific bladder carcinogens present in the work environment warrants further investigations. To conclude our analysis, a decision tree was implemented to create a disease prediction model. This tree has allowed us to define the risk groups most genetically likely to develop BCa. According to the established decision tree, we note the importance of both smoking status (≥ 20PY) and the genotype of CYP1A2 (rs762551) in the development of BCa. The decision-tree analysis produced a three major BCa class. The first major class (58/249) is defined by the presence of CYP1A2 CC or CYP1A2 CA genotype and also defined by others 8 variations: ARNT C > G (rs2228099), CYP1B1 T > C (rs162555), SOD1 T > C (rs2173962), ROCK1 T > C (rs288980), IL10 A > T (rs1800890), LY96 T > C (rs17226566), AICDA T > G (rs11046349) and

MAP2K4 C > T (rs4791489). The second major class of BCa group (31/249) is only defined by the intensity of tobacco use ≥ 20PY and the inheritance of the **homozygous genotype for** rs762551 in CYP1A2 (CYP1A2 A/A), XPC GG or AG genotype (rs2228000, G > A), and MAP2K4 CC or CT genotype (rs4791489, C > T). This decision tree confirms the crucial role of tobacco consumption in the etiology of BCa. Indeed BCa is considered as a smoking-related cancer [61]. This risk was attributed to many compounds of tobacco such as 4-Aminobiphenyl, 3-Amino-1,4-dimethyl-5H-pyrido [4,3-b] indole (Trp-P-1), Toluene, Benzo[a]pyrene, Benzene… [62]. In the other hand CYP1A2 is an enzyme responsible for the metabolism of caffeine and some tobacco compounds. Rs762551 variation in *CYP1A2* gene encodes for the CYP1A2*1F allele. The baseline activity of the enzyme is similar in CYP1A2*1F allele carriers and non-carriers. Moreover it has been reported that he presence of rs762551 (A) codes for the "high inducibility" form the CYP1A2 enzyme, characterized by higher enzyme activity in the presence of an inducer such as smoking or heavy coffee consumption[63]. To explain our result we have reanalyzed the CYP1A2 C > A genotype distribution between cases and controls and according to tobacco status. As result we have found that the inheritance of CYP1A2 (CC) or CYP1 A2 (CA) genotype were respectively associated with 6.89 and 9.04 –fold increased risk of BCa in only heavy smokers patients (≥ 20PY) compared to heavy smokers controls. However we have not data about coffee consumption.

Conclusion

We have conducted the first study in Tunisian population to evaluate systematically the association between genetic variations in BCa and environmental factors. We also determined the effect of studied pathway SNPs in comparison with environmental exposition. Once validated, these findings may provide urologists additional genetic information that may help for clinical assessment and treatment decisions. Nevertheless, the underlying mechanisms through which these genes or SNPs affect the clinical behavior of BCas require further studies. Future investigations in our populations and detailed functional characterization are needed to establish predictive or prognostic markers for BCa.

Abbreviations

ACTRT3
Actin Related Protein T3; 3q26.2
APOBR
Apolipoprotein B Receptor
ARNT
Aryl hydrocarbon receptor nuclear translocator
BCa
Bladder cancer
BER
base excision repair
BLNK
B cell linker
COMT
Catechol-O-methyltransferase
CYP1B1
cytochrome P450 family 1 subfamily B member 1
CYP3A4
Cytochrome P450 3A4
dbSNP
database of single nucleotide polymorphisms
DNA
deoxyribonucleic acid
DRC
DNA Repair Capacity
DSBR
double-strand break repair
EDTA
ethylene diamine tetra-acetic acid
ERCC4
Excision Repair Cross-Complementation group 4
GSTM1
Glutathione-s-transferase M1
GWAS
Genome-Wide Association Study
GSS
Glutathione Synthase
GSTA4
Glutathione S-transferase Alpha 4
HG

High-Grade
IARC
International Agency for Research on Cancer
IL27
Interleukin 27
KEGG
Kyoto Encyclopedia of Genes and Genomes
LG
Low-Grade
MIBC
muscle-invasive BCa
MMR
mismatch repair
MPG
N-methylpurine DNA glycosylase
NEIL2
Nei-like DNA glycosylase 2
NAT2
N-acetyltransferase 2
NER
nucleotide excision repair
NGS
Next-Generation Sequencing
NMIBC
non-muscle-invasive BCa
PRKAR2B
Protein Kinase cAMP-dependent type II regulatory subunit beta
ROCK1
Rho associated coiled-coil containing protein kinase 1
SNP
nucleotide polymorphisms
TCR
transcription-coupled repair
TP63
Tumor Protein p63
VPS37C
vacuolar protein sorting-associated protein 37C
WHO
World Health Organization
XPC
Xeroderma Pigmentosum, Complementation group C
XPF
excision repair cross-complementing rodent repair deficiency, complementation group 4

Declarations

Ethics approval and consent to participate

- This trial protocol and recruitment were approved and carried out after the agreement of the ethics committee of the Charles Nicolle Hospital and approved by an Ethics Committee (IEC Project No. 17-35) and an MTA (MATERIAL TRANSFER AGREEMENT MTA/ 2017 / IMP / GCS)/ 0356) from IARC.

-Informed consent was obtained from all participants (in both languages: Arabic/French) prior to enrollment and participation in this study..

- All samples are coded and no patient names appear in the study. All data whether clinical or personal remains anonymous and secret.

Consent for publication

Not applicable. This study did not use identifying images and any clinical and personal details despite written informed consent for publication of their clinical details and/or clinical

Availability of data and material

The dataset is available upon reasonable request to the corresponding author

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

IH: Collecting clinical samples and data, designed the panel, molecular analysis, drafted the manuscript and bioinformatics analyzes. SB, HD and FH: Help in Bioinformatics and statistical analyzes. GD and CV: Technical assistance for NGS analyzes. HA: Clinical characterization of the studied population. SZ, ZN and MA: help in collecting samples. MC and JM: designed the study and revised the manuscript. FL: drafted and revised the manuscript. SO: designed the study, performed the statistical analysis and drafted and revised the manuscript. All authors read and approved the final version of the manuscript.

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References

1. Antoni S, Ferlay J, Soerjomataram I, et al (2017) Bladder Cancer Incidence and Mortality: A Global Overview and Recent Trends. European Urology 71:96–108. https://doi.org/10.1016/j.eururo.2016.06.010
2. Wong MCS, Fung FDH, Leung C, et al (2018) The global epidemiology of bladder cancer: a joinpoint regression analysis of its incidence and mortality trends and projection. Scientific Reports 8:. https://doi.org/10.1038/s41598-018-19199-z
3. Cancer today. http://gco.iarc.fr/today/home. Accessed 15 Jun 2020
4. Bray F, Ferlay J, Soerjomataram I, et al (2018) Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: A Cancer Journal for Clinicians 68:394–424. https://doi.org/10.3322/caac.21492
5. Cassell A, Yunusa B, Jalloh M, et al (2019) Non-Muscle Invasive Bladder Cancer: A Review of the Current Trend in Africa. World J Oncol 10:123–131. https://doi.org/10.14740/wjon1210
6. Burger M, Catto JWF, Dalbagni G, et al (2013) Epidemiology and risk factors of urothelial bladder cancer. Eur Urol 63:234–241. https://doi.org/10.1016/j.eururo.2012.07.033
7. Colin P, Koenig P, Ouzzane A, et al (2009) Environmental factors involved in carcinogenesis of urothelial cell carcinomas of the upper urinary tract. BJU International 104:1436–1440. https://doi.org/10.1111/j.1464-410X.2009.08838.x
8. Skipper PL, Kim MY, Sun H-LP, et al (2010) Monocyclic aromatic amines as potential human carcinogens: old is new again. Carcinogenesis 31:50–58. https://doi.org/10.1093/carcin/bgp267
9. Rouissi K, Ouerhani S, Hamrita B, et al (2011) Smoking and Polymorphisms in Xenobiotic Metabolism and DNA Repair Genes are Additive Risk Factors Affecting Bladder Cancer in Northern Tunisia. Pathol Oncol Res 17:879. https://doi.org/10.1007/s12253-011-9398-3
10. Matic M, Pekmezovic T, Djukic T, et al (2013) GSTA1, GSTM1, GSTP1, and GSTT1 polymorphisms and susceptibility to smoking-related bladder cancer: A case-control study. Urologic Oncology: Seminars and Original Investigations 31:1184–1192. https://doi.org/10.1016/j.urolonc.2011.08.005
11. García-Closas M, Malats N, Silverman D, et al (2005) NAT2 slow acetylation and GSTM1 null genotypes increase bladder cancer risk: results from the Spanish Bladder Cancer Study and meta-analyses. Lancet 366:649–659. https://doi.org/10.1016/S0140-6736(05)67137-1
12. Kumar N, Moreno NC, Feltes BC, et al (2020) Cooperation and interplay between base and nucleotide excision repair pathways: From DNA lesions to proteins. Genetics and Molecular Biology 43:. https://doi.org/10.1590/1678-4685-gmb-2019-0104
13. Greenman C, Stephens P, Smith R, et al (2007) Patterns of somatic mutation in human cancer genomes. Nature 446:153–158. https://doi.org/10.1038/nature05610
14. Pleasance ED, Cheetham RK, Stephens PJ, et al (2010) A comprehensive catalogue of somatic mutations from a human cancer genome. Nature 463:191–196. https://doi.org/10.1038/nature08658
15. He J, Shi T-Y, Zhu M-L, et al (2013) Associations of Lys939Gln and Ala499Val polymorphisms of the XPC gene with cancer susceptibility: A meta-analysis. International Journal of Cancer 133:1765–1775. https://doi.org/10.1002/ijc.28089
16. Rouissi K, Bahria IB, Bougatef K, et al (2011) The effect of tobacco, XPC, ERCC2 and ERCC5 genetic variants in bladder cancer development. BMC Cancer 11:101. https://doi.org/10.1186/1471-2407-11-101
17. Leibovici D, Grossman HB, Dinney CP, et al (2005) Polymorphisms in Inflammation Genes and Bladder Cancer: From Initiation to Recurrence, Progression, and Survival. JCO 23:5746–5756. https://doi.org/10.1200/JCO.2005.01.598
18. Kanehisa M, Goto S (2000) KEGG: kyoto encyclopedia of genes and genomes. Nucleic Acids Res 28:27–30. https://doi.org/10.1093/nar/28.1.27
19. Kanehisa M, Sato Y, Furumichi M, et al (2019) New approach for understanding genome variations in KEGG. Nucleic Acids Res 47:D590–D595. https://doi.org/10.1093/nar/gky962

20. PANTHER version 14: more genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6323939/. Accessed 30 Sep 2020
21. Ashburner M, Ball CA, Blake JA, et al (2000) Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. Nat Genet 25:25–29. https://doi.org/10.1038/75556
22. Jassal B, Matthews L, Viteri G, et al (2020) The reactome pathway knowledgebase. Nucleic Acids Res 48:D498–D503. https://doi.org/10.1093/nar/gkz1031
23. Selinski S (2017) Discovering urinary bladder cancer risk variants: Status quo after almost ten years of genome-wide association studies. EXCLI J 16:1288–1296. https://doi.org/10.17179/excli2017-1000
24. Calvez-Kelm FL, Foll M, Wozniak MB, et al (2016) KRAS mutations in blood circulating cell-free DNA: a pancreatic cancer case-control. Oncotarget 7:78827–78840. https://doi.org/10.18632/oncotarget.12386
25. Wang M, Wang M, Zhang W, et al (2009) Common genetic variants on 8q24 contribute to susceptibility to bladder cancer in a Chinese population. Carcinogenesis 30:991–996. https://doi.org/10.1093/carcin/bgp091
26. Dally H, Edler L, J??ger B, et al (2003) The CYP3A4*1B allele increases risk for small cell lung cancer: effect of gender and smoking dose. Pharmacogenetics 13:607–618. https://doi.org/10.1097/00008571-200310000-00004
27. Božina N, Bradamante V, Lovrić M (2009) Genetic Polymorphism of Metabolic Enzymes P450 (CYP) as a Susceptibility Factor for Drug Response, Toxicity, and Cancer Risk. Archives of Industrial Hygiene and Toxicology 60:217–242. https://doi.org/10.2478/10004-1254-60-2009-1885
28. Windmill KF, Gaedigk A, Hall PM, et al (2000) Localization of N-acetyltransferases NAT1 and NAT2 in human tissues. Toxicol Sci 54:19–29. https://doi.org/10.1093/toxsci/54.1.19
29. Yamada S, Tang M, Richardson K, et al (2009) Genetic variations of NAT2 and CYP2E1 and isoniazid hepatotoxicity in a diverse population. Pharmacogenomics 10:1433–1445. https://doi.org/10.2217/pgs.09.66
30. Pande JN, Pande A, Singh SPN (2003) Acetylator status, drug metabolism and disease. Natl Med J India 16:24–26
31. Quan L, Chattopadhyay K, Nelson HH, et al (2016) Differential association for N-acetyltransferase 2 genotype and phenotype with bladder cancer risk in Chinese population. Oncotarget 7:40012–40024. https://doi.org/10.18632/oncotarget.9475
32. Chen Y, Yu X, Li T, et al (2016) Significant Association of Catechol-O-Methyltransferase Val158Met Polymorphism with Bladder Cancer Instead of Prostate and Kidney Cancer. Int J Biol Markers 31:110–117. https://doi.org/10.5301/jbm.5000204
33. Chen J, Lipska BK, Halim N, et al (2004) Functional Analysis of Genetic Variation in Catechol-O-Methyltransferase (COMT): Effects on mRNA, Protein, and Enzyme Activity in Postmortem Human Brain. Am J Hum Genet 75:807–821
34. Lachman HM, Papolos DF, Saito T, et al (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6:243–250. https://doi.org/10.1097/00008571-199606000-00007
35. Figueroa JD, Han SS, Garcia-Closas M, et al (2014) Genome-wide interaction study of smoking and bladder cancer risk. Carcinogenesis 35:1737–1744. https://doi.org/10.1093/carcin/bgu064
36. ARNT Gene - GeneCards | ARNT Protein | ARNT Antibody. https://www.genecards.org/cgi-bin/carddisp.pl?gene=ARNT. Accessed 14 Feb 2021
37. Ke H-L, Lin J, Ye Y, et al (2015) Genetic Variations in Glutathione Pathway Genes Predict Cancer Recurrence in Patients Treated with Transurethral Resection and Bacillus Calmette–Guerin Instillation for Non-muscle Invasive Bladder Cancer. Ann Surg Oncol 22:4104–4110. https://doi.org/10.1245/s10434-015-4431-5
38. Ateş NA, Tamer L, Ateş C, et al (2005) Glutathione S-Transferase M1, T1, P1 Genotypes and Risk for Development of Colorectal Cancer. Biochem Genet 43:149–163. https://doi.org/10.1007/s10528-005-1508-z
39. Hazra TK, Kow YW, Hatahet Z, et al (2002) Identification and Characterization of a Novel Human DNA Glycosylase for Repair of Cytosine-derived Lesions. J Biol Chem 277:30417–30420. https://doi.org/10.1074/jbc.C200355200
40. Wei H, Kamat A, Chen M, et al (2012) Association of Polymorphisms in Oxidative Stress Genes with Clinical Outcomes for Bladder Cancer Treated with Bacillus Calmette-Guérin. PLoS One 7:. https://doi.org/10.1371/journal.pone.0038533
41. Kang L, Zou X, Zhang G, et al (2019) A variant in a microRNA binding site in NEIL2 3'UTR confers susceptibility to age-related cataracts. FASEB j 33:10469–10476. https://doi.org/10.1096/fj.201802291R
42. Sugasawa K, Ng JMY, Masutani C, et al (1998) Xeroderma Pigmentosum Group C Protein Complex Is the Initiator of Global Genome Nucleotide Excision Repair. Molecular Cell 2:223–232. https://doi.org/10.1016/S1097-2765(00)80132-X
43. Zhu Y, Lai M, Yang H, et al (2007) Genotypes, haplotypes and diplotypes of XPC and risk of bladder cancer. Carcinogenesis 28:698–703. https://doi.org/10.1093/carcin/bgl201
44. Dai Y, Song Z, Zhang J, Gao W (2019) Comprehensive assessment of the association between XPC rs2228000 and cancer susceptibility based on 26835 cancer cases and 37069 controls. Biosci Rep 39:. https://doi.org/10.1042/BSR20192452
45. Zhang J-S, Zhang C, Yan X-Y, et al (2013) Effect of Xeroderma pigmentosum complementation group F polymorphisms on gastric cancer risk and associations with H.pylori infection. Asian Pac J Cancer Prev 14:1847–1850. https://doi.org/10.7314/apjcp.2013.14.3.1847
46. Shi T-Y, He J, Qiu L-X, et al (2012) Association between XPF Polymorphisms and Cancer Risk: A Meta-Analysis. PLoS ONE 7:e38606. https://doi.org/10.1371/journal.pone.0038606
47. Association between XPF Polymorphisms and Cancer Risk: A Meta-Analysis. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3388076/. Accessed 10 Mar 2020

48. Xu X-P, Hua L-Y, Chao H-L, et al (2017) Genetic association between IL-27 rs153109 polymorphism and cancer risk in Chinese population: a meta-analysis. Journal of Receptors and Signal Transduction 37:335–340. https://doi.org/10.3109/10799893.2014.986743
49. Masson-Lecomte A, Maturana EL de, Goddard ME, et al (2016) Inflammatory-Related Genetic Variants in Non–Muscle-Invasive Bladder Cancer Prognosis: A Multimarker Bayesian Assessment. Cancer Epidemiol Biomarkers Prev 25:1144–1150. https://doi.org/10.1158/1055-9965.EPI-15-0894
50. Y M, J R, E C-S, et al (1999) An essential role for BLNK in human B cell development. Science 286:1954–1957. https://doi.org/10.1126/science.286.5446.1954
51. Nakayama J, Yamamoto M, Hayashi K, et al (2009) BLNK suppresses pre–B-cell leukemogenesis through inhibition of JAK3. Blood 113:1483–1492. https://doi.org/10.1182/blood-2008-07-166355
52. Expression of BLNK in cancer - Summary - The Human Protein Atlas. https://www.proteinatlas.org/ENSG00000095585-BLNK/pathology. Accessed 13 Jul 2020
53. Bergholz J, Xiao Z-X (2012) Role of p63 in Development, Tumorigenesis and Cancer Progression. Cancer Microenviron 5:311–322. https://doi.org/10.1007/s12307-012-0116-9
54. Urist MJ, Di Como CJ, Lu M-L, et al (2002) Loss of p63 Expression Is Associated with Tumor Progression in Bladder Cancer. The American Journal of Pathology 161:1199–1206. https://doi.org/10.1016/S0002-9440(10)64396-9
55. Kiemeney LA, Thorlacius S, Sulem P, et al (2008) Sequence variant on 8q24 confers susceptibility to urinary bladder cancer. Nat Genet 40:1307–1312. https://doi.org/10.1038/ng.229
56. Lehmann M-L, Selinski S, Blaszkewicz M, et al (2010) Rs710521[A] on chromosome 3q28 close to TP63 is associated with increased urinary bladder cancer risk. Arch Toxicol 84:967–978. https://doi.org/10.1007/s00204-010-0617-6
57. Yang S, Zhao Y, Tian Y, et al (2018) Common variants of ROCKs and the risk of hypertension, and stroke: Two case-control studies and a follow-up study in Chinese Han population. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1864:778–783. https://doi.org/10.1016/j.bbadis.2017.12.007
58. Lochhead PA, Wickman G, Mezna M, Olson MF (2010) Activating ROCK1 somatic mutations in human cancer. Oncogene 29:2591–2598. https://doi.org/10.1038/onc.2010.3
59. Figueroa JD, Ye Y, Siddiq A, et al (2014) Genome-wide association study identifies multiple loci associated with bladder cancer risk. Hum Mol Genet 23:1387–1398. https://doi.org/10.1093/hmg/ddt519
60. Wang M, Chu H, Lv Q, et al (2014) Cumulative effect of genome-wide association study-identified genetic variants for bladder cancer. Int J Cancer 135:2653–2660. https://doi.org/10.1002/ijc.28898
61. Simonis K, Shariat SF, Rink M, Urothelial Cancer Working Group of the Young Academic Urologists (YAU) Working Party of the European Association of Urology (EAU) (2014) Smoking and smoking cessation effects on oncological outcomes in nonmuscle invasive bladder cancer. Curr Opin Urol 24:492–499. https://doi.org/10.1097/MOU.0000000000000079
62. Talhout R, Schulz T, Florek E, et al (2011) Hazardous Compounds in Tobacco Smoke. Int J Environ Res Public Health 8:613–628. https://doi.org/10.3390/ijerph8020613
63. Wang L, Hu Z, Deng X, et al (2013) Association between Common CYP1A2 Polymorphisms and Theophylline Metabolism in Non-smoking Healthy Volunteers. Basic & Clinical Pharmacology & Toxicology 112:257–263. https://doi.org/10.1111/bcpt.12038

Figures

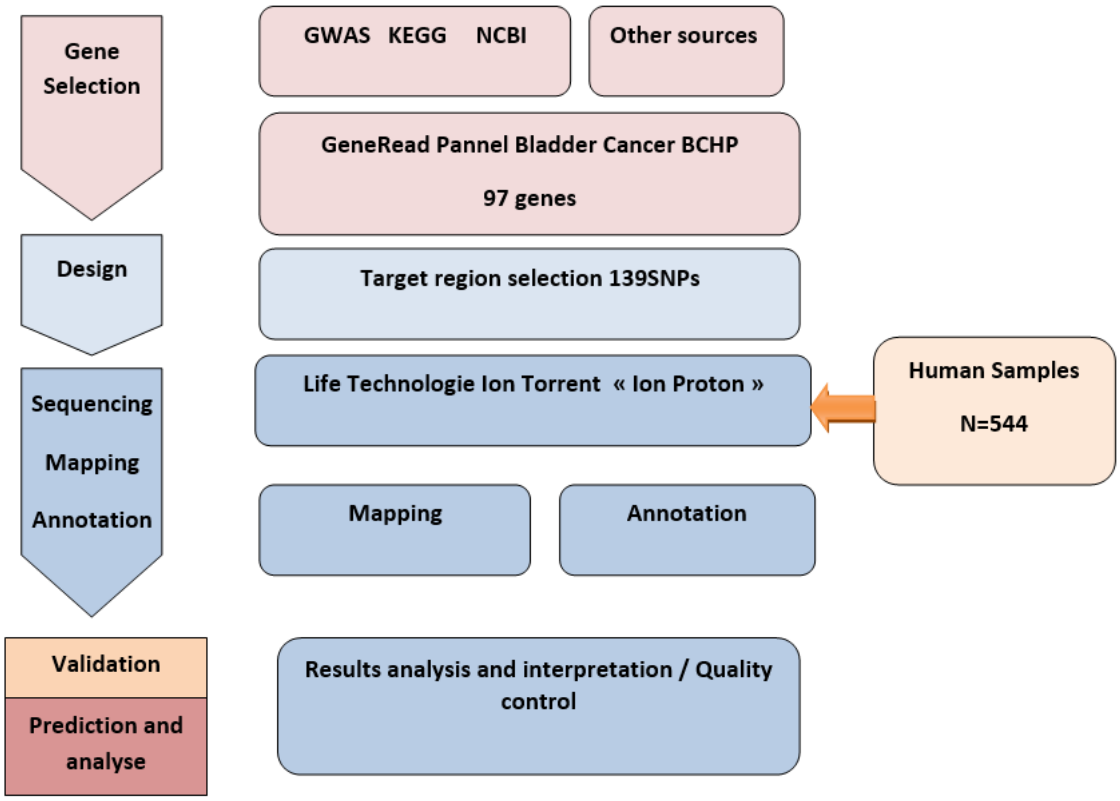


Figure 1

Overview of data analysis workflow

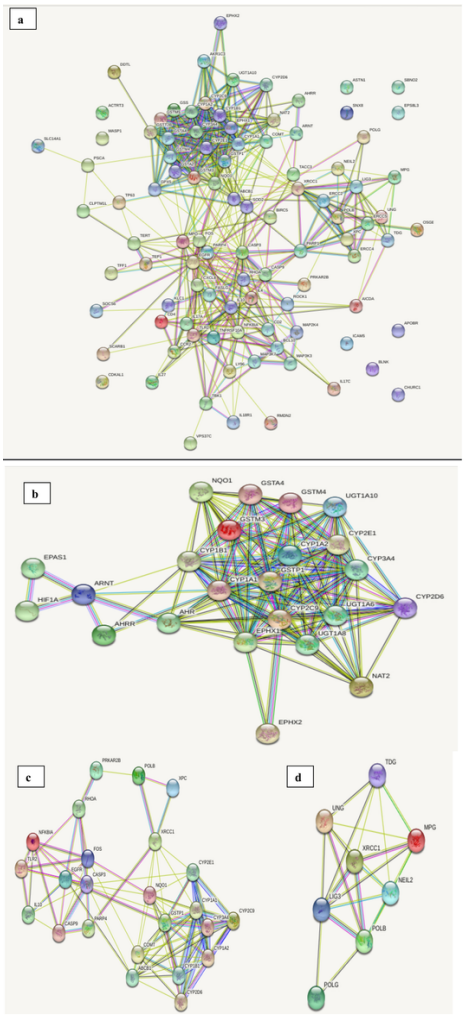


Figure 2

BCHP protein–protein interaction networks using a STRING server a: Overall BCHP panel b: Xenobiotic metabolic process c: Base-excision repair d: Response to drug

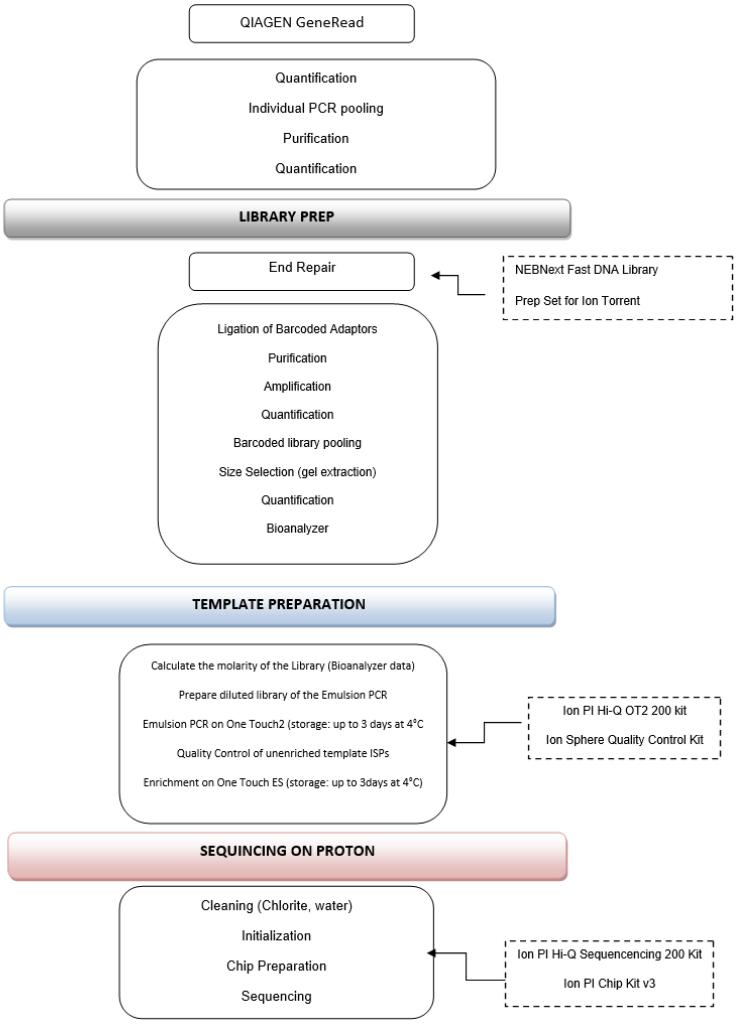


Figure 3

Proton experiment workflow

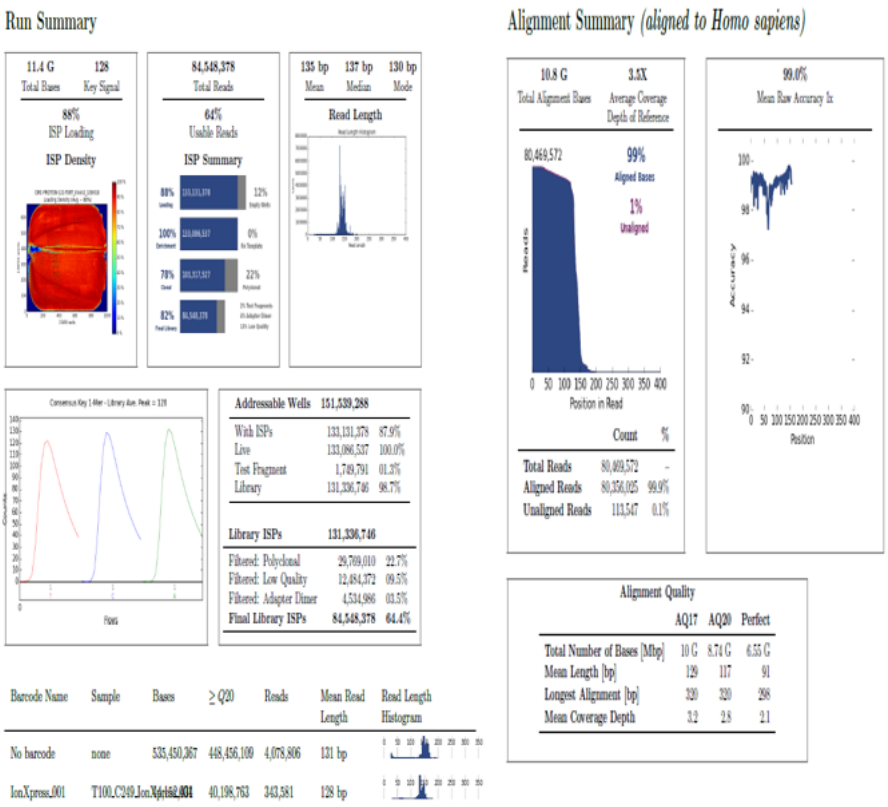


Figure 4

Run2 Report for Auto user CIRC-PROTON-121-TERT Imen2 100418 299

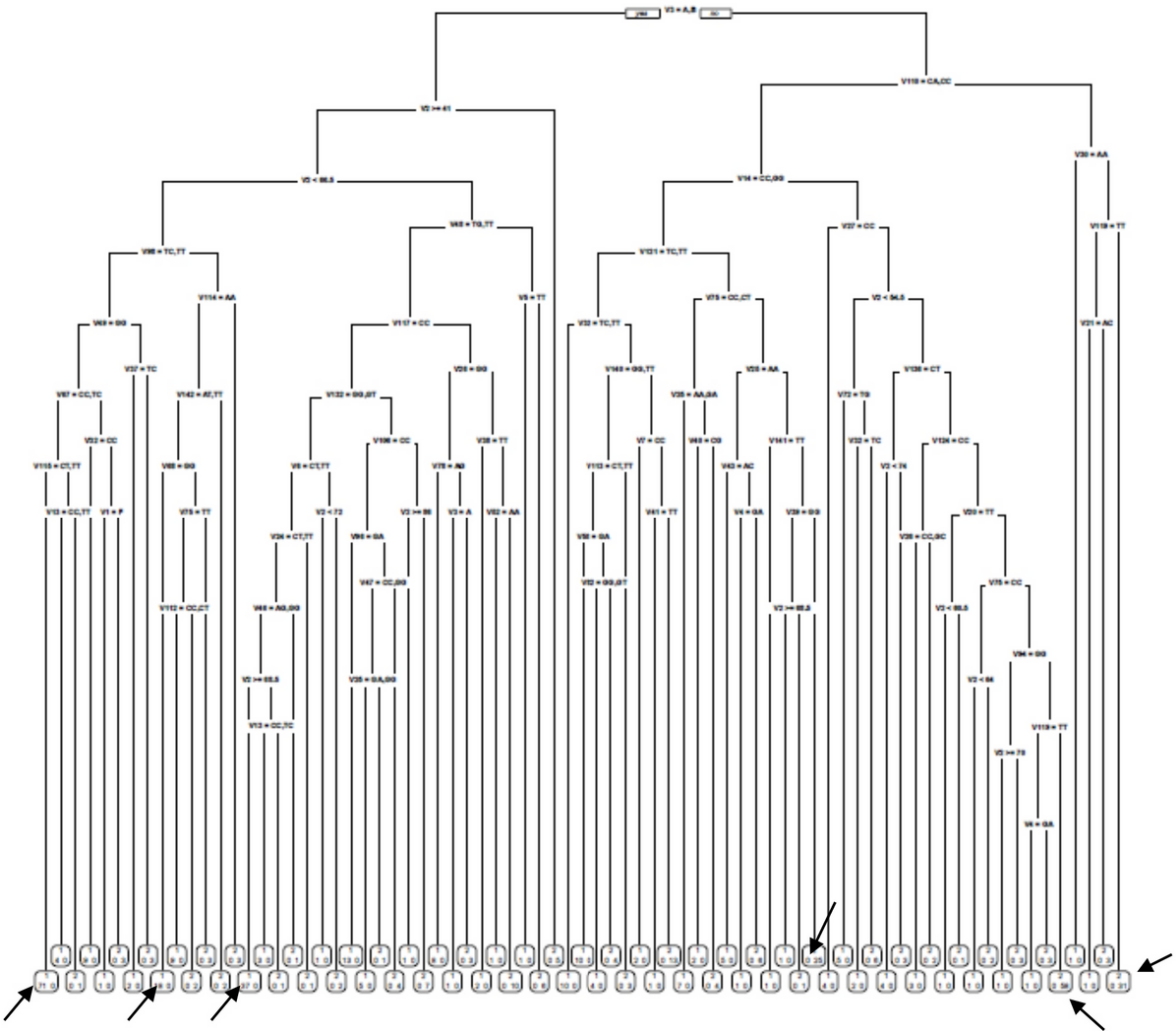


Figure 5

Decision Tree of predictive profiles of Patients and Controls V: Variable

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile.docx