RESEARCH ARTICLE

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Clinical Exome Gene Panel Analysis of a Cohort of Urothelial Bladder Cancer Patients from Sri Lanka

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Abstract

Background: Bladder cancer has a high rate of recurrence and high mortality rates in those who progress to muscle invasive disease. Biomarkers and molecular sub classification of tumours beyond standard histopathology has been proposed to address therapeutic dilemmas. The Cancer Genome Atlas project and other studies have contributed to the enhanced knowledge base of the mutational landscape of urothelial bladder cancer. Once again, these are mostly from Caucasian and Chinese patients, with data from the rest of Asia and Sri Lanka being sparse. The objective of this study was to assess the genomic variations of a cohort of urothelial bladder cancer patients in Sri Lanka. **Methods:** The molecular genetic study was conducted on formalin fixed paraffin embedded tumour samples of 24 patients, prospectively enrolled from 2013 to 2017. The samples were sequenced and variant distribution performed based on a 70-gene panel. **Results:** Total number of filtered mutations in the 24 patients was 10453. Median mutations per patient were 450 (range 22-987). The predominant mutational change was C>T and G>A. The top 5 mutated genes in our cohort were *SYNE1*, *SYNE2*, *KMT2C*, *LRP2*, and *ANK2*. The genes were clustered into 3 groups dependent on the number of mutations per patient per gene. The genes of cluster 1 and 2 mapped to Chromatin modifying enzymes and Generic Transcription Pathway. The chromatin remodelling pathway accounted for the largest proportion (22%) of mutations. **Conclusions:** Clinical exome sequencing utilising a gene panel yielded a high mutation rate in our patients. The predominant mutational change was C>T and G>A. Three clusters of genes were identified. *SYNE1* was the gene with the most mutations. The mutations comprised predominantly of genes of the chromatin remodelling pathway.

Keywords: Genetic mutations- bladder tumour- pathways- *SYNE1*

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Introduction

Bladder cancer has a high rate of recurrence and high mortality rates in those who progress to muscle invasive disease. Crucial decision making in certain patients e.g. pT1 high grade disease, muscle invasive bladder cancer (MIBC), metastatic bladder cancer, can be very challenging. While factors such as grading/ staging, comorbidities, patient preferences play an important role, there can be indecisiveness in deciding how surgery, chemo-radiation, and immunotherapy are used in particular patients.

The requirement for practical biomarkers and molecular pathology in precision medicine has been investigated and also highlighted by many (Vandekerkhove et al., 2021). In this context genomic investigation of bladder cancer has had several successes. In a study at the M.D. Anderson Cancer Center, a "p53-like" MIBC molecular subtype was identified with a wild-type p53 gene expression signature. Patients in this subtype showed resistance to neoadjuvant chemotherapy (Choi et al., 2014) Erdafitinib, an inhibitor of fibroblast growth factor receptors, was granted accelerated registration by the FDA in 2019, for patients with locally advanced or metastatic urothelial carcinoma. This oral drug is currently recommended in those with genetic alterations in *FGFR2* or *FGFR3* and

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have progressed while on platinum based chemotherapy (Thomas and Sonpavde, 2022).

During the recent past several landmark papers have attempted to clarify the molecular genetic profile of urothelial bladder cancer. The Cancer Genome Atlas Project, Pietzak et al (Weinstein et al., 2014; Pietzak et al., 2017; Robertson et al., 2017) shed light on the molecular genetics of Caucasian populations, while the data of Asian populations is mainly from Chinese populations. The findings of the above studies and Yaoting Gui's team from Shenzhen, China (Gui et al., 2011) and Zhiming Cai's (Guo et al., 2013) are summarised in supplementary material 1.

In a Chinese cohort of 112 urothelial cancer patients, Yang et al found a higher occurrence of somatic variations in *FGFR4*, *KDM5C*, *TERT*, *PDGFRB*, *FLT3*, *FLCN*, *MSH6*, *FLT1* genes when compared to the *TCGA* database (Yang et al., 2021).

Gangwar (Gangwar and Mittal, 2010), Pandith (Pandith et al., 2013), Ali (Ali et al., 2017), Mittal (Mittal et al., 2008), Ahmed (Ahmed et al., 2018) among others have evaluated the germline risk factors in urothelial bladder cancers in South Asian populations. However somatic mutations in bladder cancer of the South Asian populations has been very sparsely investigated.

Our objective was to investigate the mutational profile of a Sri Lankan cohort with urothelial bladder cancer, to identify the bladder cancer associated genes in our population and to assess the differences from Caucasian and other Asian studies.

Materials and Methods

Study sample

A urothelial bladder cancer cohort who underwent TURBT, were prospectively enrolled, by convenient sampling, from June 2013 to January 2017, from two teaching hospital urology departments of Sri Lanka. All patients provided written informed consent. The FFPE blocks of patients who had undergone their first intervention (transurethral resection of bladder tumours i.e. TURBT) for bladder tumours was obtained from the relevant pathology departments.

Genetic analysis

Genomic DNA was isolated from FFPE blocks by using Phenol:chloroform isolation method. The paired-end (PE) library was prepared from the samples using TruSeq Exome Library Preparation Kit. The pool library was sequenced on NextSeq 500. High quality reads were aligned against the Human reference genome Hg19 (Build - GRCh37, obtained from https://genome.ucsc.edu/). GATK best practices protocol was used to perform the somatic mutation calling. Using different flavour of GATK realignment, recalibration and variant calling using VarDict (v1.5.1) was performed with default parameters.

Variant classification adhered to the guidelines of next generation sequencing from American College of Medical Genetics and Genomics (ACMG) and American Congress of Obstetricians and Gynecologists (ACOG). Variants were annotated using INGENUITY® toolkit.

Variant distribution of the samples based on a 70-gene panel was done (Supplementary material 2).

The variants were filtered using the following criteria:

• All germline variants with a mean allele frequency (MAF) > 0.01 identified in 1000genome All, 1000 Genomes SAS, GenoAdd exome_ALL and GenoAdd exome _SAS databases were removed.

• All variants with a COSMIC ID were retained while all variants with an rsID were removed.

• As per CLINVAR SIG, variants that are likely benign/benign were filtered out.

• The exonic variant functions, nonsynonymous, frameshift, stop gain/loss, startloss were retained while others were removed.

• Variants in exonic regions and splice sites were retained.

• Of the 70-gene panel, 60 genes were selected as per the previously identified mutated genes in urothelial cancer from literature.

• From the Sri Lanka Genetic Variation Database variants and allele counts were extracted from the Inherited cancer patient list (n=187) to identify common germline variants in the Sri Lankan population. In the 60 genes selected as per above, variants were selected with a MAF > 0.01. The duplicated variants were removed from our variant list. To check if its non-silent mutation rate was significantly higher ($P < 0.05$) than the background we used the chi square test to remove the non-significantly mutated genes.

Results

A total number of 11033 mutations were identified. As per the variant type it comprised of 10384 single nucleotide variants, 559 deletions, 86 insertions and 4 substitutions. The functional classification of the mutations is shown in Table 1. The two commonest mutations documented in our patients was C>T and G>A. The SNV class is shown in Table 2.

From the above mutations, 60 genes were selected from our panel of 70 as per the genes identified in previous literature. To check if its non-silent mutation rate was significantly higher ($P < 0.05$) than the background we used the chi square test to remove the non-significantly

Table 1. The Functional Classification of the Mutations

The functional classification of the mutations	
Functional classification	Number of mutations
Nonsynonymous SNV (missense)	9255
Stopgain (nonsense)	848
Frameshift deletion	524
Frameshift insertion	79
Startloss	11
Stoploss	4
Frameshift substitution	2
Blanks	310
Grand Total	11033

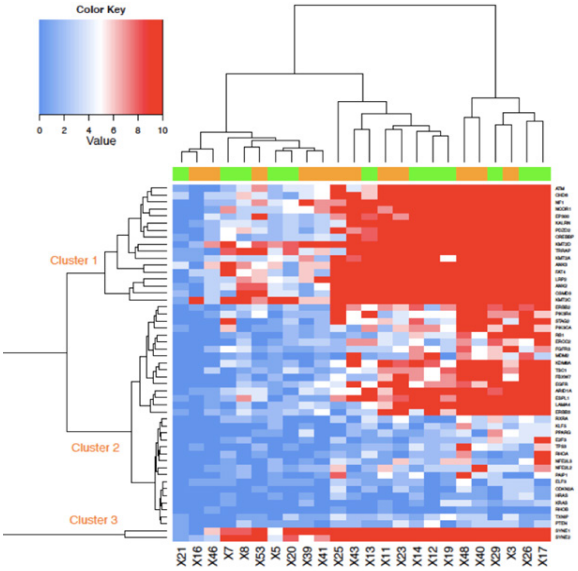

Figure 1. Clustering of the Genes as Per Number of Mutations. NB, Orange is MIBC and green is NMIBC. 0 (no expression) denoting blue and 10 (higher expression) denoting red, obtained by normalizing the number of mutations per patient per gene into the range of 0 -10.

mutated genes (Table 3).

Number of mutations per patient are detailed in Table 4. Median mutations per patient were 450 (range 22-987). There were three patients with over 900 mutations each. Two were high grade MIBC; the third was an 80-year-old male with pTa LG disease.

Total mutations in the genes, with significant mutations compared to background mutations, were 10453. The number of mutations in genes as per patient, are tabulated in table 5. The top 5 mutated genes were *SYNE1*, *SYNE2*, *KMT2C*, *LRP2*, *ANK2*.

Clustering of the genes was done dependent on the number of mutations and is displayed in Figure 1. There were 3 main clusters identified in the 51-gene list. Utilising the DAVID Gene Functional Classification Tool (https://david.ncifcrf.gov/tools.jsp updated December 2021) (Huang da et al., 2009; Sherman et al., 2022) we identified two main biological processes linked to these clusters (Supplementary material 3). The genes of cluster 1 and 2 mapped to chromatin modifying enzymes and generic transcription pathway respectively as per the Reactome pathway database.

The chromatin remodelling pathway accounted for the largest proportion (22%) of mutations of pathways implicated in urothelial cancer oncogenesis (Table 6). The following 9 genes of this pathway were present in our gene list *ARID1A*, *CHD6*, *CREBBP*, *EP300*, *KDM6A*, *KMT2A*, *KMT2C*, *KMT2D*, and *NCOR1*. The details of the mutations within these genes are given in Supplementary material 4.

Discussion

Our study population shows a high number of somatic tumour mutations with median mutations per patient of 450 (range 22-987). DNA extraction and sequencing of FFPE tumour samples can be associated with DNA fragmentation and artifacts which could lead to false positives in variant calling. Hadd et al identified that compared to cell line DNA, FFPE extracted DNA yielded almost double the number of background variations. However with bioinformatic adjustments, they achieved a 96.1% (95% CI 96.1-99.3%) accuracy in variant calling for FFPE samples across various sequencing platforms compared to cell lines (Hadd et al., 2013). In a whole exome sequencing of 20 primary bladder cancers from South Korea, they identified 14,864 exonic mutations, with a median of 231 (range 20–1515) (Kim et al., 2020). A whole exome sequencing of a 19-year-old male from India with high grade urothelial cancer with lymph node metastasis, they identified 558 exonic somatic mutations (Sharma et al., 2019).

The predominant mutational change in our 24 patients was C>T and G>A which is similar to mutations reported in other cancers (Greenman et al., 2007). In urothelial tumours an analysis by Gui et al in 9 patients with transitional cell cancers, identified that the SNV class of the mutations was dominated by a C:G>T:A pattern (Gui et al., 2011). This is also the predominant SNV class in our mutations.

In total, we identified 51 significantly mutated genes in our patients. The mutation frequency as per number of patients is very high for individual genes, when compared with the literature. E.g. *SYNE1* mutations in 95% of the patients; *SYNE2* 100%, *EP300* 91,67%, *TP53* 70.83%,

Table 2. SNV Class of the Mutations

SNV class	Number of mutations
C>T	3313
G>A	3210
C>A	989
G>T	948
A>T	443
T>A	394
A>G	315
T>C	274
G>C	216
C>-	186
C>G	179
G>-	160
A>-	116
T>-	97
A>C	54
T>G	49
->A	21
->T	19
->C	12
->G	8
->TC	2
->TT	2
Other	26
Grand Total	11033

Table 3. Identification of Genes with Significant Non Silent Mutations

Identification of genes with significant non silent mutations					
Genes	Synonymous	Nonsynonymous	X2_value	p_val	
ANK2	163	451	135.1	0	
ANK3	143	398	120.2	0	
ARID1A	134	192	10.3	0.001	
ATM	77	311	141.1	0	
BTG2	9	10	0.1	0.819	not significant
CCND1	23	30	0.9	0.336	not significant
CCND3	21	27	0.8	0.386	not significant
CDKN1A	13	12	0	0.841	not significant
CDKN2A	10	23	5.1	0.024	
CHD6	98	293	97.3	0	
CREBBP	128	272	51.8	0	
CSMD3	111	440	196.4	0	
E2F3	25	43	4.8	0.029	
EGFR	68	151	31.5	0	
ELF3	16	32	5.3	0.021	
EP300	87	262	87.8	0	
ERBB2	65	128	20.6	0	
ERBB3	53	172	62.9	0	
ERCC2	56	85	6	0.015	
ESPL1	87	177	30.7	0	
FAT4	111	365	135.5	0	
FBXW7	32	105	38.9	0	
FGFR3	53	96	12.4	0	
FOXA1	25	23	0.1	0.773	not significant
FOXQ1	21	16	0.7	0.411	not significant
HRAS	6	20	7.5	0.006	
KALRN	168	271	24.2	0	
KDM6A	43	147	56.9	0	
KLF5	21	40	5.9	0.015	
KMT2A	133	322	78.5	0	
KMT2C	133	583	282.8	0	
KMT2D	228	446	70.5	0	
KRAS	10	23	5.1	0.024	
LAMA4	72	162	34.6	0	
LRP2	165	506	173.3	0	
MDM2	12	72	42.9	0	
NCOR1	84	282	107.1	0	
NF1	103	341	127.6	0	
NFE2L2	17	66	28.9	0	
NFE2L3	23	54	12.5	0	
PAIP1	14	49	19.4	0	
PDZD2	99	247	63.3	0	
PIK3CA	20	123	74.2	0	
PIK3R4	34	115	44	0	
PPARG	23	43	6.1	0.014	
PTEN	13	40	13.8	0	
RB1	40	90	19.2	0	

Table 3. Continued

Identification of genes with significant non silent mutations					
Genes	Synonymous	Nonsynonymous	X2_value	p_val	
RHOA	12	38	13.5	0	
RHOB	22	5	10.7	0.001	
RXRA	26	47	6	0.014	
STAG2	21	120	69.5	0	
SYNE1	364	871	208.1	0	
SYNE2	265	716	207.3	0	
TP53	27	50	6.9	0.009	
TRAK1	72	80	0.4	0.516	not significant
TRRAP	198	391	63.2	0	
TSC1	47	135	42.5	0	
TXNIP	20	42	7.8	0.005	
ZFP36L1	30	25	0.5	0.5	not significant
Grand Total	4565	10676			

PTEN 79,17%. These could be due to the small patient numbers evaluated. The unavailability of further tumour tissues has precluded us from performing validation studies.

Of the genes with the highest frequency of mutations identified in our study, Spectrin Repeat Containing Nuclear Envelope Protein 1 (*SYNE1*) gene, with a total of 871 mutations, was the highest. From cBIOportal with

Table 4. Number of Mutations Per Patient and Pathological and Survival Details

Participan num	Number of mutations	Age at diagnosis	Gender	Stage	Grade	Muscle invasiveness	Survival in months as at Nov 2020 or death
3	790	73	M	5	2	1	NA
5	109		M	2	1	0	87
7	179	63	M	4	1	0	87
8	171	66	M	4	2	0	NA
11	471	69	M	5	2	1	24
12	556	66	M	4	1	0	51 (lost for FU after 2017)
13	429	55	M	2	1	0	NA
14	574	67	M	2	1	0	86
16	25	66	M	5	2	1	NA
17	948	80	M	2	1	0	3
19	558	70	M	4	1	0	81
20	128	89	F	4	2	0	15
21	22	54	M	4	1	0	78
23	581	49	M	5	2	1	11
25	488	75	F	5	2	1	NA
26	863	85	M	4	1	0	71
29	716	56	M	4	1	0	NA
39	134	62	F	5	2	1	12
40	987	68	M	5	2	1	36 (lost for FU after 2017)
41	146	58	M	5	2	1	35 (lost for FU after 2017)
43	355	84	M	5	2	1	NA
46	70	71	M	5	2	1	19 (lost for FU after 2017)
48	980	65	M	5	2	1	NA
53	173	67	M	5	2	1	19
Total	10453						

FU, follow up; Grade, (1, low grade; 2, high grade); Stage, (2, pTa; 3, CIS; 4, pT1; 5, pT2 or more)

Table 5. The Number of Mutations Per Gene Per Patient

	Participant number																									
Gene	3	5	7	8	11	12	13	14	16	17	19	20	21	23	25	26	29	39	40	41	43	46	48	53	Grand Total	Mutations in patients %
SYNE1	65	5	8	8	44	51	30	41		75	51	10	1	36	29	86	60	10	99	11	36	6	93	16	871	95.83
SYNE2	60	4	12	9	38	44	25	33	2	77	45	8	1	37	34	72	47	5	65	7	11	3	61	16	716	100.00
KMT2C	45	13	13	11	26	39	33	24	10	48	20	13	1	43	45	37	34	6	50	6	21	3	35	7	583	100.00
LRP2	46	5	3	7	24	26	23	36	1	56	24	4		20	15	53	40	6	50	10	13	3	35	6	506	95.83
ANK2	31	4	4	8	28	22	27	23		39	27	3	1	21	19	42	33	1	46	5	12	3	44	8	451	95.83
KMT2D	37	7	14	5	11	20	29	33	2	30	28	8		24	18	28	26	12	31	9	16	7	43	8	446	95.83
CSMD3	47	4	1	11	10	14	19	13		58	13	4	2	20	39	35	48	1	39	3	11	3	36	9	440	95.83
ANK3	26	6	13	7	10	27	23	24		35	26	1	4	17	35	24	26	6	37	6	12	6	22	5	398	95.83
TRRAP	22	4	8	11	25	24	16	18	1	32	24	8	1	34	3	21	31	4	36	6	10		38	14	391	95.83
FAT4	11	3	19	5	9	17	17	21	3	32	21	1		17	51	26	13	6	49	2	12	3	21	6	365	95.83
NF1	30	4	2	4	20	18	12	12		25	22	5		23	7	28	17	9	27	7	16	2	44	7	341	91.67
KMT2A	21	5	4	3	9	14	14	21	1	28	5	4	1	23	19	22	19	7	50	2	8	3	36	3	322	100.00
ATM	25	2	2	4	17	18	6	17		34	23	4	1	24	13	27	17	4	26	5	4		31	7	311	91.67
CHD6	13	2	3	6	20	14	6	18		21	21	1	3	22	13	32	18	4	21	5	9	3	34	4	293	95.83
NCOR1	11	3	7	3	7	21	11	16		24	15	5		13	10	20	25	5	32	7	14	1	29	3	282	91.67
CREBBP	39	2	5	1	14	14	8	15		21	22	3	1	15	3	24	21	3	16	4	14	2	23	2	272	95.83
KALRN	23	2	4	4	10	17	9	24	1	29	14	3		24	4	19	10	2	25	3	13	2	26	3	271	95.83
EP300	25	2	2	3	13	19	8	11		13	13	3		7	7	23	26	2	27	3	11	4	29	11	262	91.67
PDZD2	19	2	5	6	13	11	8	20		13	14	3		14	11	33	9	1	26	3	7	2	23	4	247	91.67
ARID1A	15	1	3	4	6	13	9	13	1	17	14			10	5	12	8	4	18	3	5	1	26	4	192	91.67
ESPL1	14		2	7	7	10	9	16		14	7	2	1	8	7	13	12	1	16	1	9	1	16	4	177	91.67
ERBB3	10	2	1	4	11	9	3	4		16	6	3	1	12		12	21	2	17	5	3	2	26	2	172	91.67
LAMA4	16	2		3	6	9	5	9		14	12	2		10	1	18	13	4	20	3	2	1	12		162	83.33
EGFR	10	1		3	11	7	5	5		18	7	2		16	4	15	13	3	6	5	10		8	2	151	83.33
KDM6A	6	1	4	3	11	8	5	12		17	5	2		8	5	9	10		14	2	9		15	1	147	83.33
TSC1	7	1		1	7	5	4	7		19	8	4		7	2	10	9	2	14	1	5	3	17	2	135	87.50
ERBB2	10	1	1	3	7	2	5	8	1	9	7	3		6	13	8	8	1	13	1	7		10	4	128	91.67
PIK3CA	13	1	6		2	7	7	7		6	5	2	1	6	4	11	6	1	20	2	1		14	1	123	87.50
STAG2	4	1	11		6	3	5	8		13	4	3		4	9	9	9		15	2	5		9		120	75.00
PIK3R4	7		4	2	2	5	9	5		6	5	2		4	9	12	7	2	10	2	5	1	11	5	115	87.50

Table 5. Continued

Gene	Participant number																										Total	Mutations in patients %
FBXW7	5	2		2	6	5	2	7		10	7			11	5	9	9		12	2	2		9		105	70.83
FGFR3	17	2	5	4	3	5	3	7		7	3	1		5	2	4	6	1	5	2	3	2	8	1	96	91.67
RB1	9		1	2	4	4	1	6		11	4	2		3	1	15	9	1	5	2	2		8		90	79.17
ERCC2	6	1		3	4	1	7	5		12	3	2		3	2	5	8	3	3	3	7		7		85	79.17
MDM2	4	1	1	2	2	9	1	7		3				2		8	6		5	1	13		7		72	66.67
NFE2L2	4	2	1	2	6	3	2	1		7	3	1		3	6	3	4	2	10	2		1	3		66	83.33
NFE2L3	1	2	1		1	2	1	3		9	6			6	4	5	1	1	2		3		6		54	70.83
TP53	3				2	2	2	4	1	6	3	1		1	1	1	4		5		1		11	2	50	70.83
PAIP1		1	1	2	5		1	5		5	1			2	8		3		4	1	1		9		49	62.50
RXRA	3	2	2	2	2		4	1		3				5	3	5	6		3				5	1	47	62.50
E2F3	4		1	2	1	2	1	2		9	2				3	4	6	1	3				2		43	62.50
PPARG	5	1			3	2	1	1		4	3			1		4	7	4			2		4	1	43	62.50
TXNIP	4			1	3	3	2	1		1	5	3	1	2	4	1		1	3	1	2		3	1	42	79.17
KLF5	2	2				3	3			4	2			3		4	4	1	3		2		7		40	54.17
PTEN	3	1		3	1	5	1	2		3		1	1	4	2	1	3	1	3		2		2	1	40	79.17
RHOA	1		2		1	2	4	2		10	2				2	1			1	1	1		7	1	38	62.50
ELF3	3	1	1		1		1	2	1		1	1		2	3	3	3	1			2	1	5		32	70.83
CDKN2A	3						2	1		1				3	4	3			1				4	1	23	41.67
KRAS	2		1		1			1		3	4					2	1	2	2				4		23	45.83
HRAS	3	1	1					2		1	1				3	4		1	1		1		1		20	50.00
RHOB					1										1				1			1	1		5	20.83
Grand Total	790	109	179	171	471	556	429	574	25	948	558	128	22	581	488	863	716	134	987	146	355	70	980	173	10453	

Table 6. Common Pathways Involved in Mutations

Pathways	Total mutations	Mutations as percentage of total mutations (%)
Chromatin remodelling	2799	26.78
p53 cell cycle	769	7.36
DNA damage and repair	600	5.74
RTK-RAS-MAPK	521	4.98
PI (3) K/AKT/mTOR	502	4.80

1868 samples in 10 studies of urothelial bladder cancer patients, the somatic mutation frequency was 10.8% (Cerami et al., 2012).

SYNE1 encodes for synaptic nuclear envelope protein 1 or nesprin-1 or enaptin which is an actin binding protein in humans. It has been implicated in autosomal recessive spinocerebellar ataxia. *SYNE1* encodes for several protein isoforms, which are constituents of many cellular processes including the organisation, integrity and positioning of the nucleus, and Golgi function (Doherty et al., 2010).

In a study by Shaglouf et al evaluating the expression profile of several proteins and to quantify the expressed transcripts in rats with hepatocellular carcinoma (HCC), they found *SYNE1* protein was up regulated along with two other proteins. The transcripts of these proteins showed elevated expression, at different stages of hepatocellular carcinoma from initiation to established tumour stage. It indicates the involvement of *SYNE1* proteins in HCC tumorigenesis (Faraj Shaglouf et al., 2020). They also hypothesise that the genes coding for the up regulated proteins probably act via interaction with other genes (e.g. *CUL7*, *CUL9*, *p53* and *VEGFR*) involved in cancer pathways.

The Exonic Variant rs9479297 in the *SYNE1* gene has been implicated in double primary cancer oncogenesis in urothelial carcinoma and hepatocellular carcinoma (Chu et al., 2021). *SYNE1* is a known driver gene in breast cancer. It has also been identified as one among 14 genes useful in identifying chemosensitivity in breast cancer (Al Amri et al., 2020).

In an analysis of somatic mutations in clear cell renal cell cancers from the TCGA databases Li and others identified that mutations of *SYNE1* were associated with a higher tumour mutational burden and a poorer prognosis. However *SYNE1* correlated with a better response to immune checkpoint inhibitors (Li et al., 2020).

A study utilizing the urothelial cancer datasets from the Cancer Genome Atlas (TCGA) database were evaluated using the CIBERSORT algorithm. This algorithm was used to identify signatures based on tumour immune cell infiltration patterns. Four immune subtype clusters were identified with specific genes among them, as identified by mRNA expression profiling. *SYNE1* was a gene identified in cluster 2 which had the best outcome with patients having a longer overall survival (Wu et al., 2020).

The high mutation rate in the *SYNE1* gene in our population warrants further confirmatory studies, as well as functional evaluation studies to assess its role in bladder cancer oncogenesis.

Chromatin remodelling pathway

One or more of the genes involved in the chromatin remodelling pathways were mutated in 76% of tumours in the TCGA 2014 cohort (Weinstein et al., 2014). Of the 39 significantly mutated genes in the 2017 TCGA study, 10 were from the chromatin-remodelling pathway (Robertson et al., 2017). In a Chinese cohort of 112 patients the mostly mutated pathway was the chromatin remodelling (73.21%) (Yang et al., 2021). In our study the chromatin remodelling pathway accounted for 26.21% of the total mutations seen in our cohort. The mutations of this pathway belonged to the histone acetyltransferase genes, *CREBBP* and *EP300*; SWI/SNF-related chromatin remodeling gene *ARID1A*; histone methyltransferase (HMT) genes *KMT2A* (MLL) and *KMT2C* (MLL3); *NCOR1* (which codes for a subunit possessing histone deacetylation (HDAT) activity; *CHD6* (encodes a component which remodels chromatin) (Gui et al., 2011).

Three main pathways have been implicated in urothelial cancer oncogensis. Cell proliferation and survival is altered by mutations in e.g. *FGFR3*, *RAS*, *PIK3CA*, *PTEN*, which cause activation of MAPK and PI3K/AKT signal transduction pathways. *TP53*, *RB1*, *CDKN2A* mutations affect p53-cell cycle pathway and cell cycle checkpoints. Also disruption of cell senescence via mutations in TP53, RB1 and TERT promoter gene, are also primarily implicated (Hoffmann and Schulz, 2021; Tran et al., 2021). Recent studies including TCGA (Robertson et al., 2017), and Yang et al in their study of a Chinese cohort with urothelial cancer (Yang et al., 2021), have identified consistent mutations in the chromatin remodelling pathway being implicated in bladder cancer. Urothelial bladder cancer has one of the highest mutations among all cancers within the chromatin remodelling pathway. Chromatin structure regulation is required for genome replication, transcription, mitosis, DNA repair, heterochromatin formation, and other nuclear processes.

In a recent study by Lawson et al utilising microdissected samples from 15 cadaveric transplant patients and 5 bladder cancer patients, they performed targeted whole exome and genome scanning. In this they identified that normal urothelium contains a median number of 40 mutations per exome and 1879 genomic mutations. Of the frequently mutated genes in the normal urothelium, 5 (*KMT2D*, *KDM6A*, *ARID1A*, *KMT2C*, *EP300*) of the top six genes were from the chromatin remodelling pathway (Lawson et al., 2020). This was in contrast to the genes of the PI3K/AKT and p53-cell cycle pathways which are more commoner in bladder cancer urothelium in comparison to normal urothelium. This fact and the observation that chromatin remodelling pathway genes are mutated across all tumour stages and molecular

subtypes of urothelial cancer, support a hypothesis that mutations of chromatin regulators is an early process during bladder cancer oncogenesis (Lawson et al., 2020; Hoffmann and Schulz, 2021). They further expound that mutations may enhance the capability of precursor urothelial cells to self-replicate. Our study adds to this evidence of the significant involvement of the chromatin remodelling pathway in bladder cancer tumorigenesis, and provides the first such evidence in a South Asian population.

Limitations of this study include the small patient numbers which impact the power of the study. Funding restrictions and non-availability of tumour tissue for further validation studies have restricted us from confirming or refuting the findings of this study. The gene panel based next generation sequencing of the samples were only performed on the tumour tissues. The absence of non-tumour samples for tumour-normal comparison could also be considered as a limitation in discovering the true somatic variants from the background variants. Nonetheless, we used optimized bioinformatics workflows and stringent filtering criteria to identify true somatic variants from the tumour samples. In the genetic data analysis, comparisons with several genomic databases (1,000 genome All, 1,000 Genomes SAS, GenoAdd exome_ALL and GenoAdd exome_SAS databases) were carried out including the Sri Lanka Genetic Variation Database. All variants with a minor allele frequency (MAF) > 0.01 were removed to retain the true somatic variants.

In conclusion, the clinical exome sequencing utilising a panel of 70 genes yielded a high mutation rate in our patients with a median mutation rate of 450. The predominant mutational change was C>T and G>A. Three clusters of genes were identified dependent on the number of mutations. The genes of cluster 1 and 2 mapped to Chromatin modifying enzymes and Generic Transcription Pathway. *SYNE1* was the gene with the most mutations. The mutations comprise predominantly of genes of the chromatin remodelling pathway.

Author Contribution Statement

APM, RWJ, VHWD, MDSL were involved in the protocol and project development. Data collection was conducted by APM, SASG, NDP, AA, SP. Data analysis and interpretation was by APM, NN, PS, SP, CE, DE. The initial manuscript writing was done by APM, NN, while all authors did the critical revision of the article. All authors approved the final version to be published.

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Approval by scientific bodies

Approved by the Research & Higher Degrees Committee, Faculty of Medicine, University of Colombo, Sri Lanka.

This is a subsection of the main PhD study by the corresponding author Ajith Malalasekera

Ethics approval and consent to participate

Ethical approval was obtained from the ethical review committee of the Faculty of Medicine, University of Colombo. (EC-12-088 / 19th October, 2012). The study was conducted in accordance with the ethical standards of the relevant institutional ethics committee and the Helsinki Declaration of 1975, as revised in 2000. All study participants have granted their consent as per the institutional review protocols.

Availability of data and materials

The datasets generated and/or analysed during the current study are not publicly available due to the information being private and confidential, but are available from the corresponding author on reasonable request.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

References

Ahmed T, Nawaz S, Noreen R, et al (2018). A 3' untranslated region polymorphism rs2304277 in the DNA repair pathway gene OGG1 is a novel risk modulator for urothelial bladder carcinoma. *Ann Hum Genet*, **82**, 74-87.

Al Amri WS, Baxter DE, Hanby AM, et al (2020). Identification of candidate mediators of chemoresponse in breast cancer through therapy-driven selection of somatic variants. *Breast Cancer Res Treat*, **183**, 607-16.

Ali SHB, Bangash KS, Rauf A, et al (2017). Identification of novel potential genetic predictors of urothelial bladder carcinoma susceptibility in Pakistani population. *Fam Cancer*, **16**, 577-94.

Cerami E, Gao J, Dogrusoz U, et al (2012). The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer Discov*, **2**, 401-4.

Choi W, Porten S, Kim S, et al (2014). Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. *Cancer Cell*, **25**, 152-65.

Chu YD, Kee KM, Lin WR, et al (2021). SYNE1 Exonic Variant rs9479297 Contributes to Concurrent Hepatocellular and Transitional Cell Carcinoma Double Primary Cancer. *Biomedicines*, **9**.

Doherty JA, Rossing MA, Cushing-Haugen KL, et al (2010). ESR1/SYNE1 polymorphism and invasive epithelial ovarian cancer risk: an Ovarian Cancer Association Consortium study. *Cancer Epidemiol Biomarkers Prev*, **19**, 245-50.

Faraj Shaglouf LH, Ranjpour M, Wajid S, et al (2020). Elevated expression of cellular SYNE1, MMP10, and GTPase1 and

their regulatory role in hepatocellular carcinoma progression. *Protoplasma*, **257**, 157-67.
Gangwar R, Mittal RD (2010). Association of selected variants in genes involved in cell cycle and apoptosis with bladder cancer risk in North Indian population. *DNA Cell Biol*, **29**, 349-56.
Greenman C, Stephens P, Smith R, et al (2007). Patterns of somatic mutation in human cancer genomes. *Nature*, **446**, 153-8.
Gui Y, Guo G, Huang Y, et al (2011). Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nat Genet*, **43**, 875-8.
Hadd AG, Houghton J, Choudhary A, et al (2013). Targeted, high-depth, next-generation sequencing of cancer genes in formalin-fixed, paraffin-embedded and fine-needle aspiration tumor specimens. *J Mol Diagn*, **15**, 234-47.
Hoffmann MJ, Schulz WA (2021). Alterations of Chromatin Regulators in the Pathogenesis of Urinary Bladder Urothelial Carcinoma. *Cancers (Basel)*, **13**.
Huang da W, Sherman BT, Lempicki RA (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc*, **4**, 44-57.
Kim T, Yoo J-s, Moon HW, et al (2020). Distinct mutation profiles between primary bladder cancer and circulating tumor cells warrant the use of circulating tumors cells as cellular resource for mutation follow-up. *BMC Cancer*, **20**, 1203.
Lawson ARJ, Abascal F, Coorens THH, et al (2020). Extensive heterogeneity in somatic mutation and selection in the human bladder. *Science*, **370**, 75-82.
Li P, Xiao J, Zhou B, et al (2020). SYNE1 mutation may enhance the response to immune checkpoint blockade therapy in clear cell renal cell carcinoma patients. *Aging (Albany NY)*, **12**, 19316-24.
Mittal RD, Singh R, Manchanda PK, et al (2008). XRCC1 codon 399 mutant allele: a risk factor for recurrence of urothelial bladder carcinoma in patients on BCG immunotherapy. *Cancer Biol Ther*, **7**, 645-50.
Pandith AA, Shah ZA, Khan NP, et al (2013). HRAS T81C polymorphism modulates risk of urinary bladder cancer and predicts advanced tumors in ethnic Kashmiri population. *Urol Oncol Semin Original Invest*, **31**, 487-92.
Pietzak EJ, Bagrodia A, Cha EK, et al (2017). Next-generation Sequencing of Nonmuscle Invasive Bladder Cancer Reveals Potential Biomarkers and Rational Therapeutic Targets. *Eur Urol*, **72**, 952-9.
Robertson AG, Kim J, Al-Ahmadie H, et al (2017). Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer. *Cell*, **171**, 540-56.e25.
Sharma J, Deb B, George IA, et al (2019). Somatic Mutations Profile of a Young Patient With Metastatic Urothelial Carcinoma Reveals Mutations in Genes Involved in Ion Channels. *Front Oncol*, **9**, 435.
Sherman BT, Hao M, Qiu J, et al (2022). DAVID: a web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res*, **50**, W216-21.
Thomas J, Sonpavde G (2022). Molecularly Targeted Therapy towards Genetic Alterations in Advanced Bladder Cancer. *Cancers (Basel)*, **14**.
Tran L, Xiao J-F, Agarwal N, et al (2021). Advances in bladder cancer biology and therapy. *Nat Rev Cancer*, **21**, 104-21.
Vandekerkhove G, Lavoie JM, Annala M, et al (2021). Plasma ctDNA is a tumor tissue surrogate and enables clinical-genomic stratification of metastatic bladder cancer. *Nat Commun,* **12**, 184.
Weinstein JN, Akbani R, Broom BM, et al (2014). Comprehensive molecular characterization of urothelial bladder carcinoma. *Nature*, **507**, 315-22.
Wu Z, Zhu K, Liu Q, et al (2020). Profiles of Immune Infiltration in Bladder Cancer and its Clinical Significance: an Integrative Genomic Analysis. *Int J Med Sci*, **17**, 762-72.
Yang B, Zhao X, Wan C, et al (2021). Genomic profiling of Chinese patients with urothelial carcinoma. *BMC Cancer*, **21**, 162.