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Genomic landscape of inverted urothelial papilloma and urothelial papilloma of the bladder

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Abstract

Inverted urothelial papilloma (IUP) and urothelial papilloma (UP) are rare urothelial neoplasms that typically follow a benign clinical course. Oncogenic mutations in *FGFR3, HRAS* and the *TERT* promoter have been reported in these entities but no comprehensive molecular analysis has been performed. We sought to characterize the genomic landscape of IUP and UP using whole

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Author contributions statement

SI, JS, HAA and DBS conceived and designed the study. YBC, AG, SWF, SKT, SJS, VER, and HAA performed consensus pathologic review of all cases. SI, WH, HAA and DBS analyzed and interpreted data. SI, WH, JS, HAA and DBS wrote draft of the manuscript. SI, WH, JS, YBC, AG, SWF, SKT, SJS, SJ, FLL, EJP, EKC, BHB, MFB, GI, DBS, VER and HAA provided input for critical revision of the manuscript for important intellectual content. HAA and DBS supervised the study.

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exome and targeted next generation sequencing. In IUP, 10 of 11 tumors harbored oncogenic hotspot mutations in *HRAS* and the remaining tumor had an oncogenic *KRAS* mutation. None of the IUP tumors harbored *TERT* promoter or *FGFR3* mutations. In UP, 8 of 11 tumors had oncogenic *KRAS* mutations and 2 had oncogenic *HRAS* mutations. One UP tumor had oncogenic mutations in *FGFR3, PIK3CA* and the *TERT* promoter and arose in a patient with recurrent non-invasive papillary urothelial carcinomas. In contrast to urothelial carcinoma, the APOBEC mutational signature was not present in any IUP and UP tumors and oncogenic alterations in chromatin remodeling genes were uncommon in both IUP and UP. The current study suggests that IUP and UP are driven primarily by RAS pathway activation and lack the more common genomic features of urothelial cancers.

Keywords

Genomics; Inverted Urothelial Papilloma; Urothelial Papilloma; Urothelial Carcinoma; RAS pathway

Introduction

Initially described by Paschkis in 1927 and named by Potts and Hirst in 1963, inverted urothelial papilloma (IUP) is an uncommon urothelial neoplasm that rarely recurs following complete surgical resection [1]. IUP is characterized by endophytic growth of urothelial nests and cords with normal/bland histology. In contrast, urothelial papilloma (UP), another rare urothelial neoplasm, is a benign proliferation of the urothelium characterized by discrete, exophytic papillary growth with central fibrovascular cores lined by urothelium of normal thickness and cytology [2]. IUP and UP are distinguishable from carcinomas as they lack architectural and cellular disorder or atypia. Prior studies of IUP and UP using single gene assays reported *FGFR3* and *TERT* promoter mutations at various rates [3–7]. One prior study of 5 IUP tumors using a targeted next generation sequencing panel reported activating *HRAS* mutations in 3 tumors and an oncogenic *FGFR3* mutation in one [8]. In this study, we performed whole exome and/or targeted next generation sequencing to characterize the genomic landscape of IUP and UP with the goal of identifying similarities and differences between these clinically benign entities and urothelial carcinomas.

Materials and Methods

We identified 11 IUP and 11 UP cases with sufficient tissue for molecular analysis and confirmed the diagnosis in all cases by consensus pathologic review among 7 genitourinary pathologists (YBC, AG, SWF, SKT, SJS, VER, and HAA) (Table 1). Patient ages ranged from 31 to 90 years and the male:female distribution was 16:6. For 3 IUP and 3 UP tumors in which matched normal tissue was available, we initially performed whole exome sequencing (WES) using paired tumor and germline DNA. To validate the WES results, we performed targeted deep sequencing of 468 cancer-associated genes using the MSK-IMPACT assay to a target mean coverage of 586x for the 6 cases that had undergone WES and an additional 8 IUP and 8 UP cases (11 total for each histology). For all cases, formalin-fixed, paraffin embedded (FFPE) tissue was the source of DNA used for exome as well as

targeted sequencing. Genomic alterations were annotated using the OncoKB knowledge base (www.OncoKB.org) [9]. See supplementary material, Supplementary materials and methods for additional details regarding WES and the MSK-IMPACT assay.

Results and Discussion

WES revealed a relatively low tumor mutational burden (TMB) with a median TMB for IUP and UP of 0.334 and 0.698 (mutations/Mb), respectively (Figure 1). This low TMB was significantly less than that reported for non-invasive bladder cancers by Hurst *et al* (1.64 mut/Mb based on WES of 24 tumors) [10], or muscle invasive urothelial carcinoma by the TCGA (5.8 mut/Mb based on WES of 412 tumors) [11]. Oncogenic hotspot mutations in *HRAS* were identified in all 3 IUPs and oncogenic hotspot *KRAS* mutations were present in 2 of the 3 UPs (Figure 1C). The third UP had an oncogenic *BRAF* mutation (T599dup). Based on the OncoKB annotation system [9], these were the only known or likely oncogenic driver mutations in these IUP and UP tumors (supplementary material, Table S1). Missense mutations were more prevalent than nonsense mutations, insertions or deletions and these single nucleotide variants (SNV) were predominately C>T transitions. Notably, the APOBEC (apolipoprotein B mRNA editing catalytic polypeptide-like) mutational signature prevalent in urothelial carcinomas [11] was absent in all IUP and UP cases (Figure 1C).

To validate and expand upon the WES results, we performed targeted NGS of the same 6 samples and 16 additional tumors (8 each of IUP and UP, Figure 1D). The results of WES and MSK-IMPACT were highly concordant in the cases that underwent both analyses (supplementary material, Figure S1). Similar to WES, missense mutations were more prevalent than nonsense mutations, insertions or deletions and SNVs were predominantly C>T transitions (supplementary material, Table S2). In this expanded cohort, all IUP tumors harbored oncogenic RAS mutations (10 *HRAS* and 1 *KRAS* mutations). *TERT* promoter and *FGFR3* mutations, which are common in urothelial carcinomas, were not detected in any of the IUP cases. Ten of 11 UP tumors also had oncogenic mutations in the RAS/ERK signaling pathway (8 *KRAS*, 2 *HRAS* and 1 *BRAF* mutations). Notably, one of the UP tumors had both *KRAS* and *HRAS* mutations (with similar allele frequencies of 0.39 and 0.35). Further investigation as to whether these co-occurring RAS mutations were sub-clonal was hampered by the lack of matched normal DNA for this sample.

Only one of the cases, a UP, harbored oncogenic *FGFR3* or *TERT* promoter mutations. This tumor, which also had oncogenic *PIK3CA, KMT2D*, and *CDKN1A* mutations, had a mutational profile common to that observed in urothelial carcinoma [10, 12]. While morphologic evaluation of this tumor, which consisted of a single small papillary lesion, was compatible with the diagnosis of papilloma, it arose in a patient who had several low-grade non-invasive papillary urothelial carcinomas prior and subsequent to the index UP tumor. This clinical history suggested that this tumor was likely a recurrence of the prior NMIBC despite the bland histopathologic appearance and that the correct histologic diagnosis was confounded by its small size. To explore this possibility, we performed MSK-IMPACT analysis on tumor tissue from two subsequent recurrent tumors from this patient, both diagnosed as non-invasive, low grade papillary urothelial carcinomas. The mutational profiles of the two recurrent tumors were nearly identical to the index UP and confirmed that

all three lesions were clonally related (Figure 2 and supplementary material, Table S3). The results suggest that in the presence of a prior history of papillary urothelial carcinoma, the diagnosis of UP or IUP should be made with extreme caution and that tumor molecular profiling could help clarify whether the tumor represented a recurrence of the prior low grade urothelial cancer or a new clonally unrelated primary tumor.

With the exception of the case outlined above and contrasting prior reports in the literature [3–7], none of the other IUP or UP tumors had *TERT* promoter or activating *FGFR3* mutations. Similarly, in contrast to urothelial carcinomas, oncogenic alterations in chromatin remodeling genes were uncommon in both IUP and UP, being identified in only 1 IUP and 2 UP cases. In addition to the case described above, only two other patients in this series had a concurrent or subsequent bladder tumor. In one, a low grade urothelial carcinoma was diagnosed 16 months following the resection of IUP, whereas the second had a synchronous urothelial carcinoma elsewhere in the bladder. In the latter case (supplementary material, Table S3), the concurrent tumor was a plasmacytoid variant of urothelial carcinoma, which upon tumor sequencing was found to share no somatic alterations with the IUP but was rather a second genomically unrelated primary tumor [13].

In sum, our data suggest that IUP and UP are distinct molecular entities from urothelial carcinoma. Clinically, both IUP and UP are characterized by a benign clinical course with rare recurrence if completely excised. Pathologically, IUP and UP have morphologic mimics that may make the diagnosis challenging and likely explain the lack of inter-observer agreement in prior studies. For example, Eiber *et al* [3] reported that considerable inter-observer variability exists in histopathologic diagnoses of IUP with only 62 of 89 cases (70%) having an initial IUP diagnosis confirmed by subsequent pathology review whereas 23 cases (26%) were reclassified as inverted non-invasive urothelial carcinomas. The remaining four cases were diagnosed as cystitis cystica or glandularis with Brunn nests upon secondary review. These findings highlight the challenges in establishing the correct diagnosis of papilloma in difficult cases and underscore the importance of ancillary studies such as next generation sequencing that could be applied to properly classify such urothelial proliferations.

Identifying a unique molecular profile in IUP and UP, as we have shown in this study, suggests that tumor molecular profiling can help to correctly characterize these tumors and help to distinguish them from morphologic mimics. Specifically, we identified activating RAS pathway mutations as the defining feature of IUP and UP. *HRAS* mutations were much more common in IUP, whereas *KRAS* mutations were more prevalent in UP. Mutations in both *KRAS* and *HRAS* were present at the highest allele frequencies and, therefore, are likely to be early events in these tumors. The average VAF of KRAS and HRAS mutations was 41%. In addition, the median tumor cell fractions associated with *KRAS* and *HRAS* mutations in the 6 WES samples were mostly 100%, supporting that these are clonal and initiating alterations. At the nucleotide level, mutations in RAS genes in IUP or UP did not have a specific pattern. Beyond these specific alterations, these tumors were typically genetically silent with very low TMB. In contrast to non-invasive and invasive urothelial carcinomas, IUP and UP were not characterized by frequent mutations in the *TERT* promoter or in *FGFR3, TP53, RB1* or chromatin remodeling genes or associated pathways

(Figure 3A, Figure 3B and supplementary material, Table S4). Our findings contrast with prior reports of activating *FGFR3* and *TERT* promoter mutations in IUP and UP [4–7]. Such differences could be attributed to selection bias or due to the methodologies employed in prior studies, typically single gene-based assays versus comprehensive next generation sequencing platforms. It is important to note that a small subset of urothelial carcinomas harbor hotspot mutations in *HRAS* and *KRAS* [11, 12]. These RAS mutant urothelial carcinomas, however, display considerable morphologic atypia, do not exhibit exclusive inverted growth pattern and have other genomic alterations, which distinguish them from IUP and UP (Figure 3C). While the oncogenic RAS mutations observed in IUP and UP are occasionally found in invasive urothelial carcinomas and are also common in several other highly aggressive solid tumors, the fact that none of the papillomas in this study progressed to frank urothelial carcinoma suggests that the co-mutational background or epigenetic differences in the cell of origin may dictate the likelihood that a RAS mutant cell will progress to carcinoma. Lacking a specific co-mutational background, RAS mutations in urothelial cells may induce oncogene-induced senescence and thus result in a benign lesion incapable of further progression to carcinoma [14].

One of the limitations of the current study was its power to detect infrequently mutated genes due to small sample size. This is, however, the first study to report on the genomic landscape of IUP and UP using whole exome sequencing approach as well as the largest study of IUP and UP using targeted next generation sequencing. As IUP and UP are rare entities, larger studies of IUP and UP would require multi-institutional collaboration. To facilitate such efforts, all clinical and genomic data reported here will be made available through cBioPortal for Cancer Genomics [15].

In summary, oncogenic *HRAS* and *KRAS* mutations are present in almost all IUP and UP tumors. The benign nature of IUP and UP tumors may be attributed to the lack of cooperative co-mutations in the *TERT* promoter, *TP53* and in chromatin modifying genes, oncogenic drivers that are common in urothelial carcinomas.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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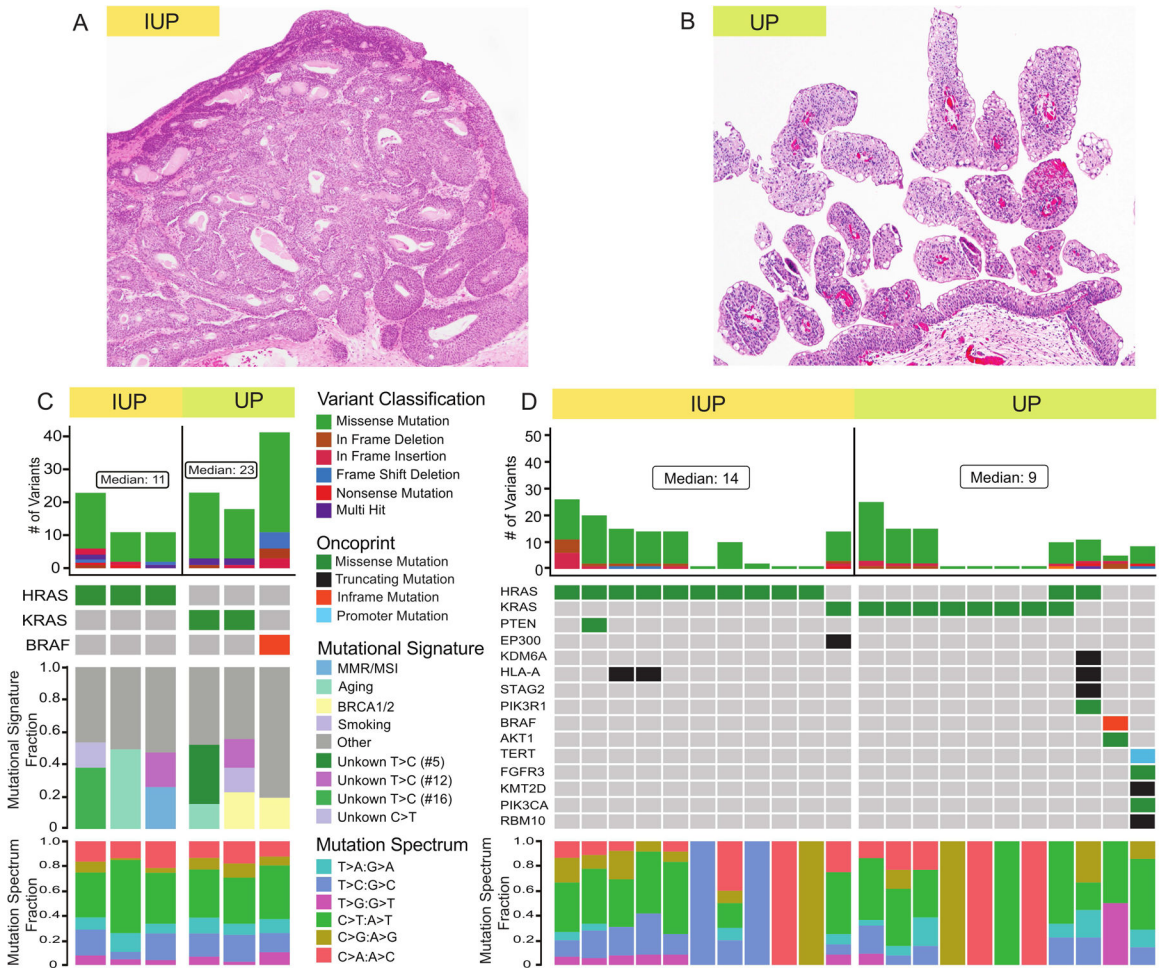

Figure 1.

Genomic landscape of inverted urothelial papilloma (IUP) and urothelial papilloma (UP). (A) H&E image of IUP at 40X magnification. (B) H&E image of UP at 40X magnification. (C) Variant classification, OncoPrint, mutational signature and mutation spectrum of 3 IUP and 3 UP cases that underwent whole exome sequencing. (D) Variant classification, OncoPrint and mutation spectrum of the 11 IUP and 11 UP cases analyzed using the MSK-IMPACT assay.

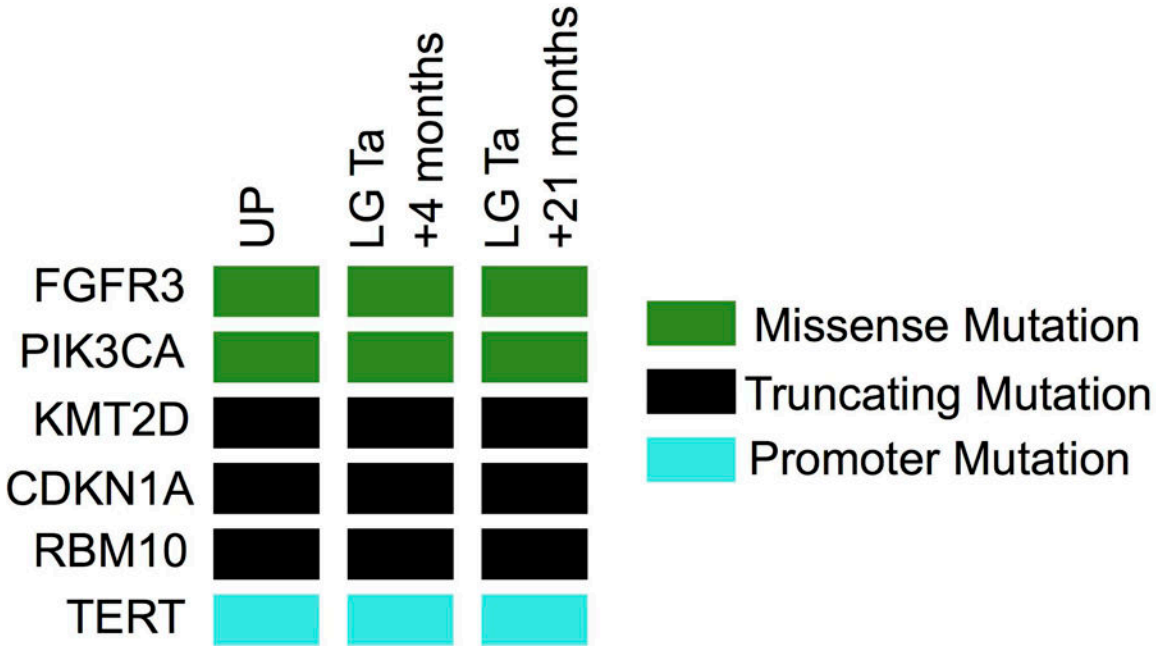

Figure 2.

OncoPrint showing clonally related identical oncogenic mutations in index UP and two subsequent recurrent low grade non-invasive tumors (LG pTa) in a patient with history of several LG pTa urothelial cancers.

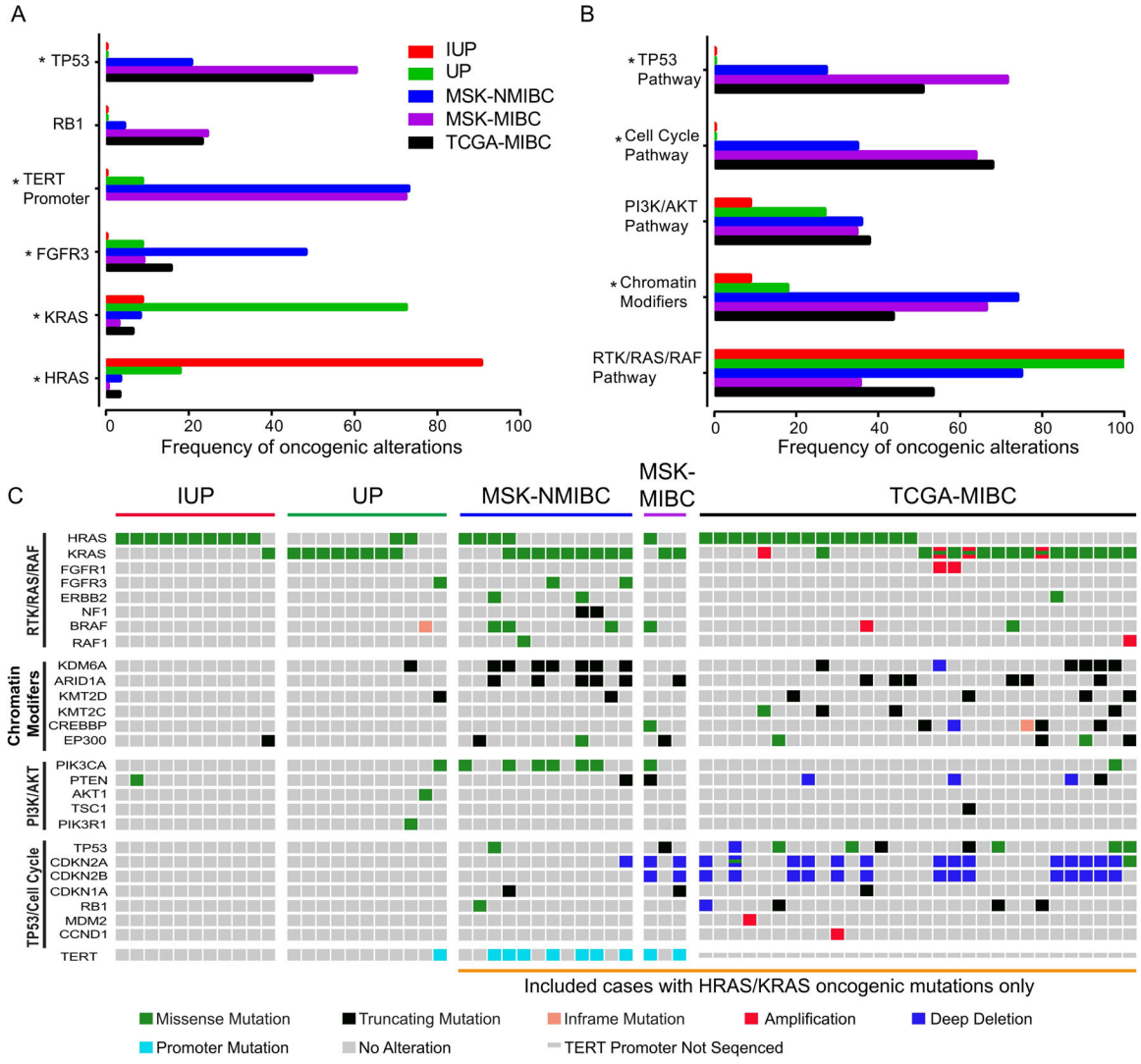

Figure 3.

Genomic comparison of inverted urothelial papilloma (IUP), urothelial papilloma (UP), non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC). (A) Frequency of *TP53*, *RB1*, *TERT* promoter, *FGFR3*, *KRAS* and *HRAS* alterations in IUP (n=11), UP (n=11), MSK-NMIBC n=105 [12], MSK-MIBC (n=117; unpublished data) and TCGA-MIBC n=412 [11]. * indicates p value <0.05. (B) Frequency of oncogenic alterations in molecular pathways in IUP (n=11), UP (n=11), MSK-NMIBC n=105 [12], MSK-MIBC (n=117; unpublished data) and TCGA-MIBC n=412 [11] cohort. * indicates p value <0.05. (C) OncoPrint showing molecular pathways in IUP (n=11), UP (n=11) and cases with oncogenic *HRAS*/*KRAS* mutations in MSK-NMIBC (n=12), MSK-MIBC (n=3) and TCGA-MIBC (n=30).

Table 1

shows clinicopathologic details on 11 IUP and 11 UP cases included in the study.

Case	Histology	Sex	Age (Years)	Matched Normal	Estimated Tumor Purity (%)	Target Mean Coverage IMPACT Assay	VAF of Driver Alterations (%)
IUP 1	Inverted Papilloma	Male	69	No	60	481	19
IUP 2	Inverted Papilloma	Male	77	No	75	501	45
IUP 3	Inverted Papilloma	Female	66	No	80	613	45
IUP 4	Inverted Papilloma	Male	57	No	80	677	46
IUP 5	Inverted Papilloma	Female	60	No	85	539	47
*IUP 6	Inverted Papilloma	Male	61	Yes	80	575	41
IUP 7	Inverted Papilloma	Male	66	No	80	594	43
IUP 8	Inverted Papilloma	Male	90	Yes	75	572	41
*IUP 9	Inverted Papilloma	Male	53	Yes	80	579	41
*IUP 10	Inverted Papilloma	Male	65	Yes	70	459	36
IUP 11	Inverted Papilloma	Male	86	No	80	690	56
UP 1	Papilloma	Male	70	No	85	600	51
UP 2	Papilloma	Male	54	No	80	711	59
UP 3	Papilloma	Female	81	No	80	405	59
*UP 4	Papilloma	Female	51	Yes	50	570	16
UP 5	Papilloma	Male	69	Yes	70	667	54
UP 6	Papilloma	Male	65	Yes	20	409	3
*UP 7	Papilloma	Male	71	Yes	60	579	31
UP 8	Papilloma	Female	31	No	70	597	39
UP 9	Papilloma	Female	62	Yes	70	671	25
*UP 10	Papilloma	Male	51	Yes	50	875	22
UP 11	Papilloma	Male	66	Yes	60	518	33

*Cases that underwent WES also