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Genomic Sequencing of Cancer-related Genes in Sinonasal Squamous Cell Carcinoma and Coexisting Inverted Papilloma

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Abstract. *Background: The genetic basis of sinonasal inverted papilloma (SNIP)-derived squamous cell carcinoma (SCC) has not yet been well characterized. Aim: To characterize the genetic abnormalities of SNIP and SNIP-derived SCC and to uncover their differences. Materials and Methods: Mutations of 409 genes were analyzed using amplicon targeted sequencing in a total of six papilloma/carcinoma samples from four patients with SNIP-derived SCC. Results: The genes that were mutated in multiple cases were epidermal growth factor receptor (EGFR) (3/6), cyclin-dependent kinase inhibitor 2A (CDKN2A) (3/6), lysine methyltransferase 2D (KMT2D) (3/6), tumor protein p53 (TP53) (3/6), neurofibromin 1 (NF1) (3/6), phosphodiesterase 4D interacting protein (PDE4DIP) (3/6), cytochrome P450 family 2 subfamily D member 6 (CYP2D6) (2/6), fms-related receptor tyrosine kinase 4 (FLT4) (2/6) and myosin heavy chain 9 (MYH9) (2/6). Of the two cases analyzed in the papilloma–oncology carcinoma pair, one did not have any common mutations; the other showed a staged functional deletion of TP53 during the process of malignant transformation from SNIP to SCC. Conclusion: CDKN2A, KMT2D, NF1, PDE4DIP, CYP2D6, FLT4, and MYH9 were identified as candidate novel SNIP-derived SCC-related genes.*

Sinonasal papillomas are generally considered benign tumors that can be cured by endoscopic surgery and are classified by the World Health Organization into three types: Inverted papilloma (IP), exophytic papilloma and oncocytic papilloma (1, 2). The most common type of papilloma is IP, with an incidence of 0.74-1.5 cases per 100,000 population per year (3). Sinonasal inverted papilloma (SNIP) is associated with squamous cell carcinoma (SCC), either synchronously or metachronously (4). The rate of carcinoma is reportedly 5 to 10% and is considered to be malignantly transformed from papilloma (5-7). SNIP-derived SCC has the same poor prognosis as de novo sinonasal SCC, with a reported 5-year postoperative survival rate of 39.6-65.7% (8-10). The preoperative diagnosis of malignant transformation of SNIP is important for radical resection. Improved tools that facilitate the preoperative diagnosis of SCC complications is a clinical issue that needs to be addressed, as imaging and markers are not well established (5, 11, 12).

The mechanism of SNIP development and its malignant transformation remain largely unknown (5). Immunohistological studies and studies focusing on certain genes have searched for risk factors for SNIP associated with SCC (13-16). In recent years, comprehensive genetic analyses using next-generation sequencing have become available and have revealed the genetic background of many carcinoma types; however, few genome-wide analyses of papilloma-derived cancer have been performed (17). Furthermore, in other types of cancer, the accumulation of genetic abnormalities during cancer evolution has been reported by comparing genetic abnormalities in precancerous and benign tumors with cancerous lesions using next-generation sequencing (18).

In this study, we identified genes that may be involved in the development of papilloma-derived SCC by analyzing the genomes of both papilloma and SCC components in papilloma-derived SCC specimens

Materials and Methods

Ethics statement. The protocol of this study was reviewed and approved by the Institutional Review Boards and Ethics Committees of Kyushu University (Protocol Number: 700-2 and 30-268). All procedures with human samples were conducted according to the principles expressed in the Declaration of Helsinki.

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Key Words: Sinonasal papilloma, squamous cell carcinoma, head and neck cancer, target sequencing.

Table I. *The clinicopathological characteristics of patients with sinonasal inverted papilloma (IP)/squamous cell carcinoma (SCC).*

Case no.	Sample type	Age, years	Gender	Primary site	TNM stage*
7	SCC	66	FemaIe	Maxillary sinus	T2N0M0
10	IP	85	Male	Frontal sinus	T4aN0M0
16	IP, SCC	83	FemaIe	Maxillary sinus	T2N0M0
17	IP, SCC	64	Male	Nasal cavity	T4bN2bM0

*According to the eighth edition of the TNM Classification of Malignant Tumors (26).

Patients. Four cases of mixed SNIP and SCC that were pathologically diagnosed at the Department of Otolaryngology, Kyushu University Hospital, between 2014 and 2019 were included in this study (Table I).

DNA extraction. Formalin-fixed and paraffin-embedded specimens of surgically resected tumor samples were separated into papilloma and SCC parts using a laser microdissection method with a Leica Laser Microdissection System (Leica Microsystems, Wetzlar, Germany); papilloma and SCC were distinguished based on the diagnosis of the pathologist (Figure 1). The extraction of genomic DNA from the formalin-fixed and paraffin-embedded tissue was conducted using QIAamp DNA Micro Kit (Qiagen, Chatsworth, CA, USA). In two cases (number 16 and 17), both the papilloma and the cancerous part of the sample were extractable but in another two (cases 10 and 7), DNA was only obtained from the papilloma and the cancerous part of the sample, respectively. Thus, we sequenced three samples of the papilloma and three samples of the cancerous part.

Targeted sequencing. Library preparation from the extracted DNA and targeted exome sequencing were performed at Cell Innovator (Fukuoka, Japan). DNA was subjected to targeted exome sequencing using an Ion AmpliSeqTM Comprehensive Cancer Panel (ThermoFisher, Waltham, MA, USA). The 409 genes included in the panel are shown in Table II. The obtained sequences were called for mutations with a mutation rate of >5% using SNPEff ver4.1 (19). Among the identified mutations, insertion, deletion and non-synonymous single nucleotide variants listed in the COSMIC Cancer Gene Census (Sanger Institute, Hinxton, UK) were considered mutant genes. Single nucleotide polymorphisms (SNPs) registered in dbSNP141 and 142 were excluded. We also excluded mutations at the same position and of the same form observed in at least three out of the four cases as sequencing errors.

Confirmation of the same case by SNP. Of the six samples from four cases, two were samples of paired SNIP and SCC. To confirm that the samples were paired without mismatch, the match rate of the SNPs identified as mutations was checked among the samples. The match rate was defined as the product set of SNPs/sum set of SNPs.

Results

The number of detected mutations ranged from 2-20 mutations per sample, with an average of 14 mutations per sample (Table III). All mutations are described in Table IV. The average numbers of indel and nonsense mutations were 4.9 and 3.5, respectively; missense mutations registered in COSMIC were only found in two genes, *EGFR* and *CDKN2A*, in case 7 SCC. The number of mutations in the IP and SCC components did not differ to a statistically significant extent.

The nine genes that were mutated in multiple cases were epidermal growth factor receptor (*EGFR*) (3/6), cyclin-dependent kinase inhibitor 2A (*CDKN2A*) (3/6), lysine methyltransferase 2D (*KMT2D*) (3/6), tumor protein p53 (*TP53*) (3/6), neurofibromin 1 (*NF1*) (3/6), phosphodiesterase 4D interacting protein (*PDE4DIP*) (3/6), cytochrome P450 family 2 subfamily D member 6 (*CYP2D6*) (2/6), fms-related receptor tyrosine kinase 4 (*FLT4*) (2/6) and myosin heavy chain 9 (*MYH9*) (2/6) (Figure 2). Among these, *KMT2D* and *TP53* mutations were found in two positions in the samples of case 7 SCC and case 17 IP, respectively. Among the recurrent mutations, there were no mutations specifically common to the three SCCs when they were compared to the three IP parts.

The SCC and IP areas within the same case were sequenced in cases 16 and 17. Confirmation of SNPs detected in cases 16 and 17 showed that the average SNP concordance rate between samples of different cases was 55%, while the concordance rate between papilloma and SCC within the same case was 96.2% and 98.0% for cases 16 and 17, respectively. Each of the tumors was confirmed to have originated from the same germline (Figure 3). Although confirmed to have originated from the same germline, none of the mutations were common to the SCC and IP components in case 16 (Figure 4).

On the other hand, in case 17, the SCC and IP had five common mutations: six SCC-specific mutations and three IP-specific mutations. Both SCC and IP of case 17 had mutations in *TP53*, p.Val73fs was common to both, while pGlu339* was only found in case 17 SCC. In addition to *TP53* (pGlu339*), five mutations, collagen type I alpha 1 chain (*COL1A1*), XPA DNA damage recognition and repair factor (*XPA*), SRY-box transcription factor 11 (*SOX11*), *CYP2D6*, and *FLT4*, were found to be specific to SCC in case 17. A *CYP2D6* mutation was also found in SCC of case 7, suggesting that mutation of *CYP2D6* may contribute to the malignant transformation of SNIP.

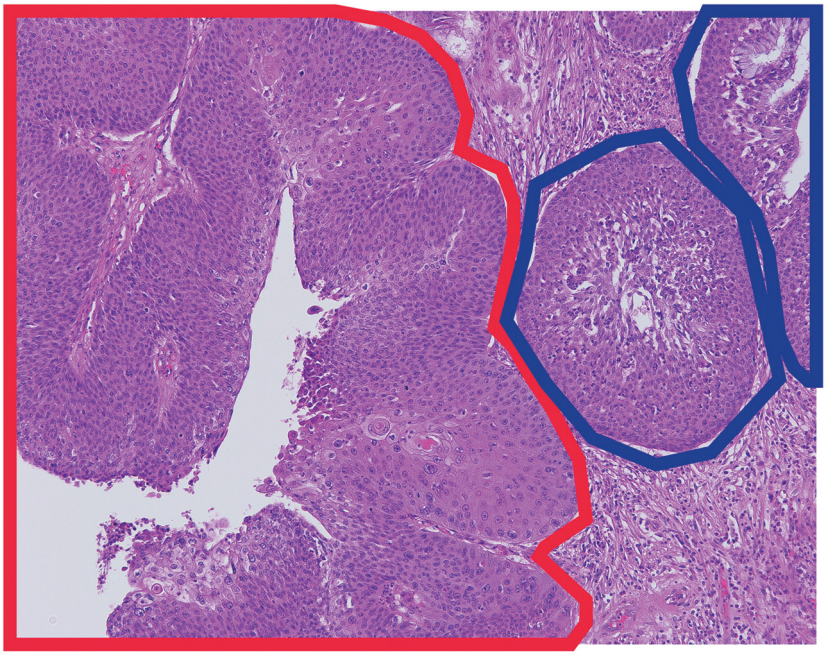

Figure 1. *Laser micro dissection to separate papilloma and carcinoma. Hematoxylin-eosin staining of sinonasal inverted papilloma-derived squamous cell carcinoma is shown (×200). Separation of cancerous (red) and papilloma (blue) areas was performed by microdissection.*

Table II. *The gene list of target sequence.*

ABL1	*BCL2*	*CDH20*	*DDB2*	*EXT1*	*GATA2*	*ING4*	*MAFB*	*MSH6*	*NSD1*	*PKHD1*	*RNF2*	*STK36*	*TRIP11*
ABL2	*BCL2L1*	*CDH5*	*DDIT3*	*EXT2*	*GATA3*	*IRF4*	*MAGEA1*	*MTOR*	*NTRK1*	*PLAG1*	*RNF213*	*SUFU*	*TRRAP*
ACVR2A	*BCL2L2*	*CDK12*	*DDR2*	*EZH2*	*GDNF*	*IRS2*	*MAGI1*	*MTR*	*NTRK3*	*PLCG1*	*ROS1*	*SYK*	*TSC1*
ADAMTS20	*BCL3*	*CDK4*	*DEK*	*FAM123B*	*GNA11*	*ITGA10*	*MALT1*	*MTRR*	*NUMA1*	*PLEKHG5*	*RPS6KA2*	*SYNE1*	*TSC2*
AFF1	*BCL6*	*CDK6*	*DICER1*	*FANCA*	*GNAQ*	*ITGA9*	*MAML2*	*MUC1*	*NUP214*	*PML*	*RRM1*	*TAF1*	*TSHR*
AFF3	*BCL9*	*CDK8*	*DNMT3A*	*FANCC*	*GNAS*	*ITGB2*	*MAP2K1*	*MUTYH*	*NUP98*	*PMS1*	*RUNX1*	*TAF1L*	*UBR5*
AKAP9	*BCR*	*CDKN2A*	*DPYD*	*FANCD2*	*GPR124*	*ITGB3*	*MAP2K2*	*MYB*	*PAK3*	*PMS2*	*RUNX1T1*	*TAL1*	*UGT1A1*
AKT1	*BIRC2*	*CDKN2B*	*DST*	*FANCF*	*GRM8*	*JAK1*	*MAP2K4*	*MYC*	*PALB2*	*POU5F1*	*SAMD9*	*TBX22*	*USP9X*
AKT2	*BIRC3*	*CDKN2C*	*EGFR*	*FANCG*	*GUCY1A2*	*JAK2*	*MAP3K7*	*MYCL1*	*PARP1*	*PPARG*	*SBDS*	*TCF12*	*VHL*
AKT3	*BIRC5*	*CEBPA*	*EML4*	*FAS*	*HCAR1*	*JAK3*	*MAPK1*	*MYCN*	*PAX3*	*PPP2R1A*	*SDHA*	*TCF3*	*WAS*
ALK	*BLM*	*CHEK1*	*EP300*	*FBXW7*	*HIF1A*	*JUN*	*MAPK8*	*MYD88*	*PAX5*	*PRDM1*	*SDHB*	*TCF7L1*	*WHSC1*
APC	*BLNK*	*CHEK2*	*EP400*	*FGFR1*	*HLF*	*KAT6A*	*MARK1*	*MYH11*	*PAX7*	*PRKAR1A*	*SDHC*	*TCF7L2*	*WRN*
AR	*BMPR1A*	*CIC*	*EPHA3*	*FGFR2*	*HNF1A*	*KAT6B*	*MARK4*	*MYH9*	*PAX8*	*PRKDC*	*SDHD*	*TCL1A*	*WT1*
ARID1A	*BRAF*	*CKS1B*	*EPHA7*	*FGFR3*	*HOOK3*	*KDM5C*	*MBD1*	*NBN*	*PBRM1*	*PSIP1*	*SEP9*	*TET1*	*XPA*
ARID2	*BRD3*	*CMPK1*	*EPHB1*	*FGFR4*	*HRAS*	*KDM6A*	*MCL1*	*NCOA1*	*PBX1*	*PTCH1*	*SETD2*	*TET2*	*XPC*
ARNT	*BRIP1*	*COL1A1*	*EPHB4*	*FH*	*HSP90AA1*	*KDR*	*MDM2*	*NCOA2*	*PDE4DIP*	*PTEN*	*SF3B1*	*TFE3*	*XPO1*
ASXL1	*BTK*	*CRBN*	*EPHB6*	*FLCN*	*HSP90AB1*	*KEAP1*	*MDM4*	*NCOA4*	*PDGFB*	*PTGS2*	*SGK1*	*TGFBR2*	*XRCC2*
ATF1	*BUB1B*	*CREB1*	*ERBB2*	*FLI1*	*ICK*	*KIT*	*MEN1*	*NF1*	*PDGFRA*	*PTPN11*	*SH2D1A*	*TGM7*	*ZNF384*
ATM	*CARD11*	*CREBBP*	*ERBB3*	*FLT1*	*IDH1*	*KLF6*	*MET*	*NF2*	*PDGFRB*	*PTPRD*	*SMAD2*	*THBS1*	*ZNF521*
ATR	*CASC5*	*CRKL*	*ERBB4*	*FLT3*	*IDH2*	*KRAS*	*MITF*	*NFE2L2*	*PER1*	*PTPRT*	*SMAD4*	*TIMP3*	
ATRX	*CBL*	*CRTC1*	*ERCC1*	*FLT4*	*IGF1R*	*LAMP1*	*MLH1*	*NFKB1*	*PGAP3*	*RAD50*	*SMARCA4*	*TLR4*	
AURKA	*CCND1*	*CSF1R*	*ERCC2*	*FN1*	*IGF2*	*LCK*	*MLL*	*NFKB2*	*PHOX2B*	*RAF1*	*SMARCB1*	*TLX1*	
AURKB	*CCND2*	*CSMD3*	*ERCC3*	*FOXL2*	*IGF2R*	*LIFR*	*MLL2*	*NIN*	*PIK3C2B*	*RALGDS*	*SMO*	*TNFAIP3*	
AURKC	*CCNE1*	*CTNNA1*	*ERCC4*	*FOXO1*	*IKBKB*	*LPHN3*	*MLL3*	*NKX2-1*	*PIK3CA*	*RARA*	*SMUG1*	*TNFRSF14*	
AXL	*CD79A*	*CTNNB1*	*ERCC5*	*FOXO3*	*IKBKE*	*POT1*	*MLLT10*	*NLRP1*	*PIK3CB*	*RB1*	*SOCS1*	*TNK2*	
BAI3	*CD79B*	*CYLD*	*ERG*	*FOXP1*	*IKZF1*	*LPP*	*MMP2*	*NOTCH1*	*PIK3CD*	*RECQL4*	*SOX11*	*TOP1*	
BAP1	*CDC73*	*CYP2C19*	*ESR1*	*FOXP4*	*IL2*	*LRP1B*	*MN1*	*NOTCH2*	*PIK3CG*	*REL*	*SOX2*	*TP53*	
BCL10	*CDH1*	*CYP2D6*	*ETS1*	*FZR1*	*IL21R*	*LTF*	*MPL*	*NOTCH4*	*PIK3R1*	*RET*	*SRC*	*TPR*	
BCL11A	*CDH11*	*DAXX*	*ETV1*	*G6PD*	*IL6ST*	*LTK*	*MRE11A*	*NPM1*	*PIK3R2*	*RHOH*	*SSX1*	*TRIM24*	
BCL11B	*CDH2*	*DCC*	*ETV4*	*GATA1*	*IL7R*	*MAF*	*MSH2*	*NRAS*	*PIM1*	*RNASEL*	*STK11*	*TRIM33*	

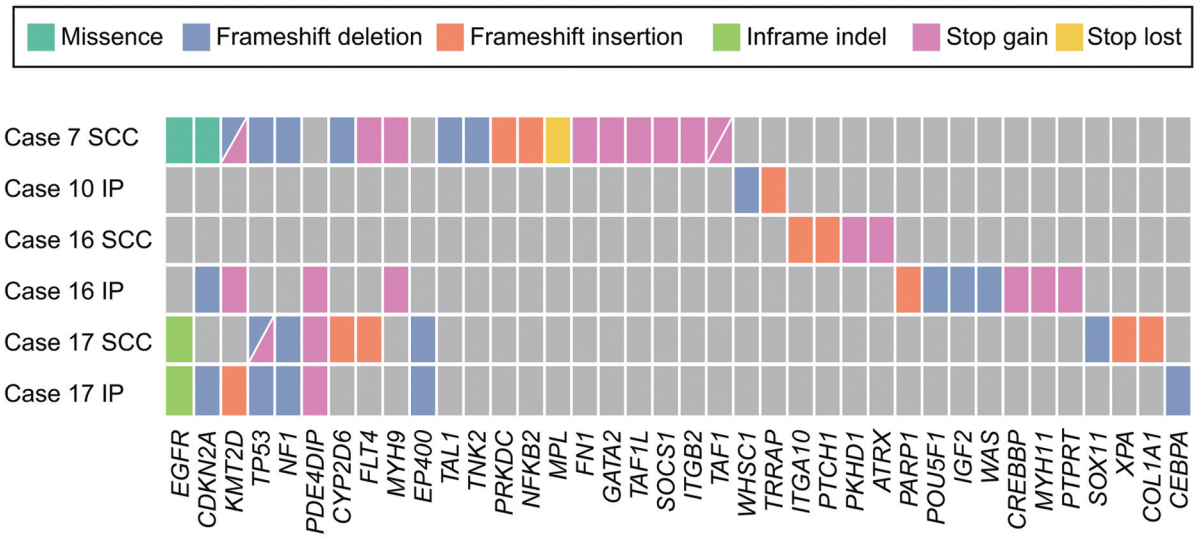

Figure 2. *Overview of mutations in sinonasal inverted papilloma (SNIP) and SNIP-derived squamous cell carcinoma (SCC). All mutated genes in SNIP and SNIP-derived SCC were color coded according to mutation type. Gene designations are given below Table III.*

Table III. *The number of mutations found in six tissue samples of inverted papilloma (IP)/squamous cell carcinoma (SCC) from four cases.*

Case no.		Mutation type, n					
	Histology	Missense	Inframe indel	Frameshift indel	Stop gain	Stop lost	Total
7	SCC	2	0	8	10	1	21
10	IP	0	0	2	0	0	2
16	SCC	0	0	2	2	0	4
	IP	0	0	5	6	0	11
17	SCC	0	1	8	2	0	11
	IP	0	1	6	1	0	8

Discussion

Radical surgical resection of papilloma-derived cancer is important for good treatment outcomes; however, complications of cancer are difficult to predict before surgery, and the discovery of markers that predict cancer complications is desired (10). Immunohistological approaches and single gene sequencing of papilloma-derived cancer suggested that *TP53* and *EGFR* abnormalities may be common findings in complicated cancer cases (15, 20). A previous study of 26 genes by Yasukawa *et al*. identified *TP53,* G protein subunit alpha q (*GNAQ*), platelet-derived growth factor receptor alpha (*PDGFRA*), *EGFR,* phosphatidylinositol-4,5-*bis*phosphate 3-kinase catalytic subunit alpha (*PIK3CA*), KRAS proto-oncogene GTPase (*KRAS*), APC regulator of WNT signaling pathway (*APC*), serine/threonine kinase 11 (*STK11*), and mutS homolog 6 (*MSH6*) as recurrent mutations in papilloma and papilloma-derived carcinoma by target sequencing. Among them, *KRAS* mutation is cancer-specific and may be a gene mutation involved in malignant transformation (17). In this study, we performed a comprehensive mutation analysis using cancer panel gene sequencing on three cancer and three papilloma samples from four papilloma-derived cancer cases. A total of 409 genes were targeted, covering most of the mutations analyzed in previous studies. In this study, we found mutations in *EGFR* and *TP53* from among those identified in previous studies. Furthermore, we found that *CDKN2A, KMT2D, NF1, PDE4DIP, CYP2D6, FLT4,* and *MYH9* were mutated in more than one case, suggesting that they may be involved in the development of SNIP and SNIP-derived SCC. Among these genes, *CDKN2A* exhibited a loss of function in more than 50% of head and neck squamous cell carcinomas of the pharynx and larynx, and may have an important role in the development and oncogenesis of SNIP-derived SCC (21). On the other hand, we did not find *KRAS* mutation, although Yasukawa *et al*. reported that it may be associated with malignant transformation of SNIP (17).

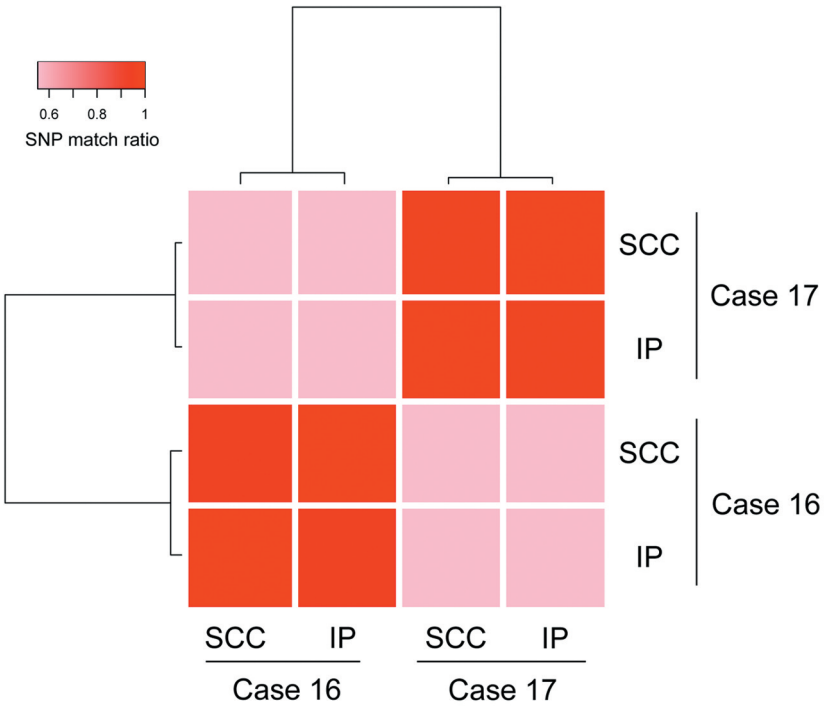

Figure 3. *Correlation of single nucleotide polymorphisms (SNPs) in cases 16 and 17. The heat map shows the match rate of SNPs detected in each sample by sequencing.*

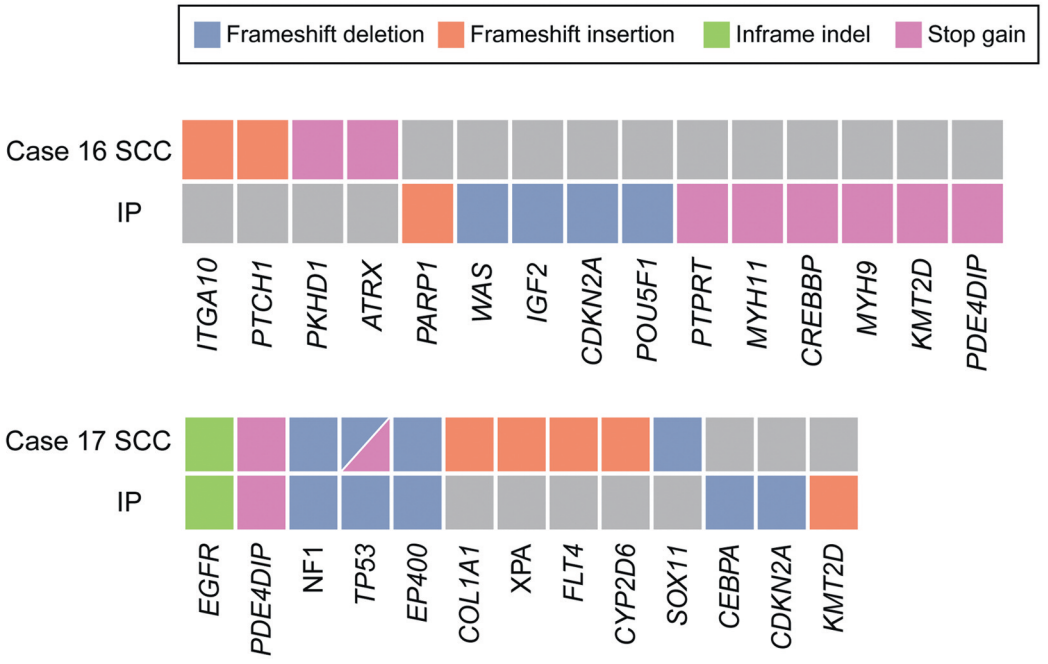

Figure 4. *Comparison of sinonasal inverted papilloma (SNIP) and SNIP-derived squamous cell carcinoma (SCC) mutations within the same case. SNIP and SNIP-derived SCC mutations were compared in cases 16 and 17. Gene designations are given below Table IV.*

Table IV. *Information on mutations found in samples of inverted papilloma (IP)/squamous cell carcinoma (SCC) from six samples from four cases*.

Case no.	Histology	Gene name	Chrom	Position	Reference	Alteration	Annotation	HGVS.c	HGVS.p	COSMIC ID
7	SCC	*MPL*	chr1	43818443	A	G	stop_lost	c.1908A>G	p.Ter636Trpext*?	.
		TAL1	chr1	47685569	GC	G	frameshift_variant	c.818delG	p.Gly273fs	.
		FN1	chr2	216293010	C	T	stop_gained	c.737G>A	p.Trp246*	.
		GATA2	chr3	128205845	C	T	stop_gained	c.30G>A	p.Trp10*	.
		TNK2	chr3	195595228	CGGGGG	CGGGG	frameshift_variant	c.2125delC	p.Pro710fs	.
		FLT4	chr5	180047969	G	A	stop_gained	c.2206C>T	p.Gln736*	.
		EGFR	chr7	55249010	G	A	missense_variant	c.2308G>A	p.Asp770Asn	COSM14068
		PRKDC	chr8	48805816	A	AG	frameshift_variant	c.3729dupC	p.Phe1244fs	.
		CDKN2A	chr9	21971159	C	T	missense_variant	c.199G>A	p.Gly67Ser	COSM12746
		TAF1L	chr9	32631839	G	A	stop_gained	c.3739C>T	p.Gln1247*	.
		TAF1L	chr9	32632938	C	T	stop_gained	c.2640G>A	p.Trp880*	.
		NFKB2	chr10	104159195	C	CG	frameshift_variant	c.1268_1269insG	p.Ser424fs	.
		KMT2D	chr12	49427621	G	A	stop_gained	c.10867C>T	p.Gln3623*	.
		KMT2D	chr12	49445148	TG	T	frameshift_variant	c.2317delC	p.Gln773fs	.
		SOCS1	chr16	11348813	G	A	stop_gained	c.523C>T	p.Gln175*	.
		TP53	chr17	7579470	CGG	CG	frameshift_variant	c.215delC	p.Val73fs	COSM46307
		NF1	chr17	29553477	ACCCC CCCG	ACCCC CCG	frameshift_variant	c.2033delC	p.Pro678fs	COSM24489
		ITGB2	chr21	46306657	C	T	stop_gained	c.2241G>A	p.Trp747*	.
		MYH9	chr22	36710279	G	A	stop_gained	c.1465C>T	p.Gln489*	.
		CYP2D6	chr22	42525755	CG	C	frameshift_variant	c.336delC	p.Phe112fs	.
		TAF1	chrX	70643066	C	T	stop_gained	c.4612C>T	p.Gln1538*	.
10	IP	*WHSC1*	chr4	1980558	GC	G	frameshift_variant	c.4028delC	p.Pro1343fs	.
		TRRAP	chr7	98508875	C	CA	frameshift_variant	c.1994dupA	p.Asn665fs	.
16	SCC	*ITGA10*	chr1	145527980	G	GT	frameshift_variant	c.220dupT	p.Tyr74fs	.
		PKHD1	chr6	51720839	A	C	stop_gained	c.7763T>G	p.Leu2588*	.
		PTCH1	chr9	98278956	G	GT	frameshift_variant	c.146dupA	p.Asn49fs	.
		ATRX	chrX	76937750	T	A	stop_gained	c.2998A>T	p.Lys1000*	.
	IP	*PDE4DIP*	chr1	144915561	G	A	stop_gained	c.1864C>T	p.Arg622*	.
		PARP1	chr1	226570839	G	GC	frameshift_variant	c.1056_1057insG	p.Gln353fs	.
		POU5F1	chr6	31138041	AG	A	frameshift_variant	c.356delC	p.Pro119fs	.
		CDKN2A	chr9	21971083	TCC	TC	frameshift_variant	c.273delG	p.Asp92fs	COSM13623
		IGF2	chr11	2154241	TGGGG GGGC	TGGGG GGC	frameshift_variant	c.680delC	p.Pro229fs	.
		KMT2D	chr12	49445068	G	A	stop_gained	c.2398C>T	p.Gln800*	.
		CREBBP	chr16	3778612	G	A	stop_gained	c.6436C>T	p.Gln2146*	.
		MYH11	chr16	15814848	G	A	stop_gained	c.4660C>T	p.Gln1554*	.
		PTPRT	chr20	41419837	G	A	stop_gained	c.484C>T	p.Gln162*	.
		MYH9	chr22	36710279	G	A	stop_gained	c.1465C>T	p.Gln489*	.
		WAS	chrX	48547170	ACC	AC	frameshift_variant	c.1058delC	p.Pro353fs	.
17	SCC	*PDE4DIP*	chr1	144916676	C	T	stop_gained	c.1679G>A	p.Trp560*	.
		SOX11	chr2	5833426	CG	C	frameshift_variant	c.578delG	p.Gly193fs	.
		FLT4	chr5	180046035	A	AG	frameshift_variant	c.2835dupC	p.Phe946fs	.
		EGFR	chr7	55249012	C	CGGG TTT	conservative_ inframe_insertion	c.2310_2311ins GGGTTT	p.Asp770_Asn 771insGlyPhe	COSM12378
		XPA	chr9	100437714	TAAA AAAT	TAAAA AAAT	frameshift_variant	c.822_*1insT		.
		EP400	chr12	132547091	CAA	CA	frameshift_variant	c.8181delA	p.Gln2727fs	.
		TP53	chr17	7574012	C	A	stop_gained	c.1015G>T	p.Glu339*	COSM11286
		TP53	chr17	7579470	CGG	CG	frameshift_variant	c.215delC	p.Val73fs	COSM46307
		NF1	chr17	29553477	ACCCC CCCG	ACCC CCCG	frameshift_variant	c.2033delC	p.Pro678fs	COSM24489
		COL1A1	chr17	48266813	AC	ATC	frameshift_variant	c.2753_2754insA	p.Pro919fs	.
		CYP2D6	chr22	42524213	C	CG	frameshift_variant	c.805dupC	p.Arg269fs	.
	IP	*PDE4DIP*	chr1	144916676	C	T	stop_gained	c.1679G>A	p.Trp560*	.
		EGFR	chr7	55249012	C	CGGG TTT	conservative_ inframe_insertion	c.2310_2311ins GGGTTT	p.Asp770_Asn 771insGlyPhe	COSM12378

Table IV. *Continued*

Table IV. *Continued*

Case no.	Histology	Gene name	Chrom	Position	Reference	Alteration	Annotation	HGVS.c	HGVS.p	COSMIC ID
		CDKN2A	chr9	21971193	GC	G	frameshift_variant	c.164delG	p.Gly55fs	.
		KMT2D	chr12	49445187	T	TC	frameshift_variant	c.2278_2279insG	p.His760fs	.
		EP400	chr12	132547091	CAA	CA	frameshift_variant	c.8181delA	p.Gln2727fs	.
		TP53	chr17	7579470	CGG	CG	frameshift_variant	c.215delC	p.Val73fs	COSM46307
		NF1	chr17	29553477	ACCCC CCCG	ACCCC CCG	frameshift_variant	c.2033delC	p.Pro678fs	COSM24489
		CEBPA	chr19	33793213	GCCCC	GCCC	frameshift_variant	c.209delG	p.Gly71fs	COSM18495

ATRX: ATRX chromatin remodeler; *CDKN2A*: cyclin-dependent kinase inhibitor 2A; *CEBPA*: CCAAT enhancer binding protein alpha; *COL1A1*: collagen type I alpha 1 chain; *CREBBP*: CREB-binding protein; *CYP2D6*: cytochrome P450 family 2 subfamily D member 6; *EGFR*: epidermal growth factor receptor; *EP400*: E1A-binding protein p400; FLT4: fms-related receptor tyrosine kinase 4; FN1: fibronectin 1; *GATA2*: GATA-binding protein 2; *IGF2*: insulin-like growth factor 2; ITGA10: integrin subunit alpha 10; ITGB2: integrin subunit beta 2; *KMT2D*: lysine methyltransferase 2D; *MPL*: MPL proto-oncogene, thrombopoietin receptor; *MYH11*: myosin heavy chain 11; *MYH9*: myosin heavy chain 9; *NF1*: neurofibromin 1; *NFKB2*: nuclear factor kappa B subunit 2; *PARP1*: poly(ADP-ribose) polymerase 1; *PDE4DIP*: phosphodiesterase 4D-interacting protein; *PKHD1*: PKHD1 ciliary IPT domain-containing fibrocystin/polyductin; *POU5F1*: POU class 5 homeobox 1; *PRKDC*: protein kinase, DNA-activated, catalytic subunit; *PTCH1*: patched 1; *PTPRT*: protein tyrosine phosphatase receptor type T; *SOCS1*: suppressor of cytokine signaling 1; *SOX11*: SRY-box transcription factor 11; *TAF1*: TATA-box binding protein-associated factor 1; *TAF1L*: TATA-box binding protein associated factor 1-like; *TAL1*: TAL bHLH transcription factor 1, erythroid differentiation factor; *TNK2*: tyrosine kinase non receptor 2; *TP53*: tumor protein p53; *TRRAP*: transformation/transcription domain-associated protein; *WAS*: WASP actin nucleation-promoting factor; *WHSC1*: Wolf-Hirschhorn syndrome candidate 1; *XPA*: XPA, DNA damage recognition and repair factor; *HGVS.c*: variants for a coding DNA sequence according to Human Genome Variation Society (HGVS); *HGVS.p*: variants for a protein sequence according to HGVS.

Malignant transformation of nasal papilloma-derived carcinoma is thought to be caused by the accumulation of genetic abnormalities in SNIP (5). *EGFR* mutation has been reported as one of the most important potential mechanisms of papilloma-derived carcinogenesis (16, 20, 22). In this study, *EGFR* mutations were found in two out of four patients with cancer. One patient also had a mutation in the IP. In nasal papilloma, *EGFR* mutations have been reported to be present in 20-91.4% of cases (14, 16, 20, 23). On the other hand, *EGFR* mutations in papilloma-derived cancer have been reported in 27-92.9% of cases. This suggests that *EGFR* may be an important factor at the time of papilloma development; however, it may not be important for malignant transformation from papilloma to cancer. Among those with papilloma-derived cancer, cases with *EGFR* mutations are reported to have a poorer prognosis in comparison to those without mutation, suggesting that *EGFR* may not be related to the grade of the cancer itself (23).

TP53 is the most well-known tumor-suppressor gene. Immunostaining studies have shown a higher rate of *TP53* positivity in SNIP derived-SCC than in IP, suggesting that it may be important for transformation (5, 15). In the present study, we observed one case in which a *TP53* mutation was common to both the papilloma and the carcinoma of origin, and one additional mutation in the cancerous region. *TP53* is known to be inactivated by loss of heterozygosity or double mutation of the mutant allele. In this case, the double mutation may have occurred during the evolution from papilloma to carcinoma, resulting in the loss of *TP53* function (18, 24, 25). Further studies are required because these findings were only observed in one case; however, malignant transformation may have occurred due to the gradual inactivation of *TP53* during IP-to-SCC transformation.

One limitation of this study is the small sample size of four cases and six samples. It is possible additional related genes might be identified by increasing the number of samples. Furthermore, case 16 did not have any mutations common to the SCC and IP parts of the sample. It is possible that SNIP and SNIP-derived SCC may have occurred simultaneously in this case, with completely different genetic backgrounds, or they may only share genes that were not analyzed in this study. Although this study analyzed mutations in 409 genes that have not previously been analyzed, a more comprehensive analysis of the entire exon sequence may be useful to determine whether a common mutation exists in this case.

Conflicts of Interest

The Authors declare that no potential conflicts of interest exist in relation to this study.

Authors' Contributions

RU and RY designed the study. RJ, HY and TH performed the experiments. RU performed the analysis and wrote the draft of the article. RU, RY, HY and TN reviewed and edited the article. TM, KS, KH, TH and TW performed collection of cases. All Authors read and approved the final article.

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