# Exome sequencing reveals candidate mutations implicated in sinonasal carcinoma and malignant transformation of sinonasal inverted papilloma

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2022-01

Viitasalo , S K E , Karhemo , P-R , Ilmarinen , T T , Väänänen , J , Katainen , R , Monni , O , Lilja , M & Aaltonen , L-M 2022 , ' Exome sequencing reveals candidate mutations implicated in sinonasal carcinoma and malignant transformation of sinonasal inverted papilloma ' , Oral Oncology , vol. 124 , 105663 . https://doi.org/10.1016/j.oraloncology.2021.105663

http://hdl.handle.net/10138/352270
https://doi.org/10.1016/j.oraloncology.2021.105663

**Abstract**

We explored somatic mutations in dysplastic sinonasal inverted papilloma (SNIP), SNIP with concomitant sinonasal squamous cell carcinoma (SNSCC), and SNSCC without preceding SNIP. Ten SNIP and SNSCC samples were analyzed with exome sequencing and tested for human papillomavirus. The identified mutations were compared to the most frequently mutated genes in head and neck squamous cell carcinoma (HNSCC) in the COSMIC database. Exome sequencing data were also analyzed for mutations not previously linked to SNSCC. Seven of the most commonly mutated genes in HNSCC and SNSCC in COSMIC harbored mutations in our data. In addition, we identified mutations in 23 genes that are likely to contribute to SNIP and SNSCC oncogenesis.

Keywords: exome sequencing, genetic mutation, head and neck squamous cell carcinoma, head and neck cancer, inverted papilloma, sinonasal papilloma, FGFR3, somatic variant analysis

Abbreviations:

SNIP sinonasal inverted papilloma

SNSCC sinonasal squamous cell carcinoma

HNSCC head and neck squamous cell carcinoma

SNV single-nucleotide variant

COSMIC Catalogue of Somatic Mutations in Cancer

HPV human papillomavirus

# Exome sequencing reveals candidate mutations implicated in sinonasal carcinoma and malignant transformation of sinonasal inverted papilloma

Sinonasal inverted papilloma (SNIP) comprises 0.5 - 4% of all primary nasal tumors. SNIP is the most common subtype of sinonasal papilloma, with an annual incidence of 0.2 – 0.7 per 100 000 individuals. SNIP is characterized by a locally aggressive growth and has the potential for malignant transformation into sinonasal squamous cell carcinoma (SNSCC). According to the literature, the malignant transformation rate of SNIP ranges considerably, from less than 1% to 53%.[1] The predisposing factors, including genetic alterations that drive malignant transformation, are incompletely elucidated. In a recent review, the overall detection rate of oncogenic HPV types (21.7%) in sinonasal carcinomas seemed to be almost equal to their prevalence in other sites of the upper aero-digestive tract and the study supports a causative role of HPV in the pathogenesis of SNSCC.[2]

We explored novel genetic alterations potentially involved in the pathogenesis of SNSCC with or without preceding SNIP, and cross-compared findings with previously identified mutations that might contribute to carcinogenesis.

We conducted exome sequencing on 10 prospectively collected fresh tissue samples: 4 from patients with SNIP with no associated malignancy, 2 from a patient with SNIP and concomitant SNSCC (identified as: SNIP 5 and SNSCC 5) and 4 from patients with SNSCC but without preceding SNIP (Table 1). DNA was extracted from matched peripheral blood lymphocytes of all patients except one SNSCC patient (#8). Ethics Committee, Surgery approved the study and patients were treated at Helsinki University Hospital, Department of Otorhinolaryngology – Head and Neck Surgery, between 2015 and 2019. Patients provided written informed consent.

Patients with SNIP that had dysplasia and a history of smoking were considered to have the highest likelihood of cancer-associated gene mutations, and thus, were selected for our study. See detailed methods in Supplementary data.

We detected a total of 111 different mutations in SNIP samples and 214 mutations in SNSCC samples with a paired normal sample. The SNIP and SNSCC groups had a total of 40 mutations in common. SNSCC samples showed higher mutation burden than SNIP samples (Supplementary Table 1). Samples showed signature associated with smoking (SBS4) and signature associated with mismatch repair (SBS15) (Supplementary Table 2). HPV was detected in two SNIP and three SNSCC samples (Table 1).

Among the most frequently mutated genes reported in HNSCC/SNSCC in the COSMIC database, seven carried mutations in our samples (Figure 1a-b). These included S77Lfs*7 in *TP53*, E267Q in *KMT2C* and N771insT in *EGFR* that were not reported in the COSMIC database (Supplementary Table 3). In addition to the novel *EGFR* mutation detected in a SNIP sample, one SNSCC sample showed a previously reported *EGFR* mutation (D770_N771insSVD). Recently, *EGFR* activating mutations, mainly exon 20 insertions, were reported in SNIP-associated SNSCC and in SNSCC [3,4]. *EGFR* mutations and HPV have been proposed as essential and alternative oncogenic mechanisms in SNIP and SNIP-associated SNSCC.[5,6] In our data, one HPV-negative SNSCC without preceding SNIP harbored an *EGFR* mutation. Two SNIP specimens in our study were negative for both *EGFR* mutation and HPV DNA.

The *TP53* mutation was detected only in an HPV-negative SNSCC sample. All the other samples were wild type for *TP53* (Figure 1a). Previously, mutations in *TP53* and *CDKN2A*

were found more frequently in HPV-negative than in HPV-positive oral cancers and in contrast, *PIK3CA* and *FGFR3* were significantly more frequently mutated in HPV-positive oral cancers.[7] Similarly, we found *TP53* and *CDKN2A* mutations in HPV-negative samples and *FGFR3* and *PIK3CA* mutations in HPV-positive samples (figure 1a). *FGFR3* mutations detected in our samples were located in a hotspot area. The same *FGFR3* mutation (R248C) was detected in both the SNIP and SNSCC samples of patient number 5. The SNSCC sample from patient 8 also showed a mutation in *FGFR3* (S249C). *FGFR3* R248C and S249C mutations are activating and transforming, which highlights their possible role in malignant transformation of SNIP.[8–13] According to COSMIC, 20% of SNSCC had *FGFR3* mutations (Figure 1a) making it the second most commonly mutated gene in SNSCC. Interestingly, samples with an *FGFR3* mutation were positive for HPV type 16 (Figure 1a). Recently, *FGFR3* mutations were detected in 17.6% of HPV-positive HNSCC tumors, and the mutations were mainly S249C mutations, consistent with our findings.[14] DeSmet et al. found in their study that FGFR3 kinase restricts HPV replication in infected epithelial cells by phosphorylating the HPV E2 protein, which coordinates viral transcription and genome replication.[15]. More importantly, *FGFR* inhibitors in treatment of tumors with *FGFR* mutations have shown promising results and their use in HPV positive SNSCC warrants further studies.[16]

We further searched the exome sequencing data for mutations that might alter the protein function, and thus, might contribute to the malignant transformation of SNIP and the carcinogenesis of SNSCC. This search yielded 23 mutated genes (Figure 1b and Supplementary Table 4). Both the SNIP and SNSCC sample from the same patient had frameshift deletion of *RFFL*. To date, *RFFL* has not been associated with sinonasal carcinogenesis. *RFFL* encodes for a protein which negatively regulates p53 and tumor necrosis factor-mediated signaling pathway.[17] In our analysis, 4/5 SNSCC samples, including the

sample with mutated *RFFL,* had no alterations in p53 indicating that additional mechanisms to inactivate p53 might play a role in sinonasal carcinogenesis. *RFFL* also negatively regulates extrinsic apoptotic signaling pathway and promotes mTOR signaling and fibroblast migration.[17]

In conclusion, we identified two mutated genes that were linked to malignant transformation of SNIP, and 22 mutated genes that were related to SNSCC without preceding SNIP. In the 23 identified genes that were previously not linked to SNSCC, twenty of the mutations were novel, not reported in the COSMIC database. The HPV statuses associated with the mutations detected in our samples were consistent with HPV associations reported in previous studies. Dysplastic SNIP specimens from patients with a history of smoking harbored only few mutations in most commonly mutated genes in HNSCC and SNSCC suggesting there are other factors driving carcinogenesis yet to be elucidated. Although these results must be verified in a larger sample set, our findings might provide new insights into potential therapeutic targets in the future.

Acknowledgments:

We thank Professor Lauri Aaltonen from the Applied Tumor Genomics Research Program and Department of Medical and Clinical Genetics, Faculty of Medicine, University of Helsinki, Helsinki, Finland, for valuable comments on this manuscript. Biomedicum Functional Genomics Unit (Helsinki Institute of Life Science, University of Helsinki and Biocenter Finland) is thanked for excellent technical support in exome sequencing.

**Figure captions**

Figure 1a. Comparison of patient data to the most commonly mutated genes in HNSCC and SNSCC according to the COSMIC database. Each row represents a gene that was found to be mutated in our data and each column represents one patient in our study. The mutations in the following genes were analyzed: TP53, PIK3CA, NOTCH1, CDKN2A, LRP1B, MET, KMT2C, NCOR2, EGFR, FAT1, NOTCH2, HLA-A, PDE4DIP, KMT2D, NSD1, CASP8, FAT4, FBXW7, HRAS, EP300, KRAS, NRAS, PTEN, JAK2, FGFR3, FGFR2, SUZ12, STK11, ATRX, PIK3R1 and FGFR1. The histology, types of mutations and HPV status (positive or negative) are color-coded. The two columns on the right show the frequency of the mutations in SNSCC and HNSCC samples in the COSMIC database. *Patient 5 had SNIP and concomitant SNSCC; †CDKN2A and KMT2C mutation frequencies were not reported in the COSMIC database for SNSCC; Abbreviations: SNIP, sinonasal inverted papilloma; HNSCC head and neck squamous cell carcinoma; SNSCC, sinonasal squamous cell carcinoma

Figure 1b. Mutations in genes previously not associated with sinonasal carcinogenesis. Each row represents a gene that was found to be mutated in our data and each column represents one patient in our study. The histology, types of mutations and HPV status (positive or negative) are color-coded. *Patient with SNIP and concomitant SNSCC; Abbreviations: SNIP, sinonasal inverted papilloma; SNSCC, sinonasal squamous cell carcinoma.

Table 1. Clinicopathologic characteristics of the patients included in this study

| Disease, Patient number | Histology | Sex | Age at sampling (years) | HPV type | Grade of dysplasia in SNIP | Smoking |
|---|---|---|---|---|---|---|
| SNIP, 1 | SNIP | Female | 38 | negative | Mild | Current |
| SNIP, 2 | SNIP | Male | 59 | negative | Mild | Current |
| SNIP, 3 | SNIP | Male | 61 | negative | Moderate | Current |
| SNIP, 4 | SNIP | Male | 69 | 6 | Severe | Current |
| SNIP, 5 * | SNIP | Male | 81 | 16 | Mild | Former |
| SNSCC, 5 * | SNSCC | Male | 82 | 16 | - | Former |
| SNSCC, 6 | SNSCC | Female | 45 | 16 | - | Current |
| SNSCC, 7 | SNSCC | Female | 56 | negative | - | Never |
| SNSCC, 8 | SNSCC | Male | 61 | 16 | - | Former |
| SNSCC, 9 | SNSCC | Male | 61 | negative | - | Former |

SNIP, Sinonasal inverted papilloma; SNSCC, Sinonasal squamous cell carcinoma; HPV, Human papilloma virus

*Patient 5 was diagnosed with SNIP in August 2015 at age of 81, and concomitant SNSCC in October 2016 at age of 82.

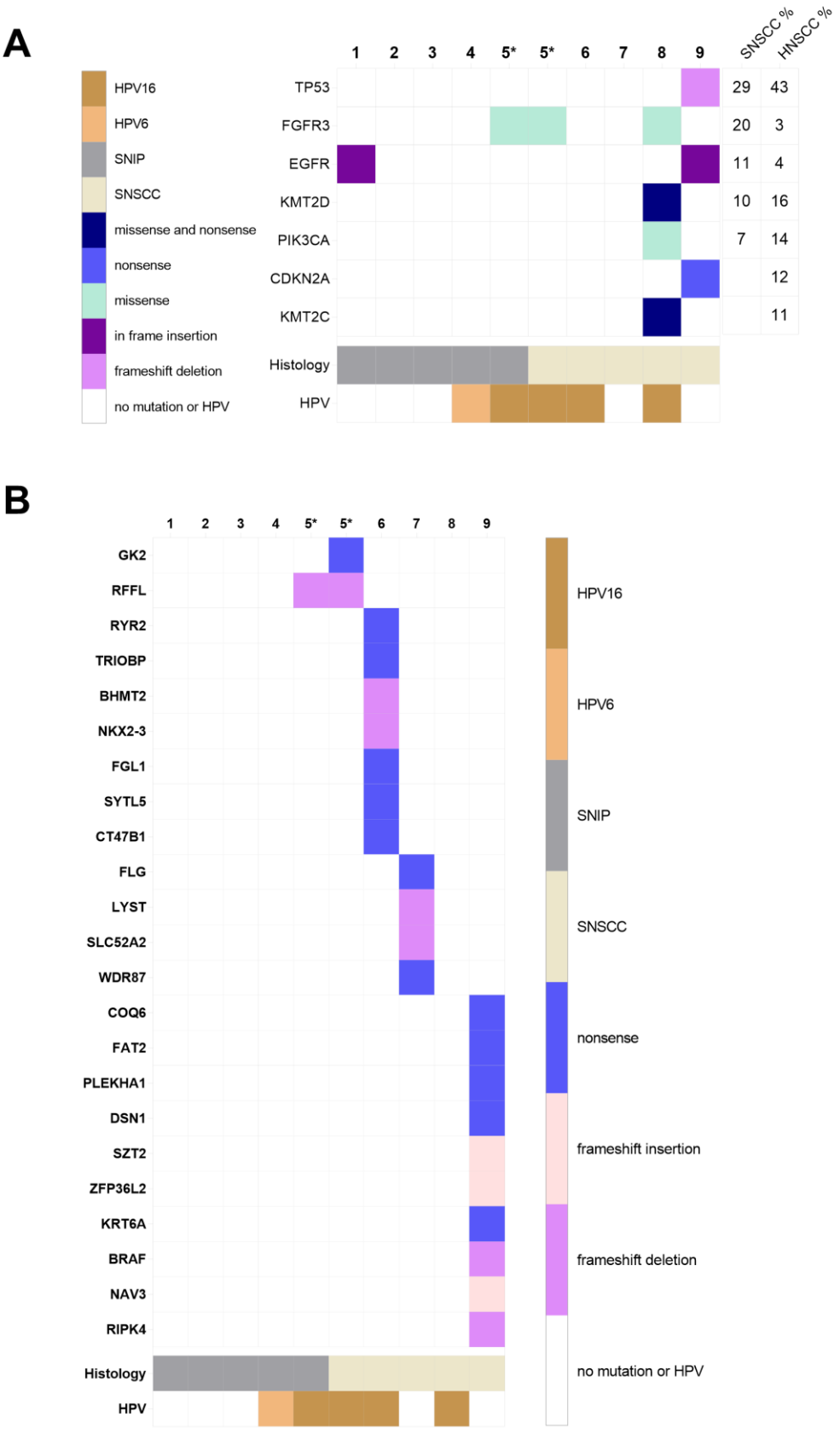
A
1 2 3 4 5* 5* 6 7 8 9
SNSCC %
HNSCC %
HPV16
HPV6
SNIP
SNSCC
missense and nonsense
nonsense
missense
in frame insertion
frameshift deletion
no mutation or HPV
TP53 29 43
FGFR3 20 3
EGFR 11 4
KMT2D 10 16
PIK3CA 7 14
CDKN2A 12
KMT2C 11
Histology
HPV
B
1 2 3 4 5* 5* 6 7 8 9
GK2
RFFL
RYR2
TRIOBP
BHMT2
NKX2-3
FGL1
SYTL5
CT47B1
FLG
LYST
SLC52A2
WDR87
COQ6
FAT2
PLEKHA1
DSN1
SZT2
ZFP36L2
KRT6A
BRAF
NAV3
RIPK4
Histology
HPV
HPV16
HPV6
SNIP
SNSCC
nonsense
frameshift insertion
frameshift deletion
no mutation or HPV

**Figure 1.**

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