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Genetic mutations in recurrent and/or metastatic nasopharyngeal carcinoma – an analysis of the Japanese national genomic profiling database

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ABSTRACT:

Introduction: Although genetic mutations have been reported in nasopharyngeal carcinoma (NPC), there are currently no scientifically validated treatments targeting these mutations. Furthermore, cancer genomic profiling tests are underutilized in clinical practice. This highlights an urgent need to explore the genomic landscape of NPC and its potential therapeutic implications.

Aim: The aim of this study is to investigate the genetic mutational landscape of recurrent and/or metastatic nasopharyngeal carcinoma (NPC).

Materials and methods: Data were analyzed for 67 consecutive patients with NPC registered at the Japan National Cancer Center, Center for Cancer Genomics and Advanced Therapeutics (C-CAT) between June 2019 and May 2024. Genetic mutations were determined by FoundationOne CDx or Liquid CDx next-generation sequencing. Survival of patients was determined by the log-rank test and a Cox proportional hazards model.

Results: The top 10 mutations in NPC were *CDKN2A* (41.8%), *CDKN2B* (31.3%), *TP53* (20.9%), *KMT2D* (20.9%), *DNMT3A* (19.4%), *NOTCH1* (17.9%), *STK11* (17.9%), *MTAP* (17.9%), *EP300* (17.9%), *TSC1* (13.4%), with 11.1 ±6.1 (mean ±SEM) mutations/individual. Mutations in *KMT2D* ($p = 0.0127$), *DNMT3A* ($p = 1.41\times10^{-7}$), *GNAS* ($p = 3.64\times10^{-11}$), *SPEN* ($p = 0.0167$), *BRCA1* ($p = 0.0379$), and *KMT2A* ($p = 2.06\times10^{-5}$) were associated with a significantly worse prognosis, as determined by the log-rank test. The hazard ratios for cases with these mutations were 0.0122 (95% CI, 1.58×10^{-4}-0.936, $p = 0.046$) for *TP53*, 1847.0 (95% CI, 4.619-7.386×10^{5}, $p = 0.014$) for *DNMT3A*, 126.7 (95% CI, 1.262-12720, $p = 0.039$) for *BRCA2*, 197.9 (95% CI, 2.844-13770, $p = 0.015$) for *ALK*, 34.22 (95% CI, 1.256-932.7, $p = 0.036$) for *SPEN*, and 6.445×10^{-4} (95% CI, 2.913×10^{-6}-0.143, $p = 7.6\times10^{-3}$) for *MYCL*.

Conclusions: This study identified genetic mutations in recurrent and/or metastatic NPC. Even in advanced cases, prognosis-related mutations were identified, underscoring the importance of cancer genomic profiling tests.

KEYWORDS: cancer genomic profiling tests, *DNMT3A*, genetic mutations, nasopharyngeal carcinoma, *SPEN*

ABBREVIATIONS

C-CAT – Center for Cancer Genomics and Advanced Therapeutics
CGP – comprehensive genome profiling
CI – confidence interval
EBV – Epstein-Barr virus
ECOG-PS – Eastern Cooperative Oncology Group Performance Status
HR – hazard ratio
NPC – nasopharyngeal carcinoma
OS – overall survival
SEM – standard error of the mean

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a malignant tumor originating from the epithelial cells of the nasopharynx and is etiologically associated with Epstein-Barr virus (EBV) infection, dietary habits, environmental factors, and genetic predisposition [1]. Its lethality is characterized by a high metastatic potential in the early stages and a high recurrence rate following radiation treatment.

Comprehensive genome profiling (CGP) was started in Japan in June 2019 in healthcare services provided by the national health insurance system. Gene cancer panel tests using CGP covered by health insurance are targeted to patients with solid tumors for which there is no standard treatment, or if standard treatment has been completed due to local progression or metastasis. Head and neck cancer is also an indication for CGP. However, although genetic mutations in NPC have been reported [2], there are no scientifically validated treatments based on these mutations, and cancer genomic profiling tests are not being utilized to their full potential. There have also been no reports examining NPC genetic mutations using FoundationOne CDx or Liquid CDx. Therefore, the purpose of this study was to investigate genetic mutations in NPC using these assays and to identify mutations associated with prognosis.

AIM

The aim of this study is to investigate the genetic mutational landscape of recurrent and/or metastatic nasopharyngeal carcinoma NPC.

MATERIALS AND METHODS

Study design and Approval

Approval for use of the Center for Cancer Genomics and Advanced Therapeutics (C-CAT) Research-Use Portal site was obtained from the ethics committee at Kagoshima University. An application form for use of C-CAT data was then submitted for review by the C-CAT Data Utilization Review Board, and once approved, a data access licensing contract was established [3].

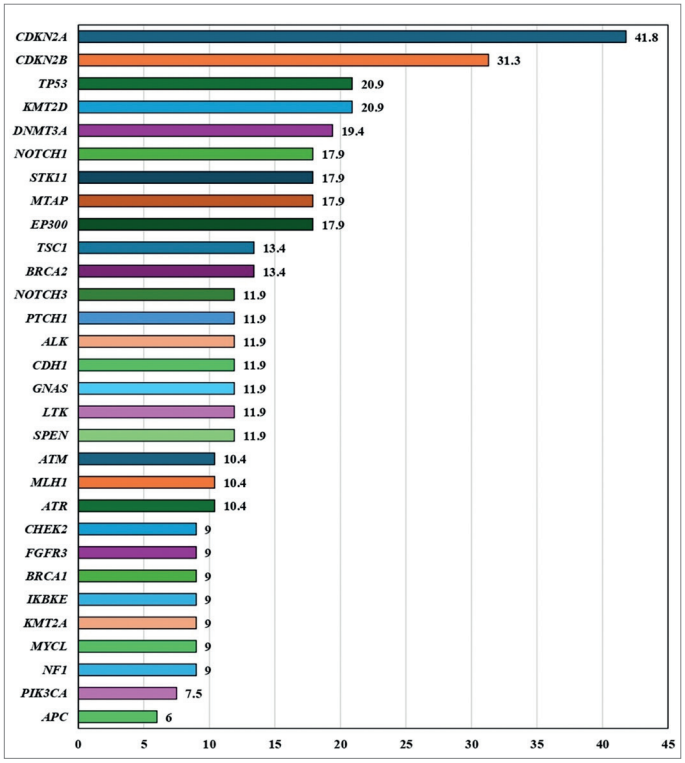

Fig. 1. Top 30 mutations in nasopharyngeal carcinoma.

Data were analyzed for 67 consecutive patients with NPC registered at the Japan National Cancer Center in C-CAT between June 2019 and March 2024. *CDKN2A* – cyclin-dependent kinase inhibitor 2A; *CDKN2B* – cyclin-dependent kinase inhibitor 2A; *TP53* – tumor protein p53; *KMT2D* – lysine methyltransferase 2D; *DNMT3A* – DNA methyltransferase 3 alpha; *NOTCH1* – notch receptor 1; *STK11* – serine/threonine kinase 11; *MTAP* – methylthioadenosine phosphorylase; *EP300* – E1A binding protein p300; *TSC1* – TSC complex subunit 1; *BRCA2* – BRCA2 DNA repair associated; *NOTCH3* – notch receptor 3; *PTCH1* – patched 1; *ALK* – ALK receptor tyrosine kinase; *CDH1* – cadherin 1; *GNAS* – GNAS complex locus; *LTK* – leukocyte receptor tyrosine kinase; *SPEN* – spen family transcriptional repressor; *ATM* – ATM serine/threonine kinase; *MLH1* – mutL homolog 1; *ATR* – ATR serine/threonine kinase; *CHEK2* – checkpoint kinase 2; *FGFR3* – fibroblast growth factor receptor 3; *BRAC1* – BRCA1 DNA repair associated; *IKBKE* – inhibitor of nuclear factor kappa B kinase subunit epsilon; *KMT2A* – lysine methyltransferase 2A; *MYCL* – MYCL proto-oncogene; bHLH transcription factor; *NF1* – neurofibromin 1; *PIK3CA* – phosphatidylinositol-4;5-bisphosphate 3-kinase catalytic subunit alpha; *APC* – APC regulator of WNT signaling pathway.

Clinical Information

Data were obtained for patient information (hospital ID, sex, age, cancer type), sample information (inspection type, tumor cell content, specimen collection site), background information (pathological diagnosis, smoking history, Eastern Cooperative Oncology Group Performance Status [ECOG-PS], family history), cancer type (presence/absence of metastasis, companion diagnostic test results) pre-/post-regimen (drug name, start/end date of administration, best overall response, adverse events) and outcomes (outcome, last survival confirmation date, date of death, cause of death).

Inclusion and Exclusion Criteria

Data were analyzed for 67 consecutive patients with NPC registered at the Japan National Cancer Center in C-CAT between June 2019 and March 2024. Genetic alterations were determined by FoundationOne CDx or Liquid CDx, (Foundation Medicine, Cambridge, MA) next-generation sequencing. Diagnosis and treatment of each disease were performed at core hospitals for

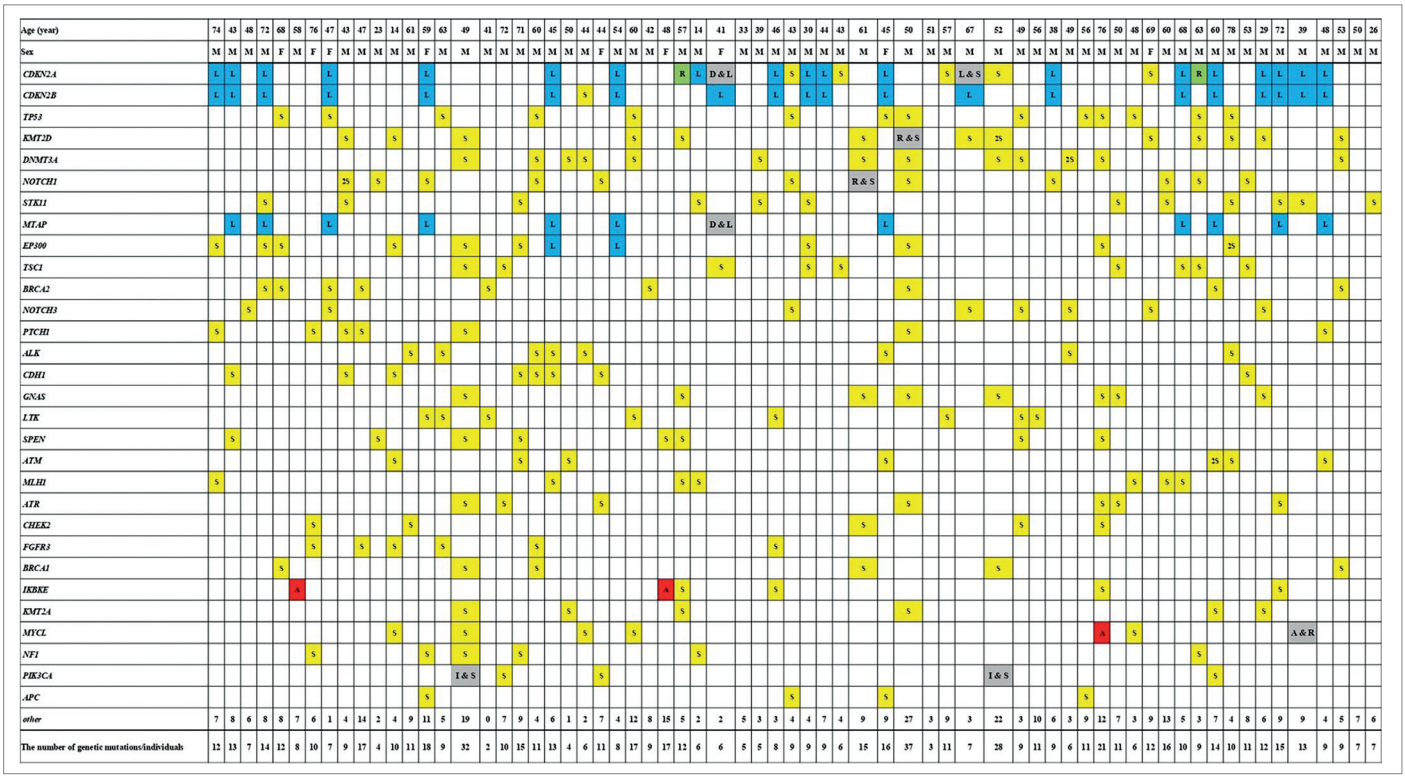

Fig. 2. Landscape of somatic mutations in nasopharyngeal carcinoma.

M – male; F – female; A – amplification; L – loss; R – rearrangement; S – small scale variant; 2S – two small scale variants; A & R – amplification and rearrangement; D & L – deletion and loss; I & S – interaction and small scale variant; L & S – loss and small scale variant; R & S – rearrangement and small scale variant; TMB – tumor mutation burden.

Tab. I. Characteristics of patients with nasopharyngeal carcinoma.

CHARACTERISTICS		**n = 67**			
Age, median (range)		51.3 (14–78)			
Sex	Male	58 (86.6%)			
	Female	9 (13.4%)			
Obervation period (days), median (range)		552.3 (35–1899)			
Cancer genomic profiling tests					
FoundationOne CDx		48 (71.6%)			
FoundationOne Liquid CDx		19 (28.4%)			
Presence of distant metastasis and lymph node metastasis					
Lymph node metastasis		33 (49.3%)			
Distant metastasis		50 (74.6%)			
Unknown and other		5 (7.5%)			
Chemotherapy					
	First-line treatment	Second-line treatment	Third-line treatment	Fourth treatment	Fifth treatment transition
Cisplatin	56	8	6	1	0
Cetuximab	7	14	18	6	5
Paclitaxel	5	11	12	6	7
Fluorouracil	23	11	5	2	1
Nivolmab	3	10	7	5	7
Pembrolizumab	9	14	5	2	3
Tegafur, Gimeracil, Oteracil Potassium	0	8	6	4	9
Carboplatin	10	7	3	2	0
Docetaxel Hydrate	6	0	1	3	2
Gemcitabine Hydrochloride	0	1	1	2	4

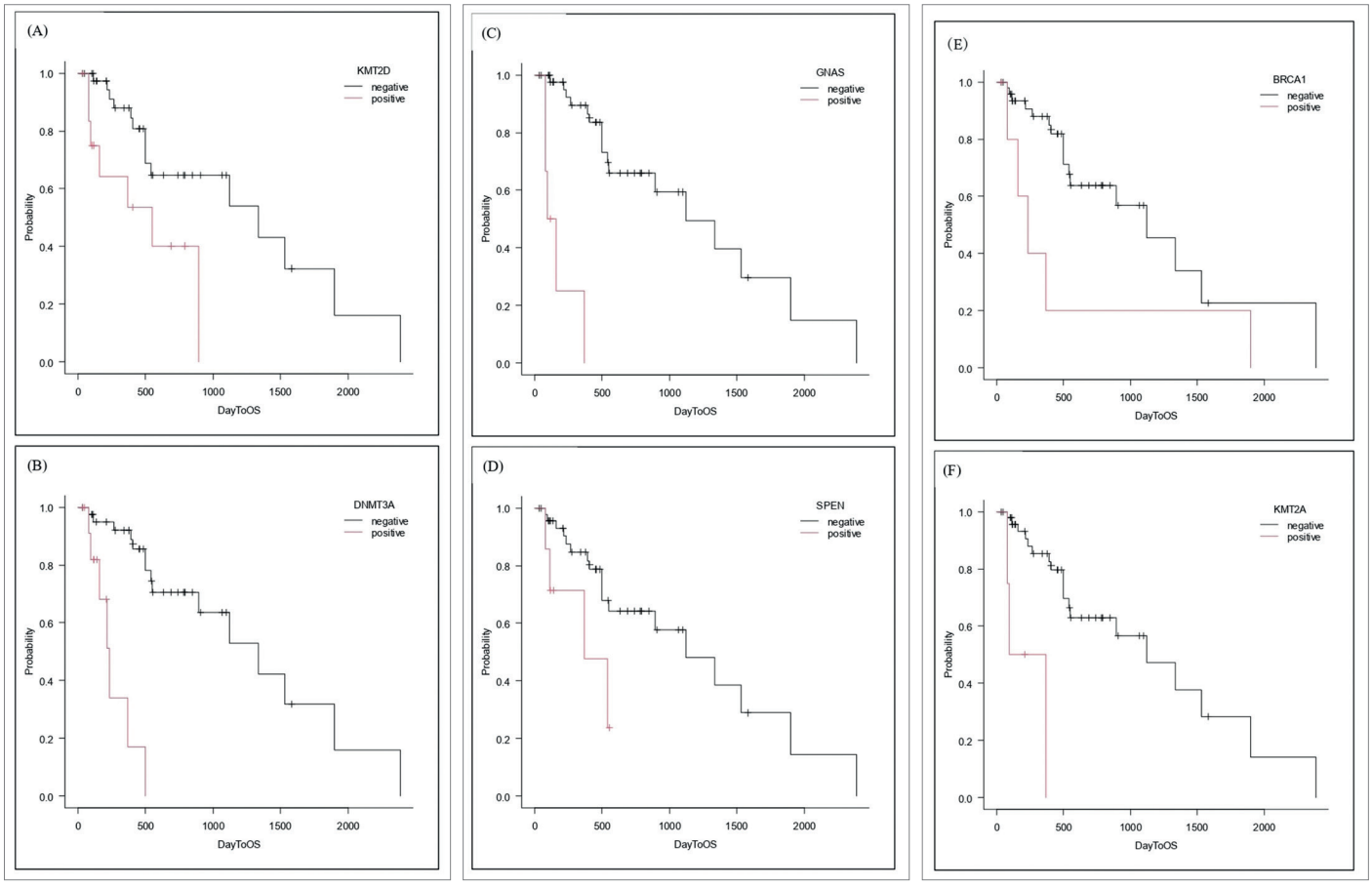

Fig. 3. (A–F) The median survival times for cases with *vs.* without each mutation.

cancer genomic medicine in Japan. Cases were excluded if the CGP test was performed using other methods (NCC OncoPanel, Guardant360 CDx, GenMineTOP). The prognostic analysis included 55 cases for which overall survival (OS) was recorded. Survival time was calculated from the date of NPC diagnosis.

Patient Background

The mean age was 51.3 (range, 14–78) years for the 67 NPC cases (58 male, 9 female). Metastases to distant organs and lymph nodes were present in 50/67 (74.6%) and 33/67 (49.3%) cases, respectively (Tab. I.).

Statistical analysis

The Kaplan-Meier method was used to calculate survival probability, with survival curves compared by the log-rank test. A Cox proportional hazards model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI). Statistical analyses were performed using EZR software (Saitama Medical Center, Jichi Medical University, Saitama, Japan) [4]. All reported P values are two-tailed, and $P < 0.05$ was considered to be significant in all analyses.

RESULTS

The top 30 mutations in recurrent and/or metastatic NPC were *CDKN2A* (28/67, 41.8%), *CDKN2B* (21/67, 31.3%), *TP53* (14/67, 20.9%), *KMT2D* (14/67, 20.9%), *DNMT3A* (13/67, 19.4%), *NOTCH1* (12/67, 17.9%), STK11 (12/67, 17.9%), *MTAP* (12/67, 17.9%), *EP300* (12/67, 17.9%), *TSC1* (9/67, 13.4%), *BRCA2* (9/67, 13.4%), *NOTCH3* (8/67, 11.9%), *PTCH1* (8/67, 11.9%), *ALK* (8/67, 11.9%), *CDH1* (8/67, 11.9%), *GNAS* (8/67, 11.9%), *LTK* (8/67, 11.9%), *SPEN* (8/67, 11.9%), *ATM* (7/67, 10.4%), *MLH1* (7/67, 10.4%), *ATR* (7/67, 10.4%), *CHEK2* (6/67, 9.0%), *FGFR3* (6/67, 9.0%), *BRCA1* (6/67, 9.0%), *IKBKE* (6/67, 9.0%), *KMT2A* (6/67, 9.0%), *MYCL* (6/67, 9.0%), *NF1* (6/67, 9.0%), *PIK3CA* (5/67, 7.5%), and *APC* (4/67, 6.0%), with 11.1 ±6.1 (mean ±SEM) mutations/individual (Fig. 1.). Details of the mutations in advanced NPC are shown in Fig. 2. Mutations, sequence variations, gene locus deletions and fusion gene transcripts are shown. Mutations were diverse, and no cases had identical mutations.

Mutations in *KMT2D* ($p = 0.0127$), *DNMT3A* ($p = 1.41\times10^{-7}$), *GNAS* ($p = 3.64\times10^{-11}$), *SPEN* ($p = 0.0167$), *BRCA1* ($p = 0.0379$), and *KMT2A* ($p = 2.06\times10^{-5}$) were associated with a significantly worse prognosis. The median survival times for cases with *vs.* without each mutation were 549 (95% CI, 80-NA) *vs.* 1335 (501-1899) days for *KMT2D*, 234 (95-NA) *vs.* 1355 (896-1899) days for *DNM3A*, 126.5 (80-NA) *vs.* 1122 (549-1899) days for *GNAS*, 369 (80-NA) *vs.* 1122 (501-1899) days for *SPEN*, 234 (80-NA) *vs.* 1122 (541-NA) days for *BRCA1*, and 232 (80-NA) *vs.* 1122 days (541-1899) for *KMT2A* (Fig. 3.A.–F.).

The HRs for cases with each mutation were 11.1 (0.109-1128, $p = 0.31$) for *CDKN2A*, 1.008 (0.0422-24.06, $p = 1.0$) for *CDKN2B*, 0.0122 (1.589×10^{-4}-0.936, $p = 0.046$) for *TP53*, 2.316 (0.0313-171.5,

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p = 0.70) for *KMT2D*, 1847.0 (4.619-7.386×10^5, p = 0.014) for *DNMT3A*, 3.0 (0.113-79.410, p = 0.51) for *NOTCH1*, 0.818 (0.0698-9.579, p = 0.87) for *STK11*, 0.0412 (8.57×10^{-4}-1.976, p = 0.10) for *MTAP*, 0.433 (0.0187-10.03, p = 0.60) for *EP300*, 6.037 (0.0807-451.7, p = 0.41) for *TSC1*, 126.7 (1.262-12720, p = 0.039) for *BRCA2*, 6.119 (0.0516-725.6, p = 0.45) for *NOTCH3*, 2.022 (0.0502-81.42, p = 0.70) for *PTCH1*, 197.9 (2.844-13770, p = 0.015) for *ALK*, 4.44 (0.0662-298.2, p = 0.48) for *CDH1*, 368.6 (0.3469-3.916×10^5, p = 0.096) for *GNAS*, 1.024 (0.0288-36.48, p = 0.99) for *LTK*, 34.22 (1.256-932.7, p = 0.036) for *SPEN*, 314.1 (0.755-1.306×10^5, p =0 .061) for *ATM*, 3.161 (0.0877-113.9, p = 0.53) for *MLH1*, 4.445 (0.041-482, p = 0.53) for *ATR*, 0.947 (0.0206-43.63, p = 0.98) for *CHEK2*, 5.606 (0.11-286.5, p = 0.39) for *FGFR3*, 0.025 (5.93×10^{-4}-1.053, p = 0.053) for *BRCA1*, 9.556 (0.0565-1615.0, p = 0.38) for *IKBKE*, 0.0195 (1.555×10^{-4}-2.435, p = 0.11) for *KMT2A*, 6.445×10^{-4} (2.913×10^{-6}-0.143, p = 7.6×10^{-3}) for *MYCL*, 6.538 (0.339-126.1, p = 0.21) for *NF1*, 37.2 (0.292-4726, p = 0.14) for *PIK3CA*, and 17.77 (0.11-2858.0, p = 0.27) for *APC* (Fig. 4).

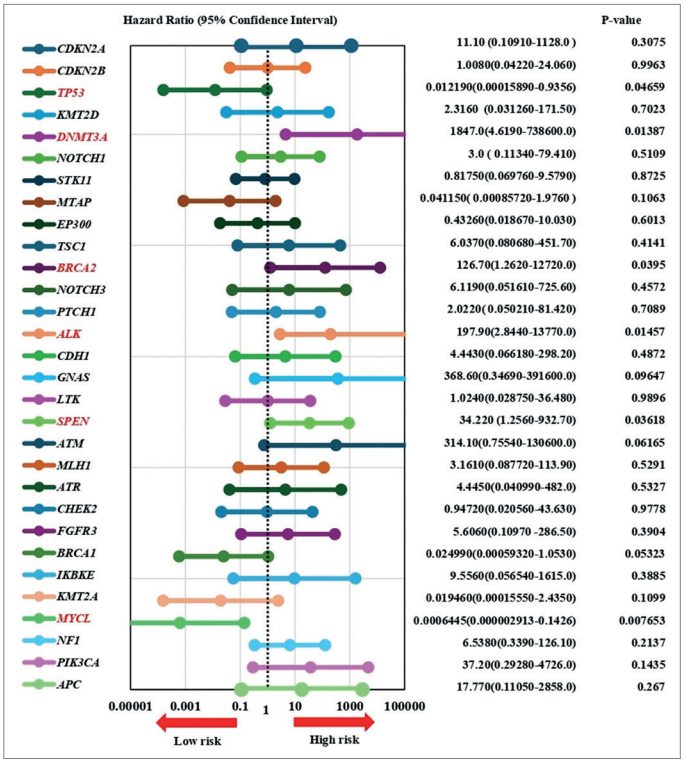

Fig. 4. Multivariate analysis of the prognostic implications of the top 30 mutations in nasopharyngeal carcinoma using a Cox proportional-hazards model.

DISCUSSION

CGP in the national health insurance system began in Japan in June 2019. CGP data and clinical information for patients are stored at C-CAT with individual informed consent [3, 5]. These data are now open for sharing with cancer genomic medicine hospitals, academic centers, and industry [5, 6]. However, the data do not indicate the pre-treatment stage or whether surgery or radiotherapy was performed. In Japan, chemoradiotherapy is the primary treatment choice for NPC, which implies that radiotherapy was used. Although CGP testing for head and neck cancer is now covered by insurance in Japan, there are few comprehensive reports on mutations in NPC. There have also been no reports using FoundationOne CDx or Liquid CDx to examine mutations in NPC.

A previous study of the genomic landscape of NPC showed that the most frequent deletion was in the 9p21 region spanning *CDKN2A* and *CDKN2B* [7]. Lung et al. detected up to 27% of tumors with mutated p53 in a small number of NPC cases [8]. In the current study, *CDKN2A*, *CDKN2B,* and *TP53* were also the most frequent mutations. Cases with *PIK3CA* and *SF3B1* mutations have worse distant metastasis-free survival than their wild-type counterparts [9]; however, an *SF3B1* mutation was barely detected in this study. The frequency of mutations may differ from other reports due to the different methods used, as well as the variations in patient backgrounds and racial differences. However, FoundationOne CDx and Liquid CDx used in this study cover a wide range of mutations, since they are pan-cancer, cfDNA-based comprehensive genomic profiling assays that detect alterations in a total panel of 324 genes [10].

In our patients, we found that *KMT2D* (p = 0.013), *DNMT3A* (p = 8.0×10^{-7}), *GNAS* (p = 2.3×10^{-10}), *SPEN* (p = 0.029), *BRCA1* (p = 0.04) and *KMT2A* (p = 4.3×10^{-5}) mutations were associated with a significantly worse prognosis in log-rank tests, while *DNMT3A* (p = 0.039), *BRCA2* (p = 0.015), *ALK* (p = 0.015), and *SPEN* (p = 7.6×10^{-3}) mutations were associated with a significantly worse prognosis in a Cox proportional hazards model. The different results may be attributed to the short observation period affecting the log-rank test and the large number of covariates impacting the Cox proportional hazards model.

DNMT3A is a DNA methyltransferase involved in de novo methylation at CpG sites in genomic DNA. DNA methylation in promoter regions prevents binding of the basal transcription machinery, resulting in reduced gene expression. *DNMT3A* mutations are common in hematologic malignancies, particularly acute myeloid leukemia and myelodysplastic syndrome. These mutations are thought to play a critical role in the pathogenesis and progression of these diseases [11, 12]. *SPEN* is a transcription factor that regulates activation of the *NOTCH*, *TCF/LEF,* and *EGFR* pathways. *SPEN* mutations are associated with breast cancer, and these mutations may play a significant role in the pathogenesis of cancers [13]. *SPEN* mRNA and protein expression are increased in NPC cells and tissues compared to nonmalignant nasopharyngeal epithelial cells and tissues, promoting the pathogenesis of NPC and being associated with poor prognosis [14].

There were no cases in which a definitive treatment method was proposed after genetic panel testing. We also propose that future clinical treatment should incorporate genetic profiling to explore targeted therapies for patients with these mutations. In the future, it will be possible to select drugs tailored to the characteristics of the tumor by performing pathological tissue tests and genetic cancer panel tests prior to head and neck cancer treatment.

Study limitations

There are several limitations in the study, including the non-uniform protocol, left-truncational bias [15], lack of information on EBV

presence/absence, and lack of detailed patient background data. Also, NPC cases cured by chemoradiotherapy were not included, so the findings may not reflect all genetic mutations. However, we believe that the findings have value, given that there is little information on genetic mutations in advanced NPC.

CONCLUSIONS

Our results show that mutations in *KMT2D*, *DNMT3A*, *GNAS*, *SPEN*, *BRCA1*, and *KMT2A* are associated with a significantly worse prognosis in recurrent and/or metastatic NPC, as determined by the log-rank test. The hazard ratios for these mutations were 1847.0 for *DNMT3A*, 126.7 for *BRCA1*, 197.9 for *ALK*, and 34.22 for *SPEN*. The significance of these mutations for prognosis, even in recurrent and/or metastatic cases, confirms the importance of use of cancer genomic profiling tests.

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