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Variant-Level Curation and Molecular Context of Therapeutically Relevant Alterations in Parathyroid Carcinoma: A Nationwide C-CAT Analysis in Japan

Jumpei Yoshida
Japanese Foundation For Cancer Research
Yasuyoshi Sato
yasuyoshi_s@hotmail.com

The University of Tokyo
Naoki Fukuda
Japanese Foundation For Cancer Research
Juro Yanagida
Japanese Foundation For Cancer Research
Kousuke Watanabe
The University of Tokyo
Mitsuhiro Fujishiro
The University of Tokyo
Yuji Miura
Japanese Foundation For Cancer Research
Katsutoshi Oda
The University of Tokyo
Shunji Takahashi
Japanese Foundation For Cancer Research

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Title: Variant-Level Curation and Molecular Context of Therapeutically Relevant Alterations in Parathyroid Carcinoma: A Nationwide C-CAT Analysis in Japan

Jumpei Yoshida[1,2,3], Yasuyoshi Sato[2], Naoki Fukuda[1], Juro Yanagida[1], Kousuke Watanabe[4], Mitsuhiro Fujishiro[2,5], Yuji Miura[1], Katsutoshi Oda[6], Shunji Takahashi[1]

[1]Department of Medical Oncology, Cancer Institute Hospital, Japanese Foundation for Cancer Research, Koto-ku, Tokyo, Japan

[2]Department of Clinical Oncology, The University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan

[3]Department of Clinical Chemotherapy, Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Koto-ku, Tokyo, Japan

[4]Next-Generation Precision Medicine Development Laboratory, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

[5]Department of Gastroenterology in Organ Pathophysiology program, Graduate School of Medicine, The University of Tokyo Hospital, Bunkyo-ku, Tokyo, Japan

[6]Division of Integrative Genomics, Graduate School of Medicine, The University of Tokyo, Bunkyo-ku, Tokyo, Japan

Short title: Variant-Level Curation and Molecular Context of Therapeutically Relevant Alterations in Parathyroid Carcinoma: A Nationwide C-CAT Analysis in Japan

Correspondence to:

Yasuyoshi Sato, Department of Clinical Oncology, The University of Tokyo Hospital, Japan, email: yasuyoshi_s@hotmail.com

Naoki Fukuda, Cancer Institute Hospital, Japanese Foundation for Cancer Research, e-mail: naoki.fukuda@jfcr.or.jp

Abstract

Introduction:

Parathyroid carcinoma (PC) is an ultra-rare malignancy with limited treatment options. Although comprehensive genomic profiling (CGP) has identified recurrent genomic alterations in PC, the pathogenicity and therapeutic relevance of individual variants and their distribution across molecular subgroups remain incompletely characterized. We aimed to characterize the genomic landscape of PC through variant-level pathogenicity curation and molecular subgroup analysis, with a focus on potentially therapeutically relevant alterations.

Methods:

We conducted a descriptive analysis of patients with pathologically confirmed PC registered in C-CAT, a Japanese nationwide cancer genomics database. Genomic alterations were systematically curated for pathogenicity using OncoKB and manual review of published and functional evidence. Molecular subgroup analyses were performed in patients with evaluable CDC73 status, classified as CDC73-altered, MEN1-altered, or other tumors.

Results:

Thirty patients were included. Among 373 unique genomic alterations detected, 114 (30.6%) were classified as pathogenic after variant-level curation. CDC73 (43.3%), TP53 (26.7%), and MEN1 (23.3%) were the most frequently altered genes. High tumor mutational burden (≥10 mutations/Mb), mismatch repair (MMR), PI3K–AKT–mTOR, and homologous recombination repair (HRR)-related alterations were identified in 23.3%, 16.7%, 23.3%, and 13.3% of patients, respectively. Only 5 of 25 unique HRR-related alterations (20.0%) were retained as pathogenic after curation. Among 29 patients evaluable for molecular subgroup analysis, therapeutically relevant alterations showed distinct distributions across CDC73-altered, MEN1-altered, and other tumors. CDC73 alterations were mutually exclusive with MEN1, TP53, and PI3K–AKT–mTOR pathway alterations, whereas TP53 alterations co-occurred with PI3K–AKT–mTOR pathway alterations.

Conclusions:

Variant-level pathogenicity curation substantially refined the genomic alterations identified by CGP in PC, particularly among HRR-related genes. Molecular stratification based on CDC73 and MEN1 revealed distinct patterns of therapeutically relevant alterations, highlighting the importance of interpreting CGP findings within disease-specific molecular contexts.

Introduction

Parathyroid carcinoma (PC) is an extremely rare malignancy, accounting for <0.005% of all cancers worldwide, with an estimated annual incidence of 3.5–5.7 cases per 10 million population.[1,2] The most frequently reported genomic alterations in parathyroid

carcinoma involve *CDC73* and *MEN1*, occurring in approximately 35–50% and 13–31% of cases, respectively.[3–10]

Approximately 20% of patients eventually develop metastatic disease.[11] PC typically follows a relatively indolent course; however, it progressively compromises patients through persistent hypercalcemia, with morbidity and mortality often driven by metabolic complications rather than tumor burden itself.[12] Because of its rarity, standardized treatment approaches have not been defined, and most available data on management are derived from case reports and small case series.[13–20] In real-world clinical practice, treatment decisions are typically based on evidence from case reports.

Recent advances in genomic profiling have provided new insights into the molecular basis of this disease, with recurrent alterations identified in pathways such as PI3K–AKT–mTOR, homologous recombination repair (HRR), and mismatch repair (MMR) pathways. [3–6,10,20–22] These observations suggest that CGP may facilitate the identification of therapeutically relevant alterations and support precision oncology approaches in this ultra-rare malignancy. However, previous genomic studies of PC have not integrated comprehensive variant-level pathogenicity curation, molecular subgroup analysis based on the major PC-associated genes CDC73 and MEN1, and systematic evaluation of potentially relevant alterations. [3–6,10,20–22] Using a nationwide CGP database in Japan, we performed curated variant-level annotation and characterized the genomic landscape of PC according to three major molecular subgroups—CDC73-altered, MEN1-altered, and other tumors—with particular emphasis on potentially therapeutically relevant genomic alterations.

Methods

Database

The Center for Cancer Genomics and Advanced Therapeutics (C-CAT) is a nationwide database in Japan that collects and integrates genomic, clinical, and pathological information from patients who undergo CGP as part of clinical care.[23] This database has been widely used in studies across various malignancies, including endocrine tumors.[22,24]

Study population

Patients with pathologically confirmed PC who underwent tissue-based CGP using either FoundationOne CDx (Foundation Medicine, Cambridge, MA, USA; tumor-only DNA-based panel targeting 324 genes), GenMineTOP (Sysmex Corporation, Kobe, Japan; paired tumor–normal sequencing using tumor tissue and matched peripheral blood with combined DNA and RNA analysis targeting 737 genes), or the OncoGuide NCC Oncopanel System (Sysmex Corporation, Kobe, Japan; paired tumor-normal sequencing using tumor tissue and matched peripheral blood), and who were registered in the C-CAT database between July 2019 and December 2025, were included. Patients who underwent liquid-based CGP were excluded from the analysis.

Statistical analysis

Gene-level alteration frequencies and descriptive genomic analyses were based on the final cohort, with genes not covered by an individual CGP assay treated as having no detected alteration. Because CDC73 status was required for molecular subgroup classification, subgroup analyses were restricted to patients with evaluable CDC73 status, who were classified as CDC73-altered, MEN1-altered, or other tumors.

Continuous variables were summarized as medians and interquartile ranges (IQRs), whereas categorical variables were summarized as counts and percentages. Comparisons among groups were performed using the Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables. All statistical analyses were performed using R version 4.4.3 (R Foundation for Statistical Computing, Vienna, Austria). All statistical tests were two-sided, and a p value <0.05 was considered statistically significant.

Variant definition

Genomic alterations were defined according to the annotations provided in the C-CAT database, including mutations (single nucleotide variants and insertions/deletions) and CNA. Pathogenicity was determined using the OncoKB: A Precision Oncology Knowledge Base.[25] Variants annotated as Oncogenic or Likely Oncogenic, truncating variants in OncoKB-defined tumor suppressor genes, and oncogenic copy number alterations were classified as pathogenic. Variants lacking sufficient annotation in OncoKB were additionally evaluated by manual review of the published literature and available functional evidence. Pathogenicity was determined based on biological annotation irrespective of variant allele frequency or copy number amplitude. All remaining alterations were classified as variants of uncertain significance. MMR pathway alterations were defined as alterations in *MLH1*, *MSH2*, *MSH6*, or *PMS2*. Alterations in the PI3K–AKT–mTOR pathway were defined as alterations in *PTEN*, *PIK3CA*, *AKT*, or *MTOR*. HRR-related gene alterations were defined as alterations in the following 18 genes involved in homologous recombination repair and related DNA damage response pathways: *BRCA1*, *BRCA2*, *PALB2*, *BARD1*, *ATR*, *ATRX*, *ATM*,

BAP1, *RAD51B*, *RAD51C*, *RAD51D*, *BRIP1*, *NBN*, *CHEK1*, *CHEK2*, *FANCA*, *FANCC*, and *MRE11*. Germline alterations identified by paired tumor–normal sequencing were classified as germline pathogenic variants (GPVs), whereas germline alterations reported by tumor-only sequencing were classified as presumed germline pathogenic variants (PGPVs).

Ethics

This study was conducted in accordance with the Declaration of Helsinki and received approval from the Ethics Committee of the Graduate School of Medicine and the Faculty of Medicine, The University of Tokyo (#2021341G) and the C-CAT Data Utilization Review Board (#CDU2022-026N). Written informed consent for registration in the C-CAT database and secondary use of clinical and genomic data was obtained from all participants at the participating institutions prior to data collection.

Results

Genomic landscape of the final cohort

A total of 3,554 patients with head and neck cancers were identified from the C-CAT database (OncoTree Level 1), among whom 31 patients had PC. After excluding one patient who underwent liquid biopsy–based CGP, 30 patients were included in the final cohort (Figure 1). Among 373 unique alterations detected across the final cohort, 114 (30.6%) were retained as pathogenic. The results of pathogenicity curation for the major genes and gene sets evaluated in this study are summarized in Supplementary Table 1. A total of 208 genes were found to be altered across the cohort, of which 65 harbored pathogenic alterations (Supplementary Table 2). The most frequently altered gene was CDC73 (43.3%), followed by TP53 (26.7%) and MEN1 (23.3%) (Figure 2). High TMB

(≥10 mutations/Mb) was observed in 7 patients (23.3%). MMR, PI3K–AKT–mTOR, and HRR-related alterations were identified in 5 (16.7%), 7 (23.3%), and 4 (13.3%) patients, respectively. Among the detected variants, only MSH2 Y619*, identified by the OncoGuide NCC Oncopanel System, was reported as a GPV, and no PGPVs were identified in this cohort. Baseline demographic and genomic characteristics are summarized in Table 1. CDC73 alterations were distributed across the entire coding region without a clear hotspot. A total of 18 alterations were identified, including 16 unique variants (Figure 3A). Truncating mutations (nonsense and frameshift) accounted for the majority (n = 14/18, 77.8%), comprising 7 nonsense and 7 frameshift events, whereas missense (n = 2/18, 11.1%) and splice-site variants (n = 2/18, 11.1%) were less frequent. MEN1 alterations were also distributed across the coding region without a clear hotspot. A total of 7 alterations were identified (Figure 3B). Truncating mutations (nonsense and frameshift) accounted for the majority (n = 6/7, 85.7%), including 1 nonsense and 5 frameshift variants, whereas only a single missense variant (n = 1/7, 14.3%) was observed.

Molecular subgroup analysis

Of the 30 patients in the final cohort, one patient was excluded from the molecular subgroup analysis because CDC73 was not covered by the assay, leaving 29 patients in the subcohort (Figure 1). Baseline characteristics of the subcohort are summarized in Table 1. High TMB, MMR, PI3K–AKT–mTOR, and HRR-related alterations showed distinct distributions across the CDC73-altered, MEN1-altered, and other subgroups (Figure 4). Pairwise analysis revealed distinct patterns of genomic mutual exclusivity

and co-occurrence (Figure 5). CDC73 alterations were mutually exclusive with MEN1, TP53, and PI3K–AKT–mTOR pathway alterations (P = 0.0084 for all comparisons). In contrast, TP53 alterations co-occurred with PI3K–AKT–mTOR pathway alterations (P = 0.038), while MEN1 alterations co-occurred with MMR alterations (P = 0.034).

Discussion

Our study represents one of the largest genomic analyses of PC and provides two key advances: comprehensive variant-level pathogenicity curation and molecular subgroup–based characterization of therapeutically relevant alterations. Of 373 unique genomic alterations detected by CGP, only 114 were classified as pathogenic after curation, underscoring the importance of variant-level interpretation. Furthermore, stratification into CDC73-altered, MEN1-altered, and other tumors revealed that therapeutically relevant alterations were non-randomly distributed across molecular subgroups, suggesting distinct genomic contexts within PC.

Consistent with prior studies, *CDC73* alterations were the most frequent genomic events, with a frequency comparable to that reported in CGP-based studies from the United States.[3–10] As reported previously, the majority of *CDC73* alterations were frameshift, nonsense, and splice-site variants.[3–10] Missense *CDC73* variants are rare and typically occur in the N-terminal region of parafibromin, which was also the case in our cohort. *MEN1* alterations were also frequently observed. Although *MEN1* S160F is not well documented in public databases, it was included in this study based on its localization within the JunD-binding domain, the highly non-conservative nature of the amino acid substitution (Grantham distance: 155), and supporting evidence from

functional analyses of neighboring codon variants demonstrating impaired menin function.[26–31] This interpretation is consistent with prior reports highlighting the limitations of ACMG-AMP classification for *MEN1* missense variants and the need for locus-specific evaluation.[31]

The PI3K–AKT–mTOR pathway has emerged as a key signaling axis and potential therapeutic target across multiple malignancies.[32–37] A recent targeted sequencing study reported PI3K–AKT–mTOR pathway alterations in 12.9% of parathyroid carcinomas.[10] In our cohort, PI3K–AKT–mTOR pathway alterations were identified in 23.3% of patients and were predominantly driven by *PTEN* alterations, which were not evaluated in the previous study. This difference may partly account for the higher frequency observed in our cohort.

High TMB and deficient MMR are potential biomarkers relevant to immune checkpoint inhibition across multiple cancer types.[38,39] In our cohort, applying a threshold of ≥10 mutations/Mb—consistent with clinical trial eligibility criteria—TMB-high tumors were identified in 23.3% of patients.[4,38] Alterations in MMR-related genes can lead to dMMR, although not all such alterations necessarily result in functional deficiency.[39] MMR alterations were identified in 5 patients (16.7%) in our cohort, including *MLH1* (n = 1, 3.3%), *MSH2* (n = 3, 10.0%), and *MSH6* (n = 1, 3.3%).

Alterations in HRR-related genes have been associated with response to PARP inhibitors across various malignancies, particularly in the presence of *BRCA* alterations.[40–42] In a phase II study from Belgium in a PC patient with a *BRCA2* alteration, olaparib achieved a partial response that was ongoing at 20 months. In another case, olaparib

resulted in a biochemical response and durable disease stabilization.[20,43] However, HRR-related alterations have not been systematically characterized at the cohort level in PC. Importantly, the detection of an alteration in an HRR-related gene does not necessarily indicate pathogenicity or therapeutic relevance. In our study, only 5 of 25 unique HRR-related alterations (20.0%) were retained as pathogenic after variant-level curation, highlighting the importance of careful variant-level interpretation when assessing potential therapeutic relevance.

RET C609_F612del was identified in one case. This in-frame deletion affects the cysteine-rich domain encoded by exon 10 and includes C609, a well-established pathogenic residue associated with *MEN2*.[44] In a recent study of medullary thyroid carcinoma, *RET* in-frame insertions/deletions were identified as recurrent oncogenic events, and both patients with *RET* indels who received selpercatinib achieved a partial response, supporting the actionability of this class of *RET* alterations.[45]

Pairwise analysis confirmed previously reported patterns of mutual exclusivity involving CDC73, including its mutual exclusivity with MEN1, TP53, and PI3K–AKT–mTOR pathway alterations.[4,22] In contrast, we observed significant co-occurrence of TP53 and PI3K–AKT–mTOR pathway alterations, a relationship that has not been well characterized in PC. Notably, both alterations occurred preferentially in CDC73-wild-type tumors, suggesting that their co-occurrence may characterize a distinct genomic context within CDC73-wild-type PC. MEN1 and MMR-related alterations also showed significant co-occurrence, although this finding should be interpreted cautiously given the small number of MMR-altered cases. These findings warrant validation in larger cohorts.

This study has several limitations. First, genomic profiling was performed using three different comprehensive genomic profiling platforms (FoundationOne CDx, GenMineTOP, and the OncoGuide NCC Oncopanel System), and differences attributable to the respective assays cannot be excluded. Because the majority of patients underwent tumor-only sequencing with FoundationOne CDx, the assessment of germline status was limited. Consequently, relatively young patients or those with high variant allele frequencies should be interpreted cautiously, as underlying germline *CDC73* or *MEN1* variants remain a possibility (Supplementary Table 3). Second, the cohort was derived from patients who underwent comprehensive genomic profiling in clinical practice, which may introduce selection bias toward more advanced or treatment-refractory cases. This cohort therefore predominantly reflects the genomic profile of aggressive phenotypes rather than localized disease. Third, the cohort consisted predominantly of Japanese patients, and the findings may not be generalizable to other populations. Fourth, the sample size was limited due to the rarity of the disease; therefore, further accumulation of cases and larger studies are warranted to validate these findings. Fifth, because of the limitations of the database, detailed treatment information and survival analyses were unavailable. Studies with comprehensive clinical data are warranted to better evaluate treatment outcomes and prognosis.

Conclusion

In conclusion, variant-level pathogenicity curation and molecular stratification based on CDC73 and MEN1 revealed distinct patterns of therapeutically relevant alterations in PC. These findings highlight the importance of interpreting CGP results within disease-

specific molecular contexts and may provide a framework for understanding the molecular pathogenesis of PC and developing molecularly guided therapeutic strategies for this ultra-rare malignancy.

Data availability statement

The data that support the findings of this study were obtained from C-CAT, Japan. Restrictions apply to the availability of these data, which were used under license for the current study and are therefore not publicly available. Researchers may apply for access to C-CAT data through the appropriate application procedures and subject to approval by C-CAT and relevant governing bodies. For inquiries regarding data access, please contact the corresponding author, Yasuyoshi Sato, who will provide guidance on the appropriate procedures for requesting access to the C-CAT data.

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No funding was received for this study.

Author contributions

J.Yo: Conceptualization, methodology, investigation, data curation, visualization, writing – review & editing.

Y.S: Conceptualization, methodology, investigation, data curation, review & editing, resources.

N.F: Conceptualization, methodology, data curation, validation, review & editing.

J.Ya: Conceptualization, methodology, review & editing.

M.F, K.W, K.O: Writing – review & editing, resources.

Y.M, S.T: Methodology, data curation, validation, review & editing, supervision.

All authors approved the final manuscript.

Competing Interests

J.Y: Honoraria for lectures from Kyowa Kirin.

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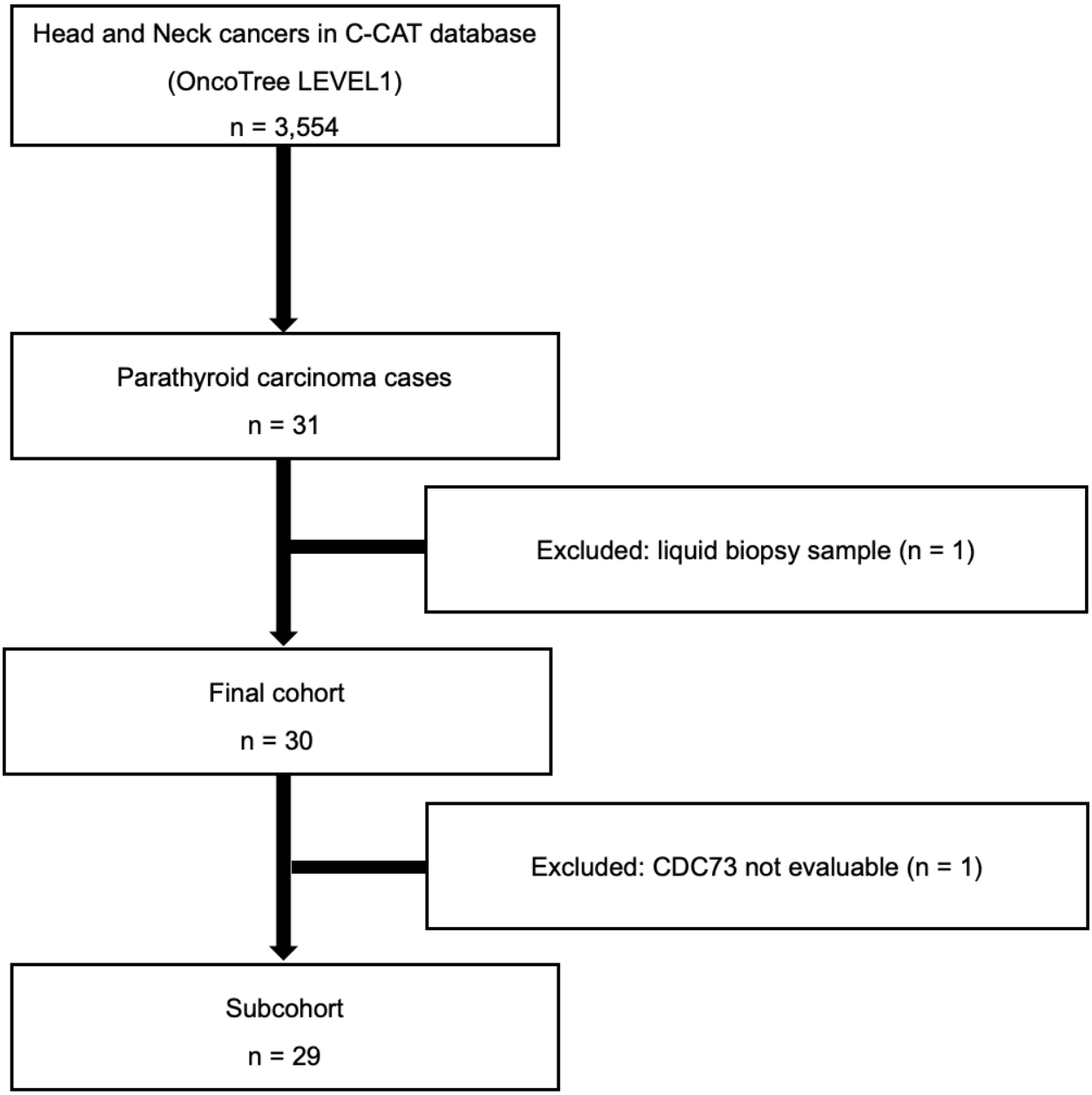


Figure 1. Study flow diagram.
From 3,554 head and neck cancer cases in the C-CAT database, 31 parathyroid carcinoma cases were identified. After excluding one case evaluated using liquid biopsy–based CGP, 30 patients were included in the final cohort. Of these, 29 patients with evaluable CDC73 status were included in the molecular subgroup subcohort.

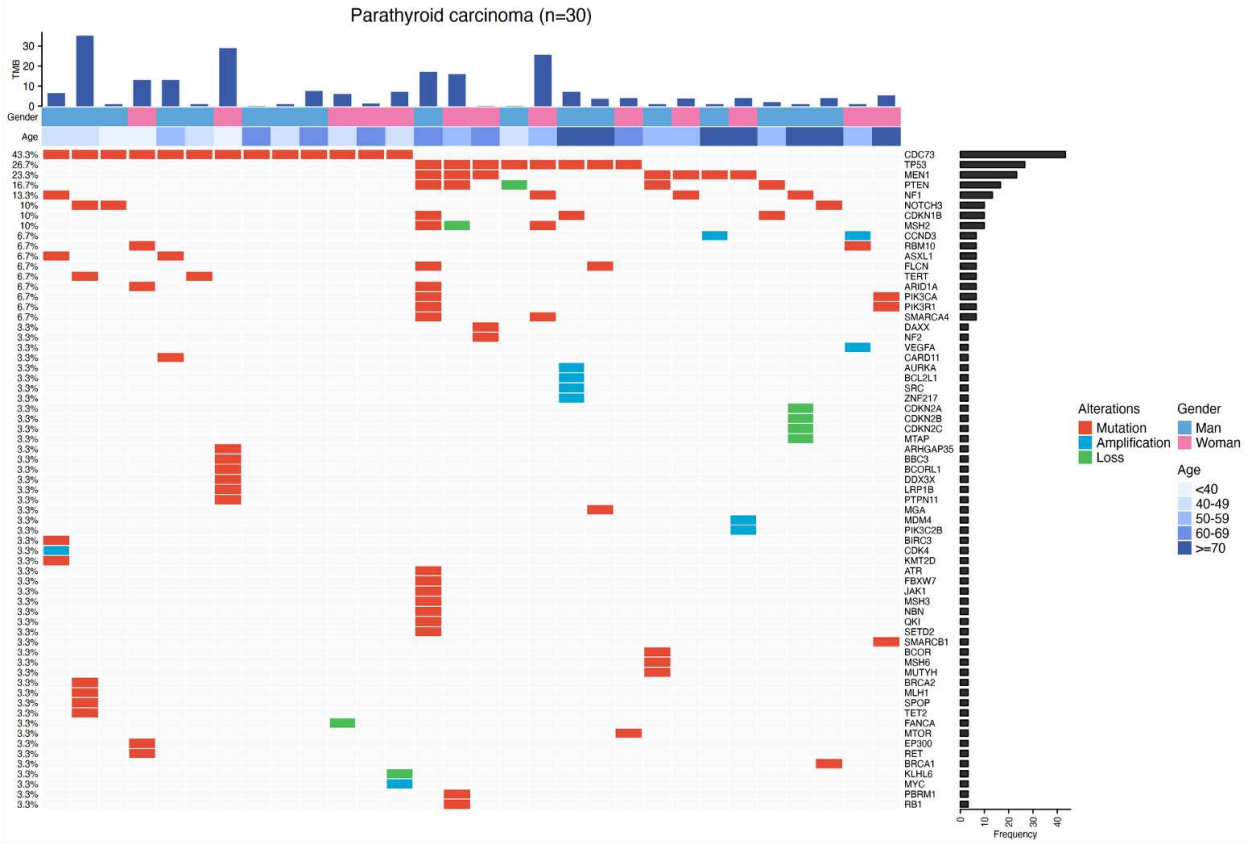


Figure 2. Integrated genomic and clinical landscape of parathyroid carcinoma (n = 30).

OncoPrint of genomic alterations in 30 patients with parathyroid carcinoma. Columns represent individual patients and rows represent genes. Mutations, amplifications, and copy number losses are shown in red, blue, and green, respectively. Tumor mutational burden (TMB) is displayed above the heatmap, together with clinical annotations for sex and age. Genes are ranked by alteration frequency, shown in the bar plot on the right.

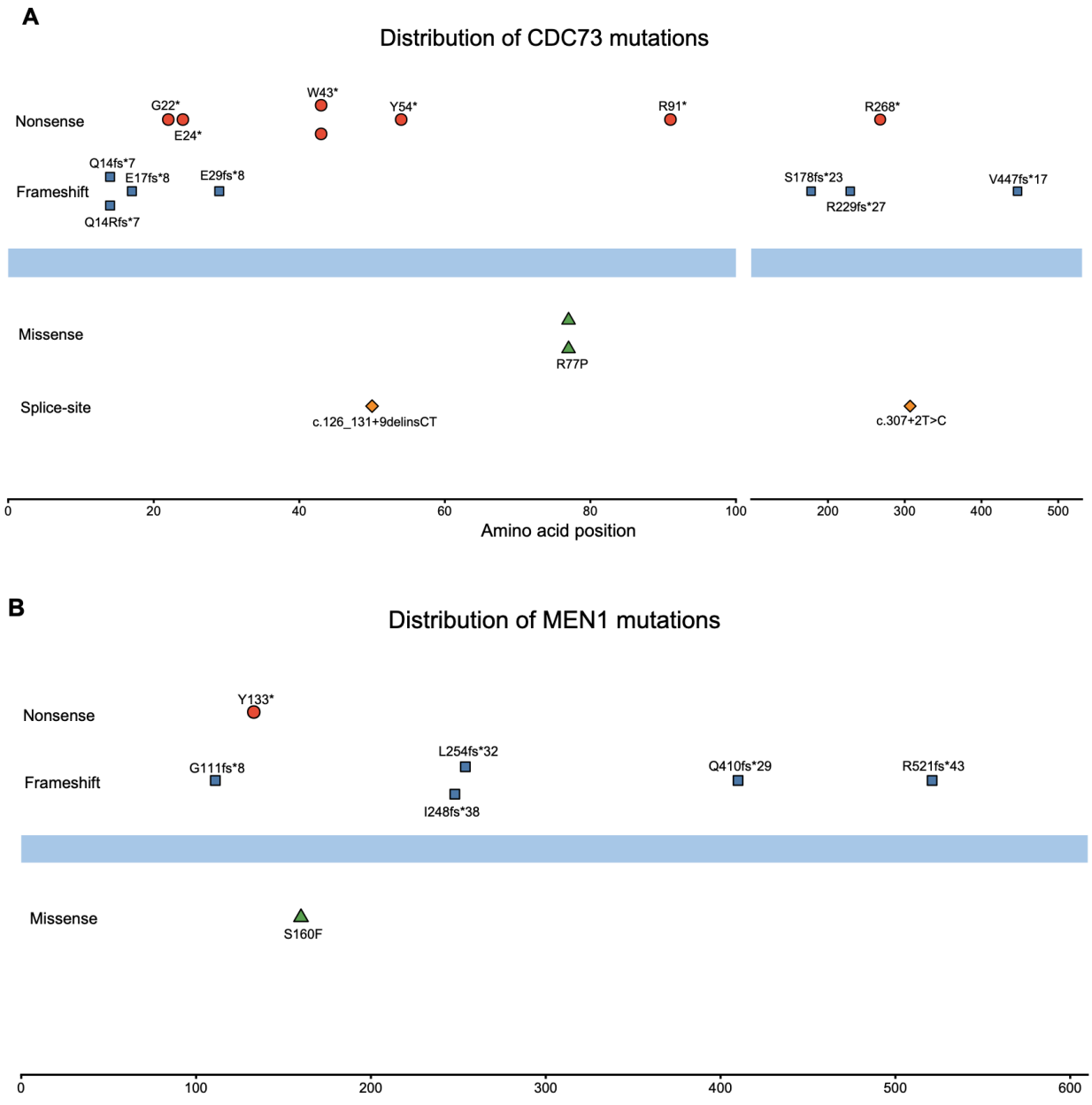


Figure 3. Distribution of key driver gene mutations in parathyroid carcinoma. Schematic representation of the distribution of genomic alterations across the coding regions of CDC73 and MEN1. Variants are categorized by mutation type, and the x-axis indicates amino acid position from the N-terminus (left) to the C-terminus (right).

(A) Distribution of CDC73 alterations. Alterations were observed throughout the coding region without a clear hotspot, with truncating mutations predominating in the N-terminal region. Amino acids 1–100 are shown at an expanded scale for clarity.

(B) Distribution of MEN1 alterations. Alterations were distributed across the coding region without an apparent hotspot, and most were truncating mutations.

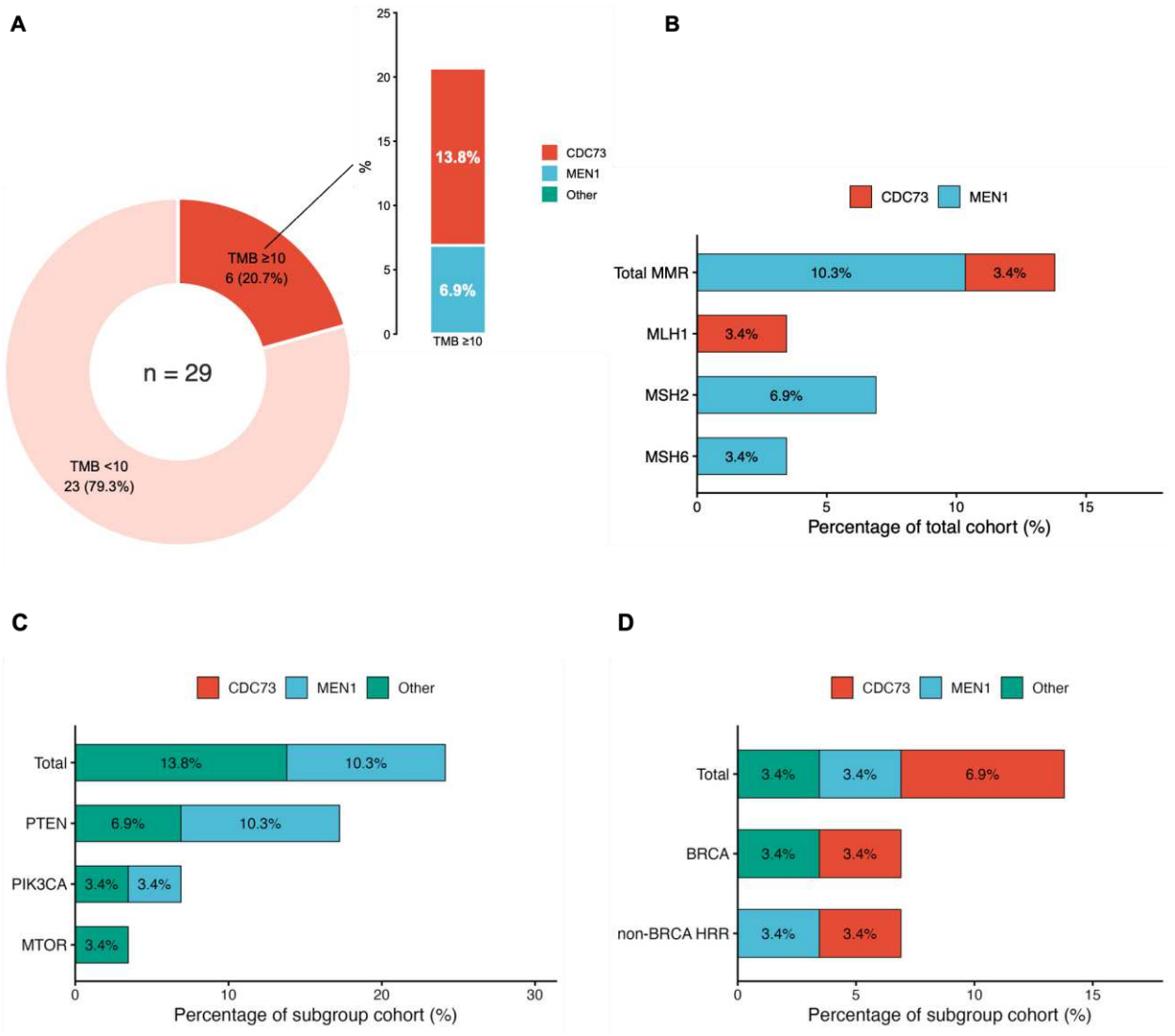


Figure 4. Distribution of therapeutically relevant genomic features across molecular subgroups.

(A) Distribution of tumor mutational burden (TMB) in the cohort (n = 29). Six patients (20.7%) had TMB-high tumors (≥10 mutations/Mb). The inset bar plot shows the distribution of TMB-high tumors across the CDC73-, MEN1-, and other molecular subgroups.

(B) Frequency of mismatch repair (MMR)–related gene alterations in the cohort. Alterations in MLH1, MSH2, and MSH6 were identified in 13.8% of patients overall, with subgroup-specific frequencies shown for the CDC73- and MEN1-altered groups.

(C) Frequency of PI3K–AKT–mTOR pathway alterations in the cohort. Alterations were identified in 24.1% of patients and were predominantly driven by PTEN alterations. Subgroup-specific contributions from MEN1-altered and other tumors are shown.

(D) Frequency of HRR gene alterations in the cohort. HRR alterations were identified in 13.8% of patients, including both BRCA and non-BRCA HRR gene alterations. Subgroup-specific contributions from CDC73-, MEN1-, and other molecular subgroups are shown.

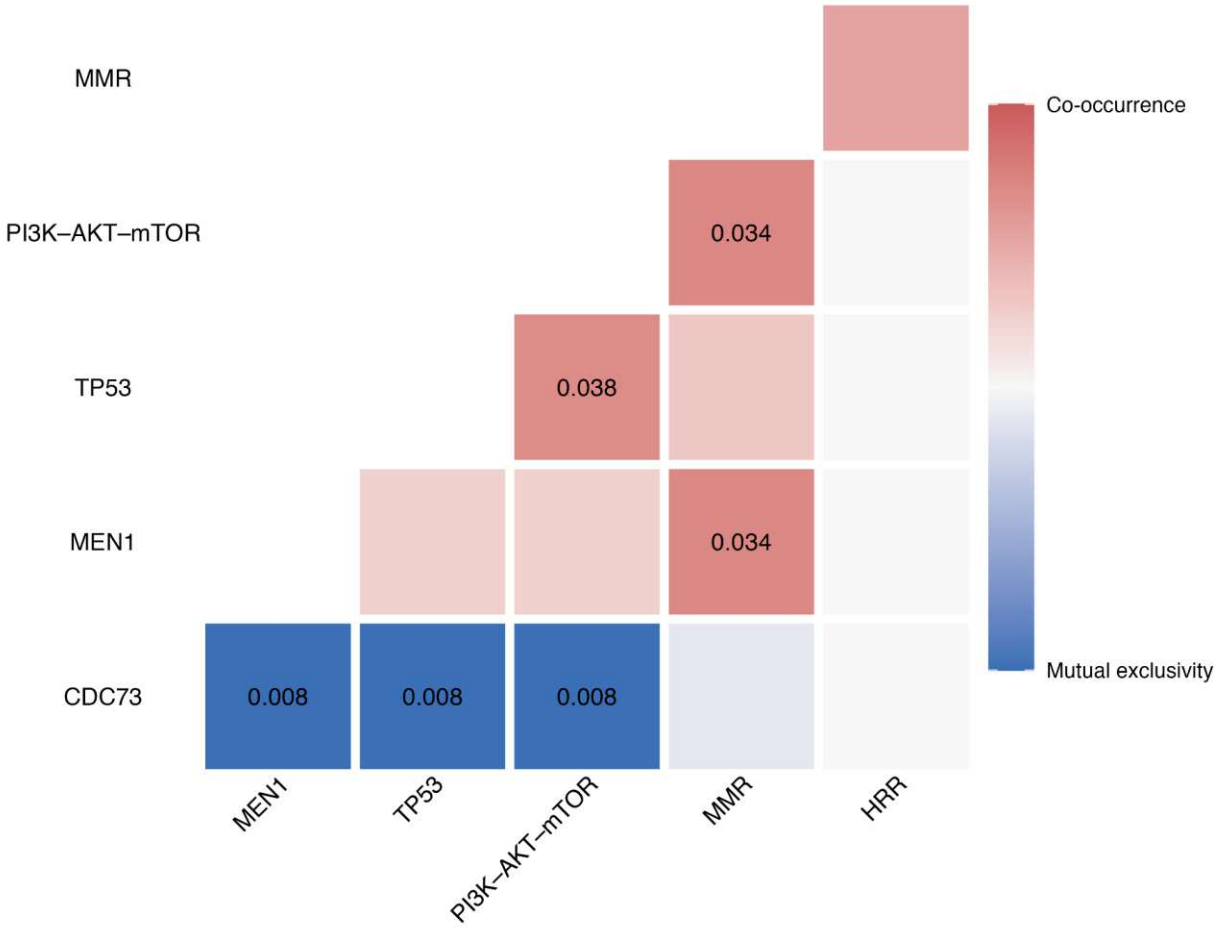


Figure 5. Pairwise associations among major genomic alterations in parathyroid carcinoma.
Pairwise associations among CDC73, MEN1, TP53, PI3K–AKT–mTOR pathway, MMR, and HRR alterations in the molecular subgroup subcohort (n = 29) were evaluated using Fisher's exact test. Blue indicates mutual exclusivity and red indicates co-occurrence,

with color intensity reflecting statistical significance. P values are shown for statistically significant associations (P < 0.05).

	All (N=30)	Molecular subgroup			
Variable		CDC73 (N=13, 44.8%)	MEN1 (N=7, 24.1%)	Other (N=9, 31.0%)	**P value**
Age (years), median (IQR)	58.0 (45.5–64.0)	45.0 (40.0–57.0)	60.0 (59.0–66.0)	71.0 (57.0–74.0)	**0.0057**
Sex, n (%)					**0.71**
Man	17 (56.7)	8 (61.5)	3 (42.9)	6 (66.7)	
Woman	13 (43.3)	5 (38.5)	4 (57.1)	3 (33.3)	
Panel type, n (%)					**0.66**
FoundationOne CDx	25 (83.3)	11 (84.6)	7 (100.0)	7 (77.8)	
GenMineTOP 1.0.0	4 (13.3)	2 (15.4)	0 (0.0)	2 (22.2)	
NCC Oncopanel	1 (3.3)	—	—	—	
TMB, median (IQR)	4.0 (1.0–7.4)	6.4 (1.0–13.0)	3.8 (1.0–10.0)	3.7 (1.0–4.0)	**0.42**

Table 1. Clinical characteristics of patients with parathyroid carcinoma.

Baseline demographic and genomic characteristics are shown for the final cohort (N = 30) and according to molecular subgroup among patients with evaluable CDC73 status (n = 29). Molecular subgroup analyses included CDC73-altered (n = 13), MEN1-altered

(n = 7), and other tumors (n = 9). Continuous variables are presented as median (interquartile range [IQR]) and categorical variables as number (%). P values represent comparisons among the three molecular subgroups and were calculated using the Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables. TMB, tumor mutational burden.

Category	All detected unique alterations	Curated pathogenic	Non-pathogenic	Pathogenic rate (%)
CDC73	16	16	0	100.0
MEN1	7	7	0	100.0
MMR	7	5	2	71.4
HRR	25	5	20	20.0
PI3K–AKT–mTOR	11	9	2	81.8
TP53	8	8	0	100.0

Supplementary Table 1. Variant-level pathogenicity curation of major genomic alterations.

The numbers of unique genomic alterations detected before and after variant-level pathogenicity curation are shown for major genes and pathways. Pathogenicity was determined using OncoKB and manual review as described in the Methods. MMR, mismatch repair; HRR, homologous recombination repair.

Gene	Variants

ARHGAP35	Q268*
ARID1A	E59*; G314fs*49
ASXL1	S430*; Y425*
ATR	I774fs*5
AURKA	amplification
BBC3	S106*
BCL2L1	amplification
BCOR	Q312del
BCORL1	E608*
BIRC3	S143*
BRCA1	truncation
BRCA2	splice site 68-1G>A
CARD11	E93D
CCND3	amplification
CDC73	E17fs*8; E29fs*8; G22*; Q14fs*7; R268*; R77P; S178fs*23; W43*; 126_131+9delinsCT; E24*; Q14Rfs*7; splice site 307+2T>C; R229fs*27; R91*; V447fs*17; Y54*

CDK4	amplification
CDKN1B	D127fs*20; H48fs*79; Q65fs*60
CDKN2A	loss
CDKN2B	loss
CDKN2C	loss
DAXX	M1I
DDX3X	M1I
EP300	E1525*
FANCA	long deletion
FBXW7	R689W
FLCN	H429fs*39; I52Nfs*48
JAK1	K860fs*16
KLHL6	loss
KMT2D	Q3913*
LRP1B	S3466*

MDM4	amplification
MEN1	G111fs*8; I248fs*38; L254fs*32; Q410fs*29; R521fs*43; S160F; Y133*
MGA	T786Pfs*31
MLH1	Q537*
MSH2	T441fs*13; loss; Y619*
MSH3	K383fs*32
MSH6	K1358fs*2
MTAP	loss
MTOR	I2500F
MUTYH	splice site 892-2A>G
MYC	amplification
NBN	R466fs*18
NF1	W267fs*24; Y489C; Y1989*; I679fs*21
NF2	W258*
NOTCH3	R1893*; truncation; S497*

PBRM1	R434*
PIK3C2B	amplification
PIK3CA	R38H; R88Q
PIK3R1	D68fs*7; E462lfs*2
PTEN	N323fs*2; R130Q; R233*; loss; splice site 77_79+3delCCTGTA; Y177C
PTPN11	S502L
QKI	K134fs*14
RB1	R787*
RBM10	E119del; splice site 2538-1G>C
RET	C609_F612del
SETD2	K629fs*2
SMARCA4	M272fs*31; R973W
SMARCB1	R377H
SPOP	F102L
SRC	amplification

TERT	promoter -146C>T
TET2	S1798*
TP53	L14fs*14; P13fs*2; R175H; R213*; S241F; T256P; E171Lfs*5; R273H
VEGFA	amplification
ZNF217	amplification

Supplementary Table 2.

Pathogenic alterations identified in this cohort. A total of 65 genes and 114 variants were identified. Truncating mutations were recorded as unspecified when precise variant details were not available.

	Age	Panel	Gene	Variant(s)	VAF (%)
1	57	FoundationOne CDx	CDC73	E17fs*8	37.3
2	61	FoundationOne CDx	CDC73	E29fs*8; R229fs*27	20.9; 34.8
3	35	FoundationOne CDx	CDC73	G22*	80.0
4	45	FoundationOne CDx	CDC73	Q14fs*7; V447fs*17	47.6; 44.3
5	47	FoundationOne CDx	CDC73	R268*; W43*	48.8; 39.4
6	45	FoundationOne CDx	CDC73	R77P; R91*	34.7; 32.3

7	48	FoundationOne CDx	CDC73	R77P	81.0
8	65	FoundationOne CDx	CDC73	S178fs*23; Y54*	38.6; 42.3
9	40	FoundationOne CDx	CDC73	W43*	42.7
10	60	FoundationOne CDx	CDC73	126_131+9delinsCT	79.7
11	30	GenMineTOP 1.0.0	CDC73	E24*	19.5
12	40	GenMineTOP 1.0.0	CDC73	Q14Rfs*7	44.3
13	34	FoundationOne CDx	CDC73	splice site 307+2T>C	94.5
14	61	FoundationOne CDx	MEN1	G111fs*8	69.0
15	80	FoundationOne CDx	MEN1	I248fs*38	66.9
16	53	FoundationOne CDx	MEN1	L254fs*32	57.6
17	59	FoundationOne CDx	MEN1	Q410fs*29	37.9
18	60	FoundationOne CDx	MEN1	R521fs*43	35.0
19	59	FoundationOne CDx	MEN1	S160F	80.8
20	71	FoundationOne CDx	MEN1	Y133*	80.1

Supplementary Table 3. Age at diagnosis and variant allele frequency (VAF) of patients harboring CDC73 or MEN1 alterations. Each row represents one patient, showing age at diagnosis, comprehensive genomic profiling panel, detected variants, and corresponding VAF. Several patients were relatively young or exhibited high VAFs, findings that may raise the possibility of underlying germline variants. However, no variants were classified as germline in the C-CAT annotation.