This chapter describes the clinical manifestations and penetrance in *CDC73*-related disorders and formulate recommendations to improve case detection in pHPT.

CDC73-Related Disorders: Clinical Manifestations and Case Detection in Primary Hyperparathyroidism

K. van der Tuin, C.M.J. Tops, M.A. Adank, J-M. Cobben, N.A.T. Hamdy, M.C.J. Jongmans, F.H. Menko, B.P.M. van Nesselrooij, R.T. Netea-Maier, J.C. Oosterwijk, G.D. Valk, B.H.R. Wolffenbuttel, F.J. Hes, H. Morreau

J Clin Endocrinol Metab. 2017 Dec 1;102(12):4534-4540

ABSTRACT

Context

Heterozygous pathogenic germline variants in *CDC73* predispose to the development of primary hyperparathyroidism (pHPT) and, less frequently, ossifying fibroma of the jaw and renal and uterine tumors. Clinical information on *CDC73*-related disorders has so far been limited to small case series.

Objective

To assess the clinical manifestations and penetrance in *CDC73*-related disorders and to improve case detection in pHPT.

Design

Nationwide retrospective Dutch cohort study.

Setting

Tertiary referral center.

Patients

We studied 89 patients with pHPT referred for germline *CDC73* analysis and 43 subsequently tested relatives who proved to be mutation carriers.

Investigation

Germline *CDC73* mutation analysis.

Mean Outcome

CDC73 mutation detection yield, referral rate and *CDC73*-related disease penetrance.

Results

Pathogenic germline *CDC73* variants were identified in 11 of the 89 referred pHPT patients (12.4%), with (suspected) hyperparathyroidism-jaw tumor (HPT-JT) syndrome (n = 3), familial isolated pHPT (n = 5), apparently sporadic parathyroid carcinoma (n = 2), and apparently sporadic parathyroid adenoma (n = 1). The estimated penetrance of *CDC73*-related disorders was 65% at age 50 years (95% confidence interval 48% to 82%) in 43 non-index mutation carriers.

Conclusions

Germline *CDC73* analysis is recommended in individuals with (suspected) HPT-JT syndrome, familial isolated pHPT, atypical or malignant parathyroid histology, and young individuals with pHPT. These criteria would increase germline *CDC73* mutation detection, thus enabling optimal clinical management of pHPT, as well as genetic counseling and surveillance for family members at risk for developing *CDC73*-related disorders. *(J Clin Endocrinol Metab 102: 4534–4540, 2017)*

INTRODUCTION

Primary hyperparathyroidism (pHPT) is a common endocrine disease with a prevalence of 1 to 4 per 1000 persons and with a peak incidence in the sixth decade of life.[1] In the majority of cases, pHPT is caused by a single parathyroid adenoma (PA) and in less than 1% by a parathyroid carcinoma (PC).[2] A genetic predisposition for pHPT can be found in ~10% of pHPT cases. This might be an underestimation because of unavailable, incomplete, or misdiagnosed family history; variable penetrance; or unknown genetic causes. To date, pathogenic variants in at least 11 genes have been found to be associated with hereditary pHPT. The most commonly identified hereditary syndromes associated with pHPT include multiple endocrine neoplasia type 1, 2a, or 4; and *CaSR*-, *GCM2*-, and *CDC73*-related disorders.[3,4] Inactivation of the *CDC73* tumor suppressor gene (formerly known as *HRPT2* and encoding parafibromin) predisposes heterozygous carriers to a spectrum of conditions: hyperparathyroidism-jaw tumor (HPT-JT) syndrome, familial isolated hyperparathyroidism (FIHP), and PC.

The penetrance of pHPT in *CDC73*-related disorders has been reported to be as high as 80% to 95%.[5] The onset is typically in late adolescence or early adulthood, although patients younger than 10 years of age have also been reported.[6,7] PC may be found in >20% of patients with germline pathogenic *CDC73* variants, which is higher than in other hereditary pHPT syndromes.[5] Distinguishing between PA, atypical adenoma (AA) and PC remains a challenge given the lack of specific differentiating clinical, biochemical and histological features among these pathologies. However, the latter is of the utmost importance because it determines the extent and radical nature of initial surgery, which is in turn the major determinant of prognosis.[5]

In addition to pHPT, patients with *CDC73*-related disorders are predisposed to developing ossifying fibromas of the mandible and/or maxilla, uterine tumors (e.g. adenofibromas, leiomyomas, adenomyosis, hyperplasia, and adenosarcomas) and less frequently, a variety of malignant and nonmalignant renal lesions [e.g., Wilms tumor, clear cell renal carcinoma (RCC), papillary renal cell tumor, renal cysts].[5]

In total, about 100 index *CDC73* mutation carriers have been reported to date, with no clearly identified phenotype-genotype relationship.[5] The majority of germline (and somatic) pathogenic *CDC73* variants are frameshift and nonsense variants, although missense variants as well as (small) deletions and insertions have been reported.[7-9]

Limited data are available on the germline *CDC73* mutation detection yield in patients with HPT-JT syndrome, FIHP and PC. In this study, we performed a nationwide evaluation of germline *CDC73* analyses undertaken in pHPT patients in the Netherlands, and characterized the clinical manifestations and penetrance of 12 families with *CDC73*-related disorders.

PATIENTS AND METHODS

Study population and design

All Dutch patients with an established diagnosis of pHPT referred for germline *CDC73* analysis in the Netherlands from February 2004 through July 2016 were included in the study. There were no specific referral criteria for germline CDC73 analysis in the Netherlands during the study period. Data on sex, diagnosis, age at diagnosis, family history, and clinical manifestations were retrieved from DNA request forms.

Referred pHPT patients were grouped in four clinical subgroups based on their personal and/or family history: (1) (suspected) HPT-JT syndrome [pHPT and at least one HPT-JT syndrome-related feature or pHPT and a close relative with (suspected) HPT-JT syndrome], (2) FIHP (pHPT

and at least one first or second degree relative with pHPT), (3) apparently sporadic PC (sPC), and (4) apparently sporadic PA (sPA). HPT-JT related features included pHPT, ossifying fibroma of mandible and/or maxilla, renal lesions and uterine tumors. According to the Dutch genetic testing strategy, before *CDC73* analysis, germline *MEN1* variants had to be excluded in patients with FIHP and sPAs diagnosed before age 35 years.

Index patients with pathogenic *CDC73* variants or variants of uncertain significance (VUS) were evaluated and counseled by a clinical geneticist in their regional university medical center. Written informed consent for collection of clinical, pathological and molecular data was obtained from all index mutation carriers. Relatives were tested for the specific pathogenic *CDC73* variant using cascade screening after counseling. All *CDC73* mutation carriers were referred for surveillance aimed at detecting pHPT or jaw-, renal- and/or uterine abnormalities. We also included in the study an extra family belonging to a Dutch index-patient with *CDC73*-related disorder who underwent genetic testing abroad, wherase genetic testing via cascade screening of relatives was performed at our laboratory.

The study was approved by the local Ethical Committee of the Leiden University Medical Center (P15.016).

DNA sequencing and data analysis

Germline *CDC73* mutation analysis was centralized in the Laboratory for Diagnostic Genome Analysis department of clinical genetics at the Leiden University Medical Center, the Netherlands, during the study period. Germline *CDC73* mutation analysis was performed with Sanger sequencing. *CDC73* deletion/duplication analysis was subsequently performed in 60 patients without pathogenic *CDC73* variant using the MRC Holland P466-A1 kit (MRC Holland, Amsterdam, the Netherlands).

Coding variants were analyzed for their effect on function with Alamut software package v2.7 (Interactive Biosoftware, Rouen, France), which incorporates, for example, Align GVGD, SIFT and PolyPhen2. Variants were annotated to the Genbank reference sequence NM_024529.4. The Leiden Open Variation Database (http://www.lovd.nl/CDC73) was consulted to find variants previously described and classified.

Histological and molecular analysis of parathyroid tumors

The overproducing parathyroid gland(s) were removed in all patients referred for germline *CDC73* mutation analysis and all *CDC73* mutation carriers diagnosed with pHPT as part of standard care. Available tumor tissue was re-examined by a referral pathologist in Leiden (H.M.). Parafibromin immunohistochemistry (IHC), somatic *CDC73* analysis and loss of heterozygosity analysis were performed on formalin-fixed, paraffin-embedded (FFPE) samples as previously described.[10] IHC was scored positive ("normal") if nuclear staining was detected in lesional cells and was only considered negative ("loss") in the presence of positive internal controls.

Statistical analysis

To describe clinical characteristics, the mean ± standard deviation (SD) with range was calculated. Continuous variables were analyzed using an independent sample t-test. Dichotomous variables were compared using the χ^2 test. Age-related penetrance of pHPT was estimated using the Kaplan–Meier method. Statistical significance was set at $P > 0.05$; analyses were conducted using SPSS 23.0 (SPSS, Chicago, IL).

RESULTS

CDC73-related disorders - case detection

Pathogenic germline *CDC73* variants were identified in 11 of 89 (12.4%) clinically heterogeneous pHPT patients referred for mutation analysis. In total, seven different nonsense or frame shift pathogenic variants were identified; two families carried an exon 1 deletion and two families carried a large deletion spanning the entire *CDC73* gene. The clinical characteristics of the study population *(CDC73* vs. non-*CDC73)* are listed in Table 1. Within the clinical subgroups, pathogenic germline *CDC73* variants were identified in 3 of 18 patients with (suspected) HPT-JT (17%), in 5 of 19 patients with FIHP (26%), in 2 of 11 patients with sPC (18%), and in 1 of 41 patients with sPA (2%). The mean age (±SD) at diagnosis of pHPT was 32 ± 15 years (range, 13 to 54 years) in *CDC73* mutation carriers and 42 ± 18 years (range, 10 to 81 years) in those without a detectable mutation (P =0.068). Ten of the 11 *CDC73* mutation carriers were male (91%), as opposed to 41% of non-mutation carriers (P= <0.01). In total, 12 patients were diagnosed with PC (11 apparently sporadic and one in the context of FIHP). Family history was positive for pHPT in 73% of *CDC73* mutation carriers, as opposed to only 24% in non-mutation carriers (P= <0.01). A personal history of Wilms tumor was reported in one *CDC73* mutation carrier and one patient carrying a variant of uncertain significance (VUS, see following section). No other index *CDC73* mutation carrier was diagnosed with renal abnormalities. In total, eight index non-mutation carriers had a personal history of renal abnormities (five with RCC and three with renal cysts).

CDC73 variant of uncertain significance

One *CDC73* variant of uncertain significance [c.14T>G, p.(Leu5Arg)] was further identified in a female aged 37 years with pHPT and a history of a Wilms tumor at age 2 years. IHC showed global loss of parafibromin staining in her PA, and loss of heterozygosity of the wild type *CDC73* allele was also seen. The Wilms tumor sample was not available for further investigation. Family

Table 1. Clinical characteristics of 89 pHPT patients referred for germline *CDC73* analysis

	Pathogenic *CDC73* variant	No pathogenic *CDC73* variant	*P* value	Yield, %
	n=11	n=78		12.4
Age mean ± SD (y)	32.3 ± 14.6	42.6 ± 18	0.068	
Range (y)	13-54	10-81		
Sex, male, n (%)	10 (91)	32 (41)	0.002	
(suspect) HPT-JT syndrome, n (%)	3 (27)	15 (19)		16.7
Familial isolated pHPT, n (%)	5 (45)[a]	14 (18)		26.3
Sporadic parathyroid carcinoma, n (%)	2 (18)	9 (12)		18.0
Sporadic parathyroid adenoma, n (%)	1 (9)	40 (51)		2.4
Familiar pHPT, n (%)	8 (73)	19 (24)	0.003	
Recurrent pHPT or multiple PA	0	12	0.162	
Renal abnormalities	1	9	0.810	
Uterine abnormalities	0	4	0.758	

[a]One of these patients was diagnosed with a PC. In total, 9 of 12 PCs were revised by a referral pathologist.

history showed a maternal cousin with pHPT aged 30 years, whereas the mother and aunt were unaffected. Segregation analysis confirmed the presence of the variant in the affected cousin. However, IHC showed positive parafibromin staining in her PA and no pathogenic somatic *CDC73* variants or loss of heterozygosity of the wild type *CDC73* allele. The c.14T>G variant has not been reported in the Single Nucleotide Polymorphism Database (dbSNP), Exome Sequencing Project (ESP,), Exome Aggregation Consortium (ExAc), Genome of the Netherlands (GoNL), or ClinVar databases and affects an evolutionarily conserved amino acid. The substitution of the leucine residue by an arginine residue results in a relatively large difference in physical and chemical properties (Grantham score 102 [range, 0 to 215])[11]. AGVGD, SIFT and PolyPhen software predicted that this amino acid change will have a major effect on protein function. *In silico* RNA splice prediction software predicted no substantial change compared with the wildtype sequence.

Clinical manifestations in families with *CDC73*-related disorders

The characteristics of the index *CDC73* mutation carriers and their tested relatives are shown in Table 2. Analysis of 77 relatives who were tested via cascade screening for their familial pathogenic

Table 2. Overview of the clinical and molecular characteristics of 12 index CDC73 mutation carriers and their tested relatives

ID	Sex	Tumors Observed (Age at Detection, y)	Family History	Phenotype	Germline *CDC73* variant	Tested Relatives	Non-index carriers (Symptomatic)	Not tested Symptomatic Relatives
A	M	PA (54)	pHPT	FIHP	c.226C>T, p.(Arg76*)	6	2 (1)	2
B	M	PC (54), RCC (57)	Negative	sPC	c.544dup, p.(Ile182Asnfs*11)	3	1 (0)	0
C	F	PA (17)	pHPT	FIHP	c.358C>T, p.(Arg120*)	1	1 (1)	1
D[a]	M	PA (34)	pHPT, Renal cysts	Suspect HPT-JT syndrome	c.687_688dellAG, p.(Arg229Serfs*37)	37	24 (14)	2
E	M	Jaw (15), PA (22)	pHPT, Wilms tumour	HPT-JT syndrome	c.3_15dup, p.(Ser6Glyfs*5)	3	3 (3)	0
F	M	PA (13)	Negative	sPA	Whole gene deletion	4	2 (0)	0
G	M	PC (45)	pHPT	FIHP	Whole gene deletion	9	4 (2)	0
H	M	Wilms tumor (8), PA (33)	pHPT, uterine fibroids	FIHP	c.3_15dup, p.(Ser6Glyfs*5)	3	1 (1)	0
I	M	PC (18)	Negative	sPC	Exon 1 deletion	0		0
J	M	PA (40)	pHPT	FIHP	Exon 1 deletion	2	1 (1)	1
K	M	PA (25)	pHPT	FIHP	c.685_688delAGAG, p.(Arg229Tyrfs*27)	0		2
L	M	PA (40)	pHPT, jaw	HPT-JT syndrome	c.760C>T, p.(Gln254*)	8	4 (1)	0

Abbreviations: M; male, F; female, Jaw; ossifying fibroma jaw, [a] Published before (Haven *et al*; 2000)

CDC73 variant revealed 43 non-index mutation carriers in 10 families. Detailed information on all *CDC73* mutation carriers can be found in Supplemental Table 1 and in pedigrees A though K (Supplemental Fig. 1). The mean age (± SD) at DNA analysis was 42 ± 20 years (range, 10 to 80 years) in the non-index *CDC73* mutation carriers. In total, 24 of 43 (56%) non-index mutation carriers were diagnosed with one or more *CDC73*-related disorder features, including pHPT (n = 20), ossifying fibroma of the jaw (n = 5), renal abnormalities (n = 8) and uterine fibroids (n=1). In non-index mutation carriers, pHPT was associated with a single PA, atypical adenoma and PC in 17 (85%), 1 (5%), and 2 (10%) cases, respectively. In addition, at least eight family members from five different families (families A, C, D, J and K) have been diagnosed with pHPT but have not (yet) been tested for the pathogenic *CDC73* variant in their family. The age-related overall penetrance values for the 43 non-index *CDC73* mutation carriers were 11% at age 25 years [95% confidence interval (CI) 2% to 20%], 65% at age 50 years (95% CI, 48% to 82%), and 83% at age 70 years (95% CI, 57% to 99%) (Fig. 1A). The mean age (± SD) at diagnosis of pHPT was 39 ± 14 years (range, 10 to 67 years) in the affected non-index mutation carriers, compared to 33 ± 15 years (range, 13–54 years) in the index mutation carriers (p=0.32). The age-related pHPT penetrance values for the 43 non-index *CDC73* mutation carriers were 8% at age 25 years (95% CI, 0% to 16), 53% at age 50 years (95% CI, 33% to74) and 75% at age 70 years (95% CI, 54% to 95%) (Fig. 1B).

DISCUSSION

Here, we report the results of a nationwide retrospective *CDC73* survey to investigate *CDC73* mutation detection yield and clinical phenotype in so far genetically unexplained pHPT patients. We identified pathogenic germline *CDC73* variants in 11 of 89 pHPT patients (12.4%). In our study population, mutation detection was associated with younger age, male sex, malignant disease and a positive family history. The estimated penetrance of *CDC73*-related disorders was 83% at

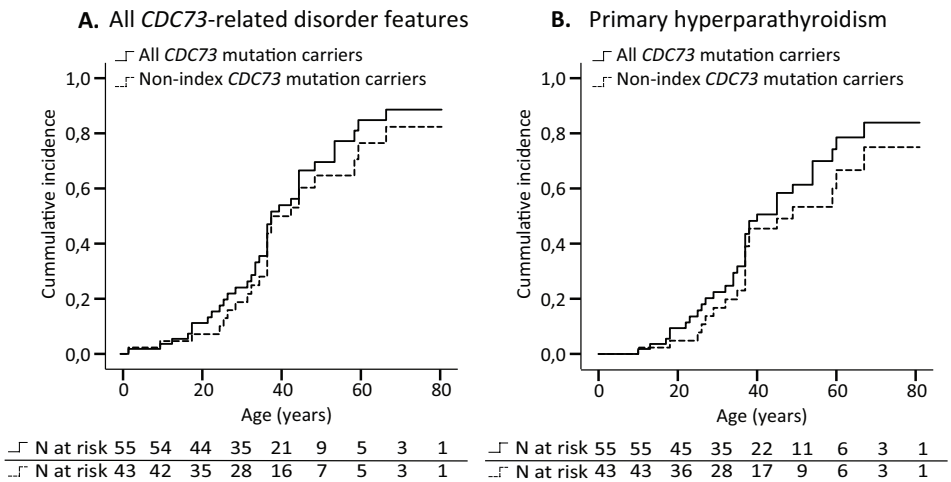


Figure 1. Age-related penetrance of *CDC73*-related disorder features in all *CDC73* mutation carriers (n = 55) vs non-index mutation carriers (n = 43). **A.** Age-related penetrance of all *CDC73*-related disorder features for all *CDC73* mutation carriers (black line) and only non-index CDC73 mutation carriers (dotted line). **B.** Age-related penetrance of pHPT for all *CDC73* mutation carriers (black line) and only non-index *CDC73* mutation carriers (dotted line).

age 70 (95% CI, 57% to 99%) in 43 non-index mutation carriers. Prospective studies in larger series of *CDC73* mutation carriers, including genotype-phenotype relationships, genetic modifiers and/or environmental factors, are required to determine the optimal age at which surveillance should be initiated and the monitoring intervals required to detect the different manifestations of *CDC73*-related disorders as they develop.

Improving future detection of *CDC73*-related disorder cases

In light of the relatively high incidence of pHPT and of the importance of genetic diagnosis, there is an unmet clinical need for development of guidelines for genetic testing. Based on data from our nationwide cohort analysis, we recommend germline *CDC73* analysis in the four clinical subgroups of patients with pHPT listed next, a recommendation that is also in line with the 2015 Consensus Report on hereditary hyperparathyroidism of the European Society of Endocrine Surgeons.[5]

- All patients with HPT-JT syndrome

First, germline *CDC73* analysis is recommended in individuals with (suspected) HPT-JT syndrome. Although the mutation detection yield (3/18, 17%) in our study population was lower than in a previous study (13/24, 54%), the high yield in the initial study might have been an overestimate due to ascertainment and selection bias.[12]

- All patients with familial pHPT (after exclusion of other gene abnormalities)

Second, germline *CDC73* analysis is recommended in patients with FIHP after exclusion of pathogenic germline *MEN1* variants. The mutation detection yield in our study population was 27% in patients with at least one first or second-degree relative with pHPT. Different mutation detection yields ranging from 0% to 28% were found in previous, mostly small, studies[13-17]

- All patients with PC or atypical histology of PA

The third subgroup of patients with pHPT in which germline *CDC73* analysis is recommended includes individuals with apparently sporadic atypical or malignant parathyroid pathology. In our study population, the mutation detection yield in s PC was 17%. The detection yield observed in previous studies varies substantially per study population; ranging from 6%, 17% to 29%, 18%, 20%, and 31% to 38% in patients from Finland[18], Italy[19-21], France[7], United States/Japan[22] and China[23,24], respectively. The study size and patient selection differed between studies and that a unequivocal morphological diagnosis can be challenging. Referral to an experienced parathyroid surgeon and an expert pathologist should be considered in all patients with suspected PC. Subsequent parafibromin IHC and somatic *CDC73* analysis could be considered for diagnostic and prognostic purposes.[25] The frequency of pathogenic germline *CDC73* variants in individuals with atypical adenoma has not been extensively studied and limited data are available on the contribution of IHC in cases with equivocal histology.

- All patients with sporadic pHPT, younger than 35 years

The fourth subgroup of patients with pHPT in which germline *CDC73* analysis is recommended includes young individuals with apparently sporadic benign pHPT, after exclusion of pathogenic germline *MEN1* variants. In our study population, one patient with sPA (a 13-year-old boy) carried a pathogenic germline *CDC73* variant. The yield of germline *CDC73* testing in patients with sPA has barely been studied; therefore, no age-specific criteria can be identified. Dutch guidelines recommend germline *MEN* analysis in patients with pHPT diagnosed before age 35 years.[26] For practical reasons, subsequent germline *CDC73* analysis should also be considered in these patients.

Gene panel testing

To date, genetic testing for germline variants in genes predisposing to hereditary pHPT involved mainly sequential testing of single genes, prioritized according to clinical features. This type of testing protocol is expensive and time-consuming because at least 11 genes are associated with hereditary pHPT. The introduction of gene panel testing using next generation sequencing would improve genetic testing for these rare disorders. However, complete analysis of *CDC73* in next generation sequencing panels will be challenging because of the presence GC-rich regions and frequent germline *CDC73* deletions (4 of 12 in our study cohort).

Limitations and strengths of the study

The main strength of the current study is that all pHPT patients referred for germline *CDC73* analysis in the Netherlands within a defined period (2004 through 2016) were included in the study. A further strength is that a total of 55 *CDC73* mutation carriers from 12 families were clinically investigated, in close collaboration with a number of Dutch University Medical Centers, representing one of the largest *CDC73*-related disorder series to date.

The study also has a number of limitations. The first is that the estimated mutation detection yield in this study was found in a retrospective diagnostic cohort, which despite being one of the largest *CDC73*-related cohorts published, might not be representative of the total patient population. Second, because we were not able to revise the histology of all parathyroid tumors from patients referred for germline *CDC73* analysis, some patients may have been misclassified. And third, a possible explanation for the relatively low penetrance for jaw, uterine and renal lesions could be inadequate surveillance and incomplete follow-up data. Alternatively, the high penetrance observed in prior studies (20% to 60%)[7,9,27,28] is likely due to ascertainment and selection bias.

In conclusion, our data demonstrate that pathogenic germline *CDC73* variants are frequently found in previously genetically-unexplained pHPT patients. Our findings further suggest that genetic testing should be recommended in individuals with pHPT and HPT-JT-syndrome related features, familial isolated pHPT, atypical or malignant parathyroid histology, and in young individuals with pHPT. Gene panel testing or consecutive gene testing, including additional deletion and Sanger sequencing testing, should be considered, depending on the phenotype and available genetic testing options. Clinical use of these criteria will enhance the identification of individuals with *CDC73*-related disorders, thus improving both early detection of tumor development and genetic counseling.

ACKNOWLEDGEMENTS

The authors thank all patients who participated in the study.

REFERENCES

1. Yeh MW, Ituarte PH, Zhou HC, et al. Incidence and prevalence of primary hyperparathyroidism in a racially mixed population. The Journal of clinical endocrinology and metabolism 2013;98:1122-9.
2. Hundahl SA, Fleming ID, Fremgen AM, et al. Two hundred eighty-six cases of parathyroid carcinoma treated in the U.S. between 1985-1995: a National Cancer Data Base Report. The American College of Surgeons Commission on Cancer and the American Cancer Society. Cancer 1999;86:538-44.
3. Simonds WF. Genetics of Hyperparathyroidism, Including Parathyroid Cancer. Endocrinology and metabolism clinics of North America 2017;46:405-18.
4. Jackson MA RT, Hu MI, Perrier ND, Waguespack SG, CDC73-Related Disorders. 2008 Dec 31 [Updated 2015 Jan 15]. GeneReviews® [Internet]. Seattle (WA):

University of Washington, Seattle; 1993-2017. Available from: https://www.ncbi.nlm.nih.gov/books/NBK3789/.

5. Iacobone M, Carnaille B, Palazzo FF, et al. Hereditary hyperparathyroidism--a consensus report of the European Society of Endocrine Surgeons (ESES). Langenbeck's archives of surgery 2015;400:867-86.
6. Pichardo-Lowden AR, Manni A, Saunders BD, et al. Familial hyperparathyroidism due to a germline mutation of the CDC73 gene: implications for management and age-appropriate testing of relatives at risk. EndocrPract 2011;17:602-9.
7. Bricaire L, Odou MF, Cardot-Bauters C, et al. Frequent large germline HRPT2 deletions in a French National cohort of patients with primary hyperparathyroidism. JClinEndocrinolMetab 2013;98:E403-E8.
8. Korpi-Hyovalti E, Cranston T, Ryhanen E, et al. CDC73 intragenic deletion in familial primary hyperparathyroidism associated with parathyroid carcinoma. The Journal of clinical endocrinology and metabolism 2014;99:3044-8.
9. Newey PJ, Bowl MR, Cranston T, et al. Cell division cycle protein 73 homolog (CDC73) mutations in the hyperparathyroidism-jaw tumor syndrome (HPT-JT) and parathyroid tumors. HumMutat 2010;31:295-307.
10. Tan MH, Morrison C, Wang P, et al. Loss of parafibromin immunoreactivity is a distinguishing feature of parathyroid carcinoma. ClinCancer Res 2004;10:6629-37.
11. Grantham R. Amino acid difference formula to help explain protein evolution. Science 1974;185:862-4.
12. Carpten JD, Robbins CM, Villablanca A, et al. HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. NatGenet 2002;32:676-80.
13. Cetani F, Pardi E, Ambrogini E, et al. Genetic analyses in familial isolated hyperparathyroidism: implication for clinical assessment and surgical management. ClinEndocrinol(Oxf) 2006;64:146-52.
14. Mizusawa N, Uchino S, Iwata T, et al. Genetic analyses in patients with familial isolated hyperparathyroidism and hyperparathyroidism-jaw tumour syndrome. ClinEndocrinol(Oxf) 2006;65:9-16.
15. Bradley KJ, Cavaco BM, Bowl MR, et al. Parafibromin mutations in hereditary hyperparathyroidism syndromes and parathyroid tumours. ClinEndocrinol(Oxf) 2006;64:299-306.
16. Villablanca A, Calender A, Forsberg L, et al. Germline and de novo mutations in the HRPT2 tumour suppressor gene in familial isolated hyperparathyroidism (FIHP). JMedGenet 2004;41:e32.
17. Warner J, Epstein M, Sweet A, et al. Genetic testing in familial isolated hyperparathyroidism: unexpected results and their implications. JMedGenet 2004;41:155-60.
18. Ryhanen EM, Leijon H, Metso S, et al. A nationwide study on parathyroid carcinoma. Acta oncologica (Stockholm, Sweden) 2017;56:991-1003.
19. Cetani F, Banti C, Pardi E, et al. CDC73 mutational status and loss of parafibromin in the outcome of parathyroid cancer. EndocrConnect 2013;2:186-95.
20. Cetani F, Pardi E, Borsari S, et al. Genetic analyses of the HRPT2 gene in primary hyperparathyroidism: germline and somatic mutations in familial and sporadic parathyroid tumors. JClinEndocrinolMetab 2004;89:5583-91.
21. Guarnieri V, Battista C, Muscarella LA, et al. CDC73 mutations and parafibromin immunohistochemistry in parathyroid tumors: clinical correlations in a single-centre patient cohort. Cellular oncology (Dordrecht) 2012;35:411-22.
22. Shattuck TM, Valimaki S, Obara T, et al. Somatic and germ-line mutations of the HRPT2 gene in sporadic parathyroid carcinoma. NEnglJMed 2003;349:1722-9.
23. Wang O, Wang C, Nie M, et al. Novel HRPT2/CDC73 gene mutations and loss of expression of parafibromin in Chinese patients with clinically sporadic parathyroid carcinomas. PLoSOne 2012;7:e45567.
24. Kong J, Wang O, Nie M, et al. [CDC73 gene mutation and parafibromin expression status of parathyroid carcinoma in Chinese]. Zhonghua YiXueZa Zhi 2013;93:3364-8.
25. Witteveen JE, Hamdy NA, Dekkers OM, et al. Downregulation of CASR expression and global loss of parafibromin staining are strong negative determinants of prognosis in parathyroid carcinoma. ModPathol 2011;24:688-97.
26. Roijers JF, de Wit MJ, van der Luijt RB, et al. Criteria for mutation analysis in MEN 1-suspected patients: MEN 1 case-finding. European journal of clinical investigation 2000;30:487-92.
27. Guarnieri V, Scillitani A, Muscarella LA, et al. Diagnosis of parathyroid tumors in familial isolated hyperparathyroidism with HRPT2 mutation: implications for cancer surveillance. JClinEndocrinolMetab 2006;91:2827-32.
28. Simonds WF, James-Newton LA, Agarwal SK, et al. Familial isolated hyperparathyroidism: clinical and genetic characteristics of 36 kindreds. Medicine (Baltimore) 2002;81:1-26.

SUPPLEMENTAL DATA

Supplementary Table 1. Clinical characteristics of all CDC73 mutation carriers in this study

ID	Sex/ Age*	Tumors observed (age at detection, years)	(Re-) examined histology	Parafibromin IHC	Somatic *CDC73* mutation	LOH
Family A; c.226C>T, p.(Arg76*)						
II.1	M/64	PA (59)	N			
II.2	**M/59**	**PA (54)**	**N**			
III.4	M/32					
Family B; c.544dup, p.(Ile182Asnfs*11)						
II.3	**M/61**	**PC (54), RCC (57)**	**Y (PC)**	**Focal loss**	**No**	No
III.1	M/30					
Family C; c.358C>T, p.(Arg120*)						
II.6	F/57	PA (25)	N			
III.7	F/25	PA (17)	N			
Family D§; c.687_688dellAG, p.(Arg229Serfs*37)						
II.1	F/80'	PA	N			
III.1	F/47'	PA (37), renal cysts, Hürthle cell adenoma thyroid, Pancreatic Ductal Adenocarcinoma (47)	N			
III.2	F/80					
III.3	M/79	PA (25), renal cysts, RCC (54)	N			
III.4	F/77	PA (29), renal cysts	N			
III.5	F/38'	PA, renal cysts	N			
III.6	F/53'	PA (32), PC (36), renal cysts, Pancreatic Ductal Adenocarcinoma (36)	Y (PC)	Global loss	No	No
III.7	M/74	PA	N			
III.9	F/69	PA	N			
IV.1	**M/53**	**PA (34)**	**Y**	**Normal**	**NA**	**NA**
IV.2	F/51	PA (18)	N			
IV.3	M/48	mixed germcell testicular tumor (30)	N			
IV.7	M/42					
IV.11	M/56	PA (37)	Y	NA	NA	NA
IV.13	M/51	Jaw* (33)	NA			
IV.14	F/45	PA (26), Jaw* (27)	Y	NA	NA	NA
IV.19	F/48					
IV.20	F/41	PA (38)	N			
IV.21	F/49	PA (49)	N			
IV.22	F/43					

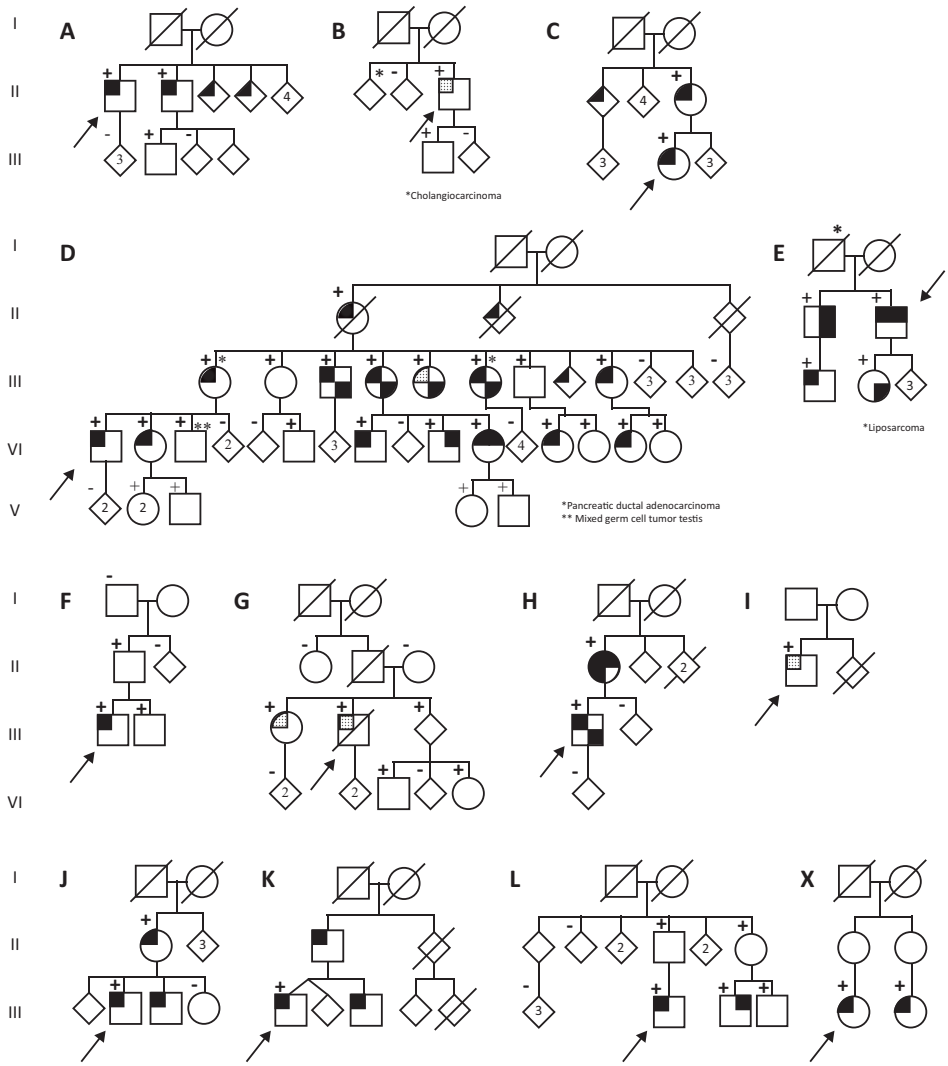
Supplementary Table 1. (continued)

ID	Sex/ Age*	Tumors observed (age at detection, years)	(Re-) examined histology	Parafibromin IHC	Somatic *CDC73* mutation	LOH
V.3	F/23					
V.4	M/21					
V.5	F/17					
V.6	F/17					
V.7	M/12					
Family E; c.3_15dup, p.(Ser6Glyfs*5)						
II.1	M/59	Jaw (43), Renal cyst	NA			
II.2	**M/57**	**Jaw (15), PA (22)**	**N**			
III.1	M/20	AA (10)	Y	Global loss	No	No
III.4	F/21	Congenital urinary tract abnormality, Wilms tumor (2)	N			
Family F; Whole gene deletion						
II.1	M/49					
III.1	**M/17**	**PA (13)**	**N**			
III.2	M/19					
Family G; Whole gene deletion						
III.1	F/54	PC (27)	Y	Normal	NA	NA
III.2	**M***	**PC (45)**	**Y**	**Focal loss**	**p.(Ser31Glyfs*7)**	
III.3	M/51	PA (45)	N			
IV.5	M/21					
IV.7	F/16					
Family H; c.3_15dup, p.(Ser6Glyfs*5)						
II.1	F/70	Uterus fibroids (36), PA (60), Jaw* (69)	Y			
III.1	**M/36**	**Wilms tumor (8), PA (33)**	**Y (PA)**	**Global loss**	**No**	**No**
Family I; Exon 1 deletion						
II.1	**M/30**	**PC (18)**	**Y**	**Global loss**	**p.(Glu29*)**	
Family J; Exon 1 deletion						
II.1	F/69	PA (67)	N			
III.2	**M/41**	**PA (40)**	**N**			
Family K; c.685_688delAGAG, p.(Arg229Tyrfs*27)						
III.1	**M/28**	**PA (25)**	**N**			
Family L; c.760C>T, p.(Gln254*)						
II.4	M/72					
II.6	F/70					
III.4	**M/45**	**PA (40)**	**N**			
III.8	M/49	Jaw	NA			
III.9	M/47					

Supplementary Table 1. (continued)

ID	Sex/ Age*	Tumors observed (age at detection, years)	(Re-) examined histology	Parafibromin IHC	Somatic *CDC73* mutation	LOH
Family X; c.14T>G, p.(Leu5Arg)^						
III.1	**F/44**	**PA (40)**	**Y**	**Global loss**	**No**	**Yes**
III.2	F/41	PA (30)	Y	Normal	No	No

IDs are according to the pedigrees (see suppl. figure 1), index mutation carriers are in bold.
Abbreviations: PA; parathyroid adenoma, PC; parathyroid carcinoma, Jaw; ossifying fibroma jaw, RCC; clear cell renal carcinoma. §Published before *(Haven et al, 2000)*; †deceased, *age last update clinical information or age of death, '= asymptomatic, detection during surveillance, ^Variant of uncertain significance.



Supplemental Figure 1. Pedigrees of 12 families with *CDC73*-related disorders (A-L) and one family (X) with a germline unclassified *CDC73* variant. Please note that the pedigree has been adjusted to protect the identity of the family without a loss of scientific integrity. Circles represent females; squares represent males; diamonds represent undisclosed gender, cross striped individuals are death, parathyroid adenoma (left top, black), parathyroid carcinoma (left top, dotted pattern), ossifying fibroma jaw (right top, black), uterine fibroids (left bottom, black), renal abnormalities (right bottom, black), *CDC73* mutation carrier (+); non-carriers (-).