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ORIGINAL ARTICLE

A large extended family with hyperparathyroidism-jaw tumor syndrome due to deletion of the third exon of *CDC73*: clinical and molecular features

Lauriane Le Collen[1,2] · Sara Barraud[1,3] · Antoine Braconnier[4] · Lucie Coppin[5] · Dominique Zachar[6] · Camille Boulagnon[6] · Sophie Deguelte[7] · Pierre François Souchon[8] · Marta Spodenkiewicz[9] · Céline Poirsier[9] · Sébastien Aubert[10] · Marie Françoise Odou[11] · Brigitte Delemer[1,3]

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Abstract

Purpose We described the phenotype of a large 4-generation family with Hyperparathyrodism-Jaw Tumor syndrome (HPT-JT) associated with a rare deletion of exon 3 of the *CDC73* gene.

Methods We collected medical, genetic data on 24 family members descended from a common ancestor carrying a heterozygous deletion of exon 3.

Results Thirteen carried the deletion, the penetrance was estimated at 50% at 40 years. Seven patients (39 ± 14.5 years) presented with HPT which could start at 13. Median plasmatic calcium and PTH levels were 3.13 ± 0.7 mmol/L and 115 ± 406 pg/ml, respectively. Kidney disease related to hypercalcemia were present in 57.1% of patients. All seven patients underwent surgery to remove a single parathyroid adenoma. One recurrence occurred 7 years post-surgery. No parathyroid carcinoma has been found to date. We found two atypical parathyroid adenomas. We described an additional somatic variant in exon 1 of gene *CDC73* in two tumors. Jaw tumors were not necessarily associated with hyperparathyroidism, as shown in one case. Two kidney cysts were also reported. Variable phenotype expressivity was emphasized by clinical presentations in 2 monozygotic twins: acute hypercalcemia, kidney failure and ossifying fibroma in one twin, versus normocalcemic parathyroid adenoma in the other one.

Conclusion We report a family carrier of a deletion of exon 3 of the *CDC73* gene. This is characterized by a high level of hypercalcemia, deleterious kidney effects and atypical parathyroid adenomas without carcinomas. Onset and intensity of HPT remain unpredictable. The additional somatic mutation found in the parathyroid tumor could lead to these phenotypical variations.

Keywords *HRPT2* · *CDC73* · Hyperparathyroidism · Jaw Tumors · Large rearrangements · Knudson two-hit hypothesis.

✉ Lauriane Le Collen
lle-collen@chu-reims.fr

✉ Brigitte Delemer
bdelemer@chu-reims.fr

[1] Department of Endocrinology Diabetology, University of Reims, Reims, France

[2] Inserm/CNRS UMR 1283/8199, Institut Pasteur de Lille, EGID, Lille, France

[3] CRESTIC EA 3804, University of Reims Champagne Ardenne, UFR Sciences Exactes et Naturelles, Moulin de La Housse, BP 1039, 51687, Reims Cedex 2, France

[4] Department of Nephrology, University of Reims, Reims, France

[5] Univ. Lille, CNRS, Inserm, CHU Lille, UMR9020-U1277 - CANTHER – Cancer - Heterogeneity Plasticity and Resistance to Therapies, F-59000 Lille, France

[6] Laboratory of Pathology, University of Reims, Reims, France

[7] Department of Surgery, University of Reims, Reims, France

[8] Department of Pediatrics, University of Reims, Reims, France

[9] Department of Genetics, University of Reims, Reims, France

[10] Laboratory of Pathology, University of Lille, Lille, France

[11] Univ. Lille, Inserm, CHU Lille, U1286 – Infinite – Institute for Translational Research in Inflammation, F-59000 Lille, France

Introduction

Primary hyperparathyroidism is a frequent endocrine disease [1, 2] with a genetic origin in ~10% of cases [3, 4], namely multiple endocrine neoplasia types 1 and 2A, familial hypocalciuric hypercalcemia and pathogenic variants in *CDC73* (or *HRPT2*). This last gene is responsible for familial isolated hyperparathyroidism, parathyroid carcinomas and Hyperparathyroidism-Jaw Tumor syndrome (HPT-JT) [5].

The first cases of familial hyperparathyroidism with ossifying fibromas of the jaw and pancreatitis were described in 1958 [6]. Thirty years were necessary to distinguish ossifying tumors from brown tumors due to the recurrent characteristics of the latter despite calcium normalization [7]. The cystic appearance of parathyroid adenomas and the possible recurrence of HPT on other parathyroid sites are other characteristics of this syndrome formerly reported in 1987 [8]. The relationship between HPT-JT syndrome and chromosome one was suspected in 1995 [9]. Seven years later, the *CDC73* gene (formerly named *HRPT2*) was identified as the cause of this disease [10].

CDC73 (OMIM * 607393) is a tumor-suppressor gene, that maps to 1q31.2, and contains of 17 exons (spanning 133 kb). It encodes the ubiquitous parafibromin protein, which is part of polymerase-associated factor 1 complex (PAF1 C) [11].

CDC73-related disorders are inherited in an autosomal dominant manner [5] (OMIM# 145001). According to the Knudson two-hit model, biallelic inactivation of the gene *CDC73* causes the loss of parafibromin expression and leads to the occurrence of a parathyroid tumor [12, 13]. One hundred and twenty different types of mutations have been reported in *CDC73* to date (HGMD database 01/21): 39 missense/ nonsense variants; 9 splicing sites; 1 splicing regulatory sequence; 32 small deletions; 15 small insertions; 3 small indels; 20 gross deletions (among them 15 take off the entire exon 3); 1 gross insertion.

Until now, no clear genotype-phenotype relationship has been formally established [5, 14]. However, new data have shown a possible genotype-phenotype correlation. It has been suggested that missense mutations might be associated with familial isolated HPT, while mutations triggering a gross parafibromin gene disruption might be responsible for the classical HPT-JT features [14]. Moreover, carriers of frameshift, deletion or nonsense variants appear to be more likely to develop parathyroid cancer than missense carriers [15].

The lack of large families and the high number of different mutations could have hindered the study of such a correlation until now. Indeed, only a few studies have been reported before in the same family [14, 16, 17].

We collected the clinical, biological, pathological and molecular data of patients from the same family, carrying a unique deletion of the third exon of gene *CDC73* over four generations. This report aims to describe the clinical and biological characteristics of this large extended family carrying a rare deletion of gene *CDC73*.

Furthermore, we were able to show that additional somatic molecular alterations of gene *CDC73* in two parathyroid tumors may contribute to the phenotype variability in this family.

Patients and methods

Patients and clinical records

In the 2000s, familial hyperparathyroidism was diagnosed in two half-brothers. The diagnosis of a familial disease was strengthened by the discovery of HPT in their sister 17 years earlier. Genetic investigations were performed for familial hyperparathyroidism and resulted, several years later, in the identification of an exon 3 deletion in gene *CDC73*.

In compliance with French legislation, the consent of patients, or of their parents in the case of an under-age child, was obtained. We collected clinical data from adult and pediatric endocrinological medical records. All *CDC73* mutation carriers were regularly followed up to detect HPT-JT related features (HPT, jaw tumors, renal or uterine lesions).

All patients related to these siblings who accepted to participate were included in a retrospective cohort study performed until January 2021. All medical events were collected and analyzed in a single center: personal medical history, age of first symptoms, presence of Jaw Tumors, calcium/PTH plasmatic levels, kidney diseases (glomerular filtration rate, presence of kidney failure, kidney ultrasound or scan), gynecological examination (pelvic ultrasound), pathological analysis after surgery (from parathyroid and thyroid resection, bone and skin biopsy) and any additional medical event.

Genetic investigations

DNA from peripheral blood leukocytes (blood samples in EDTA tube) was extracted using standard methods, for germline mutation analysis. The identification and screening for the *CDC73* deletion were first studied by real-time PCR as previously described [18], and, since 2015, by MLPA (kit P466-A1, MRC Holland©) performed on 3130 XL© sequencer (Applied Biosystems, Courtaboeuf, France) and analyzed with Coffalyser© software. To assess the pathogenicity of the variants, we used the standards and guidelines of the American College of Medical Genetics and Genomics [19].

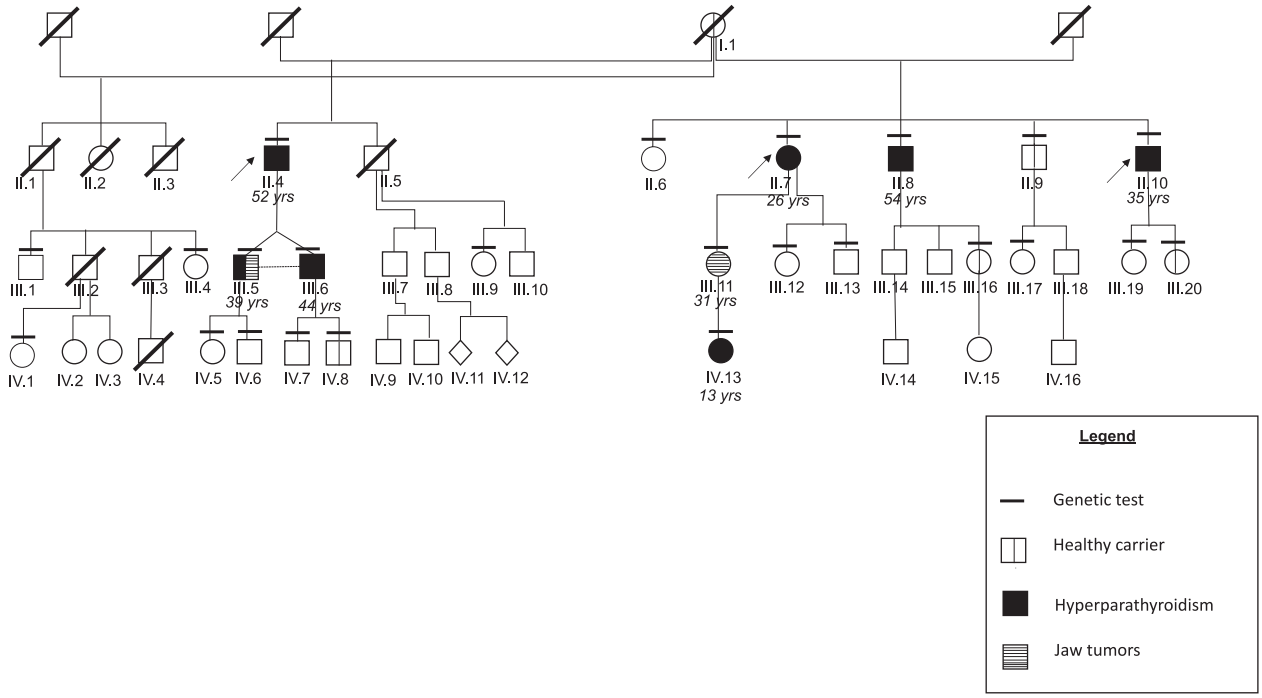

Fig. 1 Hyperparathyroidism-jaw tumor syndrome due to deletion of the third exon of gene *CDC73* represented on the genealogical tree. The circle represents the feminine gender, the square the masculine gender and the rhombus an indeterminate gender. The line above the square/circle represents the presence of genetic research. Asymptomatic cases are represented by a line in the middle of the circle/square. Hyperparathyroidism is represented by a black square/circle and the cross-hatching indicates the presence of jaw tumors. Deceased patients are shown with a strikethrough square/circle in this figure

For somatic analysis of gene *CDC73*, DNA was extracted from formalin-fixed paraffin-embedded (FFPE) samples from parathyroid tumors. After the removal of paraffin with deparaffinization solution, DNA was extracted with the QIAamp FFPE Tissue Kit© (Qiagen, Courtaboeuf, France) according to the manufacturer's instructions. Sanger sequencing of the 17 coding exons and of the flanking intronic junctions of gene *CDC73* was performed by PCR amplification using specific primers (available on demand) followed by sequencing on an automated genetic analyzer sequencer (3730XL©, Applied Biosystems, ThermoFisher Scientific, Courtaboeuf, France). Sequences were then compared to the gene *CDC73* DNA reference sequence (NM_024529; NG_012681) using Seqscape software (Applied Biosystems, Courtaboeuf, France) to identify sequence variations.

Pathological data

The overproducing parathyroid glands were surgically removed in all germline *CDC73* pathogenic variant carriers with HPT. The resected specimens were analyzed at the Laboratory of Pathology of Reims University Hospital and classified according to the WHO 2017 classification. Borderline or atypical cases were reviewed by a referent endocrine pathologist (S.A.) at Lille Academic Hospital.

Parafibromin immunohistochemistry was performed on a Ventana Benchmark Ultra autostainer (Roche, Indianapolis, IN, USA), using VENTANA reagents according to the manufacturer's instructions. Briefly, 3 µm sections of the parathyroid tumors were deparaffinized, then incubated with the primary anti-parafibromin monoclonal antibody (2H1: sc-33638, Santa Cruz Biotechnology, Dallas, TX, USA) which was used at 1:100 dilution. This antibody is directed against amino acid positions 87–100. Epitope retrieval was accomplished with CC1 solution at high temperature (100 °C). Normal parathyroid was used as a positive control. Tumors were scored as negative when no tumor cells showed specific nuclear staining.

Results

Family description

This family includes 47 members over four generations. In the genealogical tree represented in Fig. 1, three branches of the family come from three remarriages of a common ancestor.

The mother of the three patients identified initially probably carried the familial deletion. The high number of remarriages in four succeeding generations has enriched their genetic diversity. Twenty-four patients underwent a

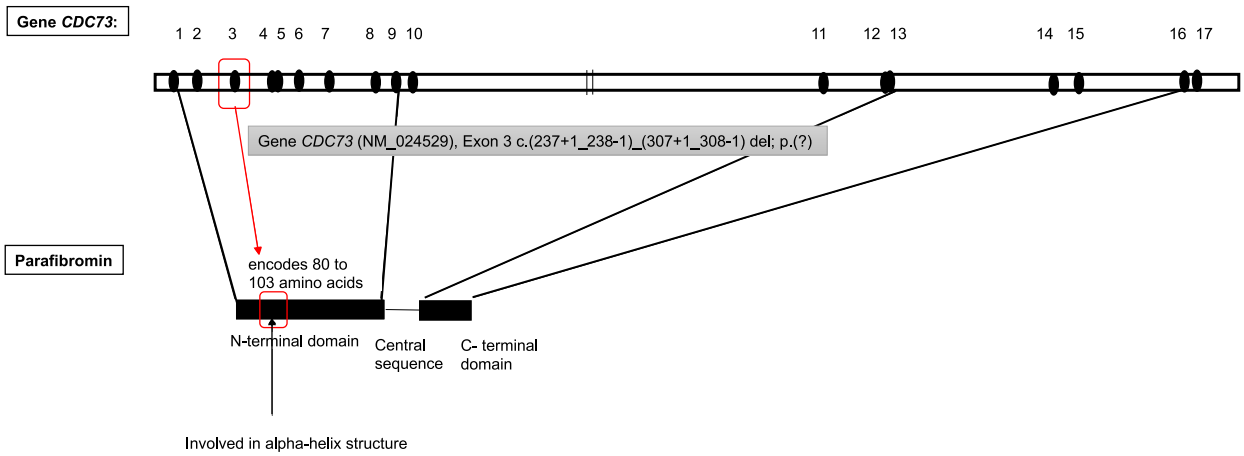

Fig. 2 Deletion of exon 3 of gene *CDC73* and its putative impact on the encoded protein. The first part of the figure at the top represents the CDC73 gene exons represented by several black dots. The third exon deleted in this family is circled in red. Parafibromin is shown below the gene with these different domains: N-terminal domain (first rectangle), Central sequence (the black line) and C-terminal sequence domain (the second one). The part encoded by the exon 3 deleted in this family is circled in red. The different black lines represent the encoding boundaries of each exon for its protein domains

genetic investigation, thirteen of them carried the deletion of the third exon of gene *CDC73*. One patient was the mandatory carrier (the mother of the three identified patients), 13 can be regarded as *CDC73* carriers (7 men and 6 women). Eight of them were symptomatic (hyperparathyroidism and/or ossifying fibroma of the jaw) with a median age of 37 ± 13.6 years at onset of HPT-JT syndrome. Five patients were still asymptomatic at the time of the study at 10, 14, 28, 56 and 91 years old (the common ancestor). The overall age-related penetrance of HPT-JT syndrome in this family was estimated to be 20% before age 30, 50% before age 40, 60% before age 50 and 88.9% before age 70. The earliest cases occurred in three women of the same branch at 13, 26 and 31 years over three generations (Fig. 1).

The first genetic analysis, allowing only SNV (Single Nucleotide Variation) identification, did not reveal any mutation. However, using a supplemental technique of Real-Time PCR, allowing large rearrangements and CNV (Copy number Variation) identification, a large deletion of exon 3 was characterized: gene *CDC73* (NM_ 024529), Exon 3 c. (237 + 1_238-1) _ (307 + 1_308-1) del; p.(?) (Fig. 2). This deletion removes the 3rd exon entirely and part of the 2nd and 3rd introns. However, the technique used does not permit to identify the precise boundaries. According to ACMG criteria, this deletion is a likely pathogenic variant.

Monitoring of HPT-JT syndrome-related clinical features

Hyperparathyroidism (HPT)

HPT was found in almost 53.8% of mutation carriers with 5 male and 2 female cases (Fig. 1 and Table 1). Seven mutated patients had HPT with a median onset age of hypercalcemia at 39 ± 14.5 years (range from 13 to 54 years) and a mean age of 38 (range from 13 to 54). The common ancestor and one of her sons (patients I.1 and II.9) had normal calcium values but presented multiple episodes of urinary lithiasis probably related to a possible Cacchi and Ricci disease. At diagnosis, serum calcium levels were highly variable ranging from moderate hyperparathyroidism (patient III.6) to severe hyperparathyroidism. One of them had hypercalcemia of 4.48 mmol/L complicated by acute renal failure and confusion (patient III.5).

The median calcium plasmatic level at diagnosis was 3.13 ± 0.7 mmol/L and the PTH median was 115 ± 406 pg/ml (Table 1). There was no correlation between serum calcium/PTH levels and the size of the parathyroid tumor. In this family, we observe the disease in 2 monozygotic twins (patients III.5 and III.6). III.5 was the patient with the more severe form of acute hypercalcemia occurring at 39 years whereas his brother presented a moderate HPT at age 44 with a visible parathyroid adenoma on sonography 2 years before an increase in calcium levels (III.6). HPT was surgically resolved in the seven patients after surgery of a single parathyroid adenoma. Recurrence of moderate fluctuating hypercalcemia occurred in only one patient after 7 years of postsurgical evolution (patient II.4) (Table 1). Overall, patients were monitored during a median of 5 years (range from 5 months to 31 years).

Four patients presented with kidney *complications of hypercalcemia* (57.1% of HPT): three with cases of urinary lithiasis (patients II.7, II.8 and III.5) (42.9% of the HPT), two cases of nephrocalcinosis (patients II.7 and II.10) (28.6%), one with chronic kidney failure (14.3%) progressing to end-stage kidney failure in a recently transplanted patient (II.10). We did not find osteoporosis on bone

Table 1 Calcium and PTH serum level values and recurrence of hypercalcemia in patients with hyperparathyroidism

Patients	General characteristics of the patients	Blood test		Adenoma		Follow up after surgery	
	Age of onset of primary hyperparathyroidism	Calcemia (mmol/L)	PTH (pg/ml)	Size (mm)	Weight (g)	Recurrence of hypercalcemia	Post-surgical follow-up time (months)
II.4[a]	52	2.7	79.5	15	1.36	To 84 months	192
II.7[a]	26	?	?	?	?	?	372
II.8	54	3	78	15	0.67	Absence	48
II.10[a]	35	3.32	98.7	20	1.87	Absence	204
III.5	39	4.48	1100	18	?	Absence	60
III.6	44	2.88	148.1	25	1.367	Absence	5
IV.13	13	3.26	132	10	?	Absence	36
Median values	39 ± 14.5	3.13 ± 0.7	115 ± 406	16.5 ± 5.1	1.36 ± 0.49		60
Mean values	38	3	272	17	1.3		131

Normal serum level values: Calcemia: 2.2–2.6 mmol/L

PTH :14–80.1 pg/ml

Normal calcium values are included between 2.2 and 2.6 mmol/L and PTH values are included between 14 and 80.1 pg/ml

The "a" represents the index cases and the question mark the unknown data

?: unknown

densitometry among three patients with primary hyperparathyroidism (patients II.7, II.10, IV.13).

Other lesions

Two out of thirteen patients (15.4% of the carriers) presented with ossifying fibromas of the maxilla/mandible at 31 (III.11) and 39 years (III.5), but not the twin brother. Jaw tumors were associated with HPT in patient III.5 and occurred independently in patient III.11. No recurrences of jaw tumors were reported. Two kidney cysts were present in patient II.9 and II.10 (15.4% of the carriers), one uterine polyp was noted for patient II.7. Interestingly, Cacchi and Ricci disease was suspected in a non-carrier patient with urinary lithiasis (patient III.17). This diagnosis was made on a renal ultrasound performed in this situation. Furthermore, the common ancestor of the three branches had urinary lithiasis without any calcium disorder. Cacchi and Ricci disease, and deletion of the third exon did not appear to be connected because they occurred equally in the family without necessarily being associated.

We found two malignant lesions: one non-encapsulated papillary thyroid microcarcinoma with a major axis equal to 4 mm, classified as pT1a (patient II.8), and one case of prostate cancer (patient II.4) were discovered.

Pre-symptomatic patient care

Until the present, none of the five asymptomatic carriers has developed any clinical features related to HPT-JT syndrome during their regular monitoring.

We should emphasize the difficulties encountered when performing genetic screening in this family. Although the deletion of gene *CDC73* was the known factor of the pathology in the family with the hereditary transmission, the genetic testing was not conducted in some of the children until the first symptoms (patients III.5, III.11, IV.13).

However, for only two of the patients carrying the familial deletion (patient II.8 and III.6), the HPT was discovered via regular biological monitoring of calcium plasmatic levels during the pre-symptomatic period. We observed that calcium levels took only a few months to increase, as in the case of patient III.6 (2.38–2.88 mmol/l in 4 months) and that of patient II.8 (2.54–3 mmol/L in 4 months).

Pathological findings

Anatomopathological features

Results were available for all patients except for patient II.7 (Fig. 3). The pathological findings of these six cases were:

- Two atypical adenomas with fibrous band and necrosis: III.5 (with minority oxyphil cells) and III.6 (with chief cells).
- Four parathyroid cystic adenomas without atypical features. More precisely, these adenomas shared cystic appearance and oxyphil cells (II.4, IV.13, II.8 and II.10) and among them, one contained a chief cell (IV.13).

The Ki67 proliferation index was <1%. Parafibromin immunohistochemistry was performed on five samples and parafibromin expression was lost on all these five tumors (Fig. 3).

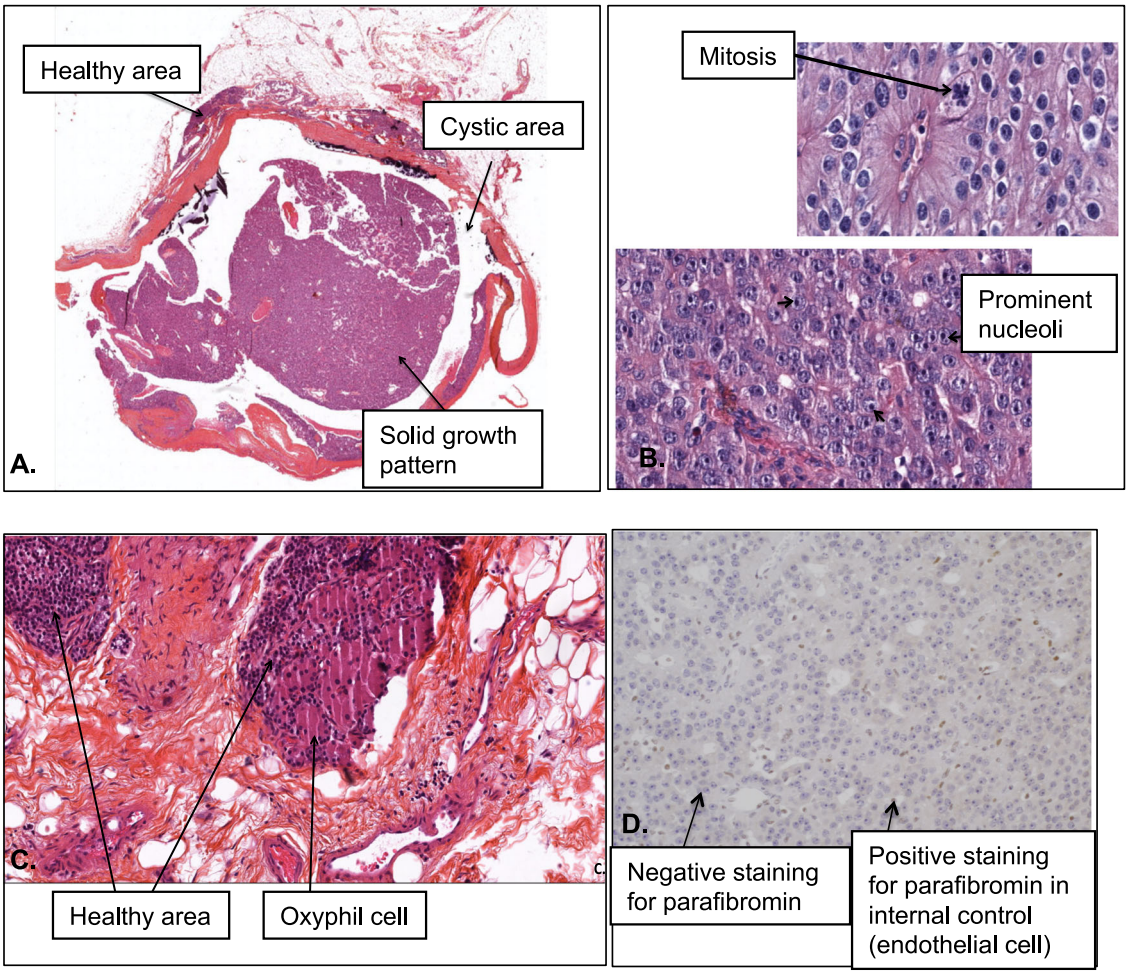

Fig. 3 Specific morphological features including cystic change (**A**), cytologic atypia (**B**), oxyphil cell (**C**) and negative staining for parafibromin in *CDC73* mutation carrier (**D**)

Somatic DNA analysis

DNA was extracted in adenomas from three patients (patients III.5, III.6, IV.13) and analyzed. Unfortunately, the analysis could not be performed optimally for patient III.5 due to the poor quality of the sample. However, no sequence variation was observed in the analyzed exons (1–6, 8–12, 15–17). In the other two patients, 2 different additional somatic deletions in exon 1 were revealed: Patient III.6: gene *CDC73* exon 1, c.94del, p. (Trp32Glyfs*5); for patient IV.13: gene *CDC73* exon 1 c.9del, p. (Asp3Glufs*18). The resulting proteins were truncated because of the presence of a stop codon for both.

Discussion

In this study we report a large extended family of 47 members with three branches carrying a rare large deletion of exon 3 in gene *CDC73* responsible for Hyperparathyroidism Jaw-Tumor syndrome. Thirteen out of 24 screened patients are carriers of the deletion. Hyperparathyroidism was present for 53.8% of mutation carriers due to a single parathyroid adenoma and 57.1% of them had kidney complications of hypercalcemia that can lead to kidney transplantation. We found that 15.4% of the carriers presented with ossifying fibromas of the mandible, less than the 30% frequently reported [14]. Indeed, the presence of ossifying fibromas is not correlated with hyperparathyroidism as illustrated by two cases in this family. Kidney abnormalities (as cysts) [5] and uterine tumors [5, 20, 21] may also be part of clinical features, but we found only two kidney cysts in this family.

Attention must be paid to the risk of multiple cancers, in particular, cancers of the thyroid, colon, pancreas carcinomas and hematological malignancies [5, 22]. 15.3% of the carriers had malignant lesions: one non-encapsulated thyroid papillary microcarcinoma and one prostate cancer. However, the most frequent occurrence of carcinoma remains parathyroid cancer in 15% of cases [5, 23] up to 23% in a recent review [14]. No case was found in this family. Five out of thirteen carriers are

still asymptomatic between 10 and 91 years of age (the deceased common ancestor).

The results of our family's penetrance of HPT-JT syndrome are similar in some points to those described by the Dutch cohort in 12 families [17]: 20% before age 30, 50% before age 40, 60% before age 50 and 88.9% before age 70. The previously estimated penetrance of HPT-JT syndrome was nearly 80–95% [5, 14, 17] with an increase according to age.

However, the age of onset of first symptoms appears to be later than in previous studies [14, 17, 18] with an estimated median age of HPT at 39 ± 14.5 years in our family and a mean age of 38 (range 13–54). Bricaire et al. [18] previously reported an earlier median age than ours for diagnosis of HPT than ours at 27 years (range 12–58) and Van Der Tuin et al. [17] described an earlier mean age of HPT at 32 ± 15 years (range: 13–54 years). Nevertheless, the expressivity of this disease is very variable and we were able to identify a rare case in a young patient, similar to the youngest case described by Van Der Tuin et al. [17] at 13 years old. Clinical and biological monitoring must be regular as HPT-JT syndrome can occur at any age. At present, the youngest case described by Pichardo-Lowden, occurred at 7 years [24] and in this family, at 13 years. These early cases are arguments for performing genetic screening starting at ~5 years of age.

In this family, hypercalcemia is often severe with more than 3 mmol/L and can deteriorate quickly from normocalcemia to a severe condition in a few months (in 4 months for two cases). As expected [10, 25], all of our cases of HPT were due to a single parathyroid adenoma with two cases of atypical adenoma occurring in the two twin brothers, which perfectly illustrates the *variable expressiveness of this disease*. It is currently recognized that the blood calcium values associated with atypical parathyroid adenomas are higher than for typical adenomas [26]. It is interesting to note that in the case of exon 3 deletion, the atypical adenoma is not necessarily associated with severe hypercalcemia (patient III.6). The occurrence of HPT-JT syndrome appears to be *unpredictable* regardless of the level of serum calcium. Furthermore, in this family, we did not observe any recurrence of parathyroid tumor or distant metastases.

Seven years after surgery, a single case of fluctuating moderate hypercalcemia without parathyroid adenoma was observed.

As expected, parafibromin immunostaining is unexpressed in the five parathyroid adenomas studied in the family. Indeed, exon 3 normally encodes the portion of the parafibromin between amino acids 80–103. Parafibromin antibody is directed against a short peptide 87–100 and might reveal the missing expression of a possible truncated or modified protein with biological activity. Other characteristics of our family are the anatomopathological features of the parathyroid adenomas. The latter previously presented with a cystic appearance as expected but also with a high frequency of oxyphilic tumors, which is a rare presentation in CDC73-associated parathyroid tumors.

The family of HPT-JT that we are reporting in this study carries a deletion of the third exon of gene *CDC73* while unclear boundaries remain (intron 2 to intron 3), already described by Bricaire et al [18].

In standard cases, the third exon is rarely involved in HPT-JT. Indeed, to date, four missense/nonsense germline mutations have been described in the exon 3 of *CDC73* (HGMD database 01/21). The rare patients presenting with other germline mutations in exon 3 [13, 18, 27–29] did not seem to present any cases of parathyroid carcinomas. However, cases of a somatic mutation of exon 3 within a parathyroid carcinoma have already been reported [30, 31].

This deleted region encodes for part of the N-terminal domain of parafibromin and presents an alpha-helix structure. It is located upstream of the protein interaction domain with PAF1. Its impact on the encoded protein could be manifold. Indeed, several hypotheses can explain its pathogenicity. Firstly, this deletion could influence the three-dimensional conformation of parafibromin, affecting the function of the protein. Secondly, the removal of an intronic region could also modify the splicing of the RNA, triggering its degradation or generating a premature termination codon encoding a truncated protein. All of these arguments lead us to classify it as a high-impact germline *CDC73* mutation.

Indeed, recently, Li et al. [15] reported *the role of high-impact* germline *CDC73* mutations that resulted in disruption of the C-terminal domain (CTD) or loss of expression of parafibromin, which were associated with a 6.6-fold higher risk of parathyroid carcinoma compared to low impact mutations. In our case, we do not know if the deletion of exon 3 conserves the CTD or not but it *led to the loss of parafibromin expression*. However, even if this deletion could be considered as a *high-impact mutation*, we *did not encounter any parathyroid carcinoma in our study*, which remain rare events. The deletion of exon 3 might be less damaging than those already reported by Li et al [15].

Moreover, we were fortunate to study the possibility of an additional somatic variant of gene *CDC73* in the tumors of three patients of our cohort. In two out of three cases, we characterized a variant in exon 1 which could encode a truncated protein or no protein at all. Interestingly, although suffering from an atypical adenoma, both twins carried a different somatic variant of gene *CDC73*. This additional somatic mutation is necessary for tumor development according to the Knudson two-hit hypothesis [12, 13, 32]. Its nature may explain the phenotypic variability observed in this family. The clinical presentation of these two twins is very different: with one suffering from

severe hypercalcemia with ossifying fibroma, while the second developed hyperparathyroidism more mildly and at a later age.

To date, no well-established follow-up guidelines have been proposed. Based on the last review of Toressan et al., *CDC73*-related disorders should be suspected in these situations [5, 14]: familial HPT and HPT-JT; HPT before age 40; sporadic parathyroid carcinoma; absence of parafibromin staining of a PTH adenoma, or in the presence of coexistence ossifying jaw fibroma, renal, or uterine tumors. Screening should be undertaken early, starting at ~5 years of age, due to the possible occurrence of the first symptom at 7 years of age. Moreover, the delay between the genetic testing and biological monitoring must be as short as possible to prevent delay in treatment with a regular follow-up. Regular and lifelong monitoring of serum calcium/PTH/creatinine levels, parathyroid/renal and pelvic ultrasound examinations, panoramic dental X-rays is recommended for *CDC73* mutation carriers [5, 14]. We can propose a calcium evaluation at least every 6 months, given the rapidly increasing calcium levels observed in some of our patients.

Clear information must be given to all the patient carriers and primary care physicians.

Concerning surgery, total prophylactic parathyroidectomy was recommended for HPT to reduce the risk of recurrences of parathyroid carcinoma in HPT-JT. However, morbidity due to permanent postsurgical hypoparathyroidism should not be underestimated and adenoma resection should be given priority [33]. The absence of recurrence in this family is an argument in favor of minimally invasive surgery.

These arguments, when taken together, make this syndrome a very distinct entity from sporadic hyperparathyroidism. In the latter case, onset of the disease is more frequent in women and occurs at a later age than in HPT-JT syndrome.

Our study reports the biological and clinical features of a new large extended family carrying a large deletion of the third exon of gene *CDC73*.

We found a high frequency of hyperparathyroidism mostly with isolated adenoma (53.8% of carriers) and significant renal impairment that can lead to transplantation. We have also described the occurrence of two ossifying fibromas, which are not necessarily associated with hyperparathyroidism. Despite very severe cases of hypercalcemia, no parathyroid carcinoma was found. The somatic mutations in the first exon described within the adenomas studied could play a role in the phenotypical variability. We have described two atypical adenomas in two monozygotic twins that do not carry the same somatic mutation and whose clinical evolution differed greatly, highlighting the variable expressivity of this disease.

Moreover, it is crucial to monitor these patients regularly because of the possibility of an increase in blood calcium levels within a few months. In order to prevent complications from HPT-JT, genetic counselling of all relatives, regardless of age, should be offered, on identification of the *CDC73* variant, in order to prevent complications from HPT-JT.

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Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

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References

1. W.D. Fraser, Hyperparathyroidism. Lancet Lond. Engl. **374**, 145–58 (2009)
2. M.W. Yeh, P.H.G. Ituarte, H.C. Zhou, S. Nishimoto, I.-L.A. Liu, A. Harari, P.I. Haigh, A.L. Adams, Incidence and prevalence of primary hyperparathyroidism in a racially mixed population. J. Clin. Endocrinol. Metab. **98**, 1122–9 (2013)
3. J.M. Sharretts, W.F. Simonds, Clinical and molecular genetics of parathyroid neoplasms. Best. Pract. Res. Clin. Endocrinol. Metab. **24**, 491–502 (2010)
4. D.J. Marsh, M.A. Hahn, V.M. Howell, A.J. Gill, Molecular diagnosis of primary hyperparathyroidism in familial cancer syndromes. Expert Opin. Med. Diagn. **1**, 377–92 (2007)
5. S.M. Hyde, T.A. Rich, S.G. Waguespack, N.D. Perrier, and M. I. Hu, In *GeneReviews®*, ed. by M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace, L. J. Bean, K. Stephens, and A. Amemiya (University of Washington, Seattle, Seattle (WA), 1993).
6. C.E. Jackson, Hereditary hyperparathyroidism associated with recurrent pancreatitis. Ann. Intern. Med. **49**, 829–36 (1958)
7. C.E. Jackson, R.A. Norum, S.B. Boyd, G.B. Talpos, S.D. Wilson, R.T. Taggart, L.E. Mallette, Hereditary hyperparathyroidism and multiple ossifying jaw fibromas: a clinically and genetically distinct syndrome. Surgery **108**, 1006–12 (1990)
8. L.E. Mallette, S. Malini, M.P. Rappaport, J.L. Kirkland, Familial cystic parathyroid adenomatosis. Ann. Intern. Med. **107**, 54–60 (1987)
9. J. Szabó, B. Heath, V.M. Hill, C.E. Jackson, R.J. Zarbo, L.E. Mallette, S.L. Chew, G.M. Besser, R.V. Thakker, V. Huff, Hereditary hyperparathyroidism-jaw tumor syndrome: the endocrine tumor gene HRPT2 maps to chromosome 1q21-q31. Am. J. Hum. Genet. **56**, 944–50 (1995)
10. J.D. Carpten, C.M. Robbins, A. Villablanca, L. Forsberg, S. Presciuttini, J. Bailey-Wilson, W.F. Simonds, E.M. Gillanders, A. M. Kennedy, J.D. Chen, S.K. Agarwal, R. Sood, M.P. Jones, T.Y. Moses, C. Haven, D. Petillo, P.D. Leotlela, B. Harding, D.

Cameron, A.A. Pannett, A. Höög, H. Heath, L.A. James-Newton, B. Robinson, R.J. Zarbo, B.M. Cavaco, W. Wassif, N.D. Perrier, I.B. Rosen, U. Kristoffersson, P.D. Turnpenny, L.-O. Farnebo, G. M. Besser, C.E. Jackson, H. Morreau, J.M. Trent, R.V. Thakker, S.J. Marx, B.T. Teh, C. Larsson, M.R. Hobbs, HRPT2, encoding parafibromin, is mutated in hyperparathyroidism–jaw tumor syndrome. Nat. Genet. **32**, 676–80 (2002)
11. O. Rozenblatt-Rosen, C.M. Hughes, S.J. Nannepaga, K.S. Shanmugam, T.D. Copeland, T. Guszczynski, J.H. Resau, M. Meyerson, The parafibromin tumor suppressor protein is part of a human Paf1 complex. Mol. Cell. Biol. **25**, 612–20 (2005)
12. A.G. Knudson, Mutation and cancer: statistical study of retinoblastoma. Proc. Natl Acad. Sci. U. S. A **68**, 820–823 (1971)
13. F. Cetani, E. Pardi, E. Ambrogini, P. Viacava, S. Borsari, M. Lemmi, L. Cianferotti, P. Miccoli, A. Pinchera, A. Arnold, C. Marcocci, Different somatic alterations of the HRPT2 gene in a patient with recurrent sporadic primary hyperparathyroidism carrying an HRPT2 germline mutation. Endocr. Relat. Cancer **14**, 493–9 (2007)
14. F. Torresan, M. Iacobone, Clinical Features, Treatment, and Surveillance of Hyperparathyroidism-Jaw Tumor Syndrome: an Up-to-Date and Review of the Literature. Int J. Endocrinol. **2019**, 1761030 (2019)
15. Y. Li, J. Zhang, P.R. Adikaram, J. Welch, B. Guan, L.S. Weinstein, H. Chen, W.F. Simonds, Genotype of CDC73 germline mutation determines risk of parathyroid cancer. Endocr. Relat. Cancer **27**, 483–94 (2020)
16. C.J. Haven, F.K. Wong, E.W. van Dam, R. van der Juijt, C. van Asperen, J. Jansen, C. Rosenberg, M. de Wit, J. Roijers, J. Hoppener, C.J. Lips, C. Larsson, B.T. Teh, H. Morreau, A genotypic and histopathological study of a large Dutch kindred with hyperparathyroidism-jaw tumor syndrome. J. Clin. Endocrinol. Metab. **85**, 1449–54 (2000)
17. K. Van der Tuin, C.M.J. Tops, M.A. Adank, J.-M. Cobben, N.A. T. Hamdy, M.C. 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, CDC73-Related Disorders: clinical Manifestations and Case Detection in Primary Hyperparathyroidism. J. Clin. Endocrinol. Metab. **102**, 4534–40 (2017)
18. L. Bricaire, M.-F. Odou, C. Cardot-Bauters, B. Delemer, M.-O. North, S. Salenave, D. Vezzosi, J.-M. Kuhn, A. Murat, P. Caron, J.-L. Sadoul, C. Silve, P. Chanson, A. Barlier, E. Clauser, N. Porchet, L. Groussin, Frequent large germline HRPT2 deletions in a French National cohort of patients with primary hyperparathyroidism. J. Clin. Endocrinol. Metab. **98**, E403–E408 (2013)
19. S. Richards, N. Aziz, S. Bale, D. Bick, S. Das, J. Gastier-Foster, W.W. Grody, M. Hegde, E. Lyon, E. Spector, K. Voelkerding, H. L. Rehm; ACMG Laboratory Quality Assurance Committee. Genet. Med. J. Am. Coll. Med. Genet. **17**, 405 (2015)
20. M. Fujikawa, K. Okamura, K. Sato, T. Mizokami, K. Tamaki, T. Yanagida, M. Fujishima, Familial isolated hyperparathyroidism due to multiple adenomas associated with ossifying jaw fibroma and multiple uterine adenomyomatous polyps. Eur. J. Endocrinol. **138**, 557–61 (1998)
21. K.J. Bradley, M.R. Hobbs, I.D. Buley, J.D. Carpten, B.M. Cavaco, J.E. Fares, P. Laidler, S. Manek, C.M. Robbins, I.S. Salti, N.W. Thompson, C.E. Jackson, R.V. Thakker, Uterine tumours are a phenotypic manifestation of the hyperparathyroidism-jaw tumour syndrome. J. Intern. Med. **257**, 18–26 (2005)
22. M.H. Tan, B.T. Teh, Renal neoplasia in the hyperparathyroidism-jaw tumor syndrome. Curr. Mol. Med. **4**, 895–7 (2004)
23. D. Betea, I. Potorac, A. Beckers, Parathyroid carcinoma: challenges in diagnosis and treatment. Ann. Endocrinol. **76**, 169–77 (2015)
24. A.R. Pichardo-Lowden, A. Manni, B.D. Saunders, M.J. Baker, Familial hyperparathyroidism due to a germline mutation of the CDC73 gene: implications for management and age-appropriate testing of relatives at risk. Endocr. Pract. J. Am. Coll. Endocrinol. Am. Assoc. Clin. Endocrinol. **17**, 602–9 (2011)
25. T.G. Kelly, T.M. Shattuck, M. Reyes-Mugica, A.F. Stewart, W.F. Simonds, R. Udelsman, A. Arnold, T.O. Carpenter, Surveillance for early detection of aggressive parathyroid disease: carcinoma and atypical adenoma in familial isolated hyperparathyroidism associated with a germline HRPT2 mutation. J. Bone Miner. Res. J. Am. Soc. Bone Miner. Res. **21**, 1666–71 (2006)
26. A. Galani, R. Morandi, M. Dimko, S. Molfino, C. Baronchelli, S. Lai, F. Gheza, C. Cappelli, C. Casella, Atypical parathyroid adenoma: clinical and anatomical pathologic features. World J. Surg. Oncol. **19**, 19 (2021)
27. L.M. Panicker, J.-H. Zhang, P.K. Dagur, M.J. Gastinger, W.F. Simonds, Defective nucleolar localization and dominant interfering properties of a parafibromin L95P missense mutant causing the hyperparathyroidism–jaw tumor syndrome. Endocr. Relat. Cancer **17**, 513–24 (2010)
28. Y. Shibata, M. Yamazaki, M. Takei, S. Uchino, A. Sakurai, M. Komatsu, Early-onset, severe, and recurrent primary hyperparathyroidism associated with a novel CDC73 mutation. Endocr. J. **62**, 627–32 (2015)
29. L.F. Starker, T. Akerström, W.D. Long, A. Delgado-Verdugo, P. Donovan, R. Udelsman, R.P. Lifton, T. Carling, Frequent germline mutations of the MEN1, CASR, and HRPT2/CDC73 genes in young patients with clinically non-familial primary hyperparathyroidism. Horm. Cancer **3**, 44–51 (2012)
30. K.J. Bradley, B.M. Cavaco, M.R. Bowl, B. Harding, T. Cranston, C. Fratter, G.M. Besser, M. Conceição Pereira, M.W.J. Davie, N. Dudley, V. Leite, G.P. Sadler, A. Seller, R.V. Thakker, Parafibromin mutations in hereditary hyperparathyroidism syndromes and parathyroid tumours. Clin. Endocrinol. (Oxf.) **64**, 299–306 (2006)
31. W. Yu, J.R. McPherson, M. Stevenson, R. van Eijk, H.L. Heng, P. Newey, A. Gan, D. Ruano, D. Huang, S.L. Poon, C.K. Ong, T. van Wezel, B. Cavaco, S.G. Rozen, P. Tan, B.T. Teh, R.V. Thakker, H. Morreau, Whole-exome sequencing studies of parathyroid carcinomas reveal novel PRUNE2 mutations, distinctive mutational spectra related to APOBEC-catalyzed DNA mutagenesis and mutational enrichment in kinases associated with cell migration and invasion. J. Clin. Endocrinol. Metab. **100**, E360–E364 (2015)
32. N. Hatabu, N. Katori, T. Sato, N. Maeda, E. Suzuki, O. Komiyama, H.Tsutsui, T. Nagao, H. Nakauchi-Takahashi, T. Matsunaga, T. Ishii, T. Hasegawa, K. Yamazawa, A Familial Case of a Whole Germline CDC73 Deletion Discordant for Primary Hyperparathyroidism. Horm. Res. Paediatr **92**, 56–63 (2019). Epub 8 Feb 2019. https://doi.org/10.1159/000495800
33. M. Iacobone, G. Masi, L. Barzon, A. Porzionato, V. Macchi, F.A. Ciarleglio, G. Palù, R. De Caro, G. Viel, G. Favia, Hyperparathyroidism–jaw tumor syndrome: a report of three large kindred. Langenbecks Arch. Surg. **394**, 817–25 (2009)