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RESEARCH LETTER

A rare S33C mutation of *CTNNB1* encoding β-catenin in a parathyroid adenoma found in an Italian primary hyperparathyroid cohort

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Introduction

Primary hyperparathyroidism (PHPT) is one of the most common endocrine disorders with a prevalence of 21/1000 in women between 55 and 75 years of age, corresponding to a 3/1000 prevalence in the general population [1]. Sporadic (non-familial) PHPT accounts for 90–95% of all cases. While activation of the protooncogene cyclin D1 (*CCDN1*) and inactivation of the tumor suppressor menin gene (*MEN1*) contribute to parathyroid adenomatosis, and inactivation of the parafibromin gene (*CDC73*) contributes to parathyroid carcinogenesis, the molecular pathogenesis of these tumors is incompletely understood. Dysregulated Wnt signaling and activation of β-catenin is involved in several cancers and consequently there has been recent interest in whether this is implicated in parathyroid tumorigenesis. In the absence of Wnt, cytosolic β-catenin phosphorylation catalyzed by glycogen synthase kinase (GSK)-β on serine/threonine residues (S33, S37, T41) encoded by exon 3 of the *CTNNB1* gene leads to the ubiquitination and to degradation mediated by the proteasome. Mutation of the serine/threonine residues causes stabilization of the β-catenin protein and localization to the nucleus where it activates gene transcription. Several studies have been reported examining *CTNNB1* gene mutations and aberrant β-catenin localization in sporadic parathyroid adenomas. While studies from Sweden found the homozygous S37A mutation in ~7% of 124 parathyroid adenomas and aberrant β-catenin localization in all cases [2, 3], other studies from Japan [4, 5], USA [6],

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Italy [7], and Sweden [8, 9] have failed to confirm these findings. Here, we report on our *CTNNB1* gene mutation analysis of parathyroid adenomas and carcinomas in a novel Italian PHPT cohort.

Patients and methods

Patients and samples

Patients were followed at the Endocrine Unit of IRCCS Casa Sollievo della Sofferenza Hospital, San Giovanni Rotondo (FG) and at the Department of Medical-Surgical Sciences and Medical Sciences, University of Milan. The studies received local ethical committee approval and the patients gave written informed consent. Fifty-nine parathyroid adenomas (57 typical and 2 atypical) and ten parathyroid carcinomas were studied. Histopathological diagnosis was made by the endocrine pathologist according to WHO criteria [10], and we defined as "atypical" an adenoma with no evidence of invasion, but showing features suspicious of malignancy, such as fibrous bands, capsular invasion, increased mitotic figures, adherence to surrounding tissues. Surgically resected tumor samples were obtained as formalin-fixed paraffin embedded blocks and DNA extracted by a xylene–phenol–chloroform protocol.

CTNNB1 sequencing

Exon 3 of *CTNNB1* was PCR amplified (forward primer: 5′-GATGGAGTTGCACATCGCC-3′ and reverse primer: 5′-CTCATACAGGACTTGGGAGG-3′), purified (GFX PCR DNA and Gel Band Purification Kit, GE Healthcare), and directly sequenced (Big Dye Terminator and ABI 3100 capillary sequencer, Applied Biosystems). As required, PCR products were cloned into the StrataClone PCR Cloning Vector pSC-A (Stratagene) and the inserts of several clones directly sequenced.

Immunohistochemistry (IHC)

Parathyroid tissue sections were subjected to an IHC antigen retrieval protocol. Immunostaining for β-catenin and cyclin D1 was performed using anti-β-catenin clone E-5 (Santa Cruz Biotechnology, Santa Cruz, CA) and anti-Cyclin D1/Bcl-1(SP4) (Thermo Scientific, Fremont, CA). Non-immune mouse serum was used as a negative control.

Results

A heterozygous *CTNNB1* exon 3 mutation, c.98C>G, p.S33C, was identified in a parathyroid adenoma from a 64-year-old female PHPT patient; albumin-adjusted serum calcium, 10.53 mg/d; (normal range 8.4–10.2), serum phosphate, 2.74 mg/dl (2.7–4.5), serum creatinine, 0.84 mg/dl (0.6–1.1), total serum alkaline phosphatase activity, 265 IU/l (80–270), parathyroid hormone, 84 pg/ml (10–72), urinary calcium, 230 mg/day (100–250). Serum gastrin, calcitonin and prolactin, urinary 5-hydroxyindole acetic acid and catecholamine levels were normal. Review of the parathyroid histopathology confirmed typical adenoma.

The *CTNNB1* exon 3 PCR product from the patient was cloned and the presence of the mutation was confirmed in ~30% of the clones examined. Genomic DNA from the peripheral blood leukocytes of the patient was negative for the mutation, consistent with a somatic origin. Tissue and leukocyte DNA of samples from all other PHPT cases, 58 adenomas and 10 carcinomas, were wild-type for the *CTNNB1* exon 3 amplicon.

IHC of tissue sections from the parathyroid adenoma of the patient harboring the *CTNNB1* S33C mutation revealed no differences in expression of β-catenin or cyclin D1 in comparison to mutation-negative parathyroid tissue (data not shown).

Discussion

A homozygous *CTNNB1* activating S37A mutation was reported in ~7% of 124 parathyroid adenomas in a Swedish PHPT cohort [2, 3]. However, no *CTNNB1* exon 3 mutation was found in several other adenoma panels from PHPT patients of Japanese [4, 5], American [6], and Italian [7] origin (Table 1). Moreover, no *CTNNB1* exon 3 mutation was found in an independent Swedish study [9], making it unlikely that genetic background played a significant role in the different results among studies.

To explore the issue of the presence and prevalence of such mutations further we have evaluated an independent Italian PHPT cohort. In this cohort, we found a heterozygous *CTNNB1* S33C mutation (thus different from the homozygous S37A mutation reported previously) in one case out of 57 typical sporadic parathyroid adenomas. There was nothing remarkable with respect to clinical presentation or the patient's serum and urinary biochemistries. The S33C β-catenin mutation is found in gastric, ovarian, endometrial, and brain tumors. In the majority of these cases, the mutation is associated with nuclear expression of β-catenin, and upregulation of the expression of the cyclin D1 gene, an important target of β-catenin.

The previous studies that identified *CTNNB1* mutations in the small subset of adenomas also found aberrant nuclear β-catenin immunostaining in all adenomas examined, not just those harboring a mutation [2, 3]. However, this finding was not confirmed in other studies [4–6, 8] (Table 1). In the

Table 1 Summary of *CTNNB1* exon 3 mutations and abnormal β-catenin expression in parathyroid adenomas

Study	Parathyroid adenomas # examined	*CTNNB1* S33C mutation	*CTNNB1* S37A mutation	Abnormal β-catenin expression[a]	Additional analyses
Semba et al. [4]	9	0[b]	0[b]	1/9	0/2 hyperplasia 0/1 carcinoma β-catenin abnormal expression
Ikeda et al. [5]	24	0	0	0	9/24 (38%) cyclin D1 abnormal expression
Björklund et al. [2]	37[c]	0	3/20 (15%)[d]	37/37 (100%)	
Costa-Guda and Arnold [6]	97	0	0	ND[e]	
Björklund et al. [3]	104	0	6 (5.8%)[d]	104	
Cetani et al. [7]	136[f]	0	0	0/63 typical and 0/3 atypical adenomas examined	0/18 carcinomas *CTNNB1* exon 3 mutations or abnormal expression
Juhlin et al. [8]	18	ND	ND	0	0/13 carcinomas abnormal expression
Haglund et al. [9]	98	0	0	ND	
Present study	59[g]	1 (1.8%)[h]	0	0[i]	0/10 carcinomas *CTNNB1* exon 3 mutations

[a] Elevated β-catenin cytoplasm/nuclear staining

[b] *CTNNB1* exon 3 examined in the one tumor with abnormal β-catenin expression

[c] β-Catenin staining examined in 37 and *CTNNB1* exon 3 examined in 20 adenomas

[d] Homozygous

[e] Not determined

[f] 130 typical adenomas, 6 atypical adenomas

[g] 57 typical adenomas, 2 atypical adenomas

[h] Heterozygous

[i] Parathyroid tumor with the *CTNNB1* S33C mutation

present study, we performed immunohistochemistry for β-catenin and cyclin D1 on sections of the parathyroid adenoma that had the S33C mutation. We did not detect differences in localization or expression level from either normal parathyroid or other adenomatous tissue. Possibly, the S33C mutation is not expressed equivalently in all tumor cells and the tissue sections available for the analysis expressed only low amounts of the mutant β-catenin.

In conclusion, *CTNNB1* mutations are rare in parathyroid adenomas (2.1%, 10/477 typical and atypical adenomas cases so far worldwide analyzed) and have yet to be identified in parathyroid carcinomas [2, 7, present study]. However, our finding in a population distinct from the original Swedish cohort [2, 3] means that a role for β-catenin mutations in benign parathyroid neoplasia may not be ruled out yet [11]. Nevertheless, we suggest that any significant change in clinical pathological management would be premature at present and further studies are needed.

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Conflict of interest The authors declare that they have no conflict of interest.

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