

SHORT REPORT

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# Lack of germline A339V mutation in thyroid transcription factor-1 (TTF-1/NKX2.1) gene in familial papillary thyroid cancer

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## Abstract

Thyroid cancer may have a familial predisposition but a specific germline alteration responsible for the disease has not been discovered yet. We have shown that familial papillary thyroid cancer (FPTC) patients have an imbalance in telomere-telomerase complex with short telomeres and increased telomerase activity. A germline mutation (A339V) in thyroid transcription factor-1 has been described in patients with multinodular goiter and papillary thyroid cancer. In this report, the presence of the A339V mutation and the telomere length has been studied in FPTC patients and unaffected family members. All samples analyzed displayed a pattern typical of the homozygous wild type revealing the absence of the A339V mutation. Shortening of telomeres was confirmed in all patients. We concluded that the A339V mutation in thyroid transcription factor-1 (TTF-1/NKX2.1) is not correlated with the familial form of PTC, even when the tumor was in the context of multinodular goiter.

## Findings

Familial non-medullary thyroid cancer (FNMTC), most frequently of papillary histotype (FPTC), recurs in two or more members of the same family in about 10% of the patients. The clinical form of PTC may be part of a clinical syndrome such as the Adenomatous polyposis of colon (FAP) [1], Cowden syndrome [2], Gardner syndrome [3], Werner syndrome [4] or Carney complex [5] or may be the only disease manifestation. In this case, the large majority of FPTC, no candidate gene(s) has been discovered [6]. Recently we provided evidence that FPTC display the feature of “genetic anticipation” (defined as earlier age at onset of the disease and/or increased severity in successive generations), and, at molecular level, are characterized by the presence of germinal alterations in the telomere-telomerase complex [7,8]. Our hypothesis is that the imbalance in telomerase complex may predispose to acquire a thyroid specific mutation able to trigger thyroid tumorigenesis. Recently, Ngan et al. demonstrated the presence of a new germline mutation (A339V) in thyroid transcription factor-1 (TTF-1/NKX2.1) in patients with multinodular goiter

and papillary thyroid cancer [9]. The authors identified the mutation in four out of 20 MNG/PTC patients which developed more advanced tumors compared to MNG/PTC or PTC patients without the mutation. In addition, the mutation was dominantly inherited in two families with members bearing MNG/PTC [9].

In light of these evidences, we investigated the presence of A339V mutation in patients with the occurrence of FPTC and short telomeres. Out of 408 patients affected by differentiated thyroid carcinoma (DTC), followed in the Section of Endocrinology of University of Siena (Italy) from 1978 to 2009 (306 females and 102 males; ratio F/M: 3/1, ranging 8-84 yrs), 63 patients (15.4%), belonging to 38 kindred, showed the familial recurrence of the disease possibly configuring the features of familial thyroid cancer (FTC). All patients with FTC had the papillary histotype (FPTC), 15 of them (23.8%) presented the follicular variant and 1 (1.6%) the warthin-like variant. Of these patients, 40 (63.4%) had a parent-child relationship (22 kindred), 19 (30.2%) had a sibling relationship (13 kindred) and 4 (6.4%) had an uncle-niece relationship (3 kindred). Age at diagnosis of FPTC group was  $46 \pm 15.5$  yrs (range: 15-78 yrs). Age at diagnosis of probands was  $54.3 \pm 9.3$  (range 25-77 yrs) and that of affected familial members was  $42.9 \pm 15.7$  (range 15-73

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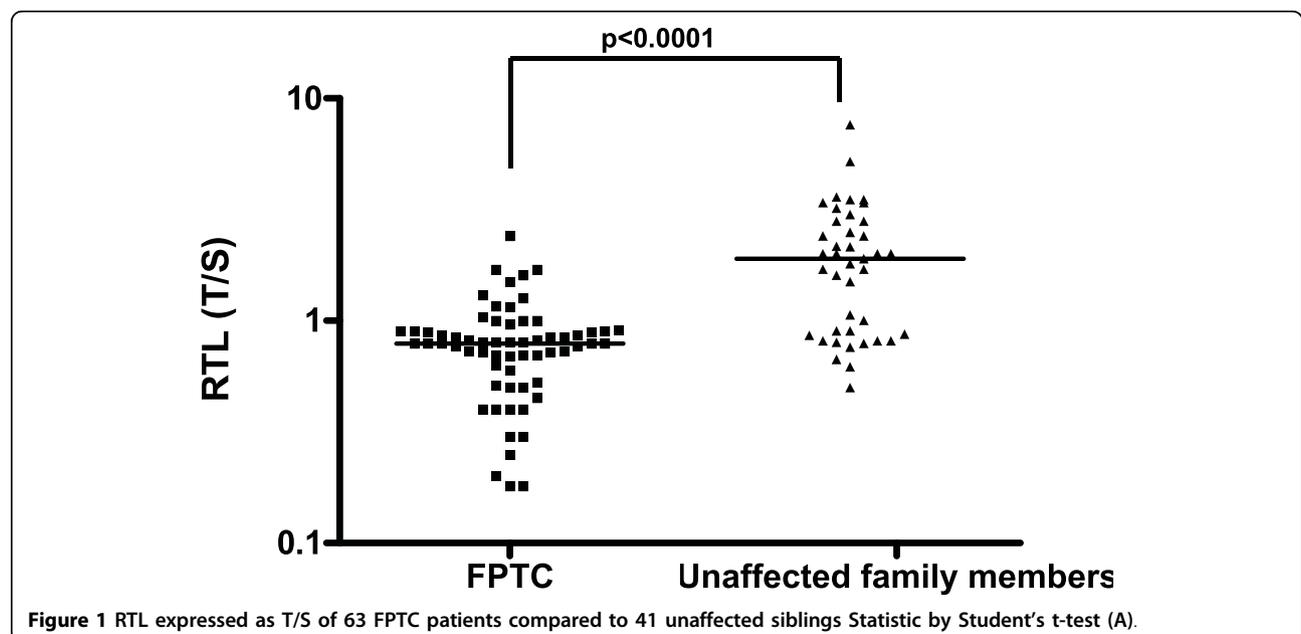
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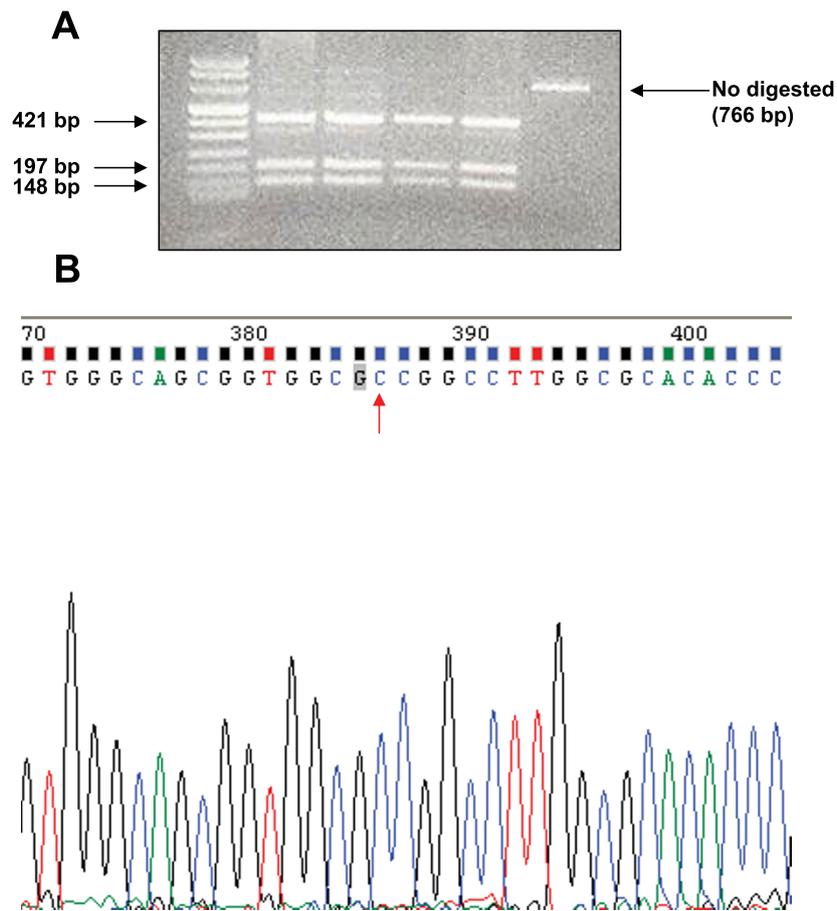
**Table 1 Clinical features of FPTC patients**

	Patients (n = 63)
<b>Age at diagnosis (yr)</b>	
Mean ± SD	46 ± 15.5
Range	15-78
<b>Sex</b>	
No. of females (%)	47 (74.6)
No. of males (%)	16 (25.4)
<b>Histotype</b>	
No. of papillary (%)	47 (74.6)
No. of papillary follicular variant (%)	15 (23.8)
No. of papillary warthin-like (%)	1 (1.6)
<b>TNM</b>	
No. of T1-T3 N0 M0(%)	40 (63.5)
No. of T1-T3 N1 M0 (%)	13 (20.6)
No. of T1-T3 N0-N1 M1 (%)	1 (1.6)
No. of T4 N0-N1 M0 (%)	3 (4.7)
Not available	6 (9.6)
<b>Outcome</b>	
Remission (%)	45 (71.4)
Persistent disease (%)	12 (19.1)
Not evaluated (%)	6 (9.5)

40 (63.5%) patients had a history of multinodular goiter. At the time of the study 45 (71.4%) were free of disease, 12 (19.1%) showed persistent disease and 6 (9.5%) were operated less than 1 year ago (follow-up not available). In the group of 41 unaffected family members, 33 (80.6%) patients had no evidence of thyroid nodule, 4 (9.7%) showed a multinodular goiter and 4 (9.7%) had a single nodule. In all cases the FNAB indicated the presence of benign lesions. We measured telomere length by Q-PCR [10] assay on genomic DNA of all subjects and compared the differences in telomere length by Student's t-test. As shown in figure 1, RTL was significantly ( $p < 0.0001$ ) shorter in FPTC patients (mean ± SD:  $0.8 \pm 0.04$ , range: 0.18-2.4) compared to unaffected siblings (mean ± SD:  $2.04 \pm 0.2$ , range: 0.7-7.6). To identify whether TITF/NKX2.1 germline point mutation (A339V) was involved with FPTC development, genomic DNA belonging to FPTC patients and unaffected siblings, was amplified using the GC-Rich PCR system followed by digestion of PCR products by restriction enzyme NaeI. This enzyme cut in correspondence of 5'-GCCGGC-3' sequence thus leading to the formation of these patterns: A) homozygous wild type characterized by three fragments of 421, 197 and 148 bp, respectively; B) mutated homozygous characterized by two PCR products of 618 and 148 bp, respectively and C) heterozygous characterized by four fragments at 618, 421, 197 and 148 bp, respectively. As shown in figure 2A, after digestion with the enzyme all the analyzed samples displayed a pattern typical of homozygous wild type. To confirm this

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**Figure 2** Representative gel illustrating the homozygous wild type digestion pattern showed by FPTC patient and unaffected siblings (A); Representative electropherogram of an FPTC patient showing the absence of mutation(s) in TITF/NKX2.1 gene (B).

result, samples were amplified twice and PCR products directly sequenced. As reported in figure 2B no mutation was found in all samples.

In conclusions, in this study we analyzed 63 FPTC patients for the presence of the A339V mutation in thyroid transcription factor-1 (TITF-1/NKX2.1) in order to correlate that mutation with the predisposition to develop familial form of thyroid cancer demonstrated by shorter telomere length which may contribute to genetic instability and thyroid cancer development. Our results confirmed that FPTC patients have significantly short telomeres compared to unaffected family members. The reason of the predisposition to develop thyroid cancer in patients displaying the imbalance in telomerase complex has not been identified yet. The A339V mutation in thyroid transcription factor-1 (TITF-1/NKX2.1) might be a candidate gene in view of recent findings [9]. All FPTC patients displayed a genetic profile typical of homozygous wild type demonstrating that A339V mutation is not necessarily associated with the development

of familial form of thyroid cancer even when the tumor was in the context of multinodular goiter.

#### Authors' contributions

SC carried out the molecular genetic studies and wrote the manuscript, SC contributed to perform the experiments, CF helped to collect clinical patient's data, MP helped with DNA extraction, MC revised the clinical data and FP revised the manuscript and coordinated the experiments. All authors read and approved the final manuscript.

#### Competing interests

The authors declare that they have no competing interests.

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