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HIGH PREVALENCE OF THYROID DISORDERS IN RELATIVES OF PATIENTS WITH FAMILIAL PAPILLARY THYROID CANCER*

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Abstract In the familial form of papillary thyroid cancer (PTC), two or more members of the same family have to be affected with PTC. Prevalence is around 5% of all PTC. We performed a clinical analysis in 79 relatives of 16 patients of 7 unrelated kindred with the diagnosis of familial papillary thyroid carcinoma (FPTC). The results were compared with a control group. Thyroid palpation and TSH and TPO-Ab assessment was carried out in the relatives without a diagnosed PTC. Additionally, molecular analysis was performed in the sixteen affected patients. Clinical screening of the 79 family members showed the presence of goiter in 22/79 (29 %). This frequency was much higher than that observed in the control group (8.7%), $p < 0.001$. Hypothyroidism was found in 4 of the relatives (5%) *vs.* 2.5% observed in the control group, $p < 0.01$, and anti-thyroid antibodies (TPO-Ab) were positive in 14% of the relative's group *vs.* 10 % in the control group, (p = NS). In the molecular analysis, only a protooncogene TRK rearrangement was observed in family # 6. In conclusion, we found a higher incidence of goiter and hypothyroidism in the relatives of patients with FPTC. Nevertheless, TPO-Ab frequency was not different. No molecular abnormalities were indicative of a specific pattern in this subset of patients with FPTC.

Key words: familial papillary thyroid cancer, relatives, prevalence, goiter, hypothyroidism

Resumen ***Elevada prevalencia de alteraciones tiroideas en familiares de pacientes con diagnóstico de carcinoma papilar familiar.*** En la forma familiar del carcinoma papilar de tiroides (CPT), dos o más miembros de la misma familia deben presentar CPT. Esta entidad ocurre en aproximadamente el 5% de todos los CPT. En este estudio, realizamos una evaluación de 79 familiares de 16 pacientes con diagnóstico de carcinoma papilar familiar (CPF) provenientes de 7 familias diferentes. Los resultados se compararon con los hallados en un grupo control. Se realizó palpación tiroidea y medición de TSH y anticuerpos anti-tiroperoxidasa (TPO-Ab) en todos los familiares. Además, se llevó a cabo el análisis molecular en los 16 sujetos que presentaban el diagnóstico de CPF. La evaluación de los 79 familiares de estos pacientes demostró la presencia de bocio en 22/79 (29%). Esta frecuencia fue mucho mayor que la observada en el grupo control (8.7%), $p < 0.001$. Se diagnosticó hipotirodismo en 4 familiares (5%) *vs.* 2.5%, observado en el grupo control, $p < 0.01$, y los TPO-Ab fueron positivos en 14% de los familiares *vs.* 10% del grupo control, (p = ns). En el análisis molecular, solamente se halló un rearreglo del protoncogen TRK en una de las 7 familias con CPF. En conclusión, hallamos una elevada prevalencia de bocio e hipotiroidismo en los familiares de pacientes con CPT. Sin embargo, la frecuencia de autoinmunidad no fue diferente. No se hallaron alteraciones moleculares distintivas en estos pacientes con CPF.

Palabras clave: carcinoma papilar familiar, familiares, prevalencia, bocio, hipotiroidismo

Papillary thyroid cancer (PTC) is the most frequent subtype of differentiated thyroid carcinoma, generally diagnosed in the adult population. It usually occurs in the sporadic form, but familial appearance is estimated in 3.5-6.2%[1]. An autosomal dominant inheritance pattern with incomplete penetrance, has been postulated[1-3]. Clinical characteristics of familial papillary thyroid cancer (FPTC) include an earlier age of onset, a more aggressive outcome, multifocality and more frequent relapses, although this situation is now a matter of controversy[4-9]. On the other side, the frequency of thyroid disorders in the relatives of patients with FPTC has not been extensively evaluated[10].

Biological molecular characteristics are uncertain. Recent papers have dealt with protooncogene *ret* / PTC1 rearrangements in human thyroid carcinoma and a high rate of activation has been reported in children who suf-

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fered papillary carcinoma after exposure to fall-out radiation from Chernobyl[11].

The aim of this study was to evaluate the frequency of thyroid disorders in the first degree relatives of patients with FPTC. We also performed a molecular analysis of the FPTC tumors searching for alterations in *ret*/PTC, and protooncogenes *trk* and *ras* assessed in DNA of these carcinomas.

Materials and Methods

Sixteen patients from seven unrelated kindred, who were not exposed to irradiation, with a diagnosis of FPTC were included in the study. FPTC was defined by the presence of two or more first degree relatives affected with papillary thyroid cancer.

On the other hand, seventy nine family members from these subjects, who did not live in an iodine deficient area and had no evidence of Gardner's syndrome, were prospectively evaluated for the presence of goiter. The thyroid function and the presence of thyroid autoimmunity were also assessed.

In patients with FPTC, mean age at diagnosis was 41.7 ± 13 years and the female/male ratio was 7/1. In the family members, mean age was 52.1 ± 19 years and female/male ratio was 5/1.

Thyroid palpation was performed in all the first degree relatives of the patients with FPTC. If thyroid weight was estimated to be higher than 20 g, these subjects were diagnosed as having goiter.

Two studies of references populations in Argentina were used to compare the frequencies of thyroid alterations for this study.

The "Goiter comparison population", comprising 542 healthy subjects was only used for detecting goiter through thyroid palpation and came from a publication performed previously from our group[12]. The second group ("Thyroid Antibody and Thyroid Function Frequency Group") consisted of a further 40 healthy individuals who attended the blood bank of our hospital. An aliquot of their donated blood was employed for measuring TPO-Ab and TSH[13].

A subject was defined as having primary thyroid disorders by one or more of the following diagnostic criteria: (i) goiter, (ii) positive anti-thyroid antibodies, and/or (iii) primary thyroid function abnormalities (TSH higher than 4.5 µUI/ml for the diagnosis of hypothyroidism and lower than 0.3 µUI/ml for hyperthyroidism).

TSH and TPO-Ab were assessed in 63 of 79 relatives (Table 1). Venous blood samples were drawn into dry tubes from fasting subjects in the morning, serum was separated from cells by centrifugation and kept frozen at –20 °C until analysis. TSH and antiperoxidase antibodies (TPO - Ab) were measured by electrochemiluminescent technology, with an automatic analyzer (*Roche Diagnostics Elecsys 2010 Immunoassay System*, Mannheim, Germany).

The molecular analysis in the sixteen FPTC patients was performed as follows: For the RNA extraction, the genetic material used was obtained from paraffin-embedded tissues, or peripheral lymphocytes. RNA isolation from paraffin-embedded tissues was performed according to the previously described procedure[11]. RNA was extracted from peripheral lymphocytes, using RNA isolation kit from *Promega*® (France), according to the manufacter's instructions. The quality and quantity of the RNAs was controlled by reverse transcription polymerase chain reaction (RT-PCR) amplification, using β-actin specific primers as described by Viglietto et al[14].

The reverse transcription reaction was performed on half the volume of RNA extracted from paraffin-embedded tissue extracts (18 ml) or 1.5 mg of total RNA from fresh tissue extracts. One fourth of the cDNA was used for PCR amplification with outer primers. A second round of PCR was done with nested primers using 1:10 of the first round PCR product. The PCR amplifications were performed as previously described[11], using an automatic thermocycler (*Gene Amp, Perkin Elmner*, France). Ten ml of PCR product were electrophoresed for control in a 2% agarose gel. PCR primer sequences used in this study, were those previously described: for *ret*/PTC1 to *ret*/PTC3 by Bonnacer et al[11]; for trkA, trkT1, trkT2 and trkT3 by Bonnacer et al[15] and for Ha-, Ki- or N-ras by Suarez et al[16].

The direct sequencing of the amplified DNA fragments was carried out by the dideoxy-nucleotide method[17], with ^{32}P-ATP, using the double strand DNA cycle sequencing system kit from *Gibco-BRL (Life Technologies*, France) and the same primers as those used in the amplification, following the manufacturer's specifications.

Statistical analysis: Results are expressed as percentages. Comparisons between-groups were made using the chi-square test. The level of significance was set at 0.05

Results

The pedigree showing the affected cases can be observed in Fig. 1. In those patients with FPTC, malignancy was bilateral in 6/16 (38%) patients. Lymph node metastases were diagnosed in 8/16 (57.1%) and distant (lung) metastases in 2/16 (14.3%). After total thyroidectomy and ^{131}I remnant ablation, and after a mean time of follow-up of 69 ± 48 months, 9 patients (56.2%), were considered free of disease. One patient died and 6/16 (37.5%) had persistent disease.

TABLE 1.– *Findings in 79 relatives of 16 patients with FPTC from 7 unrelated kindred*

Family	1	2	3	4	5	6	7	Total
Members evaluated	7	21	14	5	4	4	24	79
Nodular goiter	1	2	1	0	1	0	1	6
Diffuse goiter	2	4	6	1	0	1	3	17
Positive* TPO-Ab	2	3	2	0	0	0	2	9
TSH *>4.5 uUI/ml	0	1	1	0	0	1	1	4

** Serum TSH and TPO-Ab were evaluated in 63 family members*

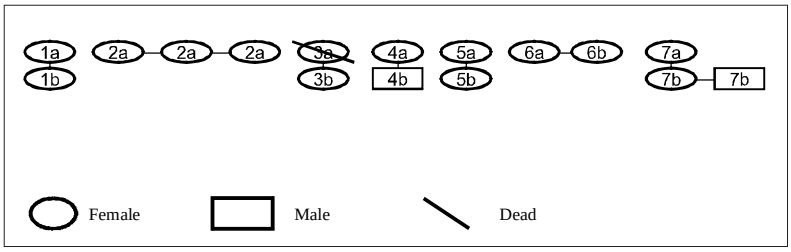

Fig. 1.– Pedigree of 16 patients with familial papillary thyroid cancer from 7 unrelated kindred included in the study.

When the molecular analysis in patients with FPTC was considered, no alterations were found by biological molecular screening, except in family # 6, in whom the presence of a rearrangement of the TRK oncogene was found. The TRK oncogene resulted from a paracentric inversion of chromosome 1 that joins the 5' end of Tropomyosin (TPM3) to NTRK1[18].

The clinical screening of the 79 family relatives of the patients with FPTC (Table 1) showed the presence of goiter in 23/79 (29%). This frequency was much higher than that observed in the "Goiter comparison population" (8.7%), $p < 0.001$. Hypothyroidism was found in 4 subjects of the relatives' group (5%) *vs.* 2.5% observed in the "Thyroid Antibody and Thyroid Function Frequency Group", ($p < 0.01$); and TPO-Ab were positive in 14% of the relatives' group *vs.* 10% in the previously mentioned population study group, (p = NS).

When the frequency of association of thyroid alterations was evaluated, it was found that 28/79 subjects (35%) had a primary thyroid alteration. From seventeen patients with diffuse goiter, 5 had positive titers of TPO-Ab and only one patient had hypothyroidism.

From the 6 subjects with nodular goiter, 2 had positive titers of TPO-Ab and none of them presented hypothyroidism.

Additionally, 3 subjects had pure thyroid autoimmunity, and 2 patients had hypothyroidism with no thyroid autoimmunity and no palpable goiter.

Discussion

The clinical characteristics of FPTC are being clarified, not only by the family studies, but also by large epidemiologic revisions. The review of the different kindred and genetic studies suggests that inheritance is autosomal dominant and that the penetrance is incomplete and increases with age[19-22]. As with sporadic PTC, women are affected approximately 2 to 3 times more frequently than men[19, 23], and the age of onset of FPTC may be younger than for sporadic papillary thyroid cancer[23].

Although efforts have been made in the last years to describe gene/s alterations responsible of FPTC, little is known on the subject. A susceptibility locus was recently shown for FPTC on chromosome 8q24[24].

As the molecular analysis in this study was performed several years ago, we did not analyze the B-RAF gene, which is frequently mutated in papillary thyroid cancer[25]. B-RAF mutations in FPTC has been shown no to be higher than that observed in patients with sporadic papillary thyroid cancer[26].

In contrast, sporadic mutations involving codons 12, 13 and 61 from *H-RAS* are rare in both follicular and papillary tumours (overall < 5%), as can also be seen in this subset of patients[27, 28]. In the present subset of patients, no alterations were found by biological molecular screening, except in one family with a TRK oncogen rearrengement.

When the prevalence of thyroid abnormalities in the relatives of patients with FPTC was considered, we found a higher frequency of goiter. We also found a higher and statistically significant difference considering thyroid function. However, the frequency of thyroid autoantibodies (TPO-Ab) was not significantly different when compared to the control group. Although not all patients were evaluated for thyroid function and TPO-Ab (63/79), when the combination of primary thyroid disorders was considered, we found that 35% of the first degree relatives of patients with FPTC had a thyroid alteration.

One recent uncontrolled study provides data derived from the use of thyroid ultrasound[10]. In this study, 149 first- and second-degree relatives of FPTC subjects were evaluated by thyroid ultrasound. The yield was 77 individuals with at least 1 nodule. All nodules were reportedly aspirated, 18 subjects underwent thyroidectomy based on aspiration results, and 15 (10% of those screened) were confirmed to have thyroid cancer (14 papillary thyroid cancer and 1 follicular thyroid cancer) at surgical resection. Three of the 18 subjects undergoing surgery had benign nodules. Therefore, in such study the yield of thyroid cancer in asymptomatic relatives of FPTC subjects seemed to be greater than that expected in the general population, where the overall prevalence is expected to be about 0.6%[29, 30].

However, there was no appropriate control group such as one composed of relatives of sporadic non FPTC subjects.

The conclusion of our study is that there is a higher incidence of goiter and hypothyroidism in the relatives of patients with FPTC when compared to a control group. No molecular abnormalities were indicative of a specific pattern in this subset of patients with FPTC.

Conflict of interest: Nothing to declare

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