CLINICAL STUDY

At least three genes account for familial papillary thyroid carcinoma: *TCO* and *MNG1* excluded as susceptibility loci from a large Tasmanian family

J D McKay¹, J Williamson², F Lesueur³, M Stark³, A Duffield¹, F Canzian³, G Romeo³ and L Hoffman¹Departments of ¹Diabetes and Endocrinology, and ²Cytogenetics and Molecular Medicine, Royal Hobart Hospital, Tasmania, Australia and³Unit of Cancer Genetic Susceptibility, International Agency for Research on Cancer, Lyon, France

(Correspondence should be addressed to L Hoffman, Department of Diabetes and Endocrinology Services, Royal Hobart Hospital, Campbell St, Hobart, Tasmania, Australia 7000)

Abstract

Recent studies have mapped two susceptibility loci which appear to account for familial multinodular goitre (*MNG1*) and a variant of familial papillary thyroid cancer (PTC), with associated multinodular goitre (*TCO*). A Tasmanian family (Tas1) has been identified with an autosomal dominant form of PTC.

This study has examined the *MNG1* and *TCO* loci to determine if they are similarly predisposing the Tas1 family to PTC. Linkage analysis using identical microsatellite markers described in the two previous studies was used to determine the significance of these loci in the Tasmanian family. The resultant LOD scores were sufficiently negative using multipoint parametric analysis to exclude these two loci from involvement in the Tasmanian family.

In addition, six candidate genes, *RET*, *TRK*, *MET*, *TSHR*, *APC* and *PTEN* were also excluded as susceptibility genes in Tas1 by using microsatellites that are positioned in or in close proximity to these genes. These results suggest that there are at least three susceptibility genes that predispose families to familial PTC.

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Introduction

Papillary thyroid cancer (PTC) is the most common form of thyroid carcinoma, accounting for over 85% of the lesions reported (1). There is increasing evidence that there is a familial component to PTC, with between 3.5–6.2% of PTC patients having one or more first degree relatives with thyroid carcinoma (2–6). Familial aggregations have been noted (7–9) and we reported the first recognised families predisposed to an autosomal dominant form of PTC with associated multinodular goitre (MNG) (10) (Fig. 1).

There are a number of candidate genes which may be involved in the development of familial PTC. These include: the *MNG1* and *TCO1* loci, two genes recently mapped to chromosomes 14q31 and 19p13.2 respectively, in familial non-medullary thyroid carcinoma families (11, 12), genes associated with the sporadic form of the cancer, the *RET* (13–15), *TRK* (16, 17) and *MET* (18) tyrosine kinases, genes associated with familial syndromes with associated PTC, the *APC* and *PTEN* tumour suppressors associated with familial adenomatous polyposis coli (19, 20) and Cowden (21) syndrome respectively and finally genes such as the *TSHR* which due to its function could be implicated in

thyroid cancer, in addition to its proven involvement in other thyroid pathologies (22, 23).

We have used a large Tasmanian family (Tas1), identified by Burgess *et al.* (10), to determine the significance of the *MNG1* and *TCO* loci and whether six possible candidate genes are predisposing this family to the PTC/MNG phenotype.

Materials and methods

Patients

The clinical and pathological features of two Tasmanian families with autosomal dominant PTC have been reported previously (10). The larger of the two families, Tas1, is displayed in Fig. 1, and represents the largest reported PTC family to date. Subsequent to the original report there has been one death in the proband due to metastatic thyroid cancer and one further patient diagnosed with thyroid cancer.

Sample extraction

Blood was collected from family individuals and all participants contributed with informed consent. DNA

Figure 1 Tas1: the Tasmanian family with familial papillary thyroid cancer.

was extracted from the white blood cells using a cell lysis and precipitation method. DNA samples were stored at -80°C until use.

Markers

Microsatellite markers which were situated in or near the *RET* (sTCL2), *TRK* (D1S1595), *MET* (D7S2817), *APC* (D5S2501), *PTEN* (D10S541) and *TSHR* (D14S606) genes and linked markers in the *MNG1* (D14S749–5.0cM–D14S1030–0.1cM–D14S1054–2.7cM–D14S611) and *TCO* regions (D19S391–2.5cM–D19S916–1.1cM–D19S413–0.6cM–D19S586) were used to genotype DNA from family members.

Amplification

Markers were amplified by PCR using fluorescent primers and standard PCR techniques (24). The fluorescent products were pooled and loaded onto a 4.8% polyacrylamide gel and run on a 377A ABI PRISM automated sequencer (Perkin-Elmer, ABI; Foster City, CA, USA). Data were automatically collected and analysed by the Genescan and Genotyper programs (Perkin-Elmer, ABI).

Analysis

Two-point linkage analysis was performed with the MLINK component of the LINKAGE program (25). Analysis was modelled using an autosomal dominant inheritance pattern with a disease allele frequency of

0.001 and a penetrance of 0.75. Multipoint analysis was performed with the Vitesse program (26) using the intervals between markers described earlier.

Results

Microsatellites located inside or in close proximity to eight candidate genes were used for linkage analysis. Loci in the *MNG1* and *TCO* regions were examined for linkage in the Tasmanian family using multipoint analysis. The most informative marker for *MNG1*, D14S1030, produced a LOD (logarithm of the odds) score of -5.037 using multipoint analysis. Similarly, the LOD score at the *TCO* locus was -6.167 for the informative marker, D19S413, again indicating that this area is not linked in the Tasmanian family. Single point analysis was used with the remaining candidate genes. The LOD scores calculated were as follows: *APC*, $\text{LOD} = -3.25$ ($\theta = 0$), *PTEN*, $\text{LOD} = -3.11$ ($\theta = 0$), *MET*, $\text{LOD} = -4.94$ ($\theta = 0$), *RET*(sTCL-2), $\text{LOD} = -5.10$ ($\theta = 0$), *TRK*, $\text{LOD} = -4.81$ ($\theta = 0$) and *TSHR*, $\text{LOD} = -3.32$ ($\theta = 0$).

These negative results strongly suggest that there is no linkage between these loci and the familial PTC seen in the Tas1 family. Therefore, it is unlikely that the genes located in the immediate area of these microsatellites are susceptibility genes in the Tas1 familial PTC patients.

Discussion

Familial PTC is an established clinical entity, as demonstrated by clinical (9) and epidemiological (27)

studies. The majority of pedigrees are small in size and a number do present with additional benign thyroid features of MNG (G Romeo *et al.*, unpublished data). Larger families with many cases of non medullary thyroid cancer (NMTC) (papillary and follicular thyroid cancer) and MNG have been reported (2, 7, 8, 10). The two susceptibility loci mapped using two of these families, *MNG1* (11) and *TCO* (12), are the only candidates that have been directly linked to familial NMTC and therefore were examined in the *Tas1*/PTC linkage study.

The negative linkage results found in this study allows for the exclusion of the *MNG1* and *TCO* loci as susceptibility loci for PTC in the *Tas1* family. This suggests that there is genetic heterogeneity in familial PTC/MNG and the existence of at least a third susceptibility gene. Further evidence for this finding is the existence of marked phenotypic differences between the three families. The *Tas1* family did not present with the distinct cell oxyphilia observed in the *TCO* family and had a much higher penetrance of PTC compared with the *MNG1* family. In light of these marked differences between the phenotypes of the families, it is not surprising that there are different predisposing genes specific for each phenotype.

As a consequence of the exclusion of both the *MNG1* and the *TCO* loci, a set of candidate genes was examined. The *RET* protooncogene is rearranged in a variable proportion of sporadic PTC tumours (13–15). In addition, *RET* mutations have been implicated in the development of familial multiple endocrine neoplasia (MEN) 2A of which medullary carcinoma of the thyroid (MTC) is the main manifestation (28) although MTC and PTC may be present (29). The association of mutations in *RET* with these diseases suggest it as a possible candidate for involvement in the development of familial PTC. However, this gene is not linked to PTC in the *Tas1* family and therefore mutations in this gene are unlikely to be a predisposing factor, a result which is in support of previous results reported for other PTC families (7). The remaining genes, *TRK*, *MET*, *APC*, *PTEN* and *TSHR* have been associated with sporadic and inherited thyroid pathologies including PTC to varying degrees (16–23). However, as in the case of *RET*, each of these genes was excluded as a candidate gene for PTC due to the lack of linkage, and therefore do not appear to be responsible for the development of familial PTC in the *Tas1* family.

In conclusion, these results suggest that there are at least three genes that are responsible for familial MNG/PTC/NMTC, with a possibility of each gene being specific for a phenotypic variant. The obvious next step is to begin a genome-wide search in order to find other genes involved in the development of this phenotype.

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