ORIGINAL ARTICLE

Evidence for interaction between the *TCO* and *NMTC1* loci in familial non-medullary thyroid cancer

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Accepted for publication 21 January 2004**Background:** Familial non-medullary thyroid cancer (fNMTC) is a complex genetic disorder that is more aggressive than its sporadic counterpart. Thus far, three genetic loci have been implicated in susceptibility to fNMTC by linkage analysis.**Methods:** We used linkage analysis to test the significance of two of the known susceptibility loci for fNMTC, *TCO* on 19p13 and *NMTC1* on 2q21 in 10 fNMTC families, nine of which present with cell oxyphilia, a rare histological phenotype associated with *TCO*. Furthermore, we used two-locus linkage analysis to examine the possibility that the *TCO* and *NMTC1* loci interact to increase the risk of NMTC.**Results:** The 10 families provided evidence for linkage at both *TCO* and *NMTC1*, with LOD scores of 1.56 and 2.85, respectively. Two-locus linkage analysis, using a multiplicative risk model for the development of NMTC, achieved a maximum LOD of 3.92, with an LOD of 4.51 when assuming 70% of families were linked, indicating that the segregation in these families is consistent with an interaction model. Most of this evidence came from a large Tyrolean family that singularly achieved a two-locus LOD of 3.21.**Conclusions:** These results provide further evidence that susceptibility genes for fNMTC exist at 19p13 and 2q21, and furthermore, raise the possibility that in a subset of fNMTC pedigrees, these loci interact resulting in significantly increased risk of NMTC for patients that carry both susceptibility loci.

Non-medullary thyroid cancer (NMTC; OMIM# 188550), including papillary thyroid cancer (PTC) and follicular thyroid cancer, is the most common form of thyroid cancer accounting for 80–90% of thyroid cancer patients.¹ NMTC is associated with some of the highest familial risks among all cancer sites, with reported risks to first degree relatives of between 5- and 10-fold,^{2–4} and many multiplex pedigrees have been identified. Consequently, familial non-medullary thyroid cancer (fNMTC) has been recognised as a distinct clinical entity, characterised by a higher degree of aggressiveness and mortality compared to its sporadic counterpart.^{5–7} The mode of inheritance for fNMTC appears, on the whole, to be autosomal dominant although the majority of families are small (sibs/trios) and therefore a multigenic inheritance pattern is plausible.^{5–8}

Thus far, three susceptibility loci have been localised by genetic linkage analysis in fNMTC, with histopathological or additional clinical features playing a key role in reducing genetic heterogeneity. The *TCO* locus on 19p13.2 was identified in a large French family with a particular cellular phenotype, cell oxyphilia (or Hurthle cells), present in a number of the tumours in the family¹⁰ (OMIM# 603386). The *TCO* linkage was subsequently confirmed in another pedigree with oxyphilia⁷ and in a family without oxyphilia.¹¹ The *PRN1* locus was localised to chromosome 1q21 in a family with PTC and additional papillary renal neoplasia¹² (OMIM# 605642). Finally, we identified a susceptibility locus on 2q21, *NMTC1*, that was suggested to account for a significant proportion of fNMTC, and appeared to be associated with another histopathological feature, the follicular variant of PTC (fvPTC)¹³ (OMIM# 606240). Linkage to the *PRN1* and the *NMTC1* loci has thus far not been replicated in independent family sets. Here, we tested the significance of the *TCO* and *NMTC1* loci in 10 families using linkage analysis and examined the possibility that these two loci interact to increase the risk of non-medullary thyroid cancer.

METHODS

Subsequent to the original publication of the *TCO* linkage on chromosome 19p13.2,¹⁰ we have identified eight additional families in which at least one tumour was noted to have cell oxyphilia, including a large fNMTC pedigree from Austria (Tyrol) with six patients with NMTC and 12 patients with goitre (fig 1). On the basis of the presence of the oxyphilia phenotype, these families were excluded from our *NMTC1* mapping study.¹³ Additionally, we identified two families from our *NMTC1* mapping study¹³ as being relevant here: one small family (201; three affected) identified as having “tall cells”, a histological phenotype very similar to oxyphilia,¹⁰ and an informative Italian family (102) in which all tumours have not yet been examined in order to include/exclude the presence of oxyphilia or fvPTC. Histopathological diagnosis was confirmed in all cases. The families are summarised in table 1. Blood samples and family history were obtained from family members after informed consent and DNA extracted (Puragene Kit, Gentra Systems) The protocol for this study was approved by the French committee for the Protection of Persons in Biomedical Research.

Genotyping

As nine of the 10 families were known to have at least one tumour with cell oxyphilia (or a similar feature), we genotyped these families for the *TCO* region on chromosome 19 as well as the *NMTC1* region on chromosome 2q21. Microsatellite markers genotyped in these families included on 2q21: D2S2224, D2S2271, D2S2215, D2S2160, D2S112, AFMa272zg9, D2S2256, D2S114, D2S368, D2S2196; and on 19p13: D19S1034, D19S901, D19S884, D19S391, D19S916, D19S586, D19S394 using standard PCR techniques with

Abbreviations: fNMTC, familial non-medullary thyroid cancer; fvPTC, follicular variant of PTC; LOH, loss of heterozygosity; PTC, papillary thyroid cancer

Table 1 Summary of fNMTC pedigrees used in this study

Family	Number of NMTC cases	Number of additional patients with goitre
103	6*†	12
128	3	0
134	2	0
188	4	0
118	3	2
39	2	0
86	3*	0
224	5	3
201	3	0
102	4†	1

*Includes one case with a Hurthle cell adenoma; †includes one case with a follicular adenoma.

fluorescent primers and genotyped using a 377 Genetic Analyzer (Applied Biosystems).

Statistical analysis

Single-locus linkage analysis

A multipoint LOD score was calculated using the Vitesse analysis program¹⁴ restricted to three markers on chromosome 19p13 (D19S884–2.5 cM–D19S391–4.3 cM–D19S586) and three on chromosome 2q21 (D2S2215–3.45 cM–D2S1260–3.69 cM–D2S112) due to the size of one of the families. Genetic distances were taken from Marshfield genetic maps¹⁵ or the genetic distance database¹⁶ and the marker order was confirmed using the human genomic sequence.¹⁷ Allele frequencies were those used in our previous linkage studies of these regions^{9–13} calculated from the larger data sets using one individual chosen from each family at random.

The genetic model chosen for each locus assumed a frequency of 0.01 for a dominantly inherited susceptibility allele at each locus and a 15-fold increased risk of NMTC (including follicular and Hurthle cell adenoma) above the assumed background rate of 0.2%. In addition, we assumed that the susceptibility allele resulted in a three-fold increased risk of goitre independent of age or sex, with a prevalence in non-carriers of the allele of 5%. Although this model is no doubt an oversimplification of the true underlying genetic basis, it captures the essential features of the effects of these two loci and satisfies constraints imposed by observed familial risk and disease prevalence. Under this model, each locus would be expected to produce a familial relative risk of 2.16, which represents about 37% of the overall familial risk of 8.0 under a multiplicative model. Together these two loci are predicted to account for 58% of the total observed familial risk of NMTC under a multiplicative model, thus leaving room for one or more additional genes contributing to the familial aggregation.

Two-locus linkage analysis

Two-locus analysis was carried out by the TMLINK program.¹⁸ We assumed the joint presence of the *TCO* and *NMTC1* susceptibility loci described above, and further assumed that the loci acted multiplicatively in terms of cancer risk (that is, an individual carrying both susceptibility alleles would have a 225-fold increased risk) and additively in terms of the risk of goitre. We also performed analyses in which only patients with cancer/adenoma were considered as affected and looked at both multiplicative and additive models. In addition to the two locus analyses, we also calculated the LOD scores at each locus, assuming the other locus was in the model. Due to the size of the Tyrolean (103) family, to allow the calculation of a two-locus LOD score that used all

available genetic data, the haplotypes of the family were recoded as a single informative locus with each haplotype assumed to be rare (fig 1A,B).

RESULTS

Evidence for linkage at the *TCO* and *NMTC* loci

For the 19p31.2 region, thought to contain the *TCO* locus, the overall maximum multipoint heterogeneity LOD score for the three markers in this region for the 10 families was 1.56 at marker D19S884, with an estimated 70% of the families linked to this region, providing further evidence of the presence of a fNMTC susceptibility gene at this locus. This was not surprising, as most of the families were selected for the presence of oxyphilia, which is a known phenotypic marker of the *TCO* locus. Somewhat more surprising, however, was our finding of linkage to the *NMTC1* locus in these pedigrees. The total LOD score using the three markers tested in this region was 2.85 at marker D2S2215, with no evidence of locus heterogeneity. This provides further strong evidence for the role of a locus in this region of 2q21 for fNMTC susceptibility. A major portion of the linkage signal at both 19p12 and 2q21 was due to a large Tyrolean pedigree (family 103). On 2q21, in this family (103) a patient who was affected with goitre (patient 103.27) is a recombinant. This patient was assumed to have received the “unaffected” haplotype because if they had received the “affected” haplotype this would be in contradiction with the critical 2q21 region outlined in the *NMTC1* study.¹³ Figure 1 shows the haplotypes for both regions in this family.

Evidence for interaction between the *TCO* and *NMTC1* loci

The finding of linkage to these two previously identified regions opens up the possibility that these two loci interact to produce a high risk of NMTC and other thyroid pathologies. In order to assess simultaneously the possibility of two loci interacting in these pedigrees, we performed two-locus linkage analysis in the 10 families using the genotype data from the *TCO* and *NMTC1* loci. Table 2 summarises the results of the two-locus linkage analyses performed. Under the model with both cancer and other thyroid pathology considered as affected, the two-locus analysis resulted in an overall LOD score of 3.92, with a value of 4.51 achieved under heterogeneity at $\alpha = 0.70$, indicating that there is strong evidence in favour of a two-locus inheritance model. The large Tyrolean family again contributed significantly to the LOD score, with an individual two-locus LOD of 3.21. Comparison of the individual LOD scores at each region showed a concordance of linkage results in nine out of the 10 families, with a Spearman rank correlation between the family LOD scores of 0.73. This is also reflected in the fact that when considering cancer only, the multiplicative model seemed to fit better than the additive model, at least for the particular models examined.

The notion of two susceptibility loci interacting in these pedigrees is further supported by an examination of the segregating haplotypes of the families in which the sharing of the NMTC cases is consistent for linkage with both loci. In the large Tyrolean family (fig 1), we see that all six cases of NMTC share both the chromosome 19 and chromosome 2 haplotypes. For the potentially related phenotype of goitre, in 5/12 cases they shared both loci, in four shared *NMTC1* only but not *TCO*, and in the remaining three shared *TCO* but not *NMTC1*. Thus, all 12 cases of goitre carried at least one of the linked haplotypes. A similar haplotype segregation pattern is present among most of the other smaller linked families (fig 2 and data not shown).

Figure 1 The haplotype of the large Tyrolean family (103) across 19p13 (A) and 2q21 (B). On 2q21, for recombinant patient 6 the position of the *NMTC1* locus is further refined to the interval between D2S1260 and AFDa272zg9. Beneath each haplotype is shown the recoding used in the two-locus linkage analysis.

DISCUSSION

The linkage analysis performed in the 10 families presented here provides further evidence that 19p13 and 2q21 harbour susceptibility genes for fNMTC. Among the 10 pedigrees presented here there is significant evidence for a two-locus inheritance pattern, indicating that among a subset

of fNMTC pedigrees the *TCO* and *NMTC1* loci may interact to result in increased risk of disease. However, the *TCO* locus appears not to be relevant to the majority of fNMTC^{9, 11} and neither the French family used to map the *TCO* locus nor the Tasmanian family used to map the *NMTC1* locus presented with any evidence for linkage at the 2q21 or

Table 2 LOD scores assuming two independent loci contribute to NMTC/goitre

	Cancer multiplicative			Cancer only					
	Goitre additive			Multiplicative			Additive		
Assume:	19	2		19	2		19	2	
	Linked	Linked		Linked	Linked		Linked	Linked	
Test:	2	19	Both	2	19	Both	2	19	Both
	Linked	Linked	linked	Linked	Linked	linked	Linked	Linked	linked
103	1.63	1.62	3.21	1.00	1.00	2.05	0.28	0.27	0.93
128	0.48	0.22	0.70	0.48	0.21	0.69	0.24	0.06	0.36
134	-0.00	-0.00	-0.01	-0.01	-0.01	-0.01	-0.00	-0.00	-0.01
188	-0.52	-0.55	-1.07	-0.52	-0.55	-1.06	-0.28	-0.29	-0.45
118	0.45	0.18	0.61	0.20	0.20	0.41	0.09	0.09	0.20
39	0.17	0.17	0.34	0.18	0.18	0.35	0.09	0.09	0.20
86	0.24	-0.52	-0.29	0.23	-0.50	-0.28	0.18	-0.12	0.00
224	-0.71	-0.28	-0.96	-0.80	-0.34	-1.14	-0.30	-0.17	-0.39
201	0.23	0.23	0.46	0.23	0.23	0.46	0.10	0.10	0.23
102	0.37	0.60	0.94	0.47	0.47	0.95	0.17	0.17	0.45
Total	2.33	1.66	3.92	1.47	0.90	2.42	0.56	0.20	1.51
HLOD	2.39	1.78	4.51	1.59	1.06	3.17	0.5	0.21	1.51
(α)	(0.85)	(0.7)	(0.7)	(0.85)	(0.7)	(0.7)	(1.0)	(0.8)	(1.0)

19p13 loci, respectively.^{9 13} Therefore, it is unlikely that the *TCO* and *NMTC1* loci contribute to NMTC risk together among the majority of fNMTC pedigrees. Nevertheless, our results indicate that such an interaction may occur in a reasonably large subset of fNMTC and a two-locus mode of inheritance is consistent with the observations made of fNMTC^{5 6 8 9} and segregation analysis in other complex genetic forms of hereditary cancer fit a two-locus model.¹⁹ 2q21 has been noted as an area of interest in a genome wide search on a Canadian *MNG1* family,²⁰ and

it would be interesting to review these data in the context of the two-locus model outlined here, especially with respect to the three NMTC cases of the Canadian *MNG1* family.²⁰ Similarly 2q21 was implicated in a large family with Gardners syndrome²¹ in which NMTC has been found to be an associated tumour.²² Given the reported lack of biallelic inactivation of the *APC* gene in the thyroid carcinomas associated with familial adenomatous polyposis²³ and the reported cytogenetic alteration in the NMTC1 region,²¹ it is possible that the NMTC1 locus could

Figure 2 Family 102. This family of Italian background also presents with a haplotype that segregates on both the 2q21 and 19p13 loci. The histopathology in this family was reported to be PTC but complete information was unavailable.

be put forward as a modifier of NMTC susceptibility in this inherited syndrome. Examination of interaction models between the identified fNMTC loci and other genes that have been associated with NMTC risk, for example *RET*²⁴ and more recently thyroglobulin²⁵ in the wider familial NMTC without oxyphilia and sporadic NMTC may also be warranted.

Genetic heterogeneity has been reduced in fNMTC family sets by stratification on the presence of the histological variants of oxyphilia⁷ and fvPTC.¹³ Some families outlined here (for example families 118, 128, and 201; table 1) present with both the oxyphilia and fvPTC phenotypes. The evidence for linkage at both loci in this subset is therefore consistent with a stratification based on the presence of both the histological variants. Thorough histopathological data on all affected members of all pedigrees was not available for independent review to explore this observation further.

Loss of heterozygosity (LOH) on chromosome 2 has been noted in oxyphilic NMTC tumours²⁶ and at 2q22²⁷ suggesting further the *NMTC1* locus may be implicated in the pathology associated with the *TCO* locus. In our own material from sporadic NMTC tumours, 35/71 (49%) oxyphilic tumours and adenomas and NMTC with oxyphilic features exhibited LOH at 2q21 while only 38% of these tumours showed LOH at 19p13. Similarly in 20 follicular adenomas/carcinomas, 30% and 15% showed LOH at *TCO* and *NMTC1*, respectively (Stankov, unpublished data). However, in neither of the two sets of tumours was the number of tumours showing concordant LOH at both loci different from that expected under independence. Unfortunately, material was unavailable to test for allelic imbalance in the familial cases of this study.

These results provide further evidence for fNMTC susceptibility genes at 2q21 and 19p13.2. Furthermore, there is evidence that *TCO* and *NMTC1* may interact to increase risk in individuals that inherit both susceptibility genes. The identification of the NMTC susceptibility genes at both 2q21 and 19p13.2 will allow the contribution of these two genes to the risk of developing NMTC to be fully characterised.

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The URL for data in this report is as follows: Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for NMTC, *TCO*, *PRN1*, and *NMTC1*).

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