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

J D McKay, D Thompson, F Lesueur, K Stankov, A Pastore, C Watfah, S Strolz, G Riccabona, R Moncayo, G Romeo, D E Goldgar

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 NMTC 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 histopathological 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. 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, 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. The mode of inheritance for fNMTC appears, on the whole, to be autosomal dominant although the majority of families are small (sib/sib/stis) and therefore a multigenic inheritance pattern is plausible.

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 family (OMIM# 603386). The TCO linkage was subsequently confirmed in another pedigree with oxyphilia and in a family without oxyphilia. The PRN1 locus was localised to chromosome 1q21 in a family with PTC and additional papillary renal neoplasia (OMIM# 605642). Finally, we identified a susceptibility locus on 2q21, NMTC1 (OMIM# 606240), 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) (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, 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. Additionally, we identified two families from our NMTC1 mapping study as being relevant here: one small family (201; three affected) identified as having “tall cells”, a histological phenotype very similar to oxyphilia, 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; PTC, papillary thyroid cancer; fvPTC, follicular variant of PTC; LOH, loss of heterozygosity; PTC, papillary thyroid cancer

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room for one or more additional genes contributing to the risk of NMTC under a multiplicative model, thus leaving are predicted to account for 58% of the total observed familial risk and disease prevalence. Under this model, each 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. 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.

Table 1 Summary of fNMTC pedigrees used in this study

<table>
<thead>
<tr>
<th>Family</th>
<th>Number of NMTC cases</th>
<th>Number of additional patients with goitre</th>
</tr>
</thead>
<tbody>
<tr>
<td>103</td>
<td>6†</td>
<td>12</td>
</tr>
<tr>
<td>128</td>
<td>3</td>
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<tr>
<td>56</td>
<td>3†</td>
<td>0</td>
</tr>
<tr>
<td>224</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>201</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>102</td>
<td>4†</td>
<td>1</td>
</tr>
</tbody>
</table>

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

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 fNMTC 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 σ = 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).
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 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 19p13 loci.

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 NTMC1 locus is further refined to the interval between D2S1260 and AFMa272g9. Beneath each haplotype is shown the recombination used in the two-locus linkage analysis.
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 and segregation analysis in other complex genetic forms of hereditary cancer fit a two-locus model. 2q21 has been noted as an area of interest in a genome wide search on a Canadian MNG1 family, 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. Similarly 2q21 was implicated in a large family with Gardner’s syndrome in which NMTC has been found to be an associated tumour. Given the reported lack of biallelic inactivation of the APC gene in the thyroid carcinomas associated with familial adenomatous polyposis and the reported cytogenetic alteration in the NMTC1 region, it is possible that the NMTC1 locus could contribute to NMTC.

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

<table>
<thead>
<tr>
<th>Cancer multiplicative</th>
<th>Cancer only</th>
</tr>
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<td>Goitre additive</td>
<td>Multiplicative</td>
</tr>
<tr>
<td>Assume:</td>
<td>Linked</td>
</tr>
<tr>
<td>Test:</td>
<td>Linked</td>
</tr>
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<tr>
<td>102</td>
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<td>HLOD</td>
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<td>(x)</td>
<td>(0.85)</td>
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</tbody>
</table>

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 INMTC loci and other genes that have been associated with NMTC risk, for example RET\(^{14}\) and more recently thyroglobulin\(^{25}\) in the wider familial NMTC without oxyphilia and sporadic NMTC may also be warranted.

Genetic heterogeneity has been reduced in INMTCN family sets by stratification on the presence of the histological variants of oxyphilia and fVPTC.\(^{11}\) 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 tumours\(^{26}\) and at 2q22\(^{2}\) 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 INMTC 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 NTMC, TCO, PRN1, and NMTC1).

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Conflict of interest: none declared.

REFERENCES


