Genetic polymorphism of CYP2D6 influences susceptibility to papillary thyroid cancer

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Summary

Objective Xenobiotic-metabolizing enzymes are widely polymorphic and confer interindividual variation in the ability to detoxify carcinogens or to activate pro-carcinogens. A common polymorphism of cytochrome P450 2D6 (CYP2D6) results in lack of enzyme activity and has been associated with an altered susceptibility to several cancers. The aim of this study was to investigate the association between the CYP2D6 poor metaboliser genotype and the risk of papillary thyroid cancer (PTC).

Design Retrospective case-control study.

Patients One hundred and eighty-seven patients with PTC and 256 controls.

Measurements Genotyping was performed by PCR and restriction enzyme analysis to detect the presence of the common CYP2D6*4 poor metaboliser allele.

Results The frequency of individuals with the homozygous poor metaboliser genotype was lower in the patient group [1.6% vs. 5.5%, P = 0.037, OR = 0.28 (95% CI 0.09–0.93)]. The CYP2D6*4 allele frequency was also lower in the patient group [13.4% vs. 21.7%, P = 0.002, OR = 0.56 (95% CI 0.39–0.80)].

Conclusions The results suggest that the poor metaboliser genotype is associated with a protective effect against PTC. This could be explained by a possible role of CYP2D6 on the metabolic activation of putative environmental chemical thyroid carcinogens or by linkage to another cancer-causing gene. Further research may allow the identification of metabolic risk factors and contribute towards understanding the molecular mechanisms involved in thyroid carcinogenesis.

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Introduction

Papillary thyroid cancer (PTC) is the most common form of thyroid cancer but its aetiology is still uncertain. Exposure to radiation is a recognized causative factor for PTC but no clear role for chemical carcinogenesis has been established in humans. Several chemicals have been shown to be able to cause thyroid tumours in rodents, so it is conceivable that these or others may also play a role in the development of thyroid cancer in humans.

Xenobiotic-metabolizing enzymes constitute one of the first lines of defence against environmental chemicals, and polymorphisms of genes encoding several of these enzymes lead to interindividual differences in enzymatic activity and have been linked to different susceptibilities to several cancers. The cytochrome P450 2D6 (CYP2D6) is one of the most thoroughly studied enzymes, as lack of its activity is the basis for adverse events occurring during therapy with some drugs. About 5–10% of Caucasians lack any functional CYP2D6 alleles (poor metaboliser genotype) and this is mostly due to the presence of an inactivating intron 3 donor splice-site nucleotide variation. The CYP2D6 poor metaboliser genotype has been reported to influence susceptibility to a number of cancers, but no studies have yet been presented for thyroid cancer.

The aim of this study was to investigate the association between the CYP2D6 poor metaboliser genotype and the risk of papillary thyroid cancer.

Materials and methods

Subjects

A retrospective case-control study was performed involving 187 patients with papillary thyroid cancer (39 males 148 female patients, mean age ± SD, 44.5 ± 15.4 years) and 256 controls (150 male, 106 female patients, mean age ± SD, 36.8 ± 13.7 years) consisting of healthy volunteers recruited among blood donors and hospital and faculty staff. Age and gender have previously been shown not to influence CYP2D6 allele frequencies. Patients treated at the University Hospital of Coimbra were consecutively included in the study at the time of radioiodine therapy, during the period 1998–2003, and were selected on the basis of histologically confirmed presence...
of one of the two major subtypes of PTC: the classical form and the follicular variant of PTC. Tumour TNM classification and staging followed the International Union Against Cancer (UICC) and the American Joint Committee on Cancer (AJCC) guidelines.12,13 All subjects were Caucasian Portuguese and gave their informed consent.

Genotyping

Leucocyte DNA was isolated from peripheral blood samples using previously described methods.14 The detection of the recessive poor metaboliser allele CYP2D6*4, bearing the c.506→1G→A transition (nucleotide numbering according to GenBank cDNA sequence NM_000106, with nucleotide +1 corresponding to the A of the ATG translation start codon), was carried out as previously described.15 Briefly, genomic DNA was used in PCR amplification reactions to generate a 334 bp fragment containing the polymorphic nucleotide. PCR was performed using CYP2D6-specific primers consisting of a forward primer 5’-GCTTCGCCAACACTCCG-3’ and a reverse primer 5’-AAATCCTGTCTTCCGAGGC-3’, and using 30 cycles with an annealing temperature of 60 °C and with 1·5 mM MgCl₂. PCR products were digested with BstNI and the presence of the c.506→1G→A polymorphism was detected by loss of the restriction site, whereas absence of this polymorphism resulted in 230 and 104-bp-size fragments. Individuals were classified as homozygous extensive metabolisers (two functional alleles), heterozygous extensive metabolisers (one functional allele) or poor metabolisers (no functional alleles). Analysis of allele frequencies revealed a lower frequency of the poor metaboliser allele CYP2D6*4 in the patient group [13·4 vs. 21·7%, P = 0·002, OR = 0·56 (95% CI 0·39–0·80)]. Table 2 presents the results of the comparison of subgroups of patients with different disease severity. Genotype and allele frequencies were not significantly different between patients with the classical and the follicular variant of PTC, between patients with unifocal and multifocal tumours, or between patients with stage I/II and stage III/IV tumours.

Discussion

The results showed a lower frequency of the CYP2D6 poor metaboliser homozygous genotype in the PTC group, when compared to the control group. Thus, in our population the poor metaboliser genotype was associated with a protective effect against PTC, and individuals with this genotype appear to have an almost fourfold decrease in risk of PTC. While the functional significance of this finding is unclear, similar results have been reported for tumours at other sites, such as lung cancer and leukaemia, for which chemical carcinogens are known contributors.15,16 Other studies have suggested that the poor metaboliser genotype is associated with an increased risk for several types of cancer.8 It is possible that the risk genotype varies not only with the type of cancer, but also with ethnic and geographical contexts that reflect different genetic backgrounds and environmental exposures.

Although the cytochrome P450 enzymes are generally regarded as detoxifying enzymes that protect the organism from toxic products, it should be noted that, in many cases, this metabolism forms toxic intermediate compounds that are more harmful than the original substrate, before further transformation is carried out by additional enzymatic systems.17 Several environmental carcinogens require metabolic activation by cytochrome P450 enzymes to exert deleterious effects;18 thus, decreased transformation of pro-carcinogens by poor metabolisers could result in a protective effect against these carcinogens.

Table 1. CYP2D6 genotype and allele frequencies in patients with papillary thyroid cancer and in controls

<table>
<thead>
<tr>
<th></th>
<th>EM</th>
<th>HEM</th>
<th>PM</th>
<th>Alleles</th>
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<tbody>
<tr>
<td>Controls (n = 256)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Genotypes [n (%)]</td>
<td>159</td>
<td>83</td>
<td>14</td>
<td>401 (78·3), 111 (21·7)</td>
</tr>
<tr>
<td></td>
<td>(62·1)</td>
<td>(32·4)</td>
<td>(5·5)</td>
<td></td>
</tr>
<tr>
<td>Patients (n = 187)</td>
<td>140</td>
<td>44</td>
<td>3</td>
<td>324 (86·6), 50 (13·4)</td>
</tr>
<tr>
<td></td>
<td>(74·9)</td>
<td>(23·5)</td>
<td>(1·6)</td>
<td></td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td></td>
<td></td>
<td>0·28 (0·09–0·93)</td>
<td>0·56 (0·39–0·80)</td>
</tr>
<tr>
<td>P-value</td>
<td></td>
<td></td>
<td>0·037</td>
<td></td>
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n, number; EM, homozygous extensive metaboliser (two functional alleles); HEM, heterozygous extensive metaboliser (one functional allele); PM, poor metaboliser (no functional alleles); OR, odds ratio; CI, confidence interval. P-values were calculated by χ²-analysis.

Results

The CYP2D6 genotype and allele observed frequencies in patients and controls are presented in Table 1. Genotypes were in Hardy–Weinberg equilibrium. The frequency of individuals with the poor metaboliser genotype was lower in the patient group than in the control group [1·6 vs. 5·5%, P = 0·037, OR = 0·28 (95% CI 0·09–0·93)].
Polymorphisms of other xenobiotic-metabolizing enzymes such as glutathione S-transferase mu-1 and theta-1 have also been associated with different susceptibilities to thyroid cancer and this greatly supports the role of xenobiotic-metabolizing enzymes in thyroid carcinogenesis. A role for chemical carcinogenesis in PTC has not yet been established in humans, although animal experiments have identified several compounds with the potential to cause thyroid tumours in vivo. Chromosomal rearrangements are frequent findings in thyroid cancer cells and are considered to play a role in the aetiology of PTC. It is of interest to note that chromosomal instability is influenced by polymorphisms of xenobiotic-metabolizing enzymes.

An alternative explanation for the findings would be the existence of linkage disequilibrium of the CYP2D6 locus at chromosome 22q13.1 with other neighbouring genes with a yet unidentified role in thyroid carcinogenesis. Our study also investigated whether the presence of the protective CYP2D6 polymorphism was associated with a less aggressive type of papillary carcinoma or advanced disease. Genotype and allele frequencies between the two major histological subtypes of PTC were not different, despite the different morphology, cytogenetic and molecular characteristics, and the more aggressive clinical behaviour of the follicular variant. Similarly, no difference was found between lower and higher tumour stage, or between unifocal and multifocal PTC.

In conclusion, our findings suggest an influence of CYP2D6 polymorphisms on the susceptibility to PTC that could be explained by an effect of CYP2D6 on the metabolic activation of putative environmental chemical thyroid carcinogens or by linkage to another cancer-causing gene. Further research in this field may allow the identification of metabolic risk factors and contribute towards understanding the molecular mechanisms involved in thyroid carcinogenesis.

Table 2. Subgroup analysis according to severity of the disease

<table>
<thead>
<tr>
<th>Genotypes [%]</th>
<th>EM</th>
<th>HEM</th>
<th>PM</th>
<th>Alleles [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classical PTC (n = 140)</td>
<td>104 (74·3)</td>
<td>35 (25·0)</td>
<td>1 (0·7)</td>
<td>243 (86·8)</td>
</tr>
<tr>
<td>Follicular variant of PTC (n = 47)</td>
<td>36 (76·6)</td>
<td>9 (19·1)</td>
<td>2 (4·3)</td>
<td>81 (86·2)</td>
</tr>
<tr>
<td>Unifocal tumour (n = 108)</td>
<td>78 (72·2)</td>
<td>30 (27·8)</td>
<td>0</td>
<td>186 (86·1)</td>
</tr>
<tr>
<td>Multifocal tumour (n = 79)</td>
<td>62 (78·5)</td>
<td>14 (17·7)</td>
<td>3 (3·8)</td>
<td>138 (87·3)</td>
</tr>
<tr>
<td>Tumour stage I/II (n = 145)</td>
<td>109 (75·2)</td>
<td>33 (22·7)</td>
<td>3 (2·1)</td>
<td>251 (86·6)</td>
</tr>
<tr>
<td>Tumour stage III/IV (n = 42)</td>
<td>31 (73·8)</td>
<td>11 (26·2)</td>
<td>0</td>
<td>73 (86·9)</td>
</tr>
</tbody>
</table>

\(P\)-values were calculated by Fisher's exact test for genotype frequencies and by \( \chi^2 \)-analysis for allele frequencies.

References

CYP2D6 polymorphisms and papillary thyroid cancer


