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Proline homozygosity in codon 72 of *TP53* is a factor of susceptibility to nasopharyngeal carcinoma in Tunisia

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Abstract A common polymorphism at codon 72 of *TP53*, the gene encoding the tumor suppressor protein p53, encodes either arginine or proline. These variants may be associated with tumor susceptibility since they interfere with the ability of *TP53* to activate apoptosis, and might account for ethnic variation in cancer frequency. Using a polymerase chain reaction–restriction fragment length polymorphism assay, we tested peripheral blood samples from 115 patients with nasopharyngeal carcinoma (NPC) and from 83 healthy individuals. Patients with NPC (Arg/Arg = 38.26%, Arg/Pro = 41.73%, and Pro/Pro = 20%) showed a significantly different percentage of the Pro/Pro genotype, compared with the control population (Arg/Arg = 39%, Arg/Pro = 54%, and Pro/Pro = 7%) (P = 0.0307). No significant difference was observed between *TP53* codon 72 polymorphism and age, sex, histological grade, and metastasis. These results provide evidence that individuals with the Pro/Pro genotype have an increased risk of developing NPC in Tunisia.

1. Introduction

Nasopharyngeal carcinoma (NPC), an epithelial malignancy, occurs with a high incidence in Southeast Asia and is rare in Europe and North America [1,2]. North Africa represents an area of intermediate incidence. In Tunisia, for example, the incidence rate of NPC is 3/100,000 persons per year, with a bimodal age distribution of 10–25 (20%) and 45–50 (80%) that is not observed in the endemic region of China [3,4]

NPC is thought to be the combined result of genetic susceptibility, environmental factors such as carcinogens, and infection with Epstein–Barr Virus (EBV) [5–7]. Possible environmental or cultural factors that may be associated with NPC include the ingestion of Cantonese-style salted fish and preserved foods containing carcinogenic nitrosamines, especially during childhood [3]. Moreover, detection of the EBV nuclear antigen and viral DNA in NPC has revealed that EBV can infect epithelial cells and participate in their transformation [7].

Because of its role as a tumor suppressor, *TP53*, the gene that encodes for the p53 protein, is one of the most extensively studied human genes [8]. *TP53* is altered in 50% of human cancer, and mutations are clustered in exons 5–8, which are involved in p53–DNA interactions [9,10]. In contrast to other types of human cancer, *TP53* is rarely mutated in NPC, although the accumulation of p53 protein was reported in many NPC cases, particularly in the juvenile form [11–13]

In addition to gene mutations, several reports have focused on *TP53* polymorphisms as risk factors for malignant disease [14]. The most informative polymorphism in the *TP53* gene is located in exon 4 at codon 72, with proline (CCC) or arginine (CGC) creating three distinct genotypes: homozygous for arginine (Arg/Arg), homozygous for proline (Pro/Pro), and heterozygous (Pro/Arg) [15,16]. Dumont et al. [17] reported that this single nucleotide polymorphism at codon 72 affects function of the p53 protein. They have shown that the Pro72 variant exhibits a lower ability to induce apoptosis in vitro than does

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Arg72. The enhanced apoptotic potential of the Arg72 variant seems to be related to the localization of this variant in mitochondria [17].

Several groups have reported an association between the Arg72 variant and an increased risk for gastric [18] and breast cancers [19,20], among others. This association remains very controversial since the role of this single nucleotide polymorphism did not show any significant effect on the pathology and prognosis of pancreatic, testicular, and prostate cancers [21,22]. Data from other studies, however, indicate that this *TP53* polymorphism is related to the prognosis of some types of cancer. In fact, the Arg72 homozygosity was considered a risk factor for cervical cancer [23]; the Pro72 homozygous genotypes were related to a higher risk of thyroid [24], lung [25], and hepatocellular cancers [26]; and the heterozygous genotype was associated to an increased susceptibility for bladder [27] and smoke-induced lung adenocarcinoma [28].

Few studies have investigated the *TP53* codon 72 polymorphism and NPC susceptibility. In two independent studies performed in the south of China and in Hong Kong, the researchers found no association between the *TP53* polymorphism and cancer susceptibility [29,30]. On the other hand, a study among Taiwanese suggested that individuals with Pro/Pro genotype were at high risk of NPC development [31]. Recently, Tiwawech et al. [32] reported the absence of significant association between the *TP53* polymorphism codon 72 and NPC risk, or cancer status, in Thai patients; however, when patients and control subjects were compared by age, the Pro/Pro genotype carriers of age >40 were more prone to develop NPC than those who carried Arg/Arg or Arg/Pro genotype.

Our objective was to investigate whether the *TP53* codon 72 polymorphism can be a risk factor associated with NPC and its relationship to cancer status in Tunisian patients.

2. Materials and methods

2.1. Study subjects

Peripheral blood samples for 115 patients with NPC were collected from radiotherapy department of Sfax Hospital in Tunisia. The age of patients ranged from 11 to 72 years. Clinical parameters (stage, TNM, metastasis, clinical outcome) were available for only 50 of the patients. For all patients, the NPC was histologically proven, based on the criteria of the World Health Organization (WHO). There were 4 cases of WHO type I (squamous cell carcinoma), 6 cases of WHO type II (nonkeratinizing carcinoma), and 40 cases of WHO type III (undifferentiated carcinoma). Tumor staging was based on the TNM tumor classification, with 19 cases of NPC stages I and II and 21 cases of NPC stages III and VI. Nine patients (18%) developed metastasis, and the disease-free survival percentage was 80%. The control group consisted of 83 age-matched, healthy Tunisian people.

2.2. Polymerase chain reaction–restriction fragment length polymorphism

Genomic DNA was extracted from blood samples with the Wizard genomic DNA purification kit (Promega, Madison, WI) according to the manufacturer's instructions. Amplification of the region spanning exons 4–6 by polymerase chain reaction (PCR) was performed with forward primers 5′TCC CCC TTG CCG TCC CAA GC 3′ and reverse primers 5′GTC AGG CGG CTC ATA GG 3′ according to the method described by Baccouche et al. [33]. Reactions were done in a 50-μL mixture containing DNA aliquot (500 ng), 1× PCR buffer, 1 μmol/L of each appropriate primers, 200 μmol/L of each dNTP, 2 mmol/L $MgCl_2$, and 0.5 U GoTaq DNA polymerase (Promega). The PCR conditions were set as initial denaturation at 95°C for 5 minutes followed by 40 cycles containing 94°C at 30 seconds, 55°C for 30 seconds, and 72°C for 2 minutes. The amplicon (2,100 bp) was digested overnight by the *Bst*UI restriction enzyme (Promega) and the products were analyzed on 2% agarose gel.

2.3. Statistical analyses

Statistical analysis was performed using the χ^2 test (Web Chi Square Calculator; http://hg.wustl.edu/info/linkage/web-chi/web-chi-form5.htm). A value of $P < 0.05$ was considered statistically significant.

3. Results

We analyzed blood samples of 115 NPC patients and 83 healthy individuals, to evaluate the association between the *TP53* codon 72 polymorphism and NPC susceptibility in Tunisian patients. DNA regions from exons 4 to 6 from the *TP53* gene were amplified by PCR, then digested by *Bst*UI, to identify the *TP53* genotypes.

The distribution of the *TP53* genotypes in NPC patients and control subjects is presented in Table 1. The frequencies of the three genotypes were as follows: Arg/Arg = 38.26%, Arg/Pro = 41.73%, and Pro/Pro = 20% in NPC patients and Arg/Arg = 39%, Arg/Pro = 54%, and Pro/Pro = 7% in healthy control subjects (Table 1). The Pro/Pro genotype was significantly more frequent in NPC patients (23/115) than in healthy individuals (06/83) ($P = 0.0307$), but no significant difference was observed regarding the allele distribution: 59.13% Arg and 40.86% Pro in NPC patients and 65.6% Arg and 34.3% Pro in healthy control subjects.

The frequency of the heterozygous genotype was similar in control subjects (45/83) and patients (48/115). The homozygous genotypes were more frequent in NPC patients (67/115) than control subjects (38/83), but this difference was not statistically significant ($P < 0.1 > 0.05$).

Among the 50 patients with available clinical parameters, there was no significant association between this