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Association of NKX2-1 rs944289 SNP with DTC in sudanese population.

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Abstract

The majority of malignant thyroid tumors derive from follicular epithelial cells. The Differentiated Thyroid Cancers (DTC) are papillary (PTC: 85% of cases) and follicular (FTC: 10% of cases) subtypes, and their etiology is still not well characterized. The transformation of normal into malignant tissue is a multifactorial process involving genetic and environmental factors such as radiation exposure. Some environmental factors such as hepatitis C infection or late and multiple pregnancies have been suggested as risk factors, but they have not been confirmed. Moreover, genetic components-for example proto-oncogenes, which encode Receptor Tyrosine Kinases (RET) and small GT Pases (RAS)-may contribute to thyroid cancer formation in familial syndromes. However, none of these factors is thyroid specific, and therefore the identification of additional candidate genes is important for a better understanding of the pathogenesis of thyroid cancer.

Keywords: Thyroid tumors, Hepatitis, Pathogenesis, Antibodies.

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Introduction

In this context, key genes involved in thyroid organogenesis, for example the thyroid transcription factors (TTFs), have been identified as genetic susceptibility factors of various thyroid conditions. This illustrates the pivotal function of these genes in the development of thyroid diseases. Recently, three genome wide association (GWA) studies demonstrated associations of single nucleotide polymorphisms (SNPs) located near the TTF-1 (also known as thyroid-specific enhancer-binding protein: NKX2-1, rs944289) and TTF-2 (also known as forkhead box protein: FOXE1, rs965513) loci with an increased risk of sporadic thyroid carcinoma in Icelandic, British, and Japanese populations [1]. Radiation-related PTC of Belarusian patients showed the strongest association only with the FOXE1 gene. In order to elucidate the role of TTFs as susceptibility genes in DTC of the German population,

In the present case-control study, we investigated the SNP near FOXE1 gene (rs965513). In addition, we measured thyroid hormones, thyroglobulin and thyroid peroxidase antibodies for patients and explored whether the tumor stage in the thyroid gland modified the risk of DTC in Sudanese population.

Methods and Materials

A total of 75 patients with a pathologically confirmed diagnosis of DTC (papillary, PTC; follicular, FTC), 48 females (64%), 27 males (36%) and known tumor stage were recruited from the Nuclear Hospital in Khartoum-Sudan. and 100 healthy controls (HC), 62 females (62%), 38 males (38%) were volunteer blood donors from the staff personnel and medical students from Sharg Alneel University-Khartoum-Sudan [2]. The study protocol was approved by the Ethics Committee of the Sudan ministry of health, and informed consent was obtained from all participants.

SNP selection

In total, two SNPs (rs965513 and rs944289) previously associated with thyroid cancer susceptibility in the Icelandic population were investigated. While the rs965513 variant (A/G; Chromosome 9) is located 59 kb centromeric to the FOXE1 gene, the rs944289 variant (C/T; Chromosome 14) is situated 337 kb telomeric to the NKX2-1 gene. The SNP positions are given according to the National Center for Biotechnology Information (NCBI).

Data Analysis

Demographical data, clinical data and other baseline information were collected using semi-structured questionnaire. 3 ml venous blood samples on EDTA container from both groups. Thyroglobulin (TG) and Thyroid Peroxides (TPO) Antibodies (Abs) were measured for patients. Riming of blood samples were storage and used for genetic analysis (DNA extraction and PCR).

Measurements of thyroglobulin Ab and thyroid peroxidase Ab

Thyroglobulin (TG) and Thyroid Peroxidase (TPO) Antibodies (Abs) were measure using an enzyme-linked immunosorbent assay (reference range of Sudanes).

DNA extraction

Genomic DNA was extracted/purified from blood samples by QIAamp DNA blood Mini Kit (Qiagen Inc.), yield DNA was re-suspended in 200 µl of 1X TE-buffer and stored at -20˚C. The quality and concentration of extracted DNA was determined by Nano-drop spectrophotometer apparatus.

Genomic DNA from whole blood containing ethylene di amine tetra acetic acid (EDTA) was isolated by salting out procedure and subjected to by T-ARMS PCR in a thermal cycler (major sciences USA). The PCR reaction mixture of 25 ul in a 0.2 ml PCR tube containing 1 ul approximately 100 ng/ul of DNA template, master mix (12.5 mM $MgCl_2$) 4 µl (5X FIREPol (R) Master Mix- Solis BioDyne), 1 µl primer mix (each primer of 10 pmol/µl) and up to 19 µl water (PCR grade). PCR reaction was run according to the following conditions; holding time 95˚C for 5 min, followed by 30 cycles of denaturation at 95°C for 30 sec, annealing at 58˚C for 30 sec, extension at 72˚C for 30 sec and final extension at 72˚C for 10 minutes. The amplified products of each reaction were separated on 6% PAGE stained by 0.1% silver nitrate to visualize the bands.

Statistical Analysis

Genotype and allele distributions between groups were evaluated by chi-square test statistical package for social science (SPSS v20.00). In order to elucidate the role of TTF1 as susceptibility gene in DTC of the Sudanese population, the comparison of the genotype/ allele frequencies between cases and controls were analyzed for each group separately.

Results

In this study, patients with DTC (n=75) had 81% papillary and 19% follicular subtypes, and there were more females (64%) than males (36%). All genotypes were in Hardy–Weinberg equilibrium (p>0.05) for the two investigated SNPs. All genetic analysis in this reteach has been done for FOXE1 rs965513 SNP and NKX2-1 rs944289 SNP.

Genotype and allele analysis forNKX2-1 rs944289 and DTC

The case-controls analysis for NKX2 rs944289 SNP showed that the genotype "TT" was more frequent in patients with DTC than in HC (50.7% vs. 13%, P value=0.000). While the genotypes "CC" and "CT" (0% vs. 22% and 49.3% vs. 65.0%) respectively were less. Additionally, the allele "T" was observed more often in DTC patients than in HC (73.3% vs. 45.5%, P value=0.000).

Comparison of thyroid quantitative parameters means between the tumors stages in patients

In all DTC patients, thyroid antibodies (TPO-Ab/TG-Ab) and thyroid hormones (T3, T4 and TSH) levels were available. A comparison of TG-Ab, T3, T4, and TSH levels within tumor stages shown that there were no significant differences, P-value = (0.2, 0.2, 0.5 and 0.8) respectively. In contrast there was a significant differences in TPO-Ab levels within tumor stages P-value = 0.02. Those differences have been shown very clear which shown the significant differences between stage1 and stage2 (P-value=0.03) and stage 1 and stage3 (P-value = 0.005).

Genotype and allele analysis of NKX2-1 in DTC according to tumor stages

The patients were grouped according to the tumor stages to: (stage1, stage2, stage3 and stage4). The frequency of genotypes and alleles of rs944289 in comparison with tumor stages did not reach the level of significance, P value: 0.4 (Figure 1).

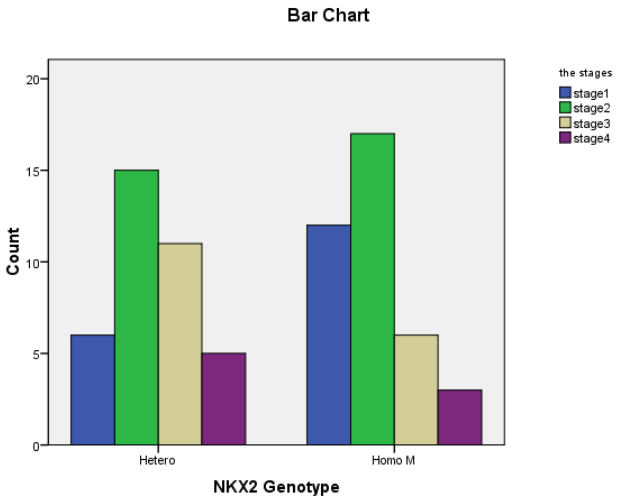

Figure 1. *Comparison of NKX2 Genotype and the stages.*

Discussion

The rs944289 SNP is situated in a 249-kb LD-region. Neighboring genes of that region are Breast Cancer Metastasis Suppressor1 like (BRMS1L), MAP3K12 binding inhibitory protein 1 (MBIP), surfactant associated 3 (SFTA3), and NKX2-1 [3]. Among these genes, NKX2-1 is a suitable candidate gene for thyroid cancer risk because of its role in thyroid development, and its altered expression in thyroid tumors.

My results demonstrated that NKX2-1 rs944289 SNP was associated with DTC in Sudan. Genetic analysis for NKX2-1 rs944289 SNP shown that genotype "TT" and allele "T" were more frequent in Sudanese DTC patients [4]. Significant association of NKX2-1 rs944289 SNP and DTC in Sudanese population agree with many studies done in different countries and populations.

We did not found any association between NKX2-1 rs944289 SNP and tumor stages. In spite there was a limited number of patients had high thyroid antibody levels, We did not found a significant differences between TG-Ab levels and thyroid hormones levels within patient's tumor stages, but there were a significant differences in TPO-Ab levels between patient's tumor stages [5]. However, there were a limited number of patients had high thyroid antibody levels, future and sufficiently powered studies must address whether the NKX2-1 rs944289 SNP was involved if DTC develops in the background of autoimmune thyroid disease.

Conclusion

Our study agreed with studies fund association of NKX2-1 rs944289 SNP with DTC in Icelandic and Japanese populations and not agree with study said no associations were found in the Belarusian. This may indicate a minor or secondary role of this variant in DTC susceptibility. Our study confirmed the association of NKX2-1 rs944289 SNP and incidence of DTC in Sudanese populations.

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