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Introduction

Hyaluronic Acid Binding Protein 2 (HABP2) is an extra-cellular matrix protein involved in cell proliferation¹. Recently, *HABP2* was proposed to be the responsible protein for the familial clustering of differentiated thyroid carcinoma (FDTC)². However, its involvement was questioned by subsequent studies revealing a high prevalence of *HABP2* polymorphisms (SNPs) in the general population, leaving its pathogenic role uncertain^{1,3,4,5}.

Objectives

To identify genetic *HABP2* variants/mutations in a series of FDTC patients and investigate their involvement in the disease.

Patients and Methods

The whole coding region of *HABP2* (13 exons) was PCR-amplified and directly sequenced from genomic DNA of six index patients with FDTC (Figure 1).

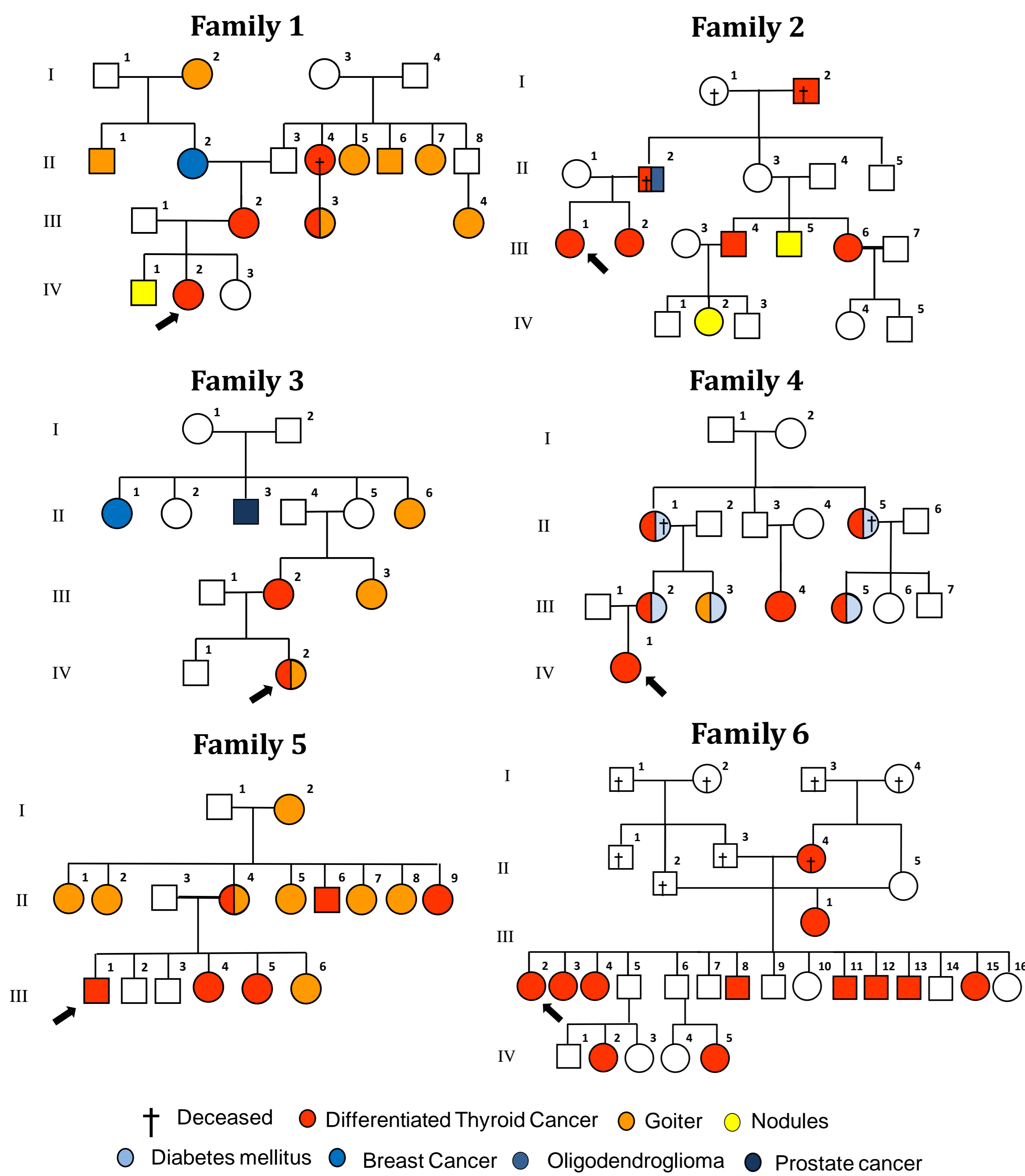


Figure 1. Clinical phenotypes of members of six FDTC family pedigrees. All families included 2 or more generations with a minimum of one PTC or FTC case per generation. Thyroid-related phenotypes are shown in red, orange and yellow and other relevant phenotypes in the blue color scale. The black arrows indicate index patients, circle for female, square for male.

Prevalence of identified variants was investigated in the Spanish population from 368 alleles of healthy control individuals. Public SNP databases were used to estimate variant prevalence in Caucasian populations. Presence of the variants was investigated in all members of Family 1 and segregation analyses performed in affected and healthy individuals. Germline mutations were screened in lymphocyte DNA. *BRAF* and *RAS* "hotspot" mutations in FDTC were studied from the paraffin-embedded thyroid tissue from index patient, through the immunofluorescent mutation test *Cobas*[®]4800.

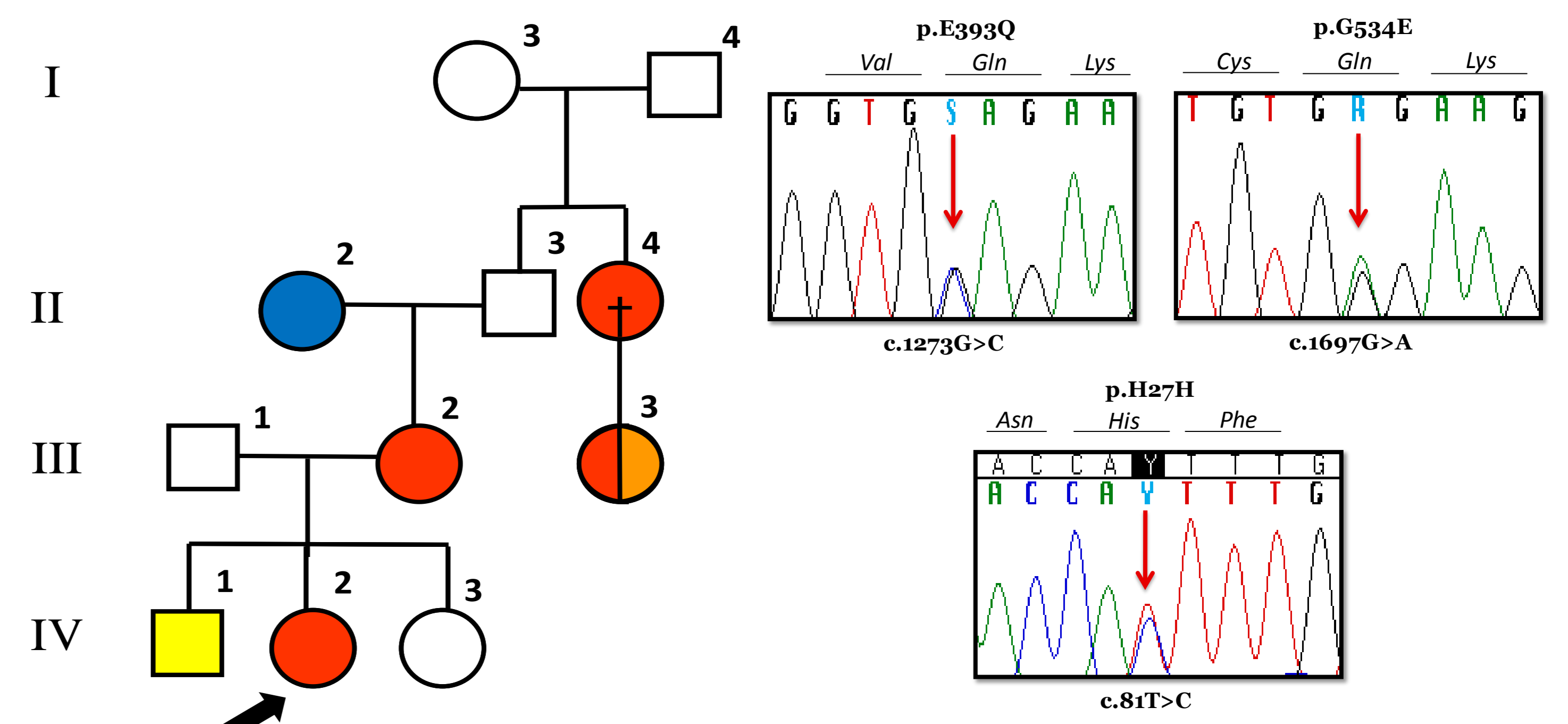
Disclosure statement

The authors report no conflicts of interest in this study.

Results

Two germline heterozygous *HABP2* variants (*p.E393Q* and *p.G534E*) located in exons 10 and 13, respectively, were investigated in 3 affected members (index patient, mother, aunt) from Family 1 and in 5 additional healthy members of the kindred (Figure 2). Both variants are present in SNP databases (*rs11575688*, *rs7080536*) with MAF's (Minor Allele Frequency) between 0.32-1.34% and 0.82-2.79%, respectively. In Spanish control alleles, the prevalence of *p.E393Q* is **0.5%** and of *p.G534E*, **5.1%**.

However, pathogenicity programs predict that the *p.G534E* variant is possibly damaging. Although 3/8 individuals in the pedigree harbored both variants, their presence does not co-segregate with the phenotype. Additionally, the index patient presented the most prevalent somatic mutation of *BRAF* (*p.V600E*) in PTC.



Genes	II.2	III.1	IV.1	IV.2	IV.3	III.2	II.3	III.3
<i>HABP2</i>	WT	<i>p.E393Q</i>	<i>p.E393Q</i>	<i>p.E393Q</i>	WT	WT	WT	WT
	WT	<i>p.G534E</i>	<i>p.G534E</i>	<i>p.G534E</i>	WT	WT	WT	WT
<i>BRAF</i>	-	-	-	<i>p.V600E</i>	-	WT	-	WT
<i>HRAS</i>	-	-	-	<i>p.H27H</i>	-	<i>p.H27H</i>	-	<i>p.H27H</i>
<i>KRAS</i>	-	-	-	WT	-	WT	-	WT
<i>NRAS</i>	-	-	-	WT	-	WT	-	WT

Figure 2. Segregation study of the non-synonymous *HABP2* variants from genomic DNA and *BRAF* and *N-, K-, HRAS* variants from available somatic DNA. Red represents non-synonymous change, green synonymous change. WT = wild type, * = ongoing study.

Conclusions

- *HABP2 p.G534E* variant is prevalent (5,1%) in the normal Spanish population, however *p.E393Q* is rare (<1%). Neither co-segregated with the FDTC phenotype in the family.
- Our findings do not support a relevant role of *HABP2* in the inherited increase of susceptibility for FDTC. Therefore, other germline defects must be responsible for the familial clustering of DTC in this pedigree.
- The finding of the most prevalent *BRAF* mutation (*P.V600E*) in thyroid DNA of VI.2 supports Knudson's "double-hit" hypothesis of cancer, suggesting the involvement of more than one gene in the expression of FDTC⁶.

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