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Does a Common Genetic Event Exist for Familial Thyroid Cancer? Results from a Large Family with fnmtc

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Abstract

Background: Despite several efforts, the genetic susceptibility of familial non medullary thyroid cancer (FNMTC), has remained still elusive.

Methods: We performed Whole Exome Sequencing (WES) in a large family with 9 available members, 6/9 (67%) affected by FNMTC.

Results: We found two missense variants, with CADD score >20: the c.C1519A (p.Pro507Thr, rs773271544) in PRKCε gene and the c.G1019A (p.R340Q) in CCZ1B gene. These alterations were absent in healthy subjects (n=40) and in 30 sporadic thyroid cancer patients. The p.P507T was possibly pathogenetic by SIFT and PRKCε is implicated with MAPK activation by STRING. When we searched for this mutation in other families, we failed to confirm this genetic event as causative of cancer in other 20 FNMTC patients belonging to 8 kindred.

Conclusions: We concluded that the PRKCε p.Pro507Thr possibly represents a private mutation even if other studies including large FNMTC family are needed to define the percentage of familial thyroid cancer cases due this alteration.

Keywords: Familial non medullary thyroid cancer; PRKCε; Whole exome sequencing; Private mutation

Introduction

Familial Non-Medullary Thyroid Cancer (FNMTC), defined as the occurrence of the disease in three or more first-degree relatives of the patient, constitutes about 5–15% of all NMTC cases, including syndromic and non-syndromic forms [1]. Among FNMTCs, papillary thyroid carcinoma (PTC), a malignant epithelial tumor showing follicular differentiation with concrete nuclear characteristics, represents the most common histological subtype [2].

The syndromic FNMTC forms (Cowden, Gardner, Carney complex, Werner, and DICER1 syndromes) are characterized by a preponderance of non-thyroidal tumors associated with Mendelian cancer syndromes, and genetic alterations have been defined. On the contrary, for the non-syndromic form, which represents the majority of all FNMTCs (95%), the genetic inheritance remains unclear, although it is believed to be either an autosomal dominant condition with incomplete penetrance and variable expressivity or a polygenic disorder likely associated with low-penetrance alleles [3-7].

In recent years, many susceptibility genes have been identified through linkage analysis, genome-wide association studies (GWAS), whole exome (WES), and next-generation sequencing [8-12]. Additionally, several

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low-penetrance risk variants have been identified [13-15], and it has been estimated that the five best-characterized SNPs identified in GWAS may contribute to 11% of PTC familiarity [1]. However, none of these mutations has been confirmed in families other than those in which they were initially identified.

The identification of predisposing genes for FNMTC is crucial to identify individuals at high risk of developing the disease because, although still debated, FNMTC is more aggressive than the sporadic counterpart, presenting a higher recurrence rate, an early age of onset, and decreased disease-free survival compared to sporadic forms [16].

Protein kinase Cε (PRKCε) belongs to the family of protein kinase C. It is overexpressed in several solid tumors and plays critical roles in pathways that lead to cancer development, such as MAPK signaling. PRKCε has been implicated in epithelial-to-mesenchymal transition (EMT) [17], cell migration, invasion, and tumor metastasis development [18-22]. Somatic rearrangements of PRKCε have been described in papillary thyroid carcinoma cell lines [23], and modulation of PRKCε by microRNA 146a results in papillary thyroid cancer development [24].

To investigate whether a common genetic event is responsible for FNMTC occurrence, we analyzed a large family with nine members using Whole Exome Sequencing (WES), with six of them being affected by FNMTC.

Materials and Methods

Patients

An Italian family with NMTC aggregation, represented by 9 members (Figure 1A) (F:6) was recruited at the Section of Endocrinology of the University of Siena, Italy. Mean age at diagnosis was 40.6±6.4 years (range 33-52 years). Eight out of 9 (88.8%) patients were submitted to total thyroidectomy. Histological examination showed the presence of papillary thyroid cancer (PTC) in 3 cases, PTC with tall cells in 1 case, PTC with tall and oxyphil cells in 2 cases and follicular adenoma in 2 cases. Lymphnode metastases were present at diagnosis in 2/6 (34%) patients. Two/6 female members (34%) had also breast cancer, in all cases diagnosed after thyroid cancer (3 and 8 years later) (Table 1). All patients are in disease remission. A second group composed by 20 FNMTC patients belonging to 8 families (17 fe-males) was analyzed to confirm the results. Among these patients, 15/20 (75%) had the classic variant of papillary thyroid cancer (PTC), 2/20 (10%) had follicular thyroid cancer (FTC) and 3/20 (15%) had the diffuse sclerosing variant of papillary thyroid carcinoma. As control groups, 40 healthy subjects and 30 sporadic PTCs (F: 21) were also included. For sporadic cases, 25/30 (84%) had PTC, 2/30 (6.7%) had PTC follicular variant and 3/30 (10%) had FTC. All procedures performed in this study were in accordance with the local ethical committee (Ethical Committee of Region Toscana, Area Vasta Sud Est, AOUS. Protocol ID: 10167). Patients signed an informed consent for FNAC, genetic analysis and surgery procedures as part of their treatment plan.

DNA extraction

Genomic DNA was extracted from peripheral blood leukocytes with QIAamp® DNA blood mini kit (Qiagen, Milan, Italy) according to manufacturer's instruction. DNA quality and concentration were assessed with Nanodrop One (Thermo Scientific, Milan, Italy).

Whole Exome Sequencing

Whole exome sequencing (WES) was performed using the Illumina platform in 9 family members (6 affected and 3 unaffected siblings). All genomic DNA samples went through quality controls for DNA concentration and quality (PicoGreen and 1% agarose gel). Library and exome capture were performed using the TrueSeq Exome Illumina kit (Illumina, Inc. San Diego, CA). Post-Library Quality

Table 1: Histological characteristics of the FNMTC patients.

	Sex	Age at diagnosis (yrs)	Histotype	Histologial variant	TNM	Second primary tumor	Site of second tumor
Patient I-1	M	67	n.a.	n.a.	n.a.	n.a.	n.a.
Patient I-2	F	63	PTC	classical	T3N0Mx	no	n.a.
Patient II-1	F	42	PTC	classical	T1N0Mx	yes	breast
Patient II-2	M	44	PTC	tall and oxyphil cells	T1N0Mx	no	n.a.
Patient II-3	F	45	PTC	tall and oxyphil cells	T3N0Mx	no	n.a.
Patient II-4	F	39	Follicular adenoma	n.a.	n.a.	yes	breast
Patient II-5	M	35	PTC	classical	T3N1Mx	no	n.a.
Patient II-6	F	35	Follicular adenoma	n.a.	n.a.	no	n.a.
Patient II-7	F	33	PTC	tall cells	T1N1Mx	yes	breast

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Controls were performed by Agilent 2100 Bioanalyzer. The clonal clusters were created on version 3 flowcells and the pair-end 2X100bp sequencing was performed on HiSeq2000, following the standard Illumina protocol. Data were filtered against dbSNP132 and control populations (1000 Genomes Project Consortium; http://www.1000genomes.org/data) using eVai software (enGenome v2.3). All variants were screened according to their frequency, location, mutation category, literature, and mutation database (ClinVar database, LOVD database, HGMD database). Polymorphisms (minor allele frequency, MAF > 0.01) and synonymous variants were excluded. Missense variants were predicted to be damaging by CADD-Phred prediction tools for functional effect prediction. Frameshift, stop-gain, and splice site variants were prioritized as pathogenic. Variants with a minor allele frequency (MAF) less than 0.01 % in 1000 genome and ExAC-nonTCGA were selected for bioinformatics evaluation.

Bioinformatics analysis

Combined Annotation Dependent Depletion (CADD) software was used and, when the score was >20, Predict SNP, a consensus classifiers for prediction of disease-related mutations, was applied (https://loschmidt.chemi.muni.cz/predictsnp/). Predict SNP elaborates a total score derived from six different software: Multivariate Analysis of Protein Polymorphism (MAPP), Sorting Intolerant from Tolerant (SIFT), Predictor of human Deleterious Single Nucleotide Polymorphisms (PhD-SNP), Polymorphism Phenotyping v1 (PolyPhen-1), Polymorphism Phenotyping v2 (PolyPhen-2) and Screening for Non-Acceptable Polymorphisms (SNAP). Swiss-Model and PyMOL software were utilized to build the three-dimensional protein structure of wild-type and mutant PRKCε protein.

PCR and Sanger sequencing

To validate the results obtained by WES and to explore the genetic variants in sporadic PTC cases, healthy subjects and a validation cohort of FNMTC patients, PCR and Sanger sequencing were performed. Primers used for CCZ1B and PRKCε are reported in Table 2. For all amplicons, PCR conditions were 1.5 mM MgCl2 with an annealing temperature of 60°C (35 cycles). For all other Sanger sequencing (i.e. the whole coding sequence of PRKCε, APC p.R414C; LRKK2 p.G2019S; AIRE p.T441M; TERC, hTERT etc.) primers are available upon request to cantara@unisi.it.

DNA sequencing was carried out using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) and BigDye Xterminator Purification Kit on automated DNA capillary sequencer (Applied Biosystems 3130xl Genetic Analyzer).

Measurements of Relative Telomere Length

Q-PCR assay was performed as previously described [25] on 50 ng/µl of genomic DNA. Telomere length quantification involved determining the relative ratio of telomere (T) repeat copy number to a single copy gene (S) copy number (T/S ratio) in experimental samples using standard curves. This ratio is proportional to the average telomere length. 36B4, encoding acidic ribosomal phosphoprotein P0, has been used as the single copy gene. Primers and PCR conditions have been as reported [25].

Statistical analysis

All statistical analyses were carried out by using the software GraphPad Prism ver-sion 5. A value of $p< 0.05$ was considered statistically significant. Statistical differences in RTL were verified by One-way Anova, followed by Bonferroni's test, to compare two or more independent groups.

Results

Identification of CCZ1B and PRKCε as candidate susceptibility genes We performed Whole-Exome Sequencing using peripheral-blood DNA from 6 affected family members and 3 unaffected siblings. We initially identify approximately 800 variants (classified as pathogenic, likely pathogenic or uncertain) per patient. Using filtering criteria (SNVs/Indel ≤ 1% in HapMap18 and 1000 Genome database, CADD-phred >20, exonic variants), we restricted to 4 variants in 4 different genes reported in Table 3: the p.R340Q in CCZ1B, the p.P507T in PRKCε, the p.R414C in APC, the p.G2019S in LRRK2 genes.

After applying other filters to identify candidate single-nucleotide variants and/or insertions/deletions that segregated with all affected members but were not present in unaffected siblings, we confirmed only the p.R340Q in CCZ1B and the p.P507T in PRKCε genes with a CADD-phred of 23.2 and 23.3, respectively. All patients were heterozygous for these mutations. The presence of these variants was confirmed by Sanger sequencing in all subjects (Figure 1B). We then applied in silico tools to investigate the pathogenic role of these mutations and found that the p.R340Q in CCZ1B resulted as a neutral variant with all applied programmes, whereas the p.P507T in PRKCε in which a proline is substituted by

Table 2: Primer sequences

Gene	PF	PR
CCZ1B	5'-CCCAGACCAGTGAAGTTTTCG-3'	5'-AAAAGCCGTTCAATGCCTGA-3'
PRKCε	5'-CTCACACTAAGCTGGCCTGT-3'	5'- TAAGAGACGAGTGGGCAGG-3'

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Table 3: Selected variants after filtering

Gene	Chr	Mutation	rs	ExAC-NFE	CADD-phred
CCZ1B	7	c.G1019A: p.R340Q	rs766911713	$9x10^{-5}$	23.2
PRKCε	2	c.C1519A: p.P507T	rs7773271544	$1x10^{-5}$	23.3
APC	5	c.C1240T: p.R414C	rs137854567	$1x10^{-3}$	34
LRRK2	12	c.G6055A: p.G2019S	rs34637584	$6x10^{-4}$	35

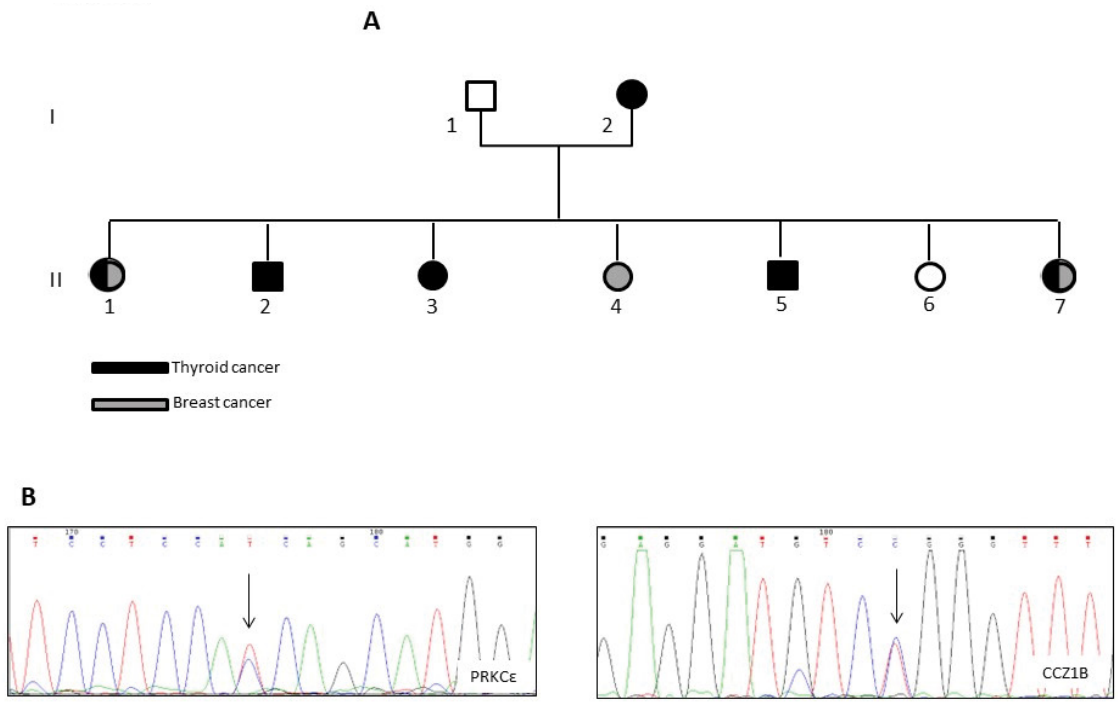

Figure 1: A) Family tree indicating relationship and diseases of the members analyzed in this pa-per. B) Electropherogram obtained by Sanger sequencing for PRKCε p.P507T and CCZ1B p.R340Q.

a threonine was considered possibly deleterious by SIFT. According with Uniprot (https://www.uniprot.org/uniprotkb/Q02156/entry), this substitution falls into the protein kinase domain which spans from aminoacid 408 to 668. Modelling of the p.P507T mutation was performed using the Swiss model server (http://swissmodel.expasy.org/) and the molecular visualization system Pymol (https://pymol.org/2/) from the crystal structure of the normal protein. Proline is a non-polar, hydrophobic amino acid, with a nitrogen atom covalently fastened within a ring, having, like that, a constrained phi angle (26) (Figure 2A). On the contrary, threonine is a linear molecule that is a polar, uncharged aminoacid due to its R group, which contains a side chain with a hydroxyl group [27] (Figure 2B). Thyroid cancer is characterized by an activation of the MAPK pathway trough RAS and BRAF phosphorylation. As shown by String (Figure 2C), PRKCε interacts with these proteins and thus, a possibly activating mutation, may result in pathway hyperfunction.

We, also, searched for this variant in 40 healthy subjects and 30 sporadic cases by Sanger sequencing. All individuals analyzed were negative for the mutation.

PRKCε p.P507T is not found in other FNMTC patients

Due to the possible implication of the PRKCε p.P507T mutation with the MAPK pathway hyper-activation, we hypothesized that it could be causative of the familial form of PTC. We then analyzed by Sanger sequencing the PRKCε p.P507T together with the whole coding sequence of the gene in 20 FNMTC patients belonging to 8 families. These patients were already screened for some of the alterations that have been described in the literature [1] to be associated with FNMTC: the p.G534E in HABP2, TERC and hTERT mutations and the p.A339V in TITF-1/NKX2.1 gene. All patients were negative for these variants. Similarly, all subjects were wild type for the PRKCε p.P507T variant. In

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addition, no other potentially impactful mutations were found in the PRKCε gene. Only 2/20 (10%) of the FNMTC patients displayed the rs1228790462 associated with a synonymous variant (p.Gln719=).

Does a susceptibility background predispose to FNMTC?

We first described that telomere length and telomere instability constitute a genetic background that predisposes to the development of familial thyroid cancer [25, 28-30]. We measured the relative telomere length (RTL) in our family and found a particularly short telomere compared to the other groups (Figure 3A). The RTL was shorter for both affected and unaffected siblings, even compared to the RTL of the FNMTC control group, indicating a progressive erosion potentially implicated in the oncogenic transformation of somatic cells. In addition, 4 members displaying thyroid diseases (both thyroid cancer or adenomas) were heterozygous for the p.R414C mutation (MAF <0.01) in the APC gene. This variant is reported to be associated with the hereditary cancer-predisposing syndrome (ClinVar: https://www.ncbi.nlm.nih.gov/snp/rs137854567/) and is deleterious by Predict SNP (87%). PRKCε and APC are both MAPK activators, as shown in Figure 3B. Similarly, although with a CAD of 13.22, all family members were heterozygous for the p.T441M mutation in the AIRE gene. This mutation is located in the zinc finger region, has a MAF <0.01, and, according to the in silico prediction tool that we have applied, is potentially deleterious. Using PROMO [31, 32], a virtual laboratory for the identification of putative transcription factor binding sites, we found that both Ets and Elk1 (activated by ERK1/2) are potential transcription factors for AIRE.

Discussion

Somatic rearrangements of PRKCε have been described in papillary thyroid carcinoma cell lines and PRKCε itself is a positive modulator of MAPK pathway. Therefore, PRKCε seems a natural candidate for cancer susceptibility particularly familial form of thyroid cancer [18-24]. FNMTC constitutes about 5–15% of all NMTC cases, including syndromic and nonsyndromic forms, and this high familial risk can be attributed to both rare, high-penetrance mutations and common, low-penetrance variants [1]. Scientific and technological advancements in genomics have allowed whole genome and exome screening to become the state-of-the-art tool for the identification of driver mutations in tumors. So far, only a few NMTC susceptible gene(s) and low-penetrance variant(s) contributing to NMTC have been described [1]. We performed WES on a large family composed of nine members, six of them affected with FNMTC. In this family

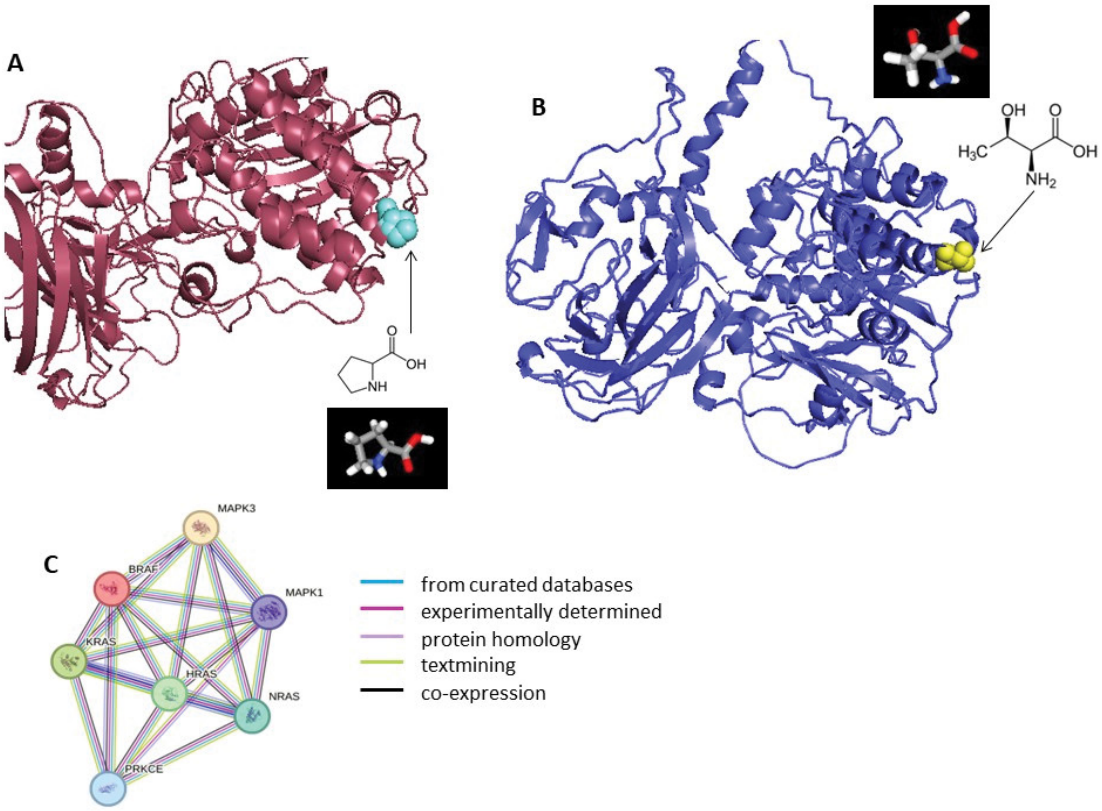

Figure 2: 3D Model of PRKCε wild type (A) and mutated (B). The Proline and Threonine aa are highlighted in light blue and yellow, respectively. C) STRING protein–protein interaction network for PRKCε and MAPK pathway.

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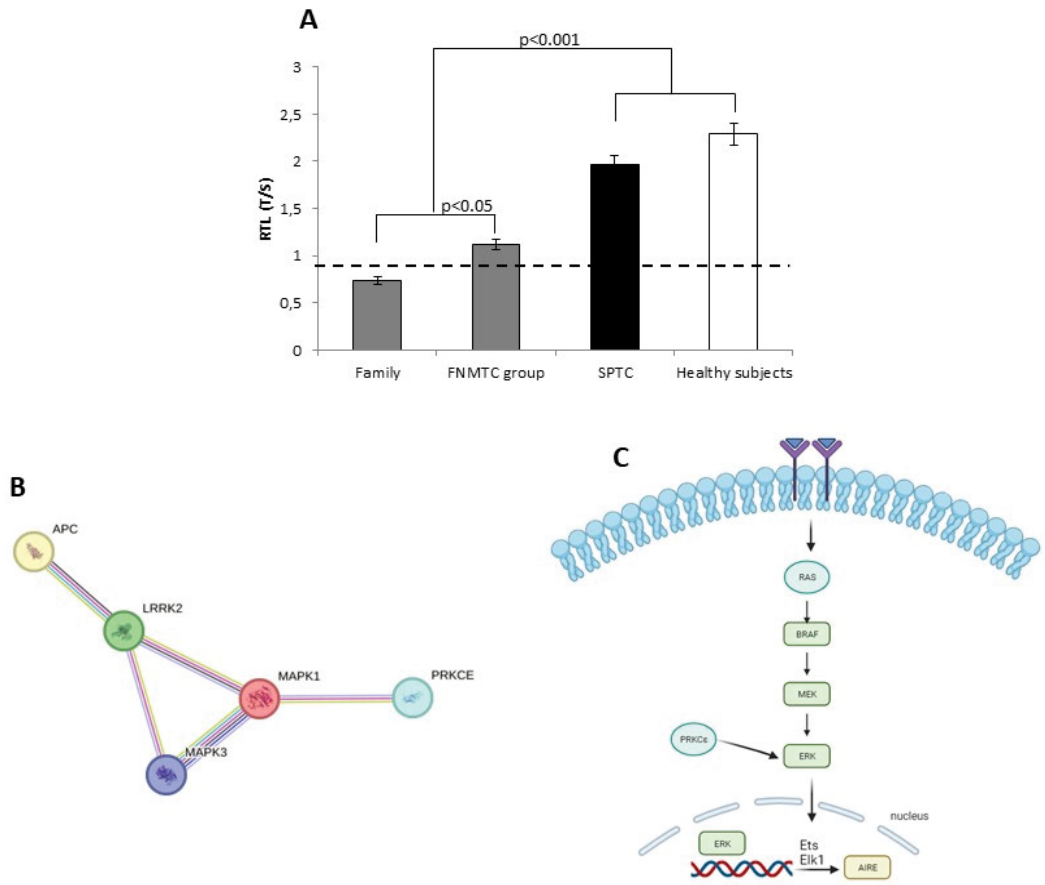

Figure 3: A) Relative telomere length measured by real time PCR for the studied family (affected members) compared to a second group of FNMTC patients, SPT and healthy subjects. Dotted line represents family unaffected siblings; B) STRING protein–protein interaction network unrevealing the relationship between genes found altered in the studied family and their link to the MAPK pathway; C) Hypothetical pathway in which activated ERK1/2 transmigrate to the nucleus and promote AIRE activation through the two transcription factors Elk1 and Ets.

we identified, for the first time, one potentially disease-causing germline variants in PRKCε gene that, in humans, encodes PRKCε protein. PRKCε, a member of the protein kinase C (PKC) family proteins, is overexpressed in most solid tumors and plays critical roles in different processes that lead to cancer development [33-37]. At the systemic level, PRKCε activation has protective roles in cardiac and brain ischemia and heat shock response while uncontrolled PRKCε activation is associated with cancer development [33-37]. The Raf-1 kinase acts downstream PRKCε regulating important intracellular signaling pathways involved with proliferation, differentiation and apoptosis. The oncogenic function of PKCε potentially occurs via activation of the Ras/Raf pathway which results in the transcription of genes involved in cell proliferation and growth [38]. Functionally PRKCε plays crucial roles in almost all aspects of tumor development, namely cell transformation, proliferation, cancer cell survival, epithelial mesenchimal transition (EMT), migration and invasion [33-37].

Some studies evaluated the role of PRKCε in thyroid cancer. Nearly 20 years ago, Knauf et al reported an isozyme-specific reduction of PRKCε, which occurs through a post-transcriptional mechanism in 8/11 PTC tissues, affecting translation or stability of the PRKCε protein. The authors concluded that the decreased abundance of PRKCε may promote tumor progression by prolonging cellular life span [23]. The same authors found a rearrangement of PRKCε in a thyroid follicular carcinoma cell line (PCCL3) suggesting that this protein may play a role in thyroid tumorigenesis. In the same cell line, the induction of RET/PTC1 or RET/PTC3 expression, a key event in the pathogenesis of PTCs, resulted in PRKCε activation. As supposed by the authors, this selective downregulation of PRKCε following prolonged RET/PTC activation promotes cell survival and clonal expansion [39]. Another study showed that the microRNA-146a, significantly overexpressed in PTC, targets PRKCε to modulate papillary thyroid tumour development through suppression of PRKCε expression and deregulation of the Ras/Raf-1 signaling

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pathway [24]. In agreement with these authors, we might suppose that PI3K/Akt, Stat3 and MAPK/ERK pathways are the likely mediators of PRKCε-induced transformation. Interestingly, when we search for the p.P507T mutation in other FNMTC patients, all subjects were wild type again indicating that FNMTC mutations represent private mutations that occur on a fragile genetic background. This genetic instability may be linked to particularly short telomeres has already demonstrated [25, 28-30] and confirmed in this family. In addition, some of the family members studied in this paper, are heterozygous for two important pathogenetic alterations: the APC p.R414C linked to the hereditary cancer-predisposing syndrome (present in 4 affected members) and the p.T441M in AIRE gene (in all affected members). AIRE, the autoimmune regulator, plays a key role in shaping central immunological tolerance by facilitating the negative selection of T cells. AIRE has been associated with the polyglandular syndrome type 1 (APS1) [40] and with cancer [41-43]. According with the literature [44] and with PROMO, a virtual laboratory for the identification of putative transcription factor binding sites, ERK1/2, which is linked to PRKCε, activates Ets and Elk1 potential TF for AIRE. We can speculate that all alterations found in this family contribute to the MAPK activation thus sustaining cell proliferation and contribute to cancer development (Figure 3C).

Limitations: Although some of the data presented in our paper seem to support that FNMTCs are characterized by a complex genetic instability associated with private mutation(s), limitations are present in the study and are linked to the absence of confirmatory functional studies for the AIRE and APC mutations and the lack of demonstration of the non-random segregation of mutations in the family. Family members analyzed, while representative of different Italian regions are all Italians and thus expanding the sample size to other countries could be beneficial to eliminate potential environmental influences. In addition, confirming these genetic alterations in tissues would be important, but unfortunately, the patients underwent surgery many years ago in different clinics, and therefore, it was not possible to retrieve the histological specimens.

Taken together our results contribute to highlight that the p.P507T in PRKCε is a potential pathogenetic mutation causative of familial thyroid cancer but, the absence of this mutation in other families lead us to conclude that FNMTC patients have a genetic instability on which, a private mutation(s) acts to induce hereditary cancer. Future work including more functional studies at protein level will improve our knowledge on the potential role of PRKCε mutation on the development of FNMTC.

Conflict of Interest: None

References

1. Capezzone M, Robenshtok E, Cantara S, et al. Familial non-medullary thyroid cancer: a critical review. J Endocrinol Invest 44 (2021): 943-950.
2. Xu B, Ghossein R. Evolution of the histologic classification of thyroid neoplasms and its impact on clinical management. Eur J Surg Oncol 44 (2018): 338-347.
3. Peiling Yang S, Ngeow J. Familial non-medullary thyroid cancer: unraveling the genetic maze. Endocr Relat Cancer 23 (2016): R577-R595.
4. Cetta F, Montalto G, Gori M, et al. Germline mutations of the APC gene in patients with familial adenomatous polyposis-associated thyroid carcinoma: results from a European cooperative study. J Clin Endocrinol Metab 85 (2000): 286-292.
5. Pilarski R, Burt R, Kohlman W, et al. Cowden syndrome and the PTEN hamartoma tumor syndrome: systematic review and revised diagnostic criteria. J Natl Cancer Inst 105 (2013):1607-1616.
6. Kamilaris CDC, Faucz FR, Voutetakis A, Stratakis CA. Carney Complex. Exp Clin Endocrinol Diabetes 127 (2019): 156-164.
7. Baloch ZW, Asa SL, Barletta JA, et al. Overview of the 2022 WHO Classification of Thyroid Neoplasms. Endocr Pathol 33 (2022): 27-63.
8. Srivastava A, Kumar A, Giangiobbe S, et al. Whole Genome Sequencing of Familial Non-Medullary Thyroid Cancer Identifies Germline Alterations in MAPK/ERK and PI3K/AKT Signaling Pathways. Biomolecules 9 (2019): 605.
9. Ye F, Gao H, Xiao L, et al. Whole exome and target sequencing identifies MAP2K5 as novel susceptibility gene for familial non-medullary thyroid carcinoma. Int J Cancer 144 (2019): 1321-1330.
10. Wang Y, Liyanarachchi S, Miller KE, et al. Identification of Rare Variants Predisposing to Thyroid Cancer. Thyroid 29 (2019): 946-955.
11. Tous C, Muñoz-Redondo C, Bravo-Gil N, et al. Identification of Novel Candidate Genes for Familial Thyroid Cancer by Whole Exome Sequencing. Int J Mol Sci 24 (2023): 7843.
12. Diquigiovanni C, Bergamini C, Evangelisti C, et al. Mutant MYO1F alters the mitochondrial network and induces tumor proliferation in thyroid cancer. Int J Cancer 143 (2018): 1706-1719.
13. Kamani T, Charkhchi P, Zahedi A, et al. Genetic

susceptibility to hereditary non-medullary thyroid cancer. Hered Cancer Clin Pract 20 (2022): 9.

14. Sánchez-Ares M, Cameselle-García S, Abdulkader-Nallib I, et al. Susceptibility Genes and Chromosomal Regions Associated With Non-Syndromic Familial Non-Medullary Thyroid Carcinoma: Some Pathogenetic and Diagnostic Keys. Front Endocrinol (Lausanne) 13 (2022): 829103.

15. Cirello V, Colombo C, Karapanou O, et al. Clinical and Genetic Features of a Large Monocentric Series of Familial Non-Medullary Thyroid Cancers. Front Endocrinol (Lausanne) 11 (2021): 589340.

16. Capezzone M, Marchisotta S, Cantara S, et al. Familial non-medullary thyroid carcinoma displays the features of clinical anticipation suggestive of a distinct biological entity. Endocr Relat Cancer 15 (2008): 1075-1081.

17. Jain K, Basu A. The Multifunctional Protein Kinase C-ε in Cancer Development and Progression. Cancers (Basel) 6 (2014): 860-878.

18. Hafeez BB, Zhong W, Weichert J, et al. Genetic ablation of PKC epsilon inhibits prostate cancer development and metastasis in transgenic mouse model of prostate adenocarcinoma. Cancer Res 71 (2011): 2318-2327.

19. Lang W, Wang H, Ding L, et al. Cooperation between PKC-alpha and PKC-epsilon in the regulation of JNK activation in human lung cancer cells. Cell Signal 16 (2004): 457-467.

20. Mohamed T, Colciago A, Montagnani Marelli M, et al. Protein kinase C epsilon activation regulates proliferation, migration, and epithelial to mesenchymal-like transition in rat Schwann cells. Front Cell Neurosci 17 (2023): 1237479.

21. Zafar S, Khan K, Badshah Y, et al. Exploring the prognostic significance of PKCε variants in cervical cancer. BMC Cancer 23 (2023):819. Erratum in: BMC Cancer 23 (2023):963.

22. Lai X, Liang Y, Jin J, et al. Protein kinase C epsilon promotes de novo lipogenesis and tumor growth in prostate cancer cells by regulating the phosphorylation and nuclear translocation of pyruvate kinase isoform M2. Exp Cell Res 422 (2023): 113427.

23. Knauf JA, Ward LS, Nikiforov YE, et al. Isozyme-specific abnormalities of PKC in thyroid cancer: evidence for post-transcriptional changes in PKC epsilon. J Clin Endocrinol Metab 87 (2002): 2150-2159.

24. Zhang X, Li D, Li M, et al. MicroRNA-146a targets PRKCE to modulate papillary thyroid tumor development. Int J Cancer 134 (2014): 257-267.

25. Capezzone M, Cantara S, Marchisotta S, et al. Short telomeres, telomerase reverse transcriptase gene amplification, and increased telomerase activity in the blood of familial papillary thyroid cancer patients. J Clin Endocrinol Metab. 2008 Oct; 93 (2008): 3950-3957.

26. Morgan AA, Rubenstein E. Proline: the distribution, frequency, positioning, and common functional roles of proline and polyproline sequences in the human proteome. PLoS One 8 (2013): e53785.

27. Debabov VG. The threonine story. Adv Biochem Eng Biotechnol 79 (2003): 113-136.

28. Pacini F, Cantara S, Capezzone M, et al. Telomerase and the endocrine system. Nat Rev Endocrinol 7 (2011): 420-430.

29. Cantara S, Pisu M, Frau DV, et al. Telomere abnormalities and chromosome fragility in patients affected by familial papillary thyroid cancer. J Clin Endocrinol Metab 97 (2012): E1327-1331.

30. Capezzone M, Cantara S, Marchisotta S, et al. Telomere length in neoplastic and nonneoplastic tissues of patients with familial and sporadic papillary thyroid cancer. J Clin Endocrinol Metab 96 (2011): E1852-1856.

31. Messeguer X, Escudero R, Farré D, et al. PROMO: detection of known transcription regulatory elements using species-tailored searches. Bioinformatics 18 (2002): 333-334.

32. Farré D, Roset R, Huerta M, et al. Identification of patterns in biological sequences at the ALGGEN server: PROMO and MALGEN. Nucleic Acids Res 31 (2003): 3651-3653.

33. Wang J, Jin J, Liang Y, et al. miR-21-5p/PRKCE axis implicated in immune infiltration and poor prognosis of kidney renal clear cell carcinoma. Front Genet 13 (2022): 978840.

34. Wang H, Zhan M, Xu SW, et al. miR-218-5p restores sensitivity to gemcitabine through PRKCE/MDR1 axis in gallbladder cancer. Cell Death Dis 8 (5): e2770.

35. Chen Z, Zhou K, Xue J, et al. Phosphorylation stabilized TET1 acts as an oncoprotein and therapeutic target in B cell acute lymphoblastic leukemia. Sci Transl Med 15 (2023): eabq8513.

36. Zafar S, Khan K, Badshah Y, et al. Exploring the prognostic significance of PKCε variants in cervical cancer. BMC Cancer 23 (2023): 819. Erratum in: BMC Cancer 23 (2023): 963.

37. Yang D, He Y, Wu B, et al. Predictions of the dysregulated competing endogenous RNA signature involved in the progression of human lung adenocarcinoma. Cancer Biomark 29 (2020): 399-416.

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38. Huang B, Fu SJ, Fan WZ, et al. PKCε inhibits isolation and stemness of side population cells via the suppression of ABCB1 transporter and PI3K/Akt, MAPK/ERK signaling in renal cell carcinoma cell line 769P. Cancer Lett 376 (2016): 148-154.

39. Knauf JA, Elisei R, Mochly-Rosen D, et al. Involvement of protein kinase Cepsilon (PKCepsilon) in thyroid cell death. A truncated chimeric PKCepsilon cloned from a thyroid cancer cell line protects thyroid cells from apoptosis. J Biol Chem 274 (1999): 23414-23425.

40. Anderson MS, Su MA. AIRE expands: new roles in immune tolerance and beyond. Nat Rev Immunol 16 (2016): 247-258.

41. van Laar GG, van Hamburg JP, Tas SW. Extrathymic AIRE-expressing cells: Friends or foes in autoimmunity and cancer? Autoimmun Rev 21 (2022): 103141.

42. Nguyen CTK, Sawangarun W, Mandasari M, et al. AIRE is induced in oral squamous cell carcinoma and promotes cancer gene expression. PLoS One 15 (2020) :e0222689.

43. Kalra R, Bhagyaraj E, Tiwari D, et al. AIRE promotes androgen-independent prostate cancer by directly regulating IL-6 and modulating tumor microenvironment. Oncogenesis 7 (2018): 43.

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