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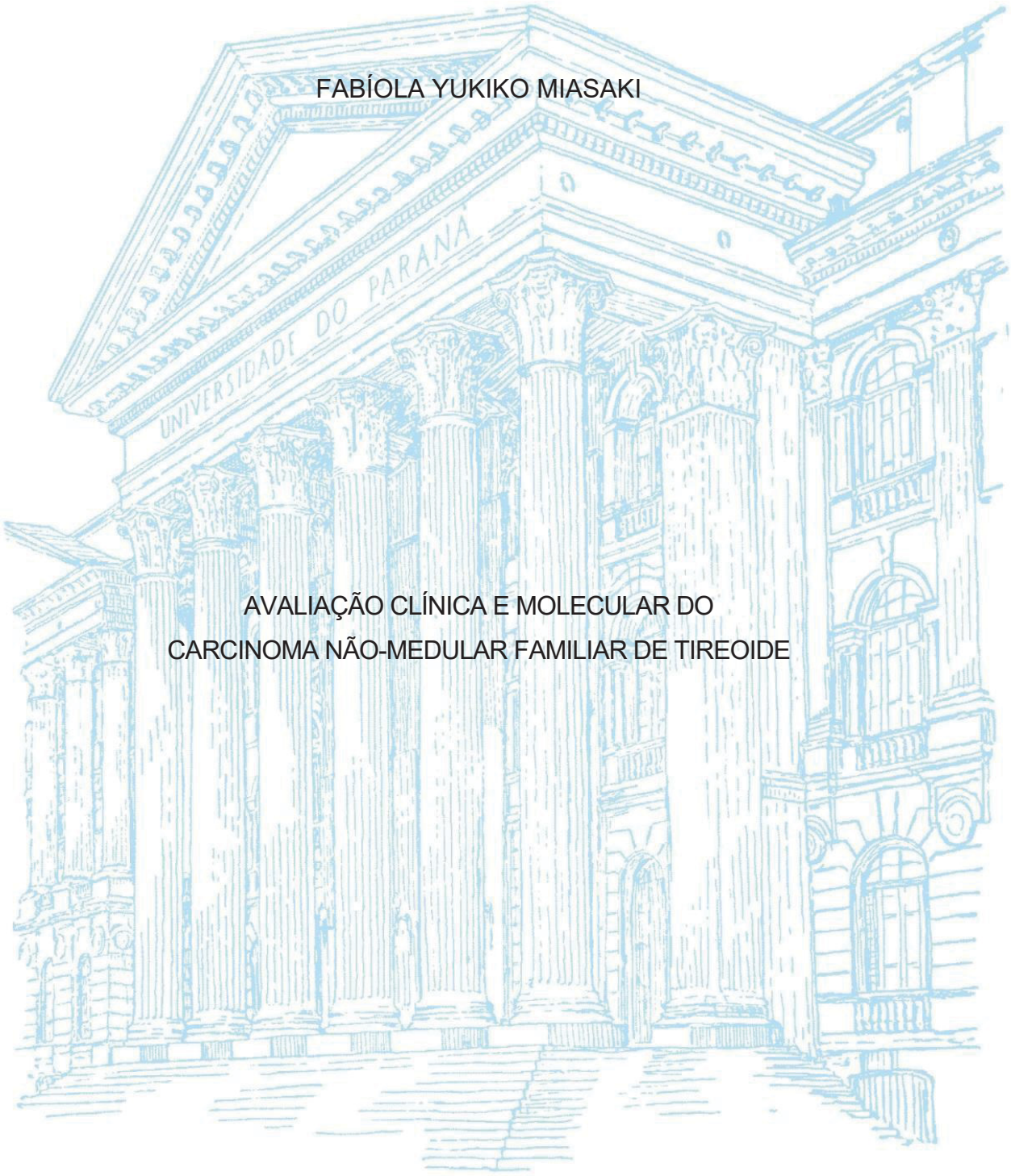

AVALIAÇÃO CLÍNICA E MOLECULAR DO CARCINOMA NÃO-MEDULAR FAMILIAR DE TIREOIDE

CURITIBA

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FABÍOLA YUKIKO MIASAKI

AVALIAÇÃO CLÍNICA E MOLECULAR DO CARCINOMA NÃO-MEDULAR FAMILIAR DE TIREOIDE

Tese apresentada ao Programa de Pós-Graduação em Medicina Interna e Ciências da Saúde, no Setor de Ciências da Saúde, na Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutora em Medicina Interna.

Orientadora: Prof.ª Dra. Gisah Amaral de Carvalho

Coorientador no exterior: Prof. Dr. Peter A. Kopp

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em MEDICINA INTERNA E CIÊNCIAS DA SAÚDE da Universidade Federal do Paraná foram convocados para realizar a arguição da tese de Doutorado de **FABIOLA YUKIKO MIASAKI** intitulada: **AVALIAÇÃO CLÍNICA E MOLECULAR DO CARCINOMA NÃO-MEDULAR FAMILIAR DE TIREOIDE.**, sob orientação da Profa. Dra. GISAH AMARAL DE CARVALHO, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

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“Et rien n'est tel que le rêve pour engendrer l'avenir.
Utopie aujourd'hui, chair et os demain.”
Victor Hugo

RESUMO

O carcinoma familiar não-medular de tireoide (CNMTF) representa cerca de 3-9% de todos os carcinomas de tireoide diferenciados: quando dois ou mais membros da mesma família são identificados com câncer papilífero, folicular, células de Hürthle e/ou anaplásico e não se identifica nenhuma característica sindrômica associada. Entretanto, não há consenso quanto à apresentação clínica do CNMTF nestes pacientes, nem quais seriam os genes envolvidos nessa predisposição. Alguns estudos mostraram uma apresentação mais agressiva nos pacientes com CNMTF do que naqueles com a forma esporádica. Mas outros trabalhos não conseguiram identificar essa diferença. Por esse motivo, um dos objetivos desse trabalho é comparar a apresentação clínica dos pacientes com a forma familiar em relação àqueles com carcinoma não-medular esporádico (CNMTE). Com essa finalidade, selecionamos 39 pacientes cujas famílias apresentavam 2 ou mais parentes de primeiro grau afetados. Foram avaliados dados sobre a idade de início do câncer, sua evolução, o grau de parentesco entre os membros afetados, a análise histológica através de material cirúrgico emblocado em parafina e dados clínicos e laboratoriais para determinação do estadiamento do câncer de tireoide. Foi, também, coletado sangue total para posterior análise genética. Paralelamente, registramos dados referentes a todos os pacientes com carcinoma de tireoide esporádicos seguidos na Unidade de Tireoide do SEMPR-HC-UFPR e selecionamos 119 deles como grupo controle. Além disso, na tentativa de identificar um padrão de variante genômica, foram selecionadas duas famílias com 8 e 5 CNMT e realizadas análise do exoma do sangue periférico, análise comparativa dos exomas e listagem de potenciais genes candidatos para avaliação do padrão de segregação dessas variantes nos demais familiares. Clinicamente, observou-se que o grupo de CNMTF tiveram diagnóstico em idade inferior ao grupo do carcinoma não-medular esporádico (CNMTE) (38,74 ± 14,48 *versus* 46,65 ± 13,77, $p < 0,001$), apresentavam um tumor de tamanho menor (18,28 mm ± 13,56 *versus* 25,27 mm ± 20,39, $p = 0,047$), e apresentavam mais frequentemente metástase linfonodal (46,2% *versus* 21,8%, $p = 0,007$) já ao diagnóstico. Apesar disso, quando avaliado o reestadiamento dinâmico, não houve diferença significativamente estatística entre a evolução do CNMTE e do CNMTF. Do ponto de vista genético, a análise bioinformática mais restrita não identificou nenhuma variante que correspondesse aos critérios estabelecidos e que segregasse em todos os pacientes afetados em uma das famílias. Na segunda família, variantes não-sinônimas nos genes *TOR1AIP1, GFI1, MUC16, GPD2, DCLRE1B, MADCAM1, NEFH, CA14, POGZ, RHBG, BCAN, OR6Y1, PFDN2, PRRC2C, AGAP5, ANAPC1, CCDC74B* e *MUC4* foram identificadas.

Palavras-chave: carcinoma familiar não-medular de tireoide, carcinoma de tireoide, exoma, genes candidatos

ABSTRACT

Familial non-medullary thyroid carcinoma (FNMTC) represents around 3-9% of all differentiated thyroid carcinoma. FNMTC is defined when non-medullary thyroid cancer (NMTC) occurs in two or more first-degree relatives and no syndromic sign is identified. NMTC includes papillary, follicular, Hürthle cell and anaplastic thyroid carcinoma. However, there is no consensus about the evolution of these patients. And, in most cases, the genetic pathways are still not known. Some studies support a worse prognosis than sporadic nonmedullary thyroid carcinoma (SNMTC). However, others do not. Therefore, one of our aims is to characterize the FNMTC patients' evolution and compare it with SNMTC. For this purpose, patients who have two or more relatives affected were recruited. Data about cancer (initial diagnosis, evolution, and paraffin blocks), kinship information and blood are being analyzed. In parallel, we collected sporadic thyroid carcinoma data followed in the Thyroid Unit of SEMPR-HC-UFPR. In FNMTC group, the age at diagnosis was earlier in FNMTC (38.74 ± 14.48 *versus* 46.65 ± 13.77, $p < 0.001$), the tumor size was smaller (18.28 mm ± 13.56 *versus* 25.27 mm ± 20.39, $p = 0.047$), and presented with more lymph node metastasis (46.2% *versus* 21.8%, $p = 0.007$) at the diagnosis. Nevertheless, when analyzed the therapy reclassification, there is no difference in outcomes between SNMTC and FNMTC. Furthermore, to identify genetic variants which confer cancer susceptibility, we selected two families with 8 and 5 thyroid cancer patients for performing: the whole exome of peripheral blood; comparative analysis of exomes and list the potential candidate genes; and selection of candidate genes and study the segregation pattern (for individualize the gene/variant is involved in this predisposition). In one of the families, the bioinformatic analysis using strict criteria did not show any candidate variant. In the second family, nonsynonymous variants in the following genes *TOR1AIP1, GFI1, MUC16, GPD2, DCLRE1B, MADCAM1, NEFH, CA14, POGZ, RHBG, BCAN, OR6Y1, PFDN2, PRRC2C, AGAP5, ANAPC1, CCDC74B,* and *MUC4* were identified.

Keywords: familial nonmedullary thyroid cancer; thyroid cancer; exoma; candidate genes

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LISTA DE ABREVIATURAS

CFT	carcinoma folicular de tireoide
CNMT	carcinoma não-medular de tireoide
CNMTE	carcinoma não-medular de tireoide esporádico
CNMTF	carcinoma não-medular de tireoide familiar
CPT	carcinoma papilífero de tireoide
DNA	ácido desoxirribonucleico
FNMTC	*familial non-medullary thyroid carcinoma*
mRNA	microRNA
NGS	*next generation sequencing*
NMTC	*non-medullary thyroid carcinoma*
RNA	ácido ribonucleico
SNMTC	*sporadic non-medullary thyroid cancer*
TCGA	*The Cancer Genome Atlas*
Tg	tireoglobulina

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1 INTRODUÇÃO

O carcinoma de tireoide é a neoplasia endocrinológica mais comum e configura como a quinta causa de carcinoma entre mulheres. Dados do *National Cancer Institute* (2020) mostram que o câncer de tireoide atinge cerca 15,7/10^5 americanos anualmente[1].

Os carcinomas de tireoide podem ter origem nas células C e, quando isso ocorre, são chamados de medulares; ou podem ainda ter origem nas células foliculares da tireoide, chamados então de não-medulares (CNMT). Nesse último grupo, 85% são carcinomas papilíferos, 12% foliculares e < 3% são pouco diferenciados [2]. Além desses, o carcinoma de células de Hürthle - após ter sido considerado por décadas uma variante do carcinoma folicular e eventualmente do carcinoma papilífero de tireoide - voltou a ser classificado como um tipo independente de CNMT em 2019 e representa cerca de 5% dos mesmos [3].

Apesar da maioria dos CNMT serem esporádicos, o CNMT configura como um dos carcinomas com maior padrão hereditário, sendo que um parente de primeiro grau apresenta um risco de 8 a 10 vezes de também desenvolver CNMT [4; 5]. Entretanto, somente 3 a 10% de todos os CNMT são familiares. Usa-se o termo carcinoma não-medular de tireoide familiar (CNMTF), quando dois ou mais parentes de primeiro grau são acometidos, sem a presença de síndromes associadas. A saber: síndrome de Carney, síndrome de Cowden, polipose adenomatosa familiar/síndrome de Gardner, síndrome de Werner, entre outras [6].

Essa definição foi questionada por Charkes (2006), já que devido à alta prevalência do carcinoma esporádico de tireoide, a probabilidade de dois parentes serem acometidos por acaso é aproximadamente de 60%, enquanto a chance desse mesmo acaso ocorrer em três parentes de primeiro grau cai para 6% e em quatro, 0,15% [7]. Apesar disso a definição clássica é ainda amplamente utilizada. Recentemente, foi ainda sugerido que o critério idade (< 45 anos) possa ser valorizado quando apenas 2 pacientes apresentem CNMT na mesma família [8].

Outro ponto pouco esclarecido é quanto à apresentação clínica e evolução da forma familiar. Enquanto, nos carcinomas medulares de tireoide, o quadro clínico já é bem estabelecido de acordo com a mutação envolvida; no caso dos CNMTF, informações sobre os polimorfismos/mutações predisponentes são ainda desconhecidos. Acredita-se que a herança seja autossômica dominante com

penetrância incompleta e expressão variável [9]. Alguns, ainda, acreditam que a herança possa ser poligênica [10].

2 HIPÓTESE E IMPORTÂNCIA DO ESTUDO

Do ponto de vista clínico e estatístico, a hipótese nula (H_0) é que os CNMTF se comportem de modo semelhante aos CNMT esporádicos (CNMTE). Sendo que H_1 indicariam que eles se comportam de modo diferente.

Determinar se o CNMTF é realmente mais agressivo é de fundamental importância para o refinamento da prática clínica no manejo do carcinoma de tireoide. Pois um comportamento mais agressivo poderia indicar uma necessidade de ultrassonografias de rotina em toda família. Ainda, num momento em que tendemos a ser mais conservadores no tratamento do CNMT, isso também poderia indicar uma abordagem cirúrgica mais radical nesses pacientes, como uma tireoidectomia total. O contrário, ou seja, um comportamento clínico semelhante do CNMTE asseguraria que tanto o rastreamento quanto condutas mais agressivas seriam desnecessárias, evitando exames e procedimentos.

Já determinar qual ou quais genes estão envolvidos na etiopatogenia do carcinoma familiar não-medular de tireoide tem aplicações bastante amplas na tireoidologia.

O impacto principal e imediato é permitir um diagnóstico precoce dos familiares carreadores da mutação. Tal ferramenta diagnóstica possibilitaria redução dos custos com ultrassonografias desnecessárias e do estresse psicológico dos pacientes não-carreadores da mutação/polimorfismo. Bem como, de medidas mais assertivas quanto ao seguimento e eventual tireoidectomia na vigência de nódulos pequenos e suspeitos.

Além disso, permitiria contribuir para a melhor compreensão da oncogênese no carcinoma papilífero de tireoide, abrindo novas linhas de pesquisa.

Não menos importantes são as oportunidades de colaboração transdisciplinar, interuniversitárias e mesmo internacionais que esse tipo de estudo proporciona.

3 OBJETIVOS

3.1 OBJETIVO GERAL

O objetivo da presente tese:

- analisar e comparar a apresentação clínica inicial dos CNMTF em relação ao CNMTE

3.2 OBJETIVOS SECUNDÁRIOS

- Analisar e comparar a evolução desses diferentes grupos através do estadiamento dinâmico.

- avaliar o padrão de herança nos membros afetados e identificar potenciais genes (polimorfismo/mutação) relacionados à susceptibilidade genética para posterior validação.

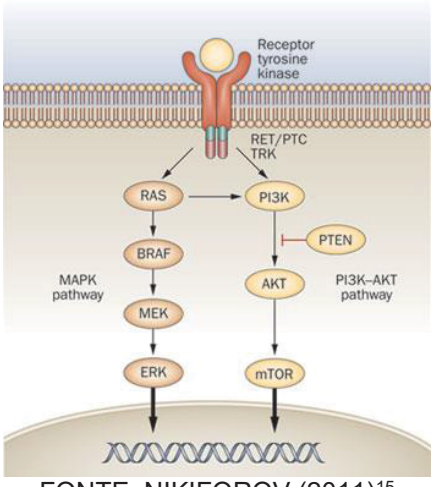
4 REVISÃO DA LITERATURA

4.1 ETIOLOGIA

O CNMT alberga uma baixa densidade de mutações e, geralmente, as mutações *drivers* são mutuamente excludentes. São ditas mutações *drivers* aquelas implicadas diretamente no processo de oncogênese e que acarretam uma vantagem no crescimento/sobrevida celular [11]. Acredita-se que essa baixa densidade de mutações seja responsável pelo comportamento relativamente indolente do CDT [12; 13].

Em aproximadamente 70% dos carcinomas papilíferos, encontram-se mutações de ponto no gene *BRAF* ou *RAS* ou rearranjos *RET*/*PTC*. Todas essas alterações resultam em proteínas fosforiladas constitutivamente que ativam a via MAPK e, portanto, estimulam o crescimento, a proliferação, a migração e a sobrevida celular. Os carcinomas pouco diferenciados/anaplásicos, por sua vez, podem albergar tais alterações, mas frequentemente também apresentam outras alterações na via MAPK, PI3K e/ou β-catenina. Já os carcinomas foliculares albergam, frequentemente, mutações *RAS* ou rearranjos *PAX8*/*PPARγ* [14].

FIGURA 1 - VIAS DE SINALIZAÇÃO (ADAPTADA)



FONTE: NIKIFOROV (2011)[15]

Recentemente, o carcinoma de células de Hürthle foi reclassificado como uma entidade à parte porque não se encaixava nas características acima: apresenta uma densidade de mutações muito maior. Alberga mutações nas vias *RAS*/*RAF*/*MAPK* e *PI3K*/*AKT*/*mTOR* em 55% dos casos, bem como em vias de dano/reparo do DNA.

Além disso, frequentemente, são vistas mutações em DNA mitocondrial e amplas deleções cromossômicas [3].

Historicamente, foi justamente analisando os CFTs associados à síndrome de Cowden, que se observou que mutações inativadoras no gene *PTEN* permitem que a via PI3K-AKT seja constitutivamente ativada. Não somente: observou-se que a via AKT é a principal via de sinalização relacionada ao CFT [16]. Já no que tange o CNMTF, o mecanismo oncogênico ainda não foi elucidado. Essa lacuna do conhecimento é causada, em parte, pela ausência de fatores de susceptibilidade bem estabelecidos nessas famílias. Os poucos genes/*loci* que foram observados foram identificados em raras famílias com alta penetrância da doença e parecem ser extremamente raros na população. Talvez por esse motivo, tais achados não foram reproduzidos em outras séries. Em síntese, o CNMTF ainda carece de marcadores genéticos de predisposição bem estabelecidos.

4.1.1 Síndromes genéticas associadas ao CNMTF

4.1.1.1 Síndrome de Cowden

É caracterizada por hamartomas generalizados (gastrointestinais, ganglioneuromas, triquelomas), podendo estar associados com melanomas, carcinomas de mama e endometrial, macrocefalia, autismo e deficiência mental [17]. Classicamente, ela é relacionada a mutações no gene "*phosphatase and tensin homolog*" (*PTEN*) no cromossoma 10q22-23. Naqueles indivíduos sem mutações em *PTEN*, foram descritas variantes nos genes *SDHB-D, SEC23B, KLLN, PARP4, AKT1, PIK3CA, USF3, TTN,*
MUTYH, RET, TSC2, BRCA1, BRCA2, ERCC2, HRAS e *RASAL1* [18].

Mutações no gene *PTEN* levam a ativação da via AKT – via frequentemente ativada nos carcinomas foliculares de tireoide, aumentando a proliferação e a migração celular, além de diminuir a morte celular [19].

4.1.1.2 Complexo de Carney

Mutações ativadoras no gene *PRKAR1A* – presente em 70% dos pacientes com complexo de Carney – aumentam a atividade da PKA dependente de AMPc,

desencadeando a proliferação de diversas células que expressam receptores acoplados à proteína G (células de Sertoli, tireócitos, melanócitos, células do córtex adrenal). Resultando, dessa forma, em tumores testiculares, carcinomas de tireoide, hiperplasia adrenal pigmentosa, bem como mixomas em diferentes localizações [20].

4.1.1.3 Síndrome de Werner

A síndrome de Werner é conhecida por ser uma das síndromes associadas à progeria: envelhecimento precoce, alterações cutâneas semelhantes à esclerodermia, cataratas bilaterais, calcificações subcutâneas, aterosclerose precoce e diabetes mellitus. Além disso, está relacionado a diferentes tipos de neoplasia como, por exemplo, meningiomas, mielodistrofias, sarcomas de tecido mole e carcinoma de tireoide [21].

Tais alterações parecem estar ligadas a mutações no gene *WRN* que regula a replicação do DNA, recombinação, reparo, transcrição e manutenção do telômero. Entretanto, não se sabe exatamente qual mecanismo está implicado na oncogênese tireoidiana nesses pacientes [22].

4.1.1.4 Polipose adenomatosa familiar

É uma doença autossômica dominante caracterizada por pólipos e câncer intestinal principalmente, podendo estar associado ao carcinoma papilífero de tireoide variante morular-cribriforme [23]. Geneticamente falando, decorre de uma mutação no gene *APC* que regula, por sua vez, a via WNT e, por conseguinte, a ativação da β-catenina [24]. Vários estudos verificaram uma segunda mutação (*second hit mutation*) no mesmo gene à nível do tecido tumoral [23].

4.1.1.5 Síndrome de ataxia-telangiectasia

A ataxia-telangiectasia propriamente dita é uma doença autossômica recessiva ligada a mutações no gene *ATM* e caracterizada por atrofia cerebelar degenerativa, telangiectasias, defeitos imunológicos e malignidade. Além disso, mesmo os parentes – portadores heterozigotos para a mutação – parecem apresentar

uma incidência maior de neoplasias malignas, principalmente carcinoma de mama, bexiga, pâncreas, pulmão, ovários, gástrico e melanoma. Já o CNMT é 2,6 mais comum nessa população do que na população geral [25]. Curiosamente, algumas mutações parecem estar mais associadas a determinados tipos de neoplasia do que outras: *ATM* c.2119T>C p.S707P (rs4986761), por exemplo, estaria associado a carcinomas de tireoide/endócrino; enquanto a mesma correlação não é observada com outras mutações nesse gene [26]. Seria de se supor que a presença de polimorfismos em *ATM* tivesse alguma influência na susceptibilidade genética ao CNMTF, mas os achados foram bastante controversos nas diferentes populações estudadas. Foram observadas tanto associações com um possível efeito protetor quanto deletério [27; 28; 29; 30]. Apesar disso, foram relatadas duas famílias com CNMTF e outras neoplasias (*ATM* p.P1054R - rs1800057 e rs149711770) recentemente [31].

4.1.1.6 Síndrome DICER1

Classicamente, os pacientes portadores de mutações em DICER1 apresentam bócio multinodular e uma maior predisposição a outras neoplasias como tumor de Sertoli-Leydig de ovário [32] e blastomas pleuropulmonares. Mais recentemente, foi observado que esses pacientes apresentam uma chance 16 vezes maior de desenvolver CNMT do que a população geral [33]. Além disso, mutações somáticas em *DICER1* foram observadas em 0,8% dos tumores catalogados no *The Cancer Genome Atlas* (TCGA), bem como em 87% carcinomas pediátricos de tireoide pouco diferenciado [34].

4.1.2 CNMTF isolado (não-sindrômico)

Dentre as alterações observadas no CNMTF, encontram-se as alterações de telômeros/telomerases. Um estudo italiano observou um aumento significativo de associações teloméricas espontâneas e fusões teloméricas no CNMTF comparado com sujeitos saudáveis e casos esporádicos [35]. Como a telomerase controla o comprimento do telômero, foi estudado alterações de *TERC* e *hTERT* (que formam a telomerase) *in vitro*. Em um estudo, foi observado aumento da amplificação de *hTERT* em leucócitos de pacientes, com respectivo aumento da expressão de mRNA e aumento da atividade da proteína hTERT [36]. Entretanto, tal observação não foi

corroborada por estudos posteriores [37; 38]. Recentemente, polimorfismos em genes relacionados ao complexo *shelterin* que está associado à manutenção dos telômeros também têm sido descritos. Mas, contrariamente ao que foi descrito previamente, uma mutação em *TINF2* foi associada a telômeros mais longos em famílias com CNMT e melanoma [39]. Reforçando isso, variantes nos genes *ACD* e *POT1* – componentes do complexo *shelterin* – foram descritos recentemente[39; 40].

Outro grupo avaliou uma grande família da Tasmânia e encontrou um aumento importante da heterogenicidade no *locus* 2q21 (NMTC1) [41], mas que não foi confirmada por estudos subsequentes [42].

Recentemente, com o uso mais amplo do *genome-wide approach study* (GWAS), foram identificados SNPs (polimorfismos de nucleotídeos isolados) em genes de pacientes com carcinoma não-medular familiar, em um total de 19 SNPs. Os achados mais robustos se concentram nas regiões 9q22 e 14q13.3 (principalmente no *locus* FOXE1); mas achados nos *loci* 2q35 e 8q12 também parecem ser importantes [43; 44; 45; 46; 47; 48; 49; 50; 51; 52; 53]. Entretanto, ainda não foi possível estabelecer-se um efeito causal dessas variantes com a etiologia do CNMTF.

TABELA 1 – VARIANTES/GENES ASSOCIADOS AO CNMT

Variante	**Gene**	**OR**	**Valor de *p***	**Referência**
rs965513[A], 9q22.33	*FOXE1, XPA, C9orf156, HEMGN*	1,75 (1,59;1,94)	$1,7x10^{-27}$	43; 45; 46; 47; 48; 49; 52; 54
rs944289[T], 14q13.3	*NKX2-1, BRMS1L, MBIP, SFTA3*	1,37 (1,24;1,52)	$2,0x10^{-9}$	43; 45; 46; 47; 48; 49; 52; 54
rs966423[C], 2q35	*DIRC3*			52; 54
rs2439302[G], 8p12	*NRG1*			49; 52

FONTE: ADAPTADO DE NAGY (2015) [53]

Outros SNPs foram descritos, mas os achados ainda não foram replicados em outras populações. A saber: rs116909374[T] (*MBIP, NKX2-1*), rs11823005 [C] (*SNX19*), rs6759952[T] (*DIRC3*), rs10238549[C] e rs7800391[T] (*IMMP2L*), rs7617304[A] (*RRARES1*), rs10781500[C] (*SNAPC4*), rs2633322[C] (*PLAU*), rs9951245[G] (*GTSCR1*), rs7267944[C] (*DHX35*), rs10136427[C] (*BATF*), rs1159444[T] (*GPD1L*), rs13184587[G] (*ARSB*), rs2245026[G] (*DACH1*), rs1220597[C] (*SPATA13*) e rs2281016[A] (*TIPRL*).

Outros genes/*loci* têm sido arrolados, mas em famílias acometidas também por bócio multinodular (MNG1), carcinomas papilíferos oxifílicos (TCO) e associação com carcinoma papilífero renal (fPTC/PRN 1q21) [53].

Nos últimos anos com o desenvolvimento do "*Next Generation Sequencing*" (NGS), várias variantes foram arroladas no papel da susceptibilidade do câncer de tireoide. Assim, variantes raras em *TDRD6, IDE, TINF2, RNF213, AGK, NHLH1, TMCC1, ALB, THBS4, C5orf15, KLH3, FGFR4, SMARCD3, GPR107, NSMF, SVIL, EIF3, RNF169, NFRB, CIS, CDH11, EDC4, FOXA3, CDS2, NAPB, SALL4, ATG14, UNC79, LZTR1, ATP13A2, CTDSP1, MAPKAPK3, AARS, KDSR, ZNF302, ZNF17, ITGAD, FGD6, PDPR*, e *EFCAB8* foram encontrada em 17 famílias com CNMT e outros tipos de neoplasias [31]. Foram também observadas nessa série, variantes em genes já estabelecidos na susceptibilidade a câncer como *CHEK2, PRF1, ATM, AKAP13* e *SLC26A11* [31]. Ainda, variantes *em ANO7, CAV2, KANK1, PIK3CB, PKD1L1, PTPRF* e *RHBDD2* foram descritas em uma série coreana [55]; bem como *FKBP10, PLEKHG5, P2RX5, SAPCD1, ANXA3, NTN4* e *SERPINA1* em uma série brasileira [56].

De modo mais detalhado, os mecanismos genéticos envolvidos nas síndromes associadas ao CNMTF, as variantes envolvidas na predisposição genética do CNMT em geral, bem como as variantes associadas ao CNMTF não-sindrômico foram revisados em artigo anexo na seção 6.1.

4.2. CARACTERÍSTICAS CLÍNICAS E EVOLUÇÃO

Várias análises retrospectivas foram realizadas na última década, mas as conclusões são bastante controversas. Em parte, isso se deve à adoção de diferentes critérios de definição e inclusão dos pacientes selecionados. Mas não explica totalmente as diferenças encontradas.

Alsanea *et al*. (2000) avaliou 48 pacientes com CNMFT provenientes do Japão e dos Estados Unidos e observou que esses pacientes apresentaram um tempo de sobrevida livre de doença menor do que seus controles com carcinoma esporádico [57]. Infelizmente, o sistema de estadiamento utilizado à época não foi capaz de prever uma evolução pior da doença [57].

Triponez *et al*. (2006) comparou a sobrevida global desses pacientes diretamente com a curva de sobrevida da população americana. Quando incluiu pacientes cujas famílias se mostravam com apenas 2 (dois) familiares acometidos, os autores não observaram diferença de sobrevida em relação à população americana em geral. Entretanto, com um critério de seleção mais restrito – utilizando como

amostra somente pacientes cujas famílias apresentavam 3 (três) ou mais pacientes acometidos – houve diferença estatística. A sobrevida desses pacientes foi significativamente menor e sugeria que a evolução dos pacientes com CNMFT fosse pior daqueles com carcinoma esporádico [58]. Entretanto, no grupo com 3 ou mais familiares, foram incluídos 3 pacientes com carcinoma anaplásico de tireoide, enquanto os outros grupos não apresentavam nenhum, limitando a interpretação dos resultados. Por outro lado, eles conseguiram separar os pacientes que foram diagnosticados antes de serem identificados como “familiar” e aqueles que procuraram seus médicos já por saberem que eram predispostos ao CNMFT. Isso permitiu concluir que a sobrevida dos pacientes com diagnóstico precoce é melhor, com menor recorrências.

Já Robenshtok *et al*. (2011) não observaram diferença nem na agressividade tumoral nos pacientes com CNMFT, nem na sobrevida livre de doença quando comparados com os pacientes controles [6]. Semelhantemente, análises em diferentes populações encontraram prevalências parecidas de doença linfonodal, invasão extratireoidiana e recorrência em pacientes com CNMT esporádico e familiar [59; 60; 61; 62].

Frente a resultados tão distintos, dados de diversos estudos foram compilados [6; 57; 59; 60; 61; 62; 63; 64; 65; 66; 67; 68] e se observou doença multicêntrica, comprometimento de linfonodos, extensão extratireoidiana e maior recorrência na forma familiar o CNMT através de meta-análise [69]. No total, 10 estudos, abrangendo 18.158 pacientes, apresentaram dados sobre recorrência. Os pacientes com CNMTF apresentaram uma taxa global de recorrência de 17,8% (range: 4.2–43.8%), enquanto o CNMTE de 10.3% (3.4–19.8%) e que foram estaticamente significantes (OR = 1.72, 95% CI: 1.34–2.20, P<0.0001). Multicentricidade foi avaliada em 9 estudos (12.770 pacientes), invasão extratireodiana em 8 estudos (6.403 pacientes) e comprometimento linfonodal em 8 estudos (9.328 pacientes). Sendo que foram mais prevalentes no grupo de CNMTF: 1,50 (1,32 a 1,71), p<0,01; 1,29 (1,02 a 1,41), p<0,03 e 1,18 (1,01 a 1,38), p=0,04, respectivamente.

5 PACIENTES, MATERIAL E MÉTODOS

5.1 ANÁLISE CLÍNICA - PACIENTES E MÉTODOS

Pacientes diagnosticados com carcinoma não-medular de tireoide familiar (CNMTF) e esporádico (CNMTE) entre 2000 e 2019 foram incluídos na análise. O CNMTF foi definido como a presença de carcinoma não-medular de tireoide (papilífero, folicular, de células de Hürthle, ou anaplásico) em dois ou mais parentes de primeiro grau, na ausência de sinais que pudessem sugerir outras síndromes associadas ao câncer de tireoide (como, por exemplo, síndrome de Cowden, síndrome de Gardner/polipose adenomatosa familiar, complexo de Carney, síndrome de Werner, síndrome da ataxia-telangiectasia). Para tanto, um questionário específico (anexo 12.4) e exame clínico foram realizados.

Já o carcinoma não-medular de tireoide esporádico foi definido quando não havia relatos de história de câncer de tireoide na família.

Os pacientes com provável CNMTF foram encaminhados por endocrinologistas de Curitiba entre 2015 e 2019 para avaliação e selecionados no ambulatório de Tireoide do SEMPR-HC-UFPR. Para tanto, o projeto foi submetido ao Comitê de Ética e Pesquisa (CAAE: 40511015.5.0000.0096) e aprovado (parecer 3.509.146). Já o banco de dados sobre câncer de tireoide existe desde 2007 e foi aprovado pelo Comitê de Ética em Pesquisa (CAAE: 48391715.8.0000.0096, parecer 1.228.761).

Alguns dos familiares dos pacientes inicialmente selecionados por CNMTF eram tratados fora de Curitiba (Rio de Janeiro, Londrina, Bogotá) e trouxeram toda a documentação necessária para a análise.

O grupo apresentando CNMTE, utilizados como controle, fizeram todo o manejo e acompanhamento no HC-UFPR. Pacientes que chegaram para seguimento no ambulatório de Tireoide por doença avançada foram excluídos. Nenhuma criança ou adolescente menor de 14 anos foi incluído nesse estudo.

Foram coletados dados sobre o tipo de cirurgia, características dos pacientes (sexo e idade do diagnóstico), assim como dados sobre as características histológicas (tipo, tamanho, presença/ausência de cápsula, invasão de cápsula, invasão angiolinfática, invasão perineural, extensão extratireoidiana grosseira, presença de envolvimento linfonodal, extensão extracapsular no linfonodo e metástases à

distância). Os dados originais foram utilizados para (re)classificação de cada caso de acordo com a 8ª edição do TNM [70].

Um patologista especialista em tireoide revisou todos os casos dúbios e excluiu um caso de neoplasia folicular não-invasiva com características semelhantes às papilares (NIFTP, do inglês *non-invasive follicular neoplasm with papillary-like features*). A resposta ao tratamento foi determinada de acordo com marcadores bioquímicos (tireoglobulina e anticorpo antitireoglobulina), bem como exames de imagem (ecografia cervical, pesquisa de corpo inteiro e/ou tomografia computadorizada), utilizando as diretrizes da *American Thyroid Association* (ATA) 2015 (resposta excelente, resposta bioquímica incompleta, resposta incompleta estrutural e resposta indeterminada) [71].

Quando o tratamento com dose terapêutica de iodo não foi realizado, uma resposta excelente foi definida quando Tg $\leq$ 0.5 ng/dl e a ecografia cervical não identificava nenhuma lesão suspeita.

Para estudarmos a hipótese do fenômeno de antecipação – surgimento da doença em idades mais precoces em cada nova geração –, foram apenas incluídas famílias cujos pais e filhos (eventualmente avós) tinham CNMTF nessa subanálise.

As análises estatísticas foram realizadas através do programa SPSS Statistics para Windows (Versão 26.0., IBM Corporation, Armonk, New York). Para a análise das variáveis qualitativas, foi o usado o teste Q-quadrado/teste exato de Fisher; enquanto para as análises quantitativas, foram usados o teste T de student, teste Mann-Whitney U ou o teste Kruskall-Wallis para amostras independentes como indicado.

5.2 REVISÃO BIBLIOGRÁFICA - GENETIC MUTATIONS AND VARIANTS IN THE SUSCEPTIBILITY OF FAMILIAL NON-MEDULLARY THYROID CANCER

Foi realizado o levantamento bibliográfico e revisados juntamente com equipe com expertise no assunto (artigo 1 na seção Resultados).

5.3 ANÁLISE EXOMA – PACIENTES, MATERIAL E MÉTODOS

5.3.1 Seleção das famílias analisadas

Dentre todas as famílias incluídas para a análise clínica, foram selecionadas duas famílias com carcinoma de tireoide não-medular, por apresentarem maior número de pacientes afetados e os blocos de parafina estarem disponíveis para estudo. Uma delas com 8 pacientes acometidos e outra com 5 pacientes afetados (figura 2 e 3).

O levantamento dos dados foi realizado através de entrevista clínica e revisão do prontuário/exames dos pacientes. Foram coletados 10 ml de sangue total e solicitados os blocos de parafina referentes à(s) cirurgia(s) de tireoide.

5.3.2 Histologia

As lâminas foram revisadas por patologista experiente e, quando necessário, novas lâminas foram confeccionadas e coradas pelo método de hematoxilina-eosina para confirmação do diagnóstico.

5.3.3 Extração de DNA genômico

A extração do DNA foi realizada usando o kit Illustra^{TM} blood genomicPrepMini Spin Kit da GE Healthcare e seguindo as orientações do fabricante.

5.3.4 Análise de exoma

DNA de todos os probandos e dos familiares considerados controle negativo foram submetidos ao sequenciamento completo do exoma utilizando-se Illumina HiSeq2500. A análise bioinformática utilizou os programas BWA, GATK e ANNOVAR para alinhamento ao genoma (hg19), detecção das variantes (SNPs, indels) e anotação das mesmas, respectivamente. Apenas variantes raras (MAF<0,1%), comuns aos probandos da mesma família e funcionalmente deletérias (de acordo com ferramentas de predição funcional: SIFT, Polyphen, Provean, CADD ≥ 15) foram consideradas para a construção de uma lista de genes candidatos.

As variantes sinônimas foram avaliadas por algoritmos de predição específicos regSNPs-splicing e TraP.

Além disso, polimorfismos já relacionados ao CPT esporádico foram sistematicamente avaliados [13; 72; 73; 74]

5.4. RELATO DE CASO

Durante a etapa de inclusão de pacientes com CNMTF foi-se identificada uma família (duas irmãs) com carcinoma ductal de mama e CPT, cujos filhos eram portadores de ataxia-telangiectasia.

Sangue total foram coletados em tubos com EDTA para extração de DNA leucocitário e sequenciamento direto do gene ATM foi realizado. Posteriormente, nova coleta foi feita para realização de exoma na Universidade de São Paulo (USP). Detalhamento da técnica do sequenciamento de exoma, *variant calling* e *annotation* podem ser encontrados na seção de 6.3 (artigo 3)

6 RESULTADOS E DISCUSSÃO

Nas próximas páginas serão apresentados os artigos submetidos e um terceiro que ainda está sendo editado.

O primeiro artigo (artigo 1), *"Genetic mutations and variants in the susceptibility of Familial Non-Medullary Thyroid Cancer*" foi publicado pela Genes (Basel). O segundo artigo (artigo 2), relacionado aos dados clínicos, está em fase de revisão para submissão e o terceiro artigo (artigo 3) *"Thyroid and breast cancer in two sisters with monoallelic mutations in the ataxia telangiectasia mutated (ATM) gene"* foi submetido ao Journal of Endocrine Society.

Após os artigos, encontram-se compilados os dados ainda não publicados da análise de exoma das famílias selecionadas.

6.1. ARTIGO 1

Review

Genetic mutations and variants in the susceptibility of Familial Non-Medullary Thyroid Cancer

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Abstract: Thyroid cancer is the most frequent endocrine malignancy with majority of cases derived from thyroid follicular cells and caused by sporadic mutations. However, when at least two or more first degree relatives present thyroid cancer, it is classified as familial non-medullary thyroid cancer (FNMTC) that may comprise 3-9% of all thyroid cancer. In this context, 5% of FNMTC are related to hereditary syndromes such as Cowden and Werner Syndromes, displaying specific genetic predisposition factors; on the other hand, the other 95% of cases are classified as nonsyndromic FNMTC. Over the last 20 years, several candidate genes emerged in different studies of families worldwide; nevertheless, the identification of a prevalent polymorphism or germinative mutation has not progressed in FNMTC. In this work, an overview of genetic alteration related to syndromic and nonsyndromic FNMTC is presented.

Keywords: thyroid cancer, thyroid neoplasms, genetic predisposition to disease, genetic variants

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1. Introduction

The most common type of thyroid cancer derives from thyroid follicular cells and is named as non-medullary thyroid cancer (NMTC) in order to be distinguished from the less frequent medullary thyroid cancer (MTC) that originates from the thyroid C-cells. The MTC occurs as sporadic and hereditary cancer, in contrast to the NMTC, which is mainly sporadic (Figure 1). The hereditary MTC can be a component of a syndrome or have a familial background. In this context, the NMTC can also be associated with syndromic conditions, such as in Cowden syndrome, Carney complex, Werner syndrome, and familial adenomatous polyposis but to a lesser extent than in MTC. Moreover, a high prevalence of NMTC in ataxia-telangiectasia, DICER1, and Pendred syndromes has been described [1,2].

Besides these well-known genetic syndromes, the characterization of the nonsyndromic form of familial non-medullary thyroid cancer (FNMTC) remains to be consolidated. In 1953, Firminger and Skelton reported the first case of papillary thyroid cancer (PTC) in twins [3]; however, the concept of FNMTC and the genetic predisposition to PTC has emerged only in recent decades. Currently, it is accepted that FNMTC occurs when two or more first-degree relatives are diagnosed with NMTC cancer [4].

The initial FNMTC studies were performed by linkage analysis and described some specific loci, although they did not identify a precise gene associated with FMNTC [5-10]. Furthermore, despite the efforts of many groups in investigating FNMTC using Sanger sequencing, no conclusive information

was found, suggesting genetic heterogeneity, multigenic and multifactorial inheritance [11]. However, a new genomic perspective emerged with the application of Next Generation Sequencing (NGS) technology that covered the whole genome. In this extent, some new insights into genetics of FNMTC have emerged by the recent genome-wide association studies (GWAS) in populations of PTC. The finding of several single nucleotide polymorphisms (SNPs), such as in *DIRC3*, *NIRG1*, *FOXE1*, *NKX2-1* and *PCNXL2*, were observed in the European, Korean, and American populations [12]. Indeed, increasing evidence suggests that genetic predisposition factors play an essential role in carcinogenesis besides environmental factors [13]. In this review, we cover the genetic findings associated with FNMTC and in syndromes related to NMTC.

Figure 1. Incidence of Sporadic and Familial Medullary Thyroid Cancer (MTC) and Non-medullary Thyroid Cancer (NMTC)

2. Syndromic causes of non-medullary thyroid cancer

Many syndromes associated with thyroid tumor predisposition have Mendelian patterns of inheritance, and they are related to mutations that may influence the mechanism of DNA repair, the microRNA processing and maturation, the genome integrity maintenance, the cell signaling, or mitochondrial regulated cellular processes (Table 1) [13]. These syndromes are characterized by several other main malignancies and sometimes lack to present thyroid cancer.

Although some authors suggest surveillance in syndromic FNMTC (see below), the 2015 American Thyroid Association (ATA) guideline does not advise for or against thyroid cancer screening [14].

Table 1. Genetic alterations in syndromes related to NMTC.

Syndrome	Gene	Inheritance pattern	Other malignant tumors	The most common thyroid tumors	Benign manifestations (continues)	Reference
Ataxia-telangiectasia syndrome *	*ATM*	AR*	Lymphocytic leukemia, lymphoma, stomach adenocarcinoma, medulloblastoma, glioma	FTC, PTC•	degenerative cerebellar atrophy, telangiectasias, immune defects	[58]
	ATM	AD	Breast cancer, digestive tract cancer, lymphoma, leukemia			[49,58]
Carney complex	*PRKAR1A*	AD	-	Follicular hyperplasia, nodular hyperplasia, FA, cystic changes, PTC, FTC	Spotty skin pigmentation (lips, conjunctiva, vaginal and penile mucosa), cutaneous and mucosal myxoma, cardiac myxoma, breast myxomatosis, primary pigmented nodular adrenocortical disease, GH-producing adenoma, large cell calcifying Sertoli cell tumors, psammomatous melanotic schwannomas	[30]
Cowden syndrome	*PTEN, SDHB-D, SEC23B, KLLN, PARP4, AKT1, PIK3CA, USF3, TTN, RASAL1*	AD	FTC, breast cancer, epithelial endometrial cancer, colon cancer, renal cell carcinoma melanoma	MNG, Hashimoto thyroiditis, FA, FTC, cPTC, FVPTC, C-cell hyperplasia	Macrocephaly	[16,18}
DICER1 syndrome	*DICER1*	AD	Pleuropulmonary blastoma, ovarian Sertoli-Leydig cell tumor, genitourinary and cerebral sarcomas	MNG, PTC, FA	MNG, cystic nephroma	[62,63]

Syndrome	Gene	Inheritance pattern	Other malignant tumors	The most common thyroid tumors	Benign manifestations (conclusion)	Reference
Familial adenomatous polyposis	*APC*	AD	Digestive tract cancers, fibrosarcomas	CMVPTC, PTC	Intestinal polyps, osteomas, fibromas, desmoid tumors, dental abnormalities, leiomyomas, congenital hypertrophy of the retinal pigment epithelium	[38,132]
Li-Fraumeni syndrome	*TP53*	AD	Breast, brain and adreno cortical cancers and sarcomas	cPTC, FVPTC		[13,81]
Werner syndrome	*WRN*	AR	Atypical melanoma, bone or soft tissue sarcomas	FTC, PTC, ATC	Ageing, bilateral cataract, type 2 diabetes mellitus, hypogonadism, meningioma	[2,133]

*Ataxia-telangiectasia syndrome occurs only in autosomal recessive pattern. However, heterozygotic carriers have an increased risk to cancer radio ionizing-induced. •An increased risk for thyroid cancer was observed in relatives of AT patients, but the histological type was not specified in those epidemiological analysis. Above information is inferred from susceptibility thyroid cancer studies [55,56].

2.1. Cowden syndrome

Cowden syndrome (OMIM #158350) is characterized by hamartomas in different parts of the body (gastrointestinal hamartomas, ganglioneuromas, trichilemmomas) associated with melanomas, breast, endometrial and thyroid cancer , macrocephaly, and eventually, autism spectrum disorder and/or mental retardation [15,16]. One of the major diagnosis criteria is FTC, but if it is not present at the diagnosis, thyroid ultrasound is advocated biannually after seven-years-old [17].

Cowden syndrome is classically associated with mutations in the *PTEN* gene on chromosome 10q22-23, although variants in several other genes have been described in patients without PTEN mutations (*SDHB-D*, *SEC23B*, *KLLN*, *PARP4*, *AKT1*, *PIK3CA*, *USF3*, *TTN*, *MUTYH*, *RET*, *TSC2*, *BRCA1*, *BRCA2*, *ERCC2*, *HRAS* and *RASAL1*) [18-21].

PTEN dephosphorylates PI(3,4,5)P3 (PIP3) and PI(3,4)P2 to PI(4,5)P2 and PI(4)P, respectively. Thus, PIP3 and PI(3,4)P2 do not activate AKT, and PI3K/AKT signaling pathway remains inhibited. PTEN mutation results in loss of function, leading to a high concentration of PI(3,4)P2 that activates AKT and enhances cell proliferation, cell migration and reduces cell death [22,23]. In addition, mechanisms that regulate PTEN expression and compartmentalization are involved in tumorigenesis [24].

Interestingly, the first correlations between PIK3-AKT pathway activation and thyroid cancer were observed in Cowden syndrome studies. As Cowden syndrome mainly presents with FTC and *PTEN* activates the PIK3-AKT pathway, some authors have postulated that PIK3-AKT activation is required for FTC oncogenesis, and these preliminary findings were further corroborated [25,26].

Another intriguing fact was the association of *RASAL1* (RAS protein activator like 1) with Cowden Syndrome. *RASAL1* is a negative modulator of the RAS signaling pathway and suppresses both MAPK and PIK3 pathways. However, *RASAL1* is frequently found methylated or mutated in sporadic follicular and anaplastic thyroid cancer [27].

In a large series of 155 patients with Cowden syndrome and thyroid cancer, 39 presented with *PTEN* germline mutations, while *RASAL1* germline alteration (*RASAL1*, c.982C>T, R328W) was observed in two patients without *PTEN* mutations [21]. In the same study the authors also analyzed the germline database of The Cancer Genome Atlas (TCGA) and discovered that 0.6% of PTC patients harbored deleterious germline *RASAL1* mutation [21].

2.2. Carney complex

Carney complex (OMIM #160980) is an autosomal dominant disorder in 70% of the cases, characterized by loss-of-function mutations in the *PRKAR1A* gene (17q22-24).

Under normal condition several endocrine related ligands, such as TSH, FSH, ACTH, GHRH, and MSH, when binding to the G-protein coupled receptor activates PKA. *PRKAR1A* encodes the R1α subunit of PKA; thus, when mutated, it increases cAMP-dependent PKA activity and drives tumorigenesis [28-30], Therefore thyrocytes, Sertoli cells, adrenocortical cells, somatotrophs, and melanocytes are directly affected by *PRKAR1a* mutation. As a result, variable endocrine tumors are observed in the Carney complex disease, including primary pigmented nodular adrenocortical disease, pituitary adenomas, testicular tumors, ovarian lesions, and myxomas and lentiginosis syndromes [30]. As thyroid cancer could also be part of this syndrome, annual long-term surveillance is recommended [30].

Interestingly, evidence shows that *PRKAR1A* acts as a tumor suppressor gene also in sporadic thyroid cancers [31]. However, the traditional thyroid cancer pathways (MAPK and PIK3-AKT pathways) are not involved in the Carney complex [32]. Instead, a recent *in vitro* study suggests that *PKA* activates AMPK through *LKB1* (also named *SKT11*) in Carney-related FTC, without inhibiting mTOR activation [33].

2.3. Werner syndrome

Werner syndrome is one of the progeroid syndromes (OMIM # 27770) characterized by early aging, scleroderma-like skin changes, bilateral cataracts, and subcutaneous calcifications, premature arteriosclerosis, diabetes mellitus. Different types of cancers are associated with this syndrome, such as meningiomas, myeloid disorders, soft tissue sarcomas, and thyroid carcinoma [1,32] and their regular surveillance is recommended [35]. The Werner Syndrome´s patients carry autosomal recessive *WRN* gene mutations on 8p11.1-21.1. *WRN* gene encodes RECQ helicases that regulate DNA replication, recombination, repair, transcription, and telomerase maintenance. Dysregulation of this pathway triggers DNA instability, telomeric fusions of homologous chromosomes, and ultimately oncogenesis [13]. However, the precise mechanisms that contributes to genome instability in Werner syndrome remains unclear [36]. In a Japanese series, mutations in the N-terminal portion of *WRN* was correlated with PTC, while mutations in C-terminal with FTC [37]. The N-terminal portion of WRN contains exonuclease activity, whereas the central part contains the DNA-dependent ATPase, 3′-5′ helicase, and annealing activity [36]. Overall, these studies suggest specific effects in WRN activity depending on the site of mutation. Moreover, an *in vitro* study showed that mutations in WRN's nuclease domain, helicase domain or DNA binding domain aborted its canonical stimulatory effect on nonhomologous end-joining (c-NHEJ) pathway during DNA double-strand break (DSB) repair [36].

2.4. Familial Adenomatous Polyposis (FAP)

The phenotype of FAP (OMIM # 175100) is characterized by numerous intestinal polyps, colon cancer, and other cancers that include thyroid cancer [2,38,39]. FAP is an autosomal dominant disorder caused by mutations in *APC* gene on chromosome 5q21. The *APC* gene is a suppressor of the Wnt signaling pathway and regulates β-catenin activation by multiple mechanisms. In normal conditions, the Axin complex (formed by APC, GSK3, and CK1) phosphorylates the amino-terminal of the free β-catenin, permitting its recognition and further ubiquitination [40,41]. By this process of continuous degradation, β-catenin remains in the cytoplasm without reaching the promoter region of target genes

in the nucleus. Thus, when APC protein is mutated or truncated, β-catenin is released from its degradation and migrates to the nucleus, activating gene transcription of oncogenic pathways. Truncated APC protein also interferes with chromosome stability and cell migration [41].

In addition to the germline mutation, biallelic inactivation of the wild-type *APC* allele is frequently necessary for tumorigenesis, and the second-hit is commonly acquired by somatic mutation [42]. In the FAP-associated thyroid cancer, the concomitant presence of germline and distinct somatic mutation were observed in several Japanese families [39,43,44]. Most of FAP-associated thyroid cancer present the histological subtype called cribriform-morular variant of PTC (CMVPTC) [2,42].

Annual thyroid ultrasound is recommended to late teen years' patients [45,46].

2.5. Ataxia-telangiectasia syndrome

Ataxia-telangiectasia (A-T) syndrome (OMIM #208900) is an autosomal recessive disorder linked to the mutation of *ATM* gene and characterized by degenerative cerebellar atrophy, telangiectasias, immune defects, and malignancy [47,48]. It is also well known that relatives of patients with ataxia-telangiectasia have an increased cancer incidence [49].

ATM protein belongs to the PI-3 kinase-like protein kinases family. Besides TP53, BRCA1, and BRCA2, ATM is considered a genome's guardian and participates directly in the DNA damage response (DDR). For its activation, MRE11-RAD50-NBS1 (MRN) complex – a sensor of DSB (double strand-break) – induces several autophosphorylations and acetylations. Activated ATM then phosphorylates different proteins involved in DSB (double-strand break) response [50]. For instance, ATM phosphorylates CHK2 and p53, which are both involved in senescence and apoptosis [50].

An increased incidence of thyroid cancer was observed in obligate *ATM* mutation carriers (RR adjusted = 2.6) [49]. Later, selective mutations in the *ATM* gene are related to thyroid cancer: *ATM* c.2119T>C p.S707P (rs4986761) heterozygotes were associated with an adjusted HR (hazard ratio for cancer) of 10 for thyroid/endocrine tumors, while no association was observed in *ATM* c.146C>G p.S49C (rs1800054) heterozygote carriers [47]. Nonetheless, recent population studies revealed that some *ATM* polymorphisms have a protective role, while others studies reported a damaging effect [51-54]. There are even controversial observations for the same polymorphism [51,52,55-57]. Despite these controversies, consistent *ATM* variants (*ATM* p.P1054R - rs1800057- and rs149711770) were recently described in families with FNMTC and other cancers (as kidney, lung, stomach, and prostate) [11].

Nonetheless, it is just recommended the current breast cancer screening in these patients [58].

2.6. DICER 1 syndrome and miRNA processing

Non-toxic multinodular goiter (MNG) is frequently diagnosed in adult population and studies correlate the presence of MNG and the development of differentiated thyroid cancer [14,59,60]. On the other hand, familial cases of MNG are a common characteristic associated with DICER1 syndrome (OMIM #601200) which predisposes patients to thyroid cancer [61], and other types of tumors such as Sertoli-Leydig cell tumors of the ovary (SLCT) [62] and pleuropulmonary blastomas [63].

DICER is an endonuclease essential for the maturation of microRNAs (miRNAs), small non-coding RNAs with ~22nt, that block mRNA translation post-transcriptionally by binding to the 3'-UTR (untranslated region) of target mRNAs, and tightly controlling cell signaling and cell biology [64]. Mutation in *DICER1* gene, especially those present in the ribonuclease domain, leads to DICER loss of function and downregulation of microRNA levels [62,65] . The correct control of miRNA expression is essential to the development of a functional thyroid gland [66]. Studies with transgenic mice with dysfunctional DICER lead to disturbance of thyroid architecture, cell proliferation and disarrangement of follicular structures, and loss of differentiation [67,68], indicating the influence of DICER loss in thyroid tumorigenesis.

A familial approach to investigate the risk of thyroid malignancy in DICER1 syndrome patients revealed a 16-fold higher risk of development of thyroid cancer when *DICER1* is mutated compared to non-mutated patients [61]. Thus, there is a suggestion for thyroid ultrasound every two-three years in patients after the age of 8-years-old to monitor thyroid status [17, 69]. Enforced evidence of *DICER1*

mutation with familial thyroid cancer was also shown in a study with 6 individuals of the same family harboring *DICER1* mutation (c.5441C>T, p.S1814L) and multiple cases of differentiated thyroid cancer and MNG [70].

TCGA database shows *DICER1* mutation in 0.8% of patients with PTC/PDTC (p.E1813G; p.D1810H; p.E1813K; p.R1906S; p.M1402T) [71-75]. A recent study revealed high prevalence of *DICER1* mutations in pediatric-adolescent poorly differentiated thyroid cancer (83%) at hotspot in the metal-ion binding sites of the RNase IIIb domain of *DICER1* (c.5113G>A, p.E1705K; c.5125G>A, p.D1709N (rs1595331264); c.5137G>A, p.1713Y; c.5437G>A, p.E1813K; c.5437G>C, p.E1813Q) [76]. Another study linked hotspot *DICER1* mutations to pediatric PTC (c.5125G>A p.D1709N; c.5428G>T p.D1801Y; c.5438A>G p.E1813G; c.5439G>C p.E1813D), with increased incidence in the patients that do not harbor MAPK classic alterations [77], suggesting a role for *DICER1* mutation detection in thyroid tumors. Interestingly, a recent study detected *DICER1* (c.5429A>T, p.D1810V; c.5437G>A, p.E1813K) and *DROSHA* mutation (c.2943C>T, p.S981S; c.3597C>T, p.Y1199Y (rs61748189)) in benign follicular adenoma, although *DICER1* mutations were not detected in follicular variant of PTC that harbored HRAS mutations [65]. On the other hand, a recent study associated MAPK alterations with germline mutations in DICER1 [78]. Altogether, these studies suggest that *DICER1* haploinsuffiency is associated to thyroid tumorigenesis.

DROSHA is another endonuclease of miRNA processing machinery and acts together with DGCR8 to form the Microprocessor complex to excise the precursor miRNA out of the primary transcript in the nucleus [64]. Then, DICER acts in the next step in the cytoplasm and cleaves the precursor miRNA to form mature functional miRNAs. In a similar extent to *DICER1* mutations, *DGCR8* mutations were also detected in familial cases of MNG and are associated with schwannoma [79]. Altogether, these studies indicate the essential role of proper miRNA processing and expression for thyroid gland physiology.

2.7. Li-Fraumeni syndrome

Li-Fraumeni syndrome is caused by heterozygous mutation in *TP53* and is typically characterized by soft tissue and bone sarcomas, breast cancers, central nervous system tumors, leukemia, and adrenal tumors. p53 interacts with a complex network and drives DNA repair, cell-cycle arrest, senescence, or apoptosis when is phosphorylated by DNA damage response (DDR) kinases [13,80]. The PTC occurs in 10% of Li-Fraumeni syndrome patients, mainly when associated with *TP53* mutation p.R337H [81]. Therefore, imaging screening for thyroid malignancy in Li-Fraumeni families has been advocated [81].

3. Nonsyndromic FNMTC

Even if FNMTC comprises only 3-9% of all thyroid cancer, the first-degree relatives of NMTC have an 8-12-fold increased risk of developing the disease [82,83]. Nonsyndromic FNMTC comprises 95% of all FNMTC and is defined by two or more first-degree relatives present with NMTC without associated syndromes. Moreover, the transmission pattern is not yet well defined seems to be autosomal dominant in most cases. Like sporadic NMTC, more than 85% are PTC, approximately 10% are FTC, and around 5% are anaplastic thyroid cancer. Furthermore, FNMTC is more aggressive, presents with nodal disease, and recurs more often. Also, thyroid cancer tends to occur earlier in subsequent generations in FNMTC, called the anticipation phenomenon [2,84,85].

3.1. Linkage analysis

From 1997 until 2006, the linkage analysis was the main method to study the familial condition. Using this approach, a positive LOD (logarithm of odds) would mean a high likelihood that locus cosegregates with the FNMTC trait, a linkage. In this way, several loci were associated to nonsyndromic FNMTC (Table 2).

Table 2. Loci and genes associated to nonsyndromic FNMTC.

Loci/gene	Localization	Characteristics	Reference
Linkage analysis			
TCO	19p13.2	Oxyphilic PTC	[6]
NMTC1	2q21		[9]
PRN1	1q21	Papillary renal cancer	[8]
MNG1/*DICER1*	14q32	MNG	[7]
Linkage analysis and NGS			
SRGAP1	12q14		[90]
	8p23.1-p22		[10]
	6q22		[88]
lncRNA inside TG	8q24	Melanoma in 1 family	[89]
Enhancer associated with POU2F1 and YY1	4q32		[87]
Other methodology			
NKX2-1	14q13.3		[92]

3.1.1. TCO locus (19p13.2)

TCO locus was identified in a French family with oxyphilic thyroid cancer in the short arm of chromosome 19 (19p13.2). This region includes several genes, such as *ICAM1* gene, which is overexpressed in thyroid cancer cells, and *JUNB* proto-oncogene [6]; however, some others genes in the locus, such as several zinc-finger-protein genes were not yet identified. Moreover, TCO locus does not seem to be involved in the majority of oxyphilic sporadic NMTC. An additional Tyrolean family with high LOD in the same locus was also described [86].

3.1.2. PRN1 locus (1q21)

Papillary thyroid cancer associated with papillary renal cancer: Linkage analysis identified this locus with the highest LOD of 3.58 in a family with three generations affected by PTC and papillary renal carcinoma. *MET* mutations, frequently associated with familial papillary renal cancer, and mutations associated with other thyroid cancer syndromes were excluded [8]. However, this finding was limited to this family.

3.1.3. NMTC1 locus (2q21)

This locus was described in a large Tasmanian family study [9], and when the authors further analyzed 17 families with FNMTC, they found an LOD heterogeneity of 4.17. At that time, it was hypothesized that multiple environmental and genetic causes could be involved in the pathogenesis of FNMTC [86].

3.1.4. 4q32 locus (an enhancer of unknown function)

A rare mutation in 4q32 was found in the linkage analysis and targeted deep sequencing in a large family with four individuals with benign thyroid disease, nine PTC patients, and one ATC patient. This nucleotide exchange in chr4:165491559 (GRCh37/hg19), named 4q32A>C, is in a highly conserved region. The ChIP assays showed that both POU2F1 and YY1 transcription factors related with specific thyroid genes and thyroid development, bind to this region. As the consequence of the allele´s change, decrease of both POUF2 and YY1 bindings were observed. Indeed, transcription factors disruption has already been associated with cancer [87].

3.1.5. 6q22 locus

The finding of 6q22 locus with LOD +3.30 was observed in 38 families of FNMTC by linkage analysis and genome-wide SNP array [88]. However, no further studies have confirmed this locus in additional families.

3.1.6. 8p23.1-p22 locus

A locus associated with FNMTC in a huge Portuguese family was identified by linkage analysis, with a maximum parametric haplotype-based LOD score of 4.41. Among seventeen candidate genes in the locus (*PPP1R3B*, *MIRN597*, *MIRN124A1*, *MSRA*, *C8orf74*, *SOX7*, *PINX1*, *MIRN598*, *C8orf15*, *C8orf16*, *MTMR9*, *C8orf13*, *NEIL2*, *CTSB*, *DUB3*, *DLC1*, *TUSC3*), no deleterious alteration was detected in those gene's coding region. [10].

3.1.7. 8q24 locus, a lncRNA inside the thyroglobulin (*TG*) gene

Linkage analysis was also performed in a group of 26 families of PTC [89] and revealed a LOD of+ 1.3 in a locus that harbors *TG* and *SLA* (Src like adaptor) genes. However, no polymorphism or mutation was found in the coding genes, suggesting that this alteration could be associated with a lncRNA related to the *TG* gene.

3.1.8. *SRGAP1* (12q14 locus)

The study of 38 families with FNMTC by genome-wide linkage analysis indicated a high peak in 12q14 in 55% (21 of 38), but with a modest OR = 1.21 (P = 0.0008). Nonetheless, it was observed six different germline mutations/variants in the *SRGAP1* gene (c.447A>C, p.Q149H; c.823G>A, p.A275T; c.1534G>A, p.V512I, rs74691643; c.1849C>T, p.R617C, rs114817817; c.2274T>C, p.S758S, rs789722; c.2624A>G, p.H875R, rs61754221). *In vitro* functional testing in thyroid cancer cells showed a decreased GAP activity in two of these *SGARP1* polymorphisms (Q149H and R617C). Interestingly, the *SRGAP1* could mediate tumorigenesis by interacting with CDC42 [90], a common signal transduction convergence point of many signaling pathways and can play a role in thyroid cancer cell migration via RAGE/Dia-1 signaling [91].

3.1.9. *NKX2-1* (14q13.3 locus)

The mutation in *NKX2-1* (c.1016 C>T; p. A339V) was described in two families associated with PTC and MNG [85]. Even though most patients had only MNG, the authors hypothesized that MNG could be the first step to malignancy [92-95].

3.1.10. MNG1 locus (14q32) - *DICER1*

The MNG1 (OMIM # 138800) locus was revealed by linkage analysis in families with multinodular goiter and NMTC [7]. Further, it was observed that MNG1 corresponded to *DICER1* gene, related with microRNA biogenesis (described in "Syndromic causes of non-medullary thyroid cancer" section).

3.2. ***Genome-wide linkage analysis in the population of PTC patients***

The sequencing of the genome by NGS uncovered the genetic variation and the potential association with several pathologies, including cancer. In particular, the GWAS (genome-wide association study) revealed numerous SNPs in the genes related to thyroid physiology and tumorigenesis (Table 3) [12].

Table 3. Genes associated with genetic predisposition of sporadic papillary thyroid cancer

Locus	Nearest gene	Population	Reference
9q22.33	*FOXE1, PTCSC2*	Belarus, Iceland, Italy, Korea, Netherlands Poland, Spain, USA	[96,102,122]
14q13.3	*PTCSC3, NKX2-1, MBIP1*	Iceland, Italy, Korea, Netherlands, Poland, Spain, USA	[96,102]
2q35	*DIRC3*	Iceland, Italy, Korea, Netherlands, Poland, Spain, UK, USA	[102,110]
8p12	*NRG1*	Iceland, Korea, Netherlands, Spain, USA	[102,109]
1q42.2	*PCNXL2*	Iceland, Korea, Netherlands, Spain, USA	[108,109]
European only			
3q26.2	*LRRC34*	Iceland, Netherlands, Spain, USA	[108]
5p15.33	*TERT*	Iceland, Netherlands, Spain, USA	[108]
5q22.1	*EPB41L4A*	Iceland, Netherlands, Spain, USA	[108]
10q24.33	*OBFC1*	Iceland, Netherlands, Spain, USA	[108]
15q22.33	*SMAD3*	Iceland, Netherlands, Spain, USA	[108]
Korean only			
12q14.3	*MSRB3*	Korea	[109]
1p13.3	*VAV3*	Korea	[109]
4q21.1	*SEPT11*	Korea	[109]
3p14.2	*FHIT*	Korea	[109]
19p13.2	*INSR*	Korea	[109]
12q24.13	*SLC8B1*	Korea	[109]

3.2.1. FOXE1/PTCSC2

Located in 9q22.3 and close to the *FOXE1* gene, rs965513 conferred an increased risk for thyroid cancer and was named *PTCSC2* (papillary thyroid carcinoma susceptibility candidate 2). The carriers of rs965513 (homozygous of allele [A]) present a 3.1-fold increased risk for thyroid cancer in a large European series [96]. The same polymorphism rs965513 was observed in Japanese and Belarusian populations, but with an OR of 1.6-1.9 [97]. Similarly, a variant in the promoter region of the *FOXE1* gene (rs1867277) was identified as a risk factor for PTC (OR = 1.49) in a Spanish series and further confirmed in an Italian one [98]. Subsequently, new studies showed a tumor suppressor effect of *FOXE1* and demonstrated that rs1867277 is involved in differential recruitment of USF1/ USF2 transcription factors, which interferes with FOXE1 expression [12,99]. Moreover, *MYH9* (myosin heavy chain-9) can bind and suppress the shared promoter of *PTCSC2* and *FOXE1* bilaterally (that includes rs1867277 region); an effect that is abolished by PTCSC2 that sequesters MYH9 [100]. Therefore, *MYH9*, a lncRNA binding protein, can also play a role in PTC susceptibility.

Interestingly, a rare *FOXE1* variant (c.743C>G; p.A248G) was identified in one of 60 Portuguese FNMTC cases and one sporadic case. Besides, polymorphisms in *FOXE1* locus (rs965513 and rs1867277) were associated with increased familial and sporadic NMTC risk [97,101].*3.2.2. NKX2-1*

A consistent finding in the 14q13.3 locus was rs944289. Located close to the *NKX2-1* gene, this VUS is in *PTCSC3*'s promoter region and regulates the lncRNA PTCSC3 expression by affecting the binding site of C/EBPα and C/EBP◎ (PTCSC3 activators). [96,102-104]. PTCSC3 downregulates S100A4, reducing cell motility and invasiveness. Thus, *PTCSC3* mutations could predispose to PTC through the S100A4 pathway [105]. Moreover, *NKX2-1* mutation (c.1016C>T; p.A339V) was observed in a family with multinodular goiter and papillary thyroid cancer [85], but this was not confirmed by another FNMTC study [106].

3.2.3. NRG1

NRG1 polymorphisms produced an association signal in GWAS for thyroid cancer. Interestingly, NRG1 is highly expressed in the thyroid and participates in cell growth pathways, mainly via ERBB-MAPK [107]. However, NRG1 expression is detected in follicular adenomas, suggesting they are linked to thyroid tumorigenesis [12].

3.2.4. DIRC3

Polymorphisms in the *DIRC3* (disrupted in renal carcinoma 3) gene have also been found in thyroid cancer GWAS [12,108,109]. *DIRC3* codifies a lncRNA that was firstly associated with renal cancer, suggesting a tumor suppressor role [110]. Interestingly, *DIRC3* and *IGFBP5* (insulin-like growth factor binding protein 5) tumor suppressor are within the same topologically associated domain. Moreover, it was observed that *DIRC3* depletion induces an increased *SOX10* (SRY-box transcription factor 10) repression of *IGFBP5* in melanoma cell cultures, corroborating the tumor suppressor role of *DIRC3* [111].

Furthermore, the TT variant of rs966423 (*DIRC3,* g.217445617C>T) has been associated with worse PTC presentation and prognosis: an increased tumor size, staging, lymph node involvement, and overall mortality was observed in TT-haplotype [112]. In a Chinese series, rs966423 was also correlated to tumor invasion and multifocality [1]. Nevertheless, no difference in these parameters was observed in a Polish series [113].

3.2.5. Polygenic contribution

Recently, an increased risk for PTC was associated with cumulative number of deleterious polymorphisms detected in the same patient. Ten different polymorphisms (rs12129938, rs11693806, rs6793295, rs73227498, rs2466076, rs1588635, rs7902587, rs368187, rs116909374, rs2289261) related to the PTC development were analyzed, and the presence of each of these SNPs increased the risk to PTC. Nevertheless, if a patient harbors all ten variants at the same time, the risk of developing thyroid cancer is 6.9-fold greater than those with no variants [114].

3.2.6. Telomere abnormalities

A decade ago, three independent groups observed that relative telomere length (RTL) is shorter in patients with FNMTC [115-117]. As telomerase controls the telomere length, one of these groups investigated *TERC* and *hTERT* (which form telomerase) alterations and observed the amplification of *hTERT* in patients' leukocytes [115]. However, this finding was not confirmed subsequently [116,117]. In the last years, many alterations in the shelterin complex's genes have been reported. The shelterin complex is formed by six proteins (POT1, ACD, TINF2, TERF1, TERF2, TERF2IP), and protects the telomere from DDR mechanisms. Along with telomerase, this complex is vital for genomic stability because telomeric ends resemble DNA double breaks. Telomeric repeat binding factor 1 (TERF1, also known as TRF1), telomeric repeat binding factor 2 (TERF2, also known TRF2), and protection of telomeres 1 (POT1) directly recognize TTAGGG repeats. In contrast, adrenocortical dysplasia protein homolog (ACD, also known as TPP1), TERF1-interacting nuclear factor 2 (TINF2, also known as TIN2), and telomeric repeat binding factor 2 interacting protein (TERF2IP, also known as RAP1) form a complex that differentiates telomeres from sites of DNA damage.

TINF2 mutation was described in a family with melanoma and thyroid cancer predisposition. Functional analysis showed that mutated *TINF2* was unable to activate *TERF2*, resulting in longer telomere lengths. All shelterin complex's genes were screened in subsequent 24 families with FNMTC, and two missense variants in *TINF2* and *ACD* genes were found, but only the *ACD* variant was predicted as deleterious [118].

Another group reported a new mutation in *POT1* (c.85G>T; p.V29L) [119] in an Italian FNMTC. *POT1* disruptions can interfere with the interaction of the POT1-ACD complex. In agreement with these findings, another *POT1* mutation (c.268A>G; p.K90E) was described in a family with predisposition to

several tumors (melanoma, breast, kidney, and thyroid cancer, pituitary tumor, and Cushing syndrome) [120]. Moreover, an association between the increased risk of thyroid cancer and the presence of an intronic variant of *POT1* (rs58722976) was also observed in a cohort of childhood cancer survivors [121].

Altogether, it suggests that telomere abnormalities and shelterin complex genes alteration may influence the predisposition to the FNMTC

3.2.7. miRNA

The miRNA-related SNPs affects the microRNA biogenesis and function. A large study evaluated approximately 80 families displaying Mendelian-like inheritance and found two candidate miRNA (let-7e and miR-181b). The variants of let-7e and miR-182b-2 were located at the 5′ end of 3p mature miRNA and the 3′ end of 5p mature miRNA, respectively, which downregulate the expression by impairing the miRNA processing [122]. The gain or loss of specific miRNAs is an important oncogenic event [66].

3.3. Whole exome/genome sequence

The whole-exome sequence (WES) or the whole genome sequence (WGS) of family members with FNMTC is another strategy besides the GWAS in large populations of DTC. Using this approach, an enormous number of variants is detected, thus, demanding some criteria to filter and select the candidate variants. In general, MAF (minor allele frequency), thyroid expression, and predictor functions (i.e., SIFT, PolyPhen, CADD, and others) are used as filters. Variants related to cancer pathways can also be used as filters. As the application of this strategy has been consolidated for genetic studies in recent years, some authors have proposed new variants involved in FNMTC, many of which are still under validation.

3.3.1.SRRM2

The association of linkage analysis and WES identified an *SRRM2* variant in a family with FNMTC [123]. However, this variant was not exclusively present in FNMTC, as it was found in sporadic NMTC cases, implying the occurrence of FNMTC may also depend on environmental factors or other genes [123].

3.3.2.NOP53

The presence of rs78530808 (*NOP53*, c.91G>C, p.D31H) was observed in one family with FNMTC when using a lesser strict filter than other studies (MAF<2%) [113] ,. NOP53 participates in ribosome biogenesis and regulates the p53 activation in case of ribosome biogenesis perturbation. The variant c.91G>C, was also identified in three out of 44 families with FNMTC [124]. In the tumor samples, NOP53 expression was increased when compared to the adjacent normal tissue. Furthermore, *NOP53* knockdown inhibited cell proliferation and colony formation *in vitro* [124]., Altogether these findings suggested that this variant could have an oncogenic role in thyroid tumorigenesis [124].

3.3.3.HABP2:

HABP2 variant is an excellent example to describe how careful we should be with possible false-positive findings. The variant G534E was described in a family with seven members with PTC [125]. However, this finding was severely criticized later by other researchers. Even though it seemed the right candidate in the beginning, further studies did not confirm it in other populations. Furthermore, as its MAF is high in the European population, we would expect a higher incidence of FNMTC [126]. Besides, the prevalence of this same variant was similar among patients with FNMTC, sporadic PTC, and controls [127,128].

3.4. Candidate variants associated with FNMTC

Recently, different groups have pinpointed a list of candidate variants in FNMTC. A Korean study identified seven candidate variants localized in *ANO7*, *CAV2*, *KANK1*, *PIK3CB*, *PKD1L1*, *PTPRF*, and *RHBDD2* genes in a family with four patients with PTC [129]. Also, a Brazilian group reported seven new variants located in *FKBP10*, *PLEKHG5*, *P2RX5*, *SAPCD1*, *ANXA3*, *NTN4*, and *SERPINA1*[130].

In a large series including 17 families with isolated FNMTC and FNMTC associated with other malignancies, 41 rare candidate variants were identified in *TDRD6*, *IDE*, *TINF2*, *RNF213*, *AGK*, *NHLH1*, *TMCC1*, *ALB*, *THBS4*, *C5orf15*, *KLH3*, *FGFR4*, *SMARCD3*, *GPR107*, *NSMF*, *SVIL*, *EIF3*, *RNF169*, *NFRB*, *CIS*, *CDH11*, *EDC4*, *FOXA3*, *CDS2*, *NAPB*, *SALL4*, *ATG14*, *UNC79*, *LZTR1*, *ATP13A2*, *CTDSP1*, *MAPKAPK3*, *AARS*, *KDSR*, *ZNF302*, *ZNF17*, *ITGAD*, *FGD6*, *PDPR*, and *EFCAB8* genes. Cancer susceptibility genes (*CHEK2*, *PRF1*, *ATM*, *AKAP13*, *SLC26A11*) were also observed [11]. As described before, the authors further correlated the presence of *TINF2* (a shelterin gene) to families with PTC and melanoma.

It was also interesting to observe that some of these genes have already been associated with thyroid cancer predisposition [51,131]. Despite these promising findings, most of the variants needs to be better investigated for its functional role in thyroid cancer risk.

4. Conclusions

It was expected that the advent of new technologies of genome study would shed new light on the genetic predisposition of FNMTC. The NGS certainly did shed light on a whole new spectrum of variants and pointed to the co-occurrence of several variants in FNMTC. However, the limiting point in this scenario is the lack of a detailed *in vitro* validation that could precisely identify the contribution of each variant for the complex FNMTC entity. Moreover, the expansion of already known genetic data in multiple cohorts is essential to establish their role in FNMTC carcinogenesis.

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Abbreviations

CMVPTC	cribriform-morular variant of PTC
cPTC	classical PTC
DDR	DNA damage response
FA	follicular adenoma
FNMTC	familial non-medullary thyroid cancer
FTC	follicular thyroid cancer
FVPTC	follicular variant of PTC
GWAS	genome-wide association studies
HR	hazard ratio
LOD	logarithm of odds
miRNAs	microRNAs
MNG	multinodular goiter
MTC	medullary thyroid cancer
NGS	Next Generation Sequencing

NMTC	non-medullary thyroid cancer
PTC	papillary thyroid cancer
SNPs	single nucleotide polymorphisms
VUS	variant of uncertain significance
WES	whole exome sequence

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6.2. ARTIGO 2

Differences in the Aggressiveness of Familial versus Sporadic Non-Medullary Thyroid Cancer: An Unresolved Controversy

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Introduction

The occurrence of thyroid cancer in twins was first reported in 1953 by Firminger and Skelton (Firminger e Skelton, 1953). However, the concept of Familial Non-Medullary Thyroid Carcinoma (FNMTC) has only been firmly established in the last decades (Grossman *et al.*, 1995). FNMTC is defined by two or more first-degree relatives affected by non-medullary thyroid carcinoma (papillary, follicular, Hürthle, anaplastic) without syndromic signs (Cowden's syndrome, Gardner syndrome/Familial Adenomatous Polyposis, Carney's complex, Werner's syndrome (Mazeh e Sippel, 2013; Guilmette e Nose, 2018).

FNMTC represents 1.5-10% of sporadic NMTC (SNMTC) (Loh, 1997; Maxwell *et al.*, 2004; Ito *et al.*, 2009; Pitoia *et al.*, 2011; Mazeh *et al.*, 2012; Park *et al.*, 2012; Jiwang *et al.*, 2015), and many controversies still surround this entity. Multicentricity and lymph node metastases were observed more commonly in FNMTC by some (Alsanea *et al.*, 2000; Uchino *et al.*, 2002; Mazeh *et al.*, 2012; Park *et al.*, 2012) but not all authors (Maxwell *et al.*, 2004; Ito *et al.*, 2009; Robenshtok *et al.*, 2011). Similarly, some but not all have reported a higher incidence of recurrence and a compromised disease-specific survival in FNMTC compared to SNMTC (Triponez *et al.*, 2006).

Syndromes related to FNMTC have well-documented genetic mechanisms: *PTEN, APC, WRN,* and *PRKAR1A* mutations were associated with Cowden syndrome, Gardner syndrome/Familial Adenomatous Polyposis, Werner syndrome, and Carney complex, respectively. On the other hand, non-syndromic FNMTC has no defined genetic predisposition. Many loci and variants have been described, but they seem to be restricted to few families. Thus, FNMTC has been associated with TCO, NMTC1, PRN1, 4q32, 6q22, 8p23.1-22, and 8q24 loci, as well as *NKX2-1, FOXE-1/PTCSC2, SRGAP1*, *SRRM2*, *NOP53*, *HAPB2* variants, and genes associated with shelterin complex (*TINF2*, *POT1, ACD*).

Given the ongoing controversies about the clinical behavior of FNTMC, the absence of high penetrance mutations outside of the syndrome forms, and the observed heterogeneity in FNMTC families in terms of age-dependent penetrance and expressivity, it remains of interest and importance to thoroughly characterize such families. This may ultimately have impact on clinical management and permit to

establish structured recommendations for screening and, in some cases, refine treatment strategies.

With the goal to contribute to a better understanding of FNMTC as a clinical entity, this study focused on the characterization of familial cases and a comparison to sporadic thyroid cancer patients.

MATERIAL AND METHODS

Patients with familial non-medullary thyroid cancer (FNMTC) and sporadic non-medullary thyroid cancer (SNMTC) diagnosed between 2000 to 2019 were included in this study. FNMTC was defined as the presence of non-medullary thyroid cancer (papillary, follicular, Hürthle, or anaplastic) in two or more first-degree relatives. Furthermore, patients with signs indicative or suggestive of syndromes associated with thyroid cancer such as Cowden syndrome, Gardner syndrome/Familial Adenomatous Polyposis, Carney complex, Werner syndrome, or ataxia-telangiectasia were excluded. A specific questionnaire (see Appendix) and clinical examination were performed to exclude syndromes associated with thyroid carcinomas. Sporadic Non-Medullary Thyroid Cancer (SNMTC) is defined as no familial history of thyroid cancer in the family. FNMTC patients were selected from databank of Thyroid Group of SEMPR – HC – UFPR (Endocrine Division (SEMPR), Department of Internal Medicine, Federal University of Parana) and from Curitiba's private clinics during 2015 to 2019. The thyroid cancer databank of SEMPR – HC - UFPR began in 2007 and was approved by Ethics Committee (CAAE: 48391715.8.0000.0096, trial: 1.228.761). In 2007, all information was collected retrospectively and, since then, prospectively. Some affected relatives of FNMTC kindreds have been treated outside of Curitiba (Rio de Janeiro, Brazil; Londrina, Brazil; Bogota, Colombia); information on these patients was obtained retrospectively from the treating physicians and/or clinical institutions.

The control cohort comprising SNMTC is formed by patients followed at the Federal University of Parana, Curitiba, a tertiary hospital, and consists of patients that have been diagnosed and followed exclusively at this institution. Patients referred to at a later time point in the course because of advanced disease were not included in the control cohort. No children on adolescents below the age of 14 years has been included in this study.

In our hospitals, lymphadenectomy was only performed when lymph node disease is observed by ultrasound and/or during the surgery.

Data about the type of surgery, patients' characteristics (sex, age at diagnosis), histological characteristics (type, size, presence/absence of capsule, capsular invasion, angio-lymphatic invasion, perineural invasion, gross extrathyroidal extension, presence of lymph node involvement, extra nodal extension, and distant metastases) were collected. Raw data was used to classify each case according TNM 8th edition.

An expert thyroid pathologist revised all cases with unclear pathology reports and excluded one case of non-invasive follicular neoplasm with papillary-like features (NIFTP). Response to therapy was determined with parameters including biochemical markers (thyroglobulin (Tg) and antithyroglobulin antibodies), as well as imaging studies (ultrasound, scintigraphy, computerized tomography), and classified according to the 2015 ATA guidelines (excellent response, biochemical incomplete response, structural incomplete response, and indeterminate response) (Haugen *et al.*, 2016). In cases with a total thyroidectomy without subsequent radioiodine treatment, an excellent response was defined as a Tg ≤ 0.2 ng/dl and a negative cervical ultrasound.

To study the possibility of an anticipation phenomenon, an earlier onset of disease manifestations in subsequent generations, we only included FNMTC kindreds with documented DTC in a parent and some of their offspring.

Approval of the Institutional Review Board was obtained (CAAE: 40511015.5.0000.0096, trial: 3.509.146).

The statistical analyses were realized using SPSS Statistics for Windows (Version 26.0., IBM Corporation, Armonk, New York). For qualitative variables, we used the Chi-square/Fisher's test; for quantitative analyses, the T-student test for independent variables, the Mann-Whitney U test, or the independent-sample Kruskal-Wallis test as indicated.

RESULTS

In total, we recruited 42 familial cases, but five were excluded. Two of them also have breast cancer and are heterozygous for *ATM* mutation (*ATM* c.3848T>C; p.L1283P) and another had a robust familial lung cancer history associated. The other two had a diagnosis before 2000.

Among the SNMTC cases, we evaluated 301 patients for enrollment. However, 62 had insufficient data, 52 were operated before 2000, and 68 were initially managed outside the hospital. Thus, the final SNMTC cohort consisted of 119 subjects (Figure 1).
Analyzing patients followed at the Federal University of Parana exclusively, the prevalence of FNMTC was 3.2 % in our historical cohort.

Baseline characteristics

In this series, there were no sex differences between the FNMTC and SNMTC groups and the majority were females (79.5% *versus* 88.2% in the FNMTC and SNMTC groups, $p = 0.187$). The patients in the two groups were also managed similarly: 100% of FNMTC and 96.6% of SNMTC patients were submitted to total thyroidectomy.
However, the subjects in the FNMTC group were younger at diagnosis than SNMTC patients (38.5 ± 14.2 *versus* 46.6 ± 13.8, $p = 0.003$). Furthermore, as described above, more patients in the FNMTC group were initially followed at private clinics, indicating that they had private health insurance, and the mean available follow-up was shorter in FNMTC patients compared to SNMTC subjects (7.0 ± 3.6 years for FNMTC versus 10.4 ± 5.3 years for SNMTC ($p < 0.001$).
The patient characteristics are summarized in TABLE 1.

Histopathological characteristics

In both groups, papillary thyroid cancer was the most common histological type: 104 (87.4%) in the SNMTC and 38 (97.4%) in the FNMTC group ($p = 0.122$). Interestingly, tumor size was significantly smaller in the FNMTC group (18.28 mm ± 13.56 in FNMTC *versus* 25.27 mm ± 20.39 in SNMTC, $p = 0.047$). For all other histopathological characteristics (capsular invasion, angiolymphatic invasion, extrathyroidal extension, multicentricity), there were no statistically significant differences between the two groups (TABLE 2).

Lymph node and distant metastatic disease

However, despite the similar characteristics in the primary tumors, FNMTC patients were found to have more positive lymph nodes at the first surgical intervention than SNMTC patients (46.2% *versus* 21.8%, $p = 0.007$). Multivariate analysis was performed to understand if other variables, as size and age, could also influence lymph

node disease. FNMTC patients had a 2.72-fold higher risk of lymph node metastasis (TABLE 3).

However, the data also showed a correlation with younger age and lymph node involvement (p = 0.004; OR = 0.96) but no correlation between tumor size and lymph node metastasis (p =0.095; OR = 1,02).

The prevalence of metastatic disease at initial diagnosis did not differ between the SNMTC and FNMTC groups (9.2% x 2.6%, *p* = 0.296) (TABLE 2).

Radioiodine therapy

The frequency of radioiodine therapy was similar between the SNMTC and FNMTC groups (*p* = 0.799). However, the administered activities were, on average, lower in the FNMTC group (5.5 ±6.03 *versus* 3.49 ±3.06 GBq, *p* = 0.054). The doses had varied from zero to 38.48 GBq in SNMTC, and from zero to 14.8 GBq in FMNTC.

Response to therapy and recurrences

Comparing SNMTC and FNMTC outcomes, the response to therapy was similar in the two groups (*p* = 0.230) and there was no difference in the incidence of recurrences, too (*p* = 0.101). Data was summarized in TABLE 5.

Comparison between families with only two versus three or more nonmedullary thyroid cancer patients

When age at diagnosis, sex and type of Health Assurance were analyzing in both groups, no statistical difference was found. Tumor size, histopathological type or other histopathological characteristics were also similar in families with two and three or more nonmedullary thyroid cancer patients (data not shown). Response to therapy reclassification and subsequent neck dissection had no statistical differences neither.

Comparison between first, second and third generations in FNMTC

On other hand, when age at diagnosis was compared between different generations, we observed that mean age is statistically significant lower in each new generation (*p* = 0.010)

DISCUSSION

In the past, NMTC was usually considered to be a sporadic cancer without an obvious genetic predisposition outside of a few syndromes. However, the concept of FNMTC, which is considered to be highly heritable nowadays, is now well established (Eng, 2000; Mazeh e Sippel, 2013; Guilmette e Nose, 2018). Whether or not the clinical behavior and outcomes differ between FNMTC and SNMTC still remains controversial (Mcdonald *et al.*, 2011; Robenshtok *et al.*, 2011; Mazeh *et al.*, 2012; Wang *et al.*, 2015). The results presented here suggest that FNMTC may be associated with more prevalent lymph node metastasis at initial diagnosis (46.2% *versus* 21.8%, p = 0.007). A similar finding was also observed by some (Pitoia *et al.*, 2011; Mazeh *et al.*, 2012; Jiwang *et al.*, 2015), but not all authors (Alsanea *et al.*, 2000; Uchino *et al.*, 2002; Capezzone *et al.*, 2008; Ito *et al.*, 2009; Moses *et al.*, 2011; Robenshtok *et al.*, 2011; Park *et al.*, 2012).

Reinforcing this finding, subsequent lymphadenectomy was repeated in some of them, showing that it was not due to a biased methodological approach by our surgeons.

The finding of more prevalent lymph node disease could be correlated with a younger age of diagnosis in the present FNMTC group, as observed in a recent SEER analysis (Wang *et al.*, 2018). In fact, the age at diagnosis in this FNMTC series is lower than in the SNMTC group (38.5 ± 14.2 *versus* 46.6 ± 13.8, *p* = 0.003). Despite this, many authors observed a younger age in FNMTC [6; 57; 59; 64; 69; 75; 76]. Additionally, younger age and an association with more aggressive behavior, even in families with only 2 affected patients, has also been described (Capezzone *et al.*, 2020).

Furthermore, an anticipation phenomenon was also postulated for FNMTC (Capezzone *et al.*, 2008). The anticipation phenomenon is classically described in

trinucleotide disorders such as Huntington's disease. It is characterized by diagnosis at an earlier age in each subsequent generation and more severe symptoms in the affected individuals. Although a similar phenomenon has not been reported in FNMTC, we observed an earlier age at diagnosis in subsequent generations (p = 0.010). Although, there is only one third-generation patient, this statistical difference persisted when first and second-generation groups are analyzed alone (p = 0,08). Moreover, the diagnosis seems to be anticipated by more than a decade in these families in subsequent generations (figure 2). However, we cannot exclude an ascertainment bias underlying this observation. When there is a thyroid cancer diagnosis in a family, it is natural that people look for health assistance and that this may lead to establishing the diagnosis at an earlier age. In addition, most of our FNMTC patients had easy access to health assurance and rapid evaluation of their thyroid. Eventually, all these factors together could explain an earlier diagnosis, and may explain the younger age at diagnosis and the fact that these patients had smaller tumors.

Corroborating the lymph node disease prevalence in our FNMTC group, a meta-analysis suggested that FNMTC might have a more aggressive behavior (Wang *et al.*, 2015). It has been suggested that the FNMTC diagnosis tends to occur at a younger age, and that FNMTC is more often multifocal and with more extensive lymph node disease than SNMTC (Wang *et al.*, 2015). However, others did not confirm this observation (Mcdonald *et al.*, 2011; Robenshtok *et al.*, 2011).

Many factors can contribute to the controversies about initial presentation and clinical behavior in SNMTC and FNMTC. For instance, according to Charkes, there is a high risk (62-69%) of misclassifying sporadic cases as familial cases when the inclusion criterion is based on only two affected individuals in a family because DTC as such has a high incidence (Charkes, 2006). Thus, differences in the definition and inclusion

criteria confound the literature on FNMTC. The presence of three or more affected individuals within a family is less common, and probably this is the reason why all the series included families with only two affected individuals, together with some kindreds with three or more subjects. In the cohort presented here, 56% of the included familial cases stemmed from families with three or more affected members. When we compared FNMTC patients from families with at least three affected members to two affected cases, we found no differences in terms of sex, age or histological characteristics (data not shown).

Despite a more prevalent lymph node disease in the FNMTC group, there were no differences between the SNMTC and FNMTC groups in terms of the ATA response to therapy classification. This finding indicates that the therapeutic interventions were appropriate for the patients in both subsets of patients. However, we acknowledge that the follow-up is relatively short (mean: 7,1 ± 3,6 years) and the number of patients is limited. Of note, however, other authors also reported similar outcomes for FNMTC patients compared to subjects with sporadic DTC (Maxwell *et al.*, 2004; Jiwang *et al.*, 2015).

Conclusion

According to the data obtained from this cohort, FNMTC seems to be associated with more prevalent lymph node metastases compared to SNMTC. Thus, as the 2015 ATA Guidelines for Thyroid Cancer advice, careful preoperative evaluation of the lateral neck is imperative, mainly in FNMTC patients.

Moreover, the present data suggest an anticipation phenomenon in FNMTC. Hence, a longitudinal monitoring with thyroid ultrasound seems to be a reasonable

recommendation in the second generation of these families, with a suggested initial evaluation at about age 15 years.

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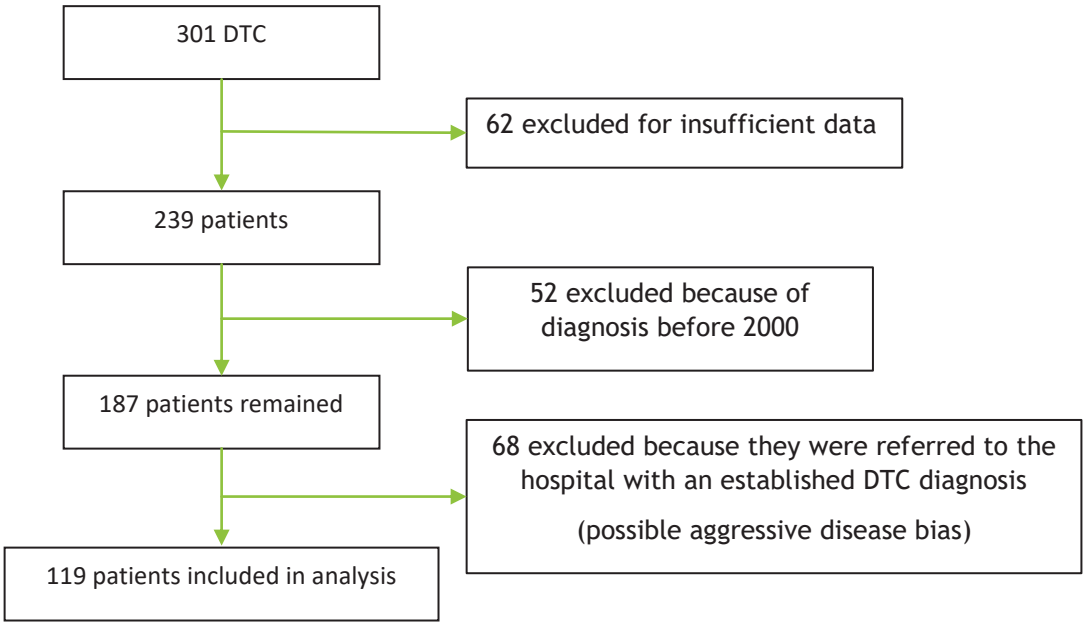
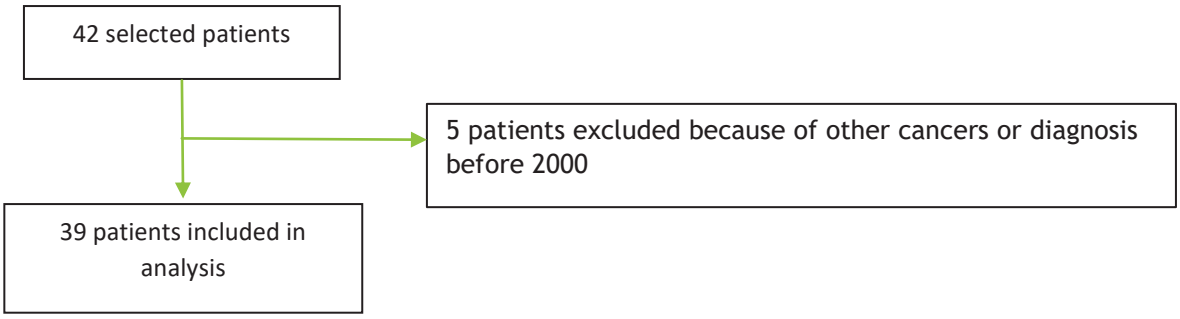
Figure 1 - Flow diagram of exclusion criteria

SNMTC

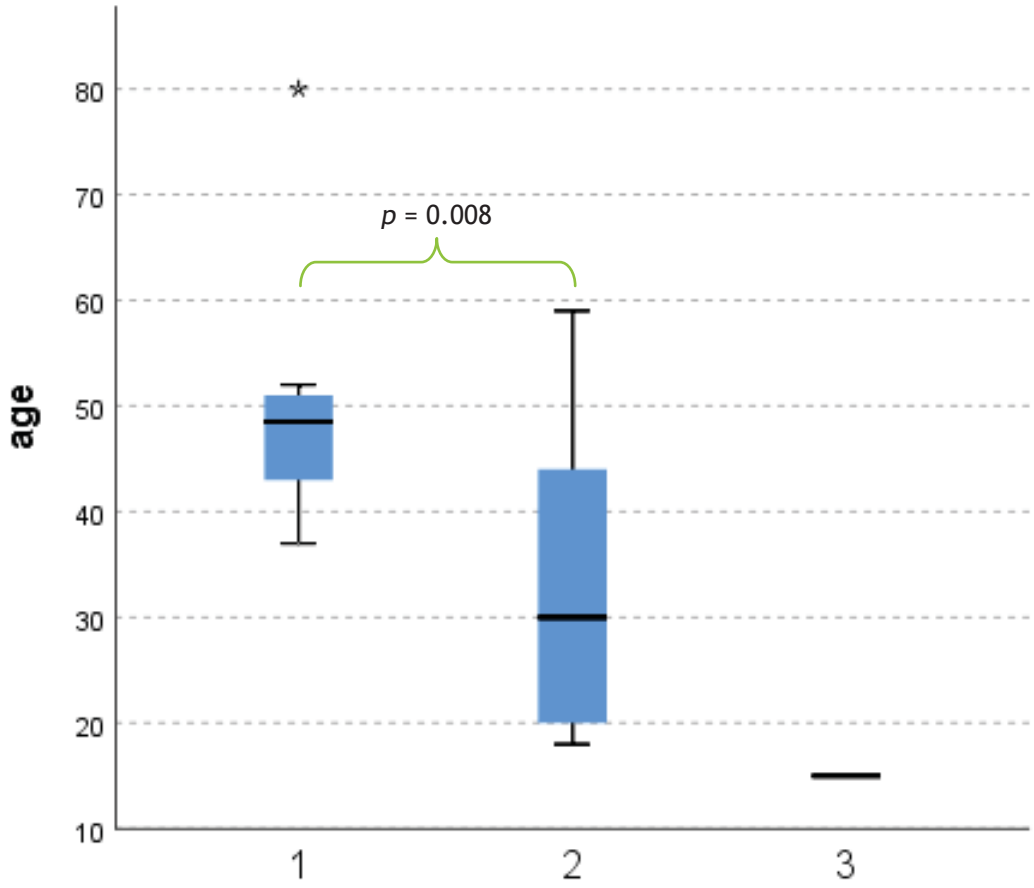


FNMTC



Legend: DTC - differentiated thyroid cancer

Figure 2 – Mean age at differentiated thyroid diagnosis in subsequent FNMTC generations:



Legend: Kruskal-Wallis test was applied to compare the three generations (p = 0.010). For comparison between the first and second generation, Mann-Whitney U test was applied

TABLE 1 – CHARACTERISTICS OF PATIENTS WITH SNMTC VERSUS FNMTC

	SNMTC (n = 119)	**FNMTC (n = 39)**	***P***
Age (years old)	46.65 ± 13.77	38.74 ± 14.48	< 0.001
Sex (female)	105 (88.2%)	31 (79.5%)	0.187
Follow-up	10.40 ± 5.32	7.05 ± 3.58	< 0.001
Public health insurance	119 (100%)	9 (23.1%)	< 0.001
Total thyroidectomy	115 (96.6%)	39 (100%)	0.573

TABLE 2 – HISTOLOGICAL CHARACTERISTICS AND INITIAL CLINICAL PRESENTATION OF SNMTC *versus* FNMTC

		SNMTC (n = 119)	**FNMTC (n = 39)**	***p***
Size (mm)		25.27 ± 20.39	18.28 ± 13.56	0.047
Histology	papillary	102 (87.4%)	38 (97.4%)	0.122
	follicular	15 (12.6%)	1 (2.6%)	
	Hürthle			
Multicentric disease		39/112 (34.8%)	18/39 (46.2%)	0.251
No capsular invasion		21/108 (19.4%)	6/37 (16.2%)	0.365
Angiolymphatic invasion		50/102 (49%)	18/36 (50%)	1.000
Gross extrathyroidal extension		7/113 (6.2%)	0/39 (0%)	0.191
Initial Clinical Presentation				
Cervical lymph node involvement		26 (21.8%)	18 (46.2%)	0.007
Distant metastases		11 (9.2%)	1 (2.6%)	0.296

TABLE 3 – RISK OF NMTC ACCORDING TO FAMILIAL HISTORY, AGE AND NODULE SIZE

Predictor	***p****	**OR**	**IC95%**
Familial history	0.019	2.72	1.17 – 6.30
Age	0.004	0.96	0.93 -0.99
Size	0.095	1.02	1 – 1.04

Note: multivariate analysis

TABLE 4 – SNMTC AND FNMTC ACCORDING TO 8TH TNM STAGING

	I	**II**	**IVa**	**IVb**	
SNMTC	101 (84.9%)	13 (10.9%)	1 (0.8%)	4 (3.4%)	p = 0.214
FNMTC	38 (97.4%)	1 (2.6%)	0 (0%)	0 (0%)	

TABLE 5 – SNMTC AND FNMTC ACCORDING TO ATA 2015 RESPONSE TO THERAPY RECLASSIFICATION

Response to Therapy Reclassification	**SNMTC**	**FNMTC**	***P***
Excellent response	82 (68,9)	32 (82,1)	0,230
Biochemical incomplete response	10 (8,4)	1 (2,6)	
Structural incomplete response	17 (14,3)	2 (5,1)	
Indeterminate response	10 (8,4)	4 (10,3)	
Subsequent neck dissection	12 (10,1)	8 (20,5)	0,101

Appendix

QUESTIONNAIRE

Familial code:

Ethnic background:

Birthday:

Place of birth:

1. Have you ever had intestinal polyps?
   ☐ yes ☐ no
2. Did some relative had intestinal polyps?
   ☐ yes ☐ no
3. Have you already done colonoscopy?
   ☐ yes ☐ no
4. Personal health history of…
   a. Osteoma
      ☐ yes ☐ no
   b. Epidermoid cyst
      ☐ yes ☐ no
   c. Hamartoma
      ☐ yes ☐ no
   d. Breast tumors
      ☐ yes ☐ no
   e. Adrenal nodule
      ☐ yes ☐ no
   f. Hypophysis disease
      ☐ yes ☐ no
   g. Myxoma (heart tumor)
      ☐ yes ☐ no
   h. Nerve tumor (schwannoma)
      ☐ yes ☐ no
   i. Nevi
      ☐ yes ☐ no
   j. Conjunctive tumor (sarcoma, osteosarcoma)
      ☐ yes ☐ no
5. Has any relative had some of the diseases above?
6. If yes, which one?

6.3. ARTIGO 3

Thyroid and breast cancer in two sisters with monoallelic mutations in the *ataxia telangiectasia mutated (ATM)* gene

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Word Count 1892

Keywords: Thyroid cancer; breast cancer; ataxia telangiectasia mutated (ATM) gene; genetic predisposition to disease; Cowden-like syndrome

Abstract

The presence of a bidirectional risk for metachronous carcinomas among women with thyroid and breast cancer is well established. However, the underlying risk factors remain poorly understood.

Two sisters developed papillary thyroid cancer (PTC) at age 32 and 34 years, followed by ductal carcinoma of the breast at 44 and 42 years. The two children of the younger sister developed ataxia-telangiectasia; the son developed lymphoblastic lymphoma, his sister died secondary to acute lymphoblastic leukemia (ALL). They were found to be compound heterozygous for *ataxia telangiectasia mutated (ATM)* gene mutations (c.3848T>C; p.L1283P and *ATM* c.802C>T p.Q268X). Exome sequencing of his mother and aunt led to the detection of the pathogenic monoallelic *ATM* mutation in both of them (c.3848T>C; minor allele frequency (MAF) < 0.01) but no other variants known to confer a risk for PTC or breast cancer.

The findings suggest that monoallelic *ATM* mutations, presumably in conjunction with additional genetic and/or non-genetic factors, can confer a risk for developing PTC and breast cancer.

Introduction

Familial non-medullary thyroid cancer (FNMTC) can present as non-syndromic or syndromic malignancy [1]. FNMTC, in particular papillary thyroid cancer, appears to have a strong genetic component. However, with the exception of syndromic forms such as Gardner's (*APC* gene mutations) or Cowden's syndrome (*PTEN* gene mutations), the search for highly penetrant causal genes has only provided limited positive results, suggesting that FNMTC is predominantly a polygenic/multifactorial disorder with variable penetrance involving a number of low-penetrant alleles [2].

Carcinomas of the thyroid and breast are among the most frequent malignancies among females [3]. Importantly, women with a history of breast cancer have an increased risk for developing metachronous thyroid carcinomas, and vice versa [4]. The mechanisms underlying this bidirectional relationship remain largely elusive but suggest an influence of common contributing factors such as genetic predisposition, hormonal factors including estrogen, obesity and lifestyle, endocrine disruptors, and in some instances secondary effects related to the therapy of the initial malignancy. Surveillance, Epidemiology, and End Results (SEER) data suggest that women with thyroid cancer who develop breast cancer tend to be younger compared to the average breast cancer patient [5], they are more likely to develop follicular thyroid cancers compared to female patients with thyroid cancer who do not develop breast cancer, and metachronous carcinomas of the breast are more likely hormone receptor–positive and of mixed ductal and lobular invasive type [5,6].

Methods

Case description

The index patient (II.1) and her sister (II.2) are two Brazilian siblings of Spanish and Portuguese ancestry (Figure 1).

Patient II.1 was diagnosed with papillary thyroid cancer (PTC) at the age of 32 years. She underwent total thyroidectomy and treatment with 100 mCi ^{131}iodine. Detailed TNM staging has not been documented in the available records. At the age of 44 years, she was diagnosed with bilateral breast cancer that was positive for estrogen and progesterone receptors (ER, PR) (Cell Marque Cat# 249R-17, RRID:AB_1158014 and Agilent Cat# M3569, RRID:AB_2532076), as well as for human epidermal growth factor receptor 2 (HER2) (Agilent Cat# M7269, RRID:AB_2246560) by immunohistochemistry. The treatment consisted in neoadjuvant chemotherapy (paclitaxel; fluorouracil, doxorubicin and cyclophosphamide; and trastuzumab), followed by bilateral mastectomy and radiation. She was subsequently treated with tamoxifen for two years, which was then replaced by anastrozole. She is deemed to be in remission.

Her sister (II.2; Figure 1) was diagnosed with PTC at the age of 34 years. She underwent total thyroidectomy with central neck dissection. The histology showed a multifocal classic papillary thyroid cancer with a dominant lesion of 1.7 cm in the right lobe with minimal extra-thyroidal extension, as well as angiolymphatic invasion. She had 4 of 10 positive lymph nodes in the lateral cervical compartments on the right, and 1 of 6 positive nodes in the central right compartment. The final staging was T1bN1bM0 (TNM/AJCC 8th edition). She was then treated with 100 mCi ^{131}iodine. Surveillance exams did not show any evidence of residual or recurrent thyroid cancer. At the age of 42 years, she was diagnosed with intraductal carcinoma of the right breast (TisN0M0),

ER, PR and HER2-positive. She opted to undergo bilateral mastectomy because she was aware to be a carrier of a mutation in the *ATM* gene (see below). She did not receive any other forms of therapy.

Because the association between thyroid and breast cancer occurs in Cowden syndrome (CS) and Cowden-like syndrome (CLS), the patients were thoroughly evaluated for the presence of associated diseases, and the only finding were head circumferences (58.5 and 59 cm) that were clearly above the 97th percentile for females after correction for height (168 and 171 cm) [7].

The two children of patient II.2 (III.1 and III.2, Figure 1) were diagnosed with ataxia-telangiectasia at the age of seven years. The boy (III.1) had more severe neurologic manifestations than his sister, in whom the diagnosis was established after completing molecular analyses in her sibling. III.2 developed acute lymphocytic leukemia (ALL) at the age of 7 years and she died at 9 years despite therapy. Her brother (III.1) developed lymphoblastic lymphoma at the age of 22 years and he currently (2020) is under conventional chemotherapy.

Informed consent

Informed consent was obtained to perform Sanger sequencing of the *ATM* gene and subsequently for performing whole exome sequencing.

Molecular analyses

Direct sequencing of the ATM gene

Because of the clinical presentation with a suspicion of ataxia telangiectasia (AT) in III.1, the *ATM* gene was submitted to Sanger sequencing using germline DNA extracted from peripheral white blood cells (primers and methodological details

available upon request). After the identification of compound heterozygous *ATM* mutations, his sister (III.2), mother (II.2) and aunt (II.1) also underwent molecular testing: the father was not available for testing.

Whole exome sequencing, variant calling, and annotation

With the knowledge that the mother (II.2) and the aunt (II.1) of the patient with ataxia-telangiectasia both harbor the same monoallelic *ATM* mutations, and because of their breast and thyroid cancer phenotype, we performed whole exome sequencing (WES) in these patients using an Illumina HiSeq2500 platform with a read length of approximately 100 x 100 bp. The on-target coverage was, on average, 100 x. Sequence reads were mapped to the human reference genome GRCh37/hg19 using Burrows-Wheeler Aligner (BWA-mem). Further details on variant calling, filtering, estimation of minor allele frequencies, and software used for the prediction of pathogenicity of variants is included in Supplemental Materials (https://doi.org/10.5061/dryad.jh9w0vtbh).

Results

Direct mutational analysis of the ATM gene

Analysis of the *ATM* gene in the siblings (III.1 and III.2) with AT revealed the presence of compound heterozygous mutations (*ATM* c.3848T>C; p.L1283P and *ATM* c.802C>T p.Q268X; MAF for both variants < 0.01). The mother (II.2) and aunt (II.1) were both found to be monoallelic carriers of the *ATM* c.3848T>C; p.L1283P (rs730881389) variant. This variant is classified as being likely pathogenic by ClinVar, SIFT (score 0) and Polyphen (score 1). The substitution of leucine at position 1283 by proline results in an alteration of the protein structure and mobility, thereby affecting its function. No

prevalence data about rs730881389 is available in the Genome Aggregation Database (gnomAD) [8] or ABraOM (Brazilian genomic variants, Arquivo Brasileiro Online de Mutações) [9].

Whole exome sequencing

Whole exome sequencing did not reveal variants in genes known to be associated with a risk familial non-medullary thyroid cancer (FNMTC), breast cancer, or Cowden-like syndrome/Cowden syndrome in the two affected sisters (Supplemental Materials, Table 1). However, they were found to have a second single nucleotide variant in the *ATM* gene (c.5557G>A, p.D1853N; rs1801516) which is predicted to be benign. The search for shared variants in other genes did not reveal any pathologic variant according ClinVar [10], but there were a few variants of uncertain significance associated with cancer according Varsome [11] (Supplemental Materials, Table 2).

Discussion

The bidirectional risk for developing metachronous breast and thyroid cancer in women is well established, but the underlying genetic and non-genetic mechanisms remain incompletely understood. In the family presented here, the two sisters (II.1 and II.2) with PTC and breast cancer were found to carry a monoallelic mutation in the *ATM* gene (c.3848T>C; p.L1283P). This sequence variant is extremely uncommon and has been previously reported in patients with AT [12]. The two children of II.2 were found to be compound heterozygous for *ATM* gene mutations (III.1 and III.2 in Figure 1) and developed a classic AT phenotype, as well as ALL (III.2) and lymphoblastic lymphoma (III.1).

The ATM protein is primarily located in the nucleus where it plays a central role as a guardian of cell division and DNA repair through activation of several enzymes. Biallelic mutations in the *ATM* gene lead to ataxia-telangiectasia (AT; Online Mendelian Inheritance in Man OMIM#208900), an autosomal recessive disorder characterized by cerebellar degeneration, telangiectasias, cancer and radiation susceptibility, as well as immunodeficiency. Monoallelic mutations in the *ATM* gene have been associated with an increased risk for developing a wide spectrum of malignancies including cancers of the breast, stomach, bladder, pancreas, lung, ovaries, and melanoma. ATM protein-truncating variants were strongly associated with ER positive breast cancers in a very recent study including 60,466 women with breast cancer [13]. In contrast, a possible correlation between the presence of ATM variants and thyroid cancer is more controversial. Certain ATM genotypes and haplotypes were reported to be associated with an increased thyroid cancer risk [14-19], although some of the proposed variants (rs373759 G > A, rs4988099 A > G, rs1801516 G > A, rs664677 T > C, and rs609429 G > C) or haplotypes could not be confirmed as susceptibility factors in the meta-analysis by Kang *et al*. [20]. Of note, some studies have suggested that *ATM* mutations might be associated with a risk for developing thyroid carcinomas, in particular FNMTC. For example, Wang *et al*. have reported *ATM* variants (rs1800057 and rs149711770) in two families with FNMTC, but without breast cancer [2]. The cBioPortal, which compiles datasets from different cancer studies, lists somatic *ATM* mutations in 2.4% of thyroid cancers and in 2.7% of breast cancers [21].

A role for ATM in thyroid carcinogenesis is indirectly supported by experimental studies [22]. In thyroid cancer, expression of HMGN4, a member of the high mobility group N (HMGN) family, is elevated in several types of thyroid carcinomas. *In vitro*, HMGN4 overexpression downregulates the expression of the tumor suppressors ATM, ATRX

(Alpha Thalassemia/Mental Retardation Syndrome X-Linked), and BRCA2 (Breast Cancer Related 2), and it leads to an increase of the DNA damage marker γH2AX. Similarly, overexpression of HMGN4 *in vivo* in transgenic mice results in the formation of preneoplastic lesions in the thyroid of transgenic mice [22].

Cowden syndrome (CS; OMIM#158350), also known as *PTEN* (phosphatase and tensin homolog) hamartoma tumor syndrome (PHTS) is associated with different types of cancer, including carcinomas of the thyroid and the breast [23]. Establishing the clinical diagnosis can be challenging and guidelines by the National Comprehensive Cancer Network (NCCN) provide a framework [24]. Clinical constellations that resemble Cowden syndrome, but do not fulfill all the criteria, are referred to as Cowden-like syndrome (CLS) [23]. Genetically, CS and CLS are heterogeneous. In addition to the initially identified mutations in the *PTEN* gene, variants in a spectrum of other genes (*SDHB-D*, *SEC23B, KLLN, PARP4, AKT1, PIK3CA, USF3, and TTN)* have been implicated in their pathogenesis [23]. Among the pleiotropic manifestations of CS and CLS, the concomitant occurrence of carcinomas of the breast and the thyroid have been reported repeatedly [3]. Although mutations in the *ataxia-telangiectasia mutated* (*ATM)* gene have not been reported as cause of CLS, the two sisters presented here have a phenotype that is reminiscient of this entity.

According to ClinVar there were no shared pathologic variants in the exomes of the two sisters [10]. Using Varsome [11], we identified a few variants of uncertain significance. Among them, it is interesting to note that mutations of *CDC27* have rarely been in thyroid cancer [25], and CDC27 alterations have also been associated with breast cancer susceptibility [26]. Moreover, CTBP2 protein overexpression has been observed in follicular thyroid cancer [27], as well as breast cancer [28].

The clinical and genomic findings in this family suggest that ATM mutations may predispose to the development of PTC and breast cancer, and that ATM mutations can play a role in FNMTC, findings that need to be corroborated. Moreover, it remains to be elucidated whether the monoallelic ATM mutation identified in the two sisters with thyroid and breast cancer may confer an increased risk through haploinsufficiency or in conjunction with other genetic or non-genetic modifiers.

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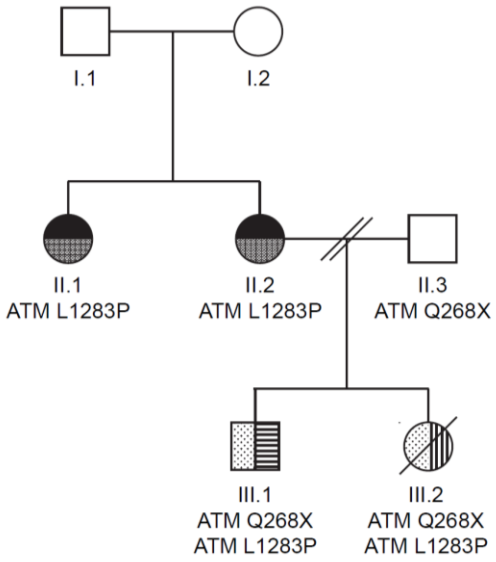
Figure Legends

Figure 1 – Pedigree



Legend: ataxia-telangiectasia; thyroid cancer; breast cancer; lymphoblastic lymphoma; acute lymphocytic leukemia

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6.4 ANÁLISE DOS EXOMAS

Na família M (figura 3, seção 10.2), não obtivemos nenhuma variante que segregasse em todos os membros da família com CNMTF.

Na família F (figura 4, seção 10.2), ao contrário, obtivemos 30 possíveis variantes candidatas das quais 9 eram mutações sinônimas, a saber:

TABELA 1 – LISTA DE POTENCIAIS VARIANTES (FAMÍLIA F)

Chr	Start	End	Func	Gene	ExonicFunc	AAChange
chr1	179870431	179870431	splicing	TOR1AIP1	.	
chr1	92944314	92944314	splicing	GFI1	.	
chr19	9006415	9006415	splicing	MUC16	.	
chr2	157368834	157368834	splicing	GPD2	.	
chr1	114448284	114448287	exonic	DCLRE1B	nonframeshift deletion	
chr19	501743	501743	exonic	MADCAM1	nonframeshift insertion	
chr22	29885567	29885567	exonic	NEFH	nonframeshift insertion	
chr1	150235797	150235797	exonic	CA14	nonsynonymous	p.V274I
chr1	151400305	151400305	exonic	POGZ	nonsynonymous	M263V
chr1	156352561	156352561	exonic	RHBG	nonsynonymous	p.I379L
chr1	156615902	156615902	exonic	BCAN	nonsynonymous	p.A19E
chr1	158517417	158517417	exonic	OR6Y1	nonsynonymous	p.T160I
chr1	161087788	161087788	exonic	PFDN2	nonsynonymous	p.K10R
chr1	171509769	171509769	exonic	PRRC2C	nonsynonymous	p.T1053M
chr10	75434641	75434641	exonic	AGAP5	nonsynonymous	p.R593W
chr2	112619981	112619981	exonic	ANAPC1	nonsynonymous	p.T416M
chr2	130902339	130902339	exonic	CCDC74B	nonsynonymous	p.H77Q
chr3	195510745	195510745	exonic	MUC4	nonsynonymous	p.A2569V
chr3	195512287	195512287	exonic	MUC4	nonsynonymous	p.S2055F
chr1	145530325	145530325	exonic	ITGA10	synonymous	
chr1	150530963	150530963	exonic	ADAMTSL4	synonymous	
chr1	171753590	171753590	exonic	METTL13	synonymous	
chr1	230561627	230561627	exonic	PGBD5	synonymous	
chr2	143742682	143742682	exonic	KYNU	synonymous	
chr2	158485112	158485112	exonic	ACVR1C	synonymous	
chr7	143417322	143417322	exonic	TCAF2	synonymous	
chr8	142228842	142228842	exonic	SLC45A4	synonymous	
chr9	41327276	41327276	exonic	SPATA31A5|SPATA31A7	synonymous	
chr1	144823917	144823917	exonic	NBPF9	nonsynonymous	

Foi avaliado a expressão das diferentes proteínas no tecido tireoidiano, bem como verificado nos bancos de dados de CPT existentes se essas mutações eram descritas.

TABELA 2 – CARACTERÍSTICAS DE EXPRESSÃO TIREOIDIANA, PROBABILIDADE DA VARIANTE NÃO-SINÔNIMA SER DELETÉRIA E PRESENÇA DE MUTAÇÕES DETECTADAS DOS GENES DETECTADOS NO TCGA

Gene	Variante não-sinônima	Expressão proteica tireoidiana	Mutações somáticas no TCGA [77]	Clinvar [78]	Varsome [79]
TOR1AIP1	rs200800578	↑	-	Likely benign	Likely benign
GFI1	rs35896485	±	0,2%	Conflicting data	Benign
MUC16	rs77874966	-	2,5%	-	Uncertain
GPD2	rs369276284	±	-	-	Likely benign
DCLRE1B	rs763030449	-	0,2%	Uncertain	Likely benign
MADCAM1	rs1555716199	-	-	-	Uncertain
NEFH	rs147489453	-	0,4%	Benign	Benign
CA14	rs1371687427	-	-	-	Uncertain
POGZ	rs574158925	↑	0,6%	-	Benign
RHBG	rs747815208	-	-	-	Uncertain
BCAN	rs200638965	-	-	-	Uncertain
OR6Y1	rs148583112	-	-	-	Likely benign
PFDN2	rs546804573	±	-	-	Uncertain
PRRC2C	rs143283766	±	0,4%	-	Uncertain
AGAP5	rs3957125	-	0,6% deletions	-	Uncertain
ANAPC1	rs201128090	±	-	-	Likely benign
CCDC74B	rs372599757	-	0,2% amplification	-	Uncertain
MUC4	rs2453138				Benign
MUC4	rs113602668	-	0,2% amplification	-	likely benign
NBPF9	?	-	-	-	?

Foram pesquisadas as variantes *MUC16* c.39607-4A>G- (rs77874966), *DCRE1B* c.76_79C (rs763030449) e *RHBG* (rs747815208) nos dados germinativos do TCGA, mas elas não foram encontradas no banco de dados dos pacientes com CPT.

6.4.1 Variantes não-sinônimas

6.4.1.1 *TOR1AIP1*

O gene *TOR1AIP1* codifica uma proteína de membrana integral tipo 2 que se liga a laminas tipo A e B. A proteína codificada se localiza na parte interna da membrana nuclear e pode estar envolvida na manutenção da ligação da membrana nuclear à lamina nuclear durante a divisão celular. A variante encontrada (*TOR1AIP1* c.653-6C>T, rs200800578), apesar de ser rara e altamente expressa nas células foliculares tireoidianas [80], é atualmente considerada como provavelmente benigna [78; 79].

6.4.1.2 *GFI1*

GFI1 (*growth factor independent 1 transcriptional repressor*) é uma proteína zinc-finger, envolvida em vários processos de hematopoiese e oncogenêse, estando implicado em mielodisplasias, linfomas, bem como carcinoma de mama e de próstata [81; 82]. A variante encontrada (rs35896485), porém, tem expressão moderada na tireoide [80]. Em geral, é considerada benigna [79], mas há dados conflitantes sobre a sua patogenicidade [78].

6.4.1.3 *MUC16*

Este gene codifica uma proteína que é um membro da família das mucinas. Acredita-se que tenha um papel na formação de uma barreira, protegendo células epiteliais dos patógenos. São hiper-expressas em carcinomas de ovário, protegendo as células tumorais dos ataques das células natural killers e das repostas imunes anti-tumorais em geral [83]. Recentemente, tem sido também relacionada à oncogênese pela ativação das vias AKT e ERK em culturas celulares de câncer de ovário [84]. A variante encontrada é considerada de significado incerto [79] e, curiosamente, mutações somáticas em *MUC16* foram encontradas em 2,5% dos CPT estudados pelo TCGA, *PanCancer Atlas* . Em condições normais, entretanto, não é expresso à nível tireoidiano [80].

6.4.1.4 *GPD2*

GPD2 parece expressar uma desidrogenase sensível ao cálcio envolvida no sensor de glicose da célula betapancreática [85]. A expressão tecidual de GPD2 (glycerol-3-phosphate dehydrogenase 2) é inespecífica e, à nível tireoidiano, é moderadamente expressa [80]. Além disso, a variante é considerada provavelmente benigna [78]. Todos esses dados tomados em conjunto tornam uma candidata improvável na susceptibilidade ao CNMTF.

6.4.1.5 *DCLRE1B*

DCLRE1B expressa uma 5’ 3’ exonuclease (Apollo) fundamental para a manutenção dos telômeros durante a fase S. Juntamente com TERF2 protege os telômeros durante a fase de replicação e parece estimular a resposta de ATM à radiação ionizante [86].

A variante rs763030449 promove uma deleção *nonframeshift* e é considerada como de significado incerto por alguns [78] e provavelmente benigna por outros grupos [79]. No TCGA, foram observados que 0,2% dos espécimes de CPT apresentavam mutação somática em *DCLRE1B* [87]. Embora *DCLRE1B* nunca tenha sido relacionado diretamente com CNMTF, tanto a extensão dos telômeros [36], como *TINF2* (envolvida no complexo *shelterin* e, portanto, na manutenção de telômeros) [31; 39], quanto *ATM* [31] que interagem com o mesmo já foram associados com CNMTF. Embora essa variante específica não tenha sido encontrada entre os dados genômicos do TCGA, essa variante pode ser interessante para posteriores avaliações.

6.4.1.6 *MADCAM1*

Mucosal addressin cell adhesion molecule 1 (MADCAM1) é um receptor de leucócitos trans-membrana que regula tanto a passagem quanto a retenção de leucócitos nas mucosas [88], através da interação com LPAM-1, L-selectina e VLA-4. Aparentemente sem relação com cânceres e não é expressa na tireoide. Ainda assim, apresenta comportamento incerto segundo a análise *in silico* [79].

6.4.1.7 *NEFH*

Tal gene expressa uma proteína componente dos filamentos intermediários dos neurofilamentos, tendo papel importante na manutenção do axônio e o do calibre neuronal [89].

A variante observada (rs147489453) é predita como benigna [78; 79], não é expressa na tireoide [80], além de não ter uma óbvia relação com oncogênese.

6.4.1.8 *CA14*

Como seu próprio nome diz, CA14 (*carbonic anhydrase* 14) expressa um anidrase carbônica que, entretanto, não é expressa na tireoide [80]. A variante identificada é de significado incerto [79].

6.4.1.9 *OGZ*

POGZ expressa uma proteína essencial para a correta ativação e dissociação da Aurora B quinase durante a fase M do ciclo celular [90]. É expressa na tireoide, ainda que tenha uma baixa especificidade tecidual [80]. Entretanto, a variante levantada parece ser benigna [79].

6.4.1.10 *RHBG*

RHBG permite o transporte de amônio nas membranas basolaterais das células renais [91]. Curiosamente, ela interage com a pendrina (SCL26A4), mas não parece ser expressa nas células foliculares tireoidianas [80]. De qualquer forma, a variante selecionada pela análise de bioinformática (rs747815208) tem significado incerto nos preditores utilizados [79] e, por isso, foi rastreada nos dados genômicos do TCGA, mas com resultado negativo.

6.4.1.11 *BCAN*

BCAN (brevican) é da família lectican da proteoglican condroítina-sulfato expressa especificamente no sistema nervoso central [80]. Parece estar envolvida na formação da matriz extra-celular do sistema nervoso central e é altamente expressa em gliomas cerebrais [92]. A alteração rs200638965 (*BCAN*, p.A19E) é predita de significado incerto [79]. Porém, às luzes do conhecimento atual (sem expressão na tireoide), parece ser improvável que tenha relação com o CNMTF.

6.4.1.12 *OR6Y1*

Codifica um receptor olfatório. Trata-se de um receptor de membrana acoplado proteína G que acoplaria as substâncias odorantes à sinalização neuronal para a percepção dos cheiros [80]. Além de não ser expresso na tireoide [80], a variante encontrada (rs148583112) é provavelmente benigna [79].

6.4.1.13 *PFDN2*

A prefoldina é um complexo molecular chaperone composta por 6 subunidades, sendo que uma delas é codificada pelo *PFDN2*. Esse complexo se liga e estabiliza polipeptídios apenas sintetizados, permitido, assim, que se dobrem adequadamente [93]. Tem uma expressão moderada em vários tecidos, inclusive na tireoide [80]. Apesar de não ter uma relação clara com oncogênese, a variante observada (rs546804573) é classificada como de significado incerto [79].

6.4.1.14 *PRRC2C*

PRRC2C tem um papel crucial na formação de grânulos de estresse. Esses juntamente com os corpos de processamento são complexos de proteínas envolvidos no processamento, transporte, translação e degradação dos mRNA [94]. Tem expressão moderada a nível tireoidiano e é considerado um fator de prognóstico desfavorável quando encontrado no câncer renal [80]. Mutações somáticas nesse gene são encontradas em 0,4% das amostras de CPTs avaliadas pelo TCGA [77]. Interessantemente, a variante relatada (rs143283766) sofreu recentemente *upstaging* e passou a ser considerada como de significado indeterminado.

6.4.1.15 *AGAP5*

AGAP5 (*ArfGAP with GTPase domain, ankyrin repeat and PH domain 5)* é anotada como uma GTP-ase ativadora e foi relacionada a atrofia muscular espinhal [95]. A expressão de RNA é ubiquitária, mas moderada na tireoide [80], enquanto a variante observada (rs3957125) é predita como de significado incerto [79].

6.4.1.16 *ANAPC1*

ANAPC1 faz parte do complexo APC/C (*anaphase promoting complex/cyclosome*) que é uma ubiquitina-ligase E3 que regula o ciclo celular. Através da ubiquitinação e degradação de suas proteínas alvos controlando a progressão para a mitose e fase G1 do ciclo celular [96]. Parece ser um marcador de mau prognóstico no carcinoma endometrial, mas tem expressão ubiquitária e moderada expressão nas células foliculares [80]. Além disso, a variante selecionada pela análise de bioinformática (rs201128090) é considerada provavelmente benigna.

6.4.1.17 *CCDC74B*

Expressa uma proteína que se liga diretamente aos microtúbulos permitindo a organização das fibras K, formação do fuso bipolar e o alinhamento cromossômico [97]. Naturalmente, é mais expressa nos testículos, mas também é expressa moderadamente na tireoide [80]. A variante encontrada (*CCDC74B*, p. H77Q, rs372599757) tem um comportamento indeterminado [79].

6.4.1.18 *MUC4*

MUC4 codifica um complexo sialomucina transmembrana que interage com ErbB2, induzindo a proliferação e a diferenciação celular de células epiteliais [98] e podendo, portanto, ter um papel na progressão tumoral. Entretanto, não parece ser expresso nas células foliculares tireoidianas [80] e as variantes observadas parecem ser benignas (rs2453138 e rs113602668).

6.4.1.19 *NBPF9*

Localizada no braço longo do cromossomo 1, *NBPF9* pertence à família de *neuroblastoma breakpoint* que corresponde a uma área de dúzias de duplicações gênicas observadas nos primatas. Não tem uma função bem estabelecida, mas foi observada que variações no número de cópias de genes nessa região 1q21.1 está relacionada com diversas alterações de desenvolvimento e neurogênicas (microcefalia, autismo, esquizofrenia, neuroblastoma), bem como insuficiência

cardíaca e alterações do trato urinário. Alterações de expressão de outros genes da mesma família foram correlacionados com outros cânceres [80]. Na tireoide, ele tem uma expressão moderada e a alteração nessa variante não é clara nesse momento.

6.4.2 Variantes sinônimas

São chamadas de variantes sinônimas aqueles polimorfismos cuja troca de base não induz a uma troca de aminoácidos e, em teoria, não afetaria a estrutura das proteínas. Entretanto, há várias evidências que variantes sinônimas possam ser patogênicas. Em alguns casos, elas podem afetar a transcrição gênica em si, alterar o *splicing*, o enovelamento cotranslacional, bem como afetar a estabilidade de mRNA [99]. Entretanto, os preditores de função proteica habituais não tem capacidade para analisar essas alterações e, por isso, lançamos mão de análises *in silico* diversas das anteriores. Apesar disso, revisões sobre o assunto ressaltam que todas as ferramentas disponíveis são falhas, visto a carência de análises *in vitro* que retroalimentem esses programas [99]. Assim, há que se ter cautela na interpretação dos resultados.

Tabela 2 – Características de expressão tireoidiana, probabilidade da variante sinônima ser deletéria e presença de mutações dos genes detectados nos bancos de dados de CPT

Gene	Variante sinônima	Expressão proteica	Mutações somáticas no TCGA	TraP	FDR (regSNPs)
ITGA10	rs781959521	?	0,4%	0,024	0,675
ADAMTSL4	rs199530808	±	0,2%	0,08	0,462
EEF1AKNMT	rs769722520	±	-	0,613	0,305
PGBD5	rs138134082	?	0,2%	-	-
KYNU	rs139634527	-	0,2%	0,028	0,904
ACVR1C	rs56177445	±	-	0,032	-
TCAF2	rs62486262	?	0,2% amplification	0,073	0,498
SLC45A4	rs61995886	±	0,4% deletion	0,011	0,498
SPATA31A5|SPATA31A7	rs1556231840	-	0,2% deletion	0,025	-

NOTA: Para predição das variantes sinônimas, é considerado FDR<0,05 como deletério, 0,05<=FDR<0,5 como potencialmente deletério e 0,5<=FDR<=1 como benigno [100]. No caso do TraP, tem se considerado como *cut-offs* 0,459 e 0,93 como possivelmente e provavelmente deletérias, respectivamente [101].

Tomando como minimamente confiáveis essas análises, observamos que a única variante sinônima classificada como possivelmente deletéria nas duas ferramentas utilizadas (TraP e regSNPs) foi em *EEF1AKNMT*.

6.4.2.1 *EEF1AKNMT*

É uma dualmetiltransferase que catalisa a metilação de *EEF1* e *EEF2*, estando, portanto, envolvida na regulação da translação do mRNA. Foi observado que canceres *RAS*-positivos utilizam *EEF1* metilado para favorecer a tumorigênese *in vivo* [102]. É moderadamente expressa na tireoide [80] e parece ser potencialmente deletéria [100] (percentil 97,5 a 99% [101]). Visto que a metilação *EEF1* parece ser importante na tumorigênese, avaliação *in vitro* seria fundamental para entender o efeito dessa variante.

6.4.3 Discussão da análise exômica

Os resultados negativos da análise bioinformática da família M podem indicar que o fator de susceptibilidade se encontre em alguma zona intrônica, podendo corresponder a um mRNA, lncRNA ou, ainda, uma área de ligação com fatores de transcrição. Contudo essa não é a única causa que poderia explicar a ausência de resultados. Quando da análise de bioinformática, o pesquisador à luz dos dados epidemiológicos vai inferir a frequência alélica para o objeto estudado. Assim, foi escolhido um MAF<0.01 visto à raridade de famílias com tantos indivíduos acometidos por CNMTF. Entretanto, considerando o aumento progressivo da incidência dos CNMT e os achados de microcarcinomas em autópsia em quase 4% dos casos, há quem questione se o CNMTF seja realmente uma doença rara [103]. Num tal caso, a MAF selecionada deveria ser maior de 1%.

Já na família F, várias variantes foram selecionadas, mas nenhuma conhecidamente patogênica. Mesmo assim, os resultados não podem ser descartados. Isso porque a construção desses preditores de função proteica/variante depende da alimentação dos seus bancos de dados. Assim, alterações nas suas classificações podem ocorrer ao longo de tempo.

Afora todo o exposto, a análise bioinformática pode ser passível de outros erros. Por exemplo, a penetrância pode ser incompleta ou um dos pacientes pode falsear a análise por ter tido um CNMT ao acaso.

Por todas essas razões, todo estudo de susceptibilidade necessidade de estudo funcional posterior.

7 CONSIDERAÇÕES GERAIS

O presente estudo corroborou com a hipótese de uma maior agressividade do CNMTF, bem como a presença do fenômeno de antecipação. Assim, na ausência de marcadores genéticos confiáveis, sugere que a ultrassonografia possa ser usada como *screening* a partir do final da adolescência, bem como na avaliação acurada desses pacientes para planejamento cirúrgico e procedimento de esvaziamento linfonodal ou não.

Além disso, continuará a contribuir com o desenvolvimento sejam das tecnologias e das análises bioinformáticas que vem acontecendo nos estudos de susceptibilidade nas diferentes doenças.

Permitiu ainda cooperação interuniversitárias e internacionais, com a colaboração de cientistas reconhecidos e, portanto, enriquecimento importante da *expertise* local.

A transdisciplinaridade promovida pela tese contribuirá para compreensão de todos os fenômenos do estudo.

8 CONCLUSÕES

O CNMTF apresenta-se inicialmente em idade mais precoce e, apesar de ter um menor tamanho, frequentemente, há envolvimento linfonodal já ao diagnóstico. Tais achados associados à predisposição genética provável suportam a indicação de tireoidectomia total quando do diagnóstico e uma acurada avaliação pré-operatória para a indicação de esvaziamento ganglionar se necessária.

Além disso, apesar do curto tempo de seguimento, com uma abordagem adequada, a evolução do CNMTF pareceu ser semelhante ao CNMTE. Não sendo necessárias, portanto, condutas adicionais no manejo dele.

Os presentes achados também corroboram para a presença do fenômeno de antecipação, com diagnóstico em média 15 anos antes da geração anterior. Esse parâmetro pode ser utilizado como indicador de ultrassonografias para rastreio enquanto não há marcadores moleculares mais precisos.

Em uma das famílias estudadas, a aparente exclusão de variantes exômicas sugere que outros mecanismos estejam envolvidos, como alterações em lncRNA que seriam evidenciadas somente por *whole genomic sequencing*. Adicionalmente, a ausência de mecanismos comuns vem em encontro ao já exposto na literatura que essas variantes/mutações possam ser extremamente raras e, portanto, de pouca reprodutibilidade. Entretanto, entre as variantes encontradas na segunda família, temos uma (*DCREL1B*) relacionada ao complexo shelterin que tem sido apontado em estudos nos últimos anos (*TINF2, POT1*) e outra (*MUC16*) que é encontrada mutada em 2,6% dos pacientes com PTC. Ambas parecem ser bons candidatos para estudos funcionais posteriores.

Ademais, observamos uma família que foi excluída do estudo clínico, portadora tanto de carcinoma ductal de mama, quanto de CPT e carreadora de uma mutação no gene *ATM*. Tal achado veio reforçar a associação dos dois tumores que tem sido sugerida nos últimos anos. Além disso, a presença de mutação no gene ATM na ausência de outras variantes que predisponham ao CPT – ao menos à luz do conhecimento atual – levantam a hipótese de que mutações no gene *ATM* possam estar relacionadas na susceptibilidade ao CNMT. Se o mecanismo envolvido estaria relacionado com haploinsuficiência ou tenha associação com outros fatores genéticos ou não-genéticos ainda precisa ser melhor explorado.

Ainda, a aparente exclusão de variantes exômicas em uma das famílias estudadas sugere que outros mecanismos estejam envolvidos, como alterações em lncRNA que seriam evidenciadas somente por *whole genomic sequencing*. Além disso, a ausência de mecanismos comuns vem em encontro ao já exposto na literatura que essas variantes/mutações possam ser extremamente raras e, portanto, de pouca reprodutibilidade. Entretanto, entre as variantes encontradas na segunda família, temos uma (*DCREL1B*) relacionada ao complexo *shelterin* que tem sido apontado em estudos nos últimos anos (*TINF2, POT1*) e outra (*MUC16*) que é encontrada mutada em 2,6% dos pacientes com PTC. Ambas parecem ser bons candidatos para estudos funcionais posteriores.

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APÊNDICE 1 - QUESTIONÁRIO DE SELEÇÃO DOS PACIENTES COM CNMTF (VERSÃO EM INGLÊS)

Familial code:

Ethnic background:

Birthday:

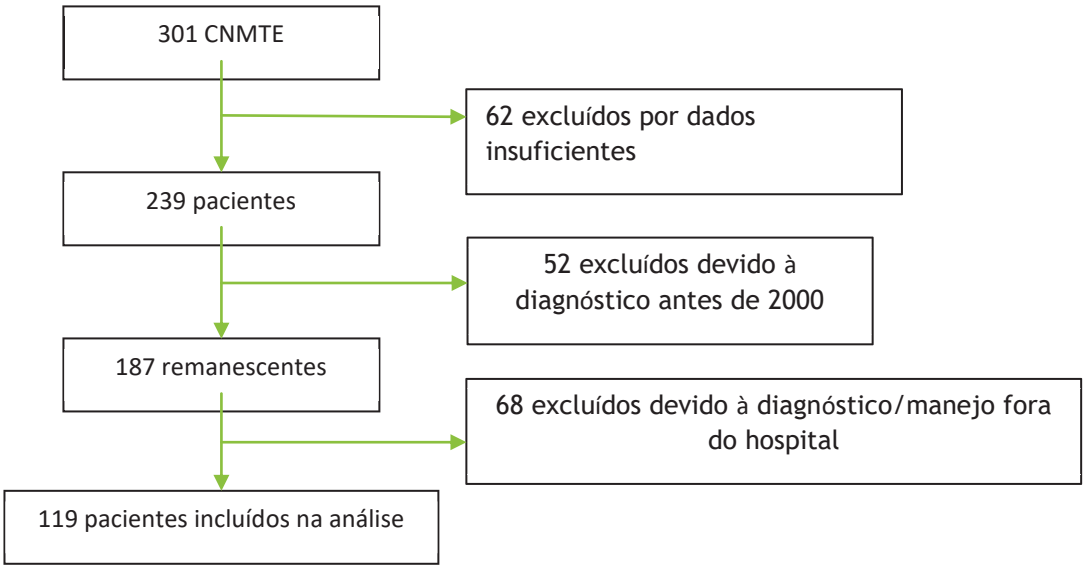
Place of birth:

1. Have you ever had intestinal polyps?
   ☐ yes ☐ no
2. Did some relative had intestinal polyps?
   ☐ yes ☐ no
3. Have you already done colonoscopy?
   ☐ yes ☐ no
4. Personal health history of…
   a. Osteoma
      ☐ yes ☐ no
   b. Epidermoid cyst
      ☐ yes ☐ no
   c. Hamartoma
      ☐ yes ☐ no
   d. Breast tumors
      ☐ yes ☐ no
   e. Adrenal nodule
      ☐ yes ☐ no
   f. Hypophysis disease
      ☐ yes ☐ no
   g. Myxoma (heart tumor)
      ☐ yes ☐ no
   h. Nerve tumor (schwannoma)
      ☐ yes ☐ no
   i. Nevi
      ☐ yes ☐ no
   j. Conjunctive tumor (sarcoma, osteosarcoma)
      ☐ yes ☐ no
5. Has any relative had some of the diseases above?
6. If yes, which one?

APÊNDICE 2 - FIGURAS DOS ARTIGOS MENCIONADAS NA TESE

FIGURA 2 - FLUXOGRAMA DE SELEÇÃO E EXCLUSÃO DOS PACIENTES (ARTIGO 3)

CNMTE



CNMTF

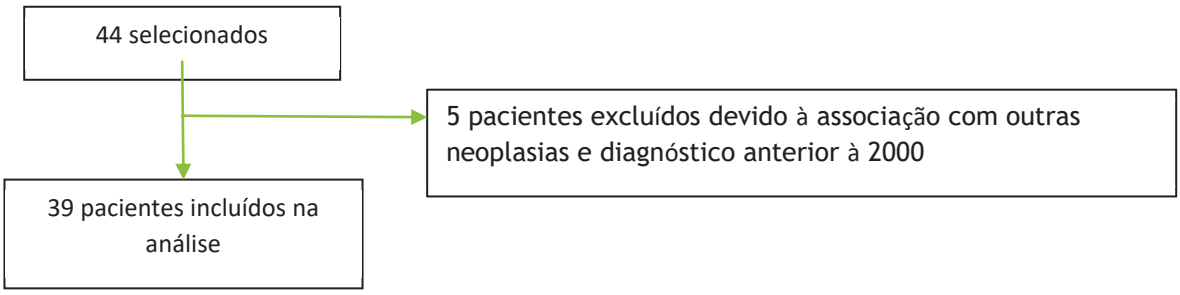


FIGURA 3 - FAMÍLIA M

M

Material	
Sangue/DNA	*
Tecido	Δ

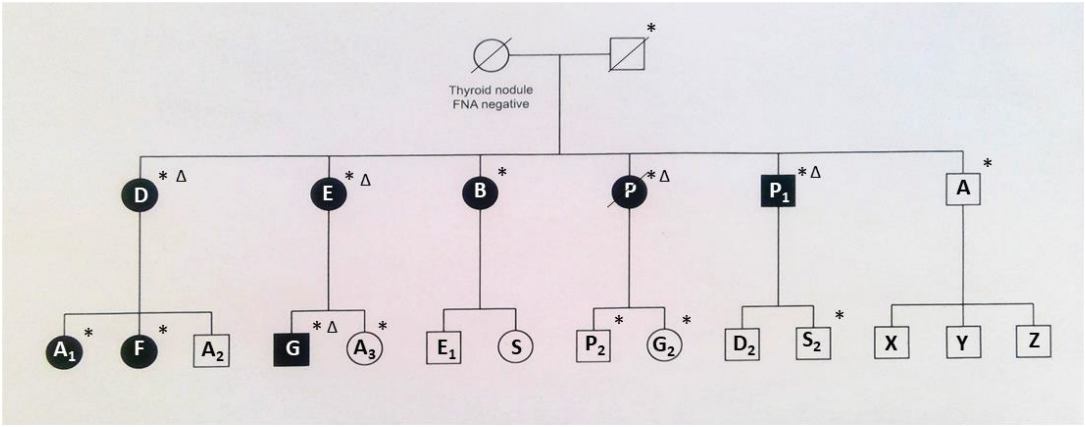
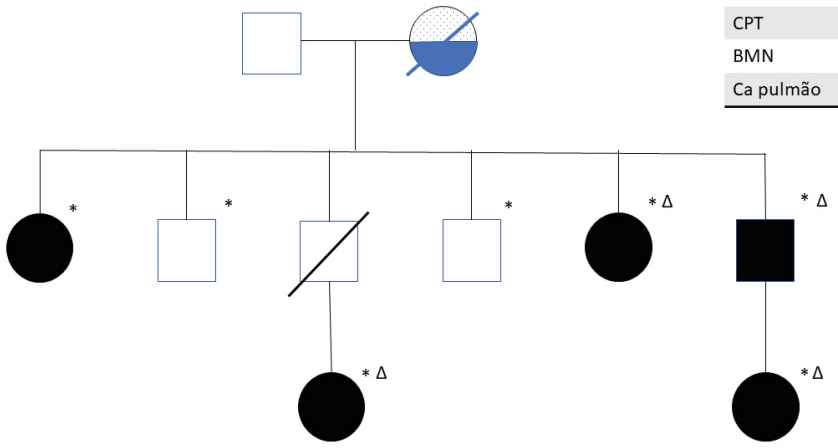


FIGURA 4 – FAMÍLIA F

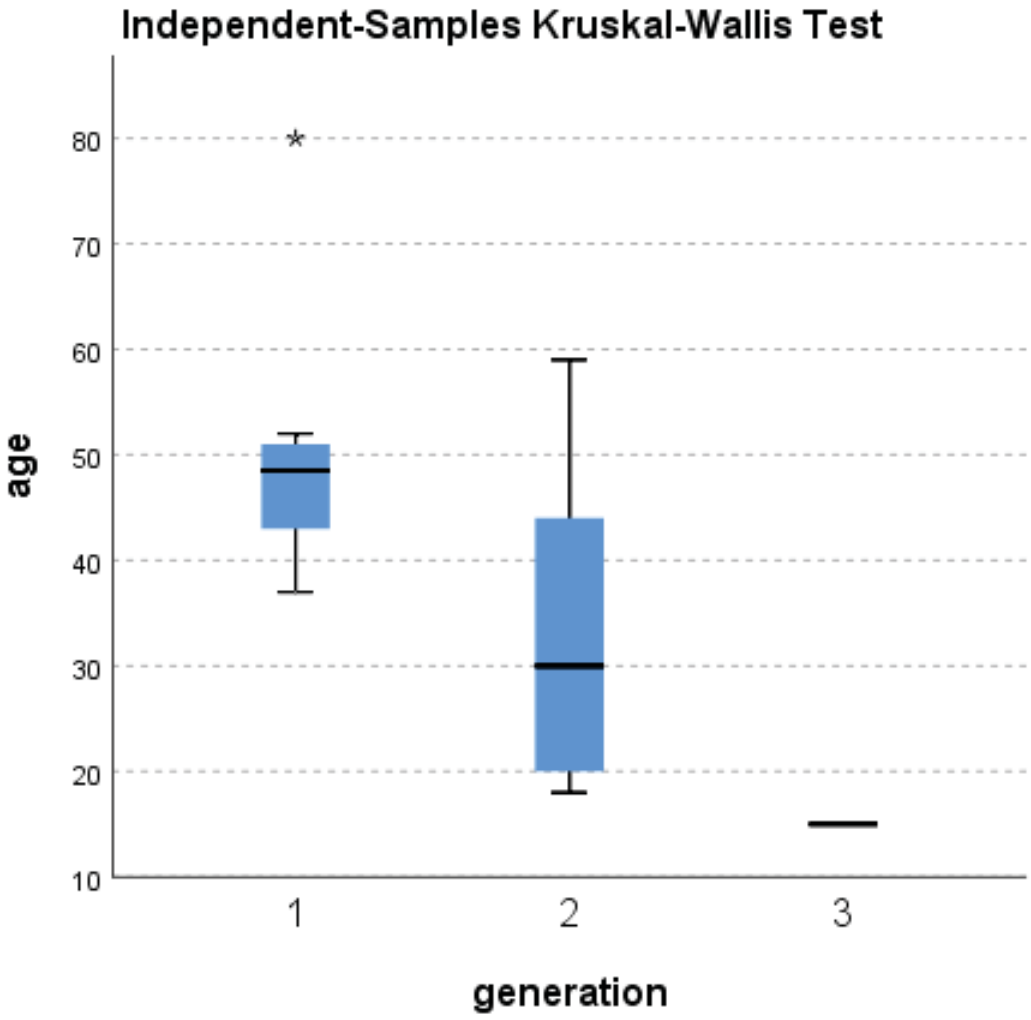
F

Legenda	
Sangue/DNA	*
Tecido	Δ
CPT	●
BMN	○
Ca pulmão	◒



APÊNDICE 3 - GRÁFICOS DOS ARTIGOS

GRÁFICO 1 – Idade média ao diagnóstico do CNMTF nas diferentes gerações



ANEXOS

REGISTRO DO ACEITE DO ARTIGO INTITULADO "*GENETIC MUTATIONS AND VARIANTS IN THE SUSCEPTIBILITY OF FAMILIAL NON-MEDULLARY THYROID CANCER*"

Gmail **Fabíola Yukiko Miasaki <fymiasaki@gmail.com>**

[Genes] Manuscript ID: genes-993666 - Accepted for Publication

1 messaggio

Paine Wei <paine.wei@mdpi.com> 16 novembre 2020 06:54

Rispondi a: Paine Wei <paine.wei@mdpi.com>, Genes Editorial Office <genes@mdpi.com>

A: Edna T Kimura <etkimura@usp.br>

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Dear Professor Kimura,

We are pleased to inform you that the following paper has been officially accepted for publication:

Manuscript ID: genes-993666
Type of manuscript: Review
Title: Genetic mutations and variants in the susceptibility of Familial Non-Medullary Thyroid Cancer
Authors: Fabíola Yukiko Miasaki, Cesar Seigi Fuziwara, Gisah Amaral de Carvalho, Edna Teruko Kimura *
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Submitted to section: Human Genomics and Genetic Diseases, https://www.mdpi.com/journal/genes/sections/HGGD
Genetic Perspectives in Thyroid Cancer https://www.mdpi.com/journal/genes/special_issues/Genetic_Thyroid_Cancer
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REGISTRO DO ACEITE DO ARTIGO INTITULADO "*THYROID AND BREAST CANCER IN TWO SISTERS WITH MONOALLEIC MUTATIONS IN THE ATAXIA TELANGIECTASIA MUTATED (ATM) GENE*"

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CC: "Fabíola Yukiko Miasaki" fymiasaki@gmail.com, "Kelly Cristina Saito" saito@icb.usp.br, "Guilherme Lopes Yamamoto" glyamamoto@gmail.com, "César Luiz Boguszewski" clbogus@uol.com.br, "Gisah Amaral de Carvalho" carvalho.gisah@gmail.com, "Edna Teruko Kimura" etkimura@usp.br

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Sincerely,

Timothy M. Beardsley, D.Phil.
Executive Editor, Endocrine Society

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