Original Article

Molecular Characterization Reveals the Importance and Diversity of Germline and Somatic RET Mutations in Cancer

Moleküler Karakterizasyonla Tespit Edilen Germline ve Somatik RET Mutasyonlarının Kanserdeki Önemi ve Çeşitliliği

İbrahim Şahin[1], Haktan Bağış Erdem[2]

[1]Department of Medical Genetics, University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Ankara, Turkey
[2]Department of Medical Genetics, University of Health Sciences, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Ankara, Turkey

ABSTRACT

Aim: Many individuals die due to cancer, and both doctors and researchers work hard to offer accurate illness, diagnosis, and prognosis monitoring, as well as resistance prediction.
Methods: A liquid biopsy and hereditary cancer panels were performed on 25 patients to examine the importance, spectrum, and diversity of RET germline and somatic mutations. Most of the patients visited the clinic with the diagnosis of advanced resistant cancers or hereditary cancer (MEN2). Two groups were formed: the first group was germline (n=7, 28%), and the second was somatic (n=18, 72%). For somatic, Tier I-II-III variants; for germline, pathogenic, likely pathogenic, and VUS variants have been included in the study.
Results: The mean age was 54.64. There were significantly more female participants (n=14, 56%) than males (n=11, 44%). In the germline group, the most common mutation was 'RET:c.2410G>A'. Nine mutations were nonsense or frameshift in the somatic group, and the most common mutations were 'RET:c.2324delinsGAC' and 'RET:c.1784A>G'. Nonsense or frameshift RET variants showed a higher incidence in the somatic group.
Conclusion: To the best of our knowledge, this is the first research to concentrate on RET mutations in the context of genetic variability between germline and somatic variants. The current of the study results indicate that patients with solid tumors, particularly breast cancer, should undergo RET sequencing to evaluate clinical features and prognosis. Discoveries about the structure and functions of RET gene will lead to more clinically relevant treatment approaches for cancer patients and will play an essential role in improving individual risk prediction, treatment, and prognosis.

Keywords: Liquid biopsy, MEN2, RET

ÖZET

Amaç: Pek çok kişi kanser nedeniyle ölmekte. Hem doktorlar hem de araştırmacılar, doğru hastalık, teşhis ve prognoz takibinin yanı sıra direnç tahmini sunmak için çok çalışıyorlar.
Gereç ve Yöntem: RET germline ve somatik mutasyonların önemini, spektrumunu ve farkını incelemek için 25 hastaya likit biyopsi ve ailesel kanser paneli uygulandı. Hastaların çoğu ileri dirençli kanser ve / veya kalıtsal kanser (MEN2) tanısıyla kliniği ziyaret etti. Toplam iki grup oluşturuldu: birinci grup germline (n=7, %28) ve ikincisi somatik (n= 8, %72). Somatik için, Tier I-II-III varyantları ve germline için patojenik, muhtemelen patojenik ve VUS varyantları çalışmaya dahil edilmiştir.
Bulgular: Ortalama yaş 54.64 idi. Kadın katılımcılar (n=14, %56) erkeklerden (n=11, %44) önemli ölçüde daha fazla idi. Germline grubunda en yaygın mutasyon "RET: c.2410G>A" idi. Somatik grupta, dokuz mutasyon nonsense veya çerçeve kaymasıydı ve en yaygın mutasyonlar "RET: c.2324delinsGAC" ve "RET: c.1784A>G" idi. Nonsense veya çerçeve kayması RET varyantları, somatik grupta daha yüksek bir insidans gösterdi.

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Sonuç: Bildiğimiz kadarıyla bu, germline ve somatik varyantlar arasındaki genetik değişkenlik bağlamında RET mutasyonlarına odaklanan ilk araştırmadır. Mevcut çalışmanın sonuçları, solid tümörlü hastaların, özellikle meme kanserinin, klinik özellikleri ve prognozu değerlendirmek için RET sekansına tabi tutulması gerektiğini göstermektedir. RET geninin yapısı ve işlevleri hakkındaki keşifler, kanser hastaları için klinik olarak daha uygun tedavi yaklaşımlarına yol açacak ve bireysel risk tahmini, tedavisi ve prognozunun iyileştirilmesinde önemli bir rol oynayacaktır.

Anahtar Kelimeler: Likit biyopsi, MEN2, RET

Introduction

Receptor tyrosine kinases regulate cell development and differentiation. Some of them have been shown to behave as oncogenes in human malignancies. RET (rearranged during transfection) is a trans-membrane receptor tyrosine kinase that may act as both a growth factor receptor and an oncogenic protein. It is triggered by a complex that includes a soluble glial cell line-derived neurotrophic factor (GDNF) family ligand (GFL) and a glycosylphospha-tidylinositol-anchored co-receptor, GDNF family receptors a (GFRa) [1]. GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN) are four distinct GFLs that can bind to and selectively activate RET through their homologous co-receptors GFRa1–4. RET has multiple activities in diverse tissues as a signal transducer of four separate ligand/co-receptor complexes. It is required for the development of the enteric nervous system as well as the regulation of the development of sympathetic, parasympa-thetic, motor, and sensory neurons [2].

The RET protein is a receptor tyrosine kinase that seems to transduce growth and differentiation signals in a variety of developmental tissues, including neural crest-derived tissues. The protein comprises an extracellular domain containing a ligand-binding domain, a cadherin-like domain, and a cysteine-rich region proximal to the cell membrane. It includes one transmembrane domain and two tyrosine kinase subdomains, TK1 and TK2 [3].

Somatic and germline mutations in the same tumor suppressor gene are widely known, as detailed in Knudson's two-mutation paradigm [4]. Similarly, somatic and germline mutations in the RET protooncogene have been discovered in a number of hereditary and non-hereditary human disorders, including multiple endocrine neoplasia (MEN) 2A and 2B, papillary thyroid cancer, and other cancers [5].

Multiple endocrine neoplasia type 2 (MEN2), sometimes referred to as Sipple's syndrome, is linked with medullary thyroid carcinoma (MTC) and hyperplasia of thyroid C cells. It is an autosomal dominant genetic disorder caused by a mutation in the RET proto-oncogene on chromosome 10, which results in the development of two or more endocrine adenomas or hyperplasia in the same patient, either simultaneously or sequentially, and resulting in the clinical condition defined by hyperfunctioning glands [6].

MEN2 is classified clinically as MEN2A, MEN2B, and familial medullary thyroid cancer, with MEN2A being the most frequent subtype [1]. Medullary thyroid cancer (MTC), pheochromocytoma (PHEO), and hyper-parathyroidism are all characteristics of MEN2A. Additionally, a tiny percentage of people develop skin lichen amyloidosis or Hirschsprung's disease. MTC is often the initial symptom of this subtype, with a near-100 percent prevalence. When patients are hospitalized, the majority have already advanced to MTC or have lymph node metastases. MTC is the leading cause of mortality in people with MEN2A, and 50% of

patients are at risk of recurrence [7]. MTC or MEN2A, on the other hand, may manifest differently in family members. Specifically, fundamental lesions may be entirely or partially manifested, lesions in the affected endocrine glands may arise at various time intervals (which may be many years), and numerous endocrine glands may sometimes be affected and demonstrate concurrent start. At the moment, individuals with MEN2A who demonstrate MTC as an early symptom are often misdiagnosed [1].

Numerous malignancies are known to be oncogene-dependent: oncogene addiction has been shown in a variety of neoplasms [8]. Somatic RET gene fusions are known to be oncogenic drivers in a variety of tumor types and are seen in 1–2% of non-squamous NSCLC patients. Fusions of the RET gene result in the formation of chimeric, cytosolic proteins containing a constitutively active RET kinase domain [9]. The recent approval of numerous tumor-agnostic medications by the Food and Drug Administration has resulted in a paradigm shift in cancer therapy away from organ/histology-specific strategies and toward biomarker-guided treatments. Selpercatinib (LOXO-292), a novel RET-specific tyrosine kinase inhibitor, has shown exceptional effectiveness in cancers with RET fusions or mutations, most notably RET fusion-positive NSCLC and RET-mutated MTC [10].

Liquid biopsy techniques have been used to treat a variety of different forms of cancer in recent years. A liquid biopsy is utilized in tumors to determine the patient's recovery, prognosis, and even diagnosis. During apoptosis, tumor cells lose fragments of biomarkers. These materials' cellular components may be examined for genetic abnormalities. This less intrusive testing procedure provides a greater likelihood of a favorable outcome and a better probability of correct findings [11,12].

In this study, we performed a liquid biopsy and hereditary cancer panel on 25 patients to examine the importance, spectrum, and difference of germline and somatic RET mutations. Our data broadens the RET mutations and provides insights for the diversity and characteristics of somatic and germline RET mutations.

Materials and methods

Patients

Consent for the publication of the study and any additional related information was taken from the patients or their parents involved in the study. The Ethics Committee approved (2021-03/1072) the study at the University of Health Sciences, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital. Twenty-five patients visited the clinic with the diagnosis of advanced resistant cancers or hereditary cancer (MEN2). Clinical histories and molecular results were reviewed for all unrelated patients examined at the Department of Medical Genetics, University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, and Department of Medical Genetics, University of Health Sciences, Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Ankara, Turkey. The patients underwent the comprehensive liquid biopsy and hereditary cancer panel between January 2018 and December 2020 at the Ankara Central Genetic Laboratory (Turkey). In the study, a total of two groups were formed. The first group was germline (n=7, 28%) and the second was somatic (n=18, 72%).

DNA Panels and NGS

From the blood samples collected in EDTA tubes, the patients' genomic DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen Inc., Hilden, Germany) by QIAcube (Qiagen Inc.,

Mississauga, ON, Canada). The DNA samples were quantified with a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific Inc., MA, USA).

Two different multigene panels have been used for liquid biopsy testing depending on the dates: ArcherDx Reveal ctDNA 28 Kit and Sophia Genetics 56 G Oncology. The Sophia Genetics 56G Oncology Solution was used at the center from 2018 to 2020, and the ArcherDx Reveal ctDNA 28 Kit has been used since 2020. The data were analyzed on the Archer Analysis Platform (ArcherDX, Inc., CO, USA) for the ArcherDx Reveal ctDNA 28 Kit and Sophia DDM software (Sophia Genetics, Saint-Sulp) for the Sophia Genetics 56G Oncology Solution.

For hereditary cancers, two different multigene panels were used depending on the dates: the Qiagen QIAseq Hereditary Custom Cancer Panel (from 2017 to 2018) and the Sophia Hereditary Cancer Solution Panel (since 2018). The sequencing was performed on an Illumina MiSeq system (Illumina Inc., San Diego, CA, USA). The data were analyzed using QIAGEN Clinical Insight (QCI™) Analyze software (Qiagen Inc., Hilden, Germany) for the Qiagen QIAseq Hereditary Custom Cancer Panel and with Sophia DDM software (Sophia Genetics, Saint-Sulp) for the Hereditary Cancer Solution (v1.1) panel. Visualization of the data was performed with IGV 2.7.2 (Broad Institute) software.

Interpretations, Descriptive Statistics & Graphics

In compliance with the recommendations issued by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology, germline variants were categorized as pathogenic, likely pathogenic, variant of uncertain significance (VUS), likely benign, and benign [13]. Pathogenic, likely pathogenic, and strong VUS (supports clinical phenotype and no other responsible mutation detected) variations were included in the study. Somatic variants were categorized as tier I, variants with strong clinical significance; tier II, variants with potential clinical significance; tier III, variants with unknown clinical significance; and tier IV, variants that are benign or likely benign, in compliance with the recommendations issued by the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists [14]. Tier I-II-III variations have been included in the study. Further, descriptive statistical calculations have been done, and the graphic has been prepared with Python 3.9.2 (IPython 7.19.0).

Results

The mean age was 54.64, with a minimum age of 35 and a maximum of 70. There were six patients below 50 years of age, and all of them were females. There were significantly more female participants (n=14, 56%) than males (n=11, 44%) (Table 1-2).

In the germline group, the mean age was 50.57, and all the mutations were missense and heterozygous. There were three pathogenic, two likely pathogenic, and two variant of uncertain significance (VUS) variants. The most common mutation was ‘RET:c.2410G>A’ (Table 1, Figure 1).

In the somatic group, the mean age was 56.22, and the variant fractions were between 0.1-10%. The majority of the patients have advanced-metastatic cancers. Nine mutations were nonsense or frameshift. The most common mutations detected were ‘RET:c.2324delinsGAC’ and ‘RET:c.1784 A>G’. The ‘RET:c.2324delinsGAC’ mutation has been observed seven times. (Figüre 1) In breast cancer, frameshift RET mutations were more predominant when compared with other groups (Table 2).

Table 1. RET germline mutations

Gender	Age	Indication	Gene	Mutation	Protein	Zygosity	Pathogenicity
F	53	colon	RET	c.1681A>T	p.Ser561Cys	heterozygous	Likely Pathogenic
F	35	MEN2	RET	c.224C>T	p. Thr75Met	heterozygous	Likely Pathogenic
F	41	MEN2	RET	c.785T>C	p.Val262Ala	heterozygous	VUS
M	67	MEN2	RET	c.341G>A	p.Arg114His	heterozygous	VUS
M	52	MEN2	RET	c.2370G>T	p.Leu790Phe	heterozygous	Pathogenic
F	56	MEN2	RET	c.2410G>A	p.Val804Met	heterozygous	Pathogenic
M	50	MEN2	RET	c.2410G>A	p.Val804Met	heterozygous	Pathogenic

Table 2. RET somatic mutations

Gender	Age	Indication	Gene	Mutation	Protein
F	53	advanced-metastatic	RET	c.1162G>A	p.Val388Ile
M	58	advanced-metastatic	RET	c.1784A>G	p.Glu595Gly
M	61	advanced-metastatic	RET	c.2071G>A	p.Gly691Ser
F	59	advanced-metastatic	RET	c.2372A>T	p.Tyr791Phe
M	66	advanced-metastatic	RET	c.1972C>T	p.His658Tyr
M	60	advanced-metastatic	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	48	breast	RET	c.1906A>C	p.Thr636Pro
M	62	advanced-metastatic	RET	c.1784A>G	p.Glu595Gly
M	51	advanced-metastatic	RET	c.1784A>G	p.Glu595Gly
F	37	breast	RET	c.2338_2339insC	p.Lys780Thrfs*64
F	69	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	46	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
M	57	advanced-metastatic	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	55	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	37	breast	RET	c.2324delinsGAC	p.Glu775Glyfs*6
M	58	advanced-metastatic	RET	c.2341C>T	p. Gln781Ter
F	70	lung	RET	c.2324delinsGAC	p.Glu775Glyfs*6
F	65	advanced-metastatic	RET	c.2657G>A	p.Arg886Gln

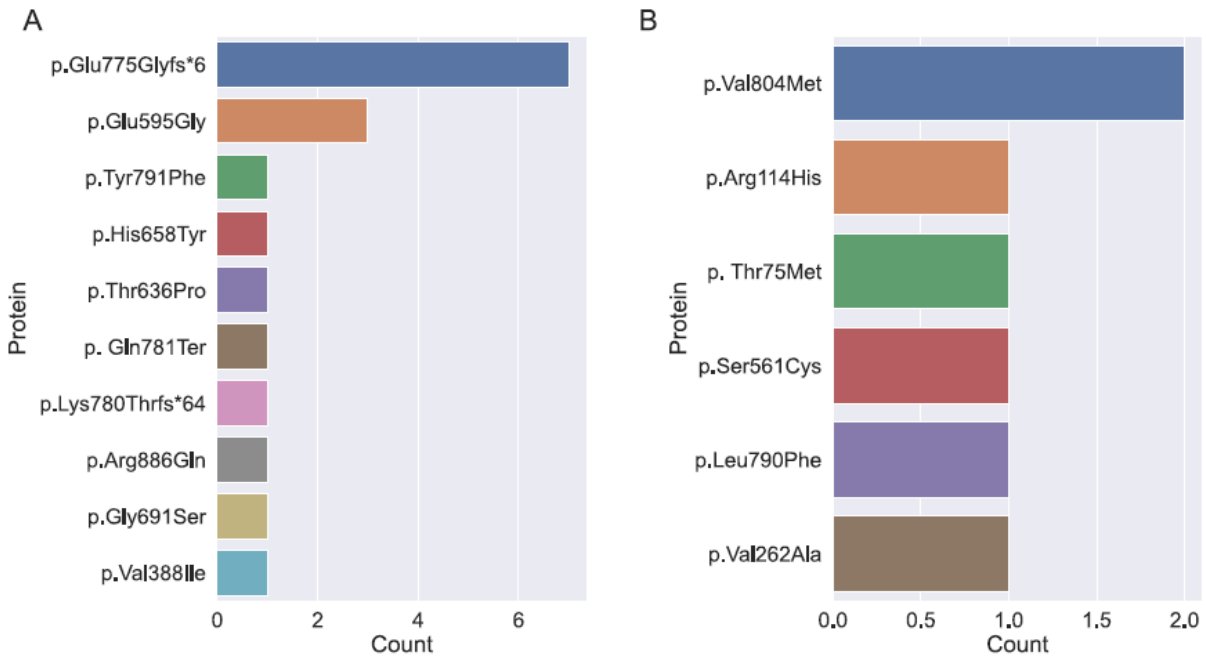

Figure 1. Somatic and germline RET mutations.
Bar plots showing the somatic (A) and germline (B) RET mutations in the study.

Discussion

Mutations in the RET gene result in various clinical symptoms and disease manifestations [2]. Based on RET's normal function, it is conceivable to identify various probable explanations for the disparate phenotypes. The signaling capability of various RET variants may be determined by subcellular location, substrate selectivity, turnover rate, percentage of activated RET, and genetic background. As a result, distinct types of clinical symptoms associated with RET may need treatment with different sorts of medications targeting specific domains of RET [2].

While germline mutations in codons 768 (exon 13), 804 (exon 14), and 891 (exon 15) are strongly related to MTC, they account for a small proportion of cases. These locations are located inside the domain of the intracellular tyrosine kinase. Exon 13 mutations are less prevalent in MEN2A/MTC (codons 790 and 791). Gatekeeper mutations in codon 804 have been found. Codon 804 mutation was found in two patients in the germline group in this study. (Figure 1) Changes at this location affect access to the RET ATP-binding domain, resulting in decreased sensitivity to some RET-targeting multi-kinase inhibitors [15]. Mutations in the intracellular TK2 domain are responsible for MEN2B-associated malignancies. A single 918 Met to Thr mutation in exon 16 accounts for almost 95% of MEN2B cases and is unique to this illness. Met 918 is a crucial component of the substrate recognition pocket found in the RET protein's tyrosine kinase catalytic core. Mutations arise as new (de novo) germline alterations in more than 50% of cases of MEN2B with codon 918 mutations. Another mutation, alanine to phenylalanine at codon 883 in exon 15, was discovered in some unrelated MEN2B relatives [16]. Dual (tandem) mutations in codons 804 and 806 or 804 and 904 may result in atypical MEN2B [17].

MEN2 RET mutations in the germline result in a gain of function. This contrasts with many other hereditary predispositions to neoplasia, which is caused by heritable "loss-of-function" mutations in tumor suppressor proteins. The functional restrictions imposed by such activating lesions are likely responsible for the rarity of RET mutations, a regulation that benefits molecular diagnostics in this condition [18].

Extensive research on large families demonstrates a clear genotype-phenotype link. MEN2B has a higher rate of morbidity and death than MEN2A. Survival is comparable between individuals with MEN2B and those with spontaneous MTC who had somatic RET mutations identical to the most prevalent germline mutations causing MEN2B. The genotype also affects the age at which MTC is first diagnosed and the result of thyroidectomy [19].

RET gene rearrangements are essential for solid tumors. In this study, nonsense and frameshift RET mutations were frequent in the somatic group, particularly breast cancer. 'RET, c.2324delinsGAC, p.Glu775Glyfs* 6' mutation was the most common. (Table 2, Figure 1) All the nonsense and frameshift RET mutations were on the 13th exon and in the kinase domain. The majority of the somatic group mutations were around the kinase domain. Most of the kinase domain RET mutations are oncogenic and associated with poor prognosis and drug resistance, particularly in thyroid cancers [20].

In contrast to the germline group, frameshift and kinase domain RET mutations were predominant in the somatic group. Many nonsense and frameshift RET mutations are also associated with gain of function according to databases (OncoKB), and they are likely oncogenic, unlike other genes. These mutations, particularly 'RET, c.2324delinsGAC, p.Glu775Glyfs*6', could be responsible for drug resistance,

progression, and metastasis. Further studies are needed to clarify the roles of these nonsense and frameshift RET mutations.

The current study's results indicate that patients with solid tumors, particularly advanced-metastatic cancers and breast cancer, should undergo RET sequencing to evaluate clinical features and prognosis. Discoveries about the structure and functions of RET gene will lead to more clinically relevant treatment approaches for cancer patients and will play an essential role in improving individual risk prediction, treatment, and prognosis.

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Corresponding author e-mail: ibrahimsahinmd@gmail.com

Orcid ID:
İbrahim Şahin 0000-0002-6050-816X
Haktan Bağış Erdem 0000-0002-4391-1387

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