CASE REPORT

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Vandetanib in a Child Affected by Neurofibromatosis Type 1 and Medullary Thyroid Carcinoma with Both *NF1* and Homozygous *RET* Proto-oncogen Germ-line Mutations

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What is already known on this topic?

Medullary thyroid carcinoma or C-cell hyperplasia are usually associated with other endocrine tumors or a patients with multiple endocrine neoplasia type 2 clinical findings. Germline mutations in both *NF1* and *RET* proto-oncogene have been reported only in a patient with thyroid C-cell hyperplasia. Although vandetanib is frequently used in thyroid medullary carcinoma in the adult age group, there is little data regarding its use in the childhood age group.

What this study adds?

This is the first report the presence of a double germline mutation involving both *NF1* and *RET* genes and treated with vandetanib.

Abstract

Cases of neurofibromatosis type 1 (NF1)-associated medullary thyroid carcinoma (MTC) or C-cell hyperplasia are rarely associated with other endocrine tumors or cases with a multiple endocrine neoplasia type 2. In these patients, mutations were detected in the *NF1* gene but no mutations were detected in the *RET* gene. Although vandetanib has been shown to improve progression-free survival in adults with advanced MTC, data in pediatric patients are limited. Herein, we report the use and outcome of vandetanib in a pediatric MTC case in which *NF1* gene and *RET* proto-oncogen mutation were identified together.
Keywords: Medullary thyroid carcinoma, vandetanib, *RET* proto-oncogene, *NF1* gene, children

Introduction

Neurofibromatosis type 1 (NF1) is a common, autosomal dominant, multi-systemic neurocutaneous disorder. An increased frequency of various endocrine pathologies, such as central precocious puberty, short stature, diencephalic syndrome, growth hormone deficiency or growth hormone hypersecretion has been reported in children. In addition, pheochromocytoma, parathyroid carcinoma, parathyroid adenoma, somatostatin producing neuroendocrine tumor, duodenal carcinoid tumor producing somatostatin, thyroid papillary carcinoma with pheochromocytoma have been described in patients with NF1 (1,2,3).

Medullary thyroid carcinoma (MTC) is a neuroendocrine tumour arising from the calcitonin producing parafollicular C-cells of the thyroid. It accounts for approximately 1-2% of all thyroid cancers. Clinically, 70-80% of MTCs are sporadic, while 20-30% are inherited in an autosomal dominant

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fashion (4). Hereditary MTCs may be part of multiple endocrine neoplasia type 2 (MEN) and occur in three different clinical forms as MEN2A, MEN2B and familial MTC. Mutated "REarrangement during Transfection" (*RET*) proto-oncogene plays a very significant role in the development of human neuroendocrine tumors and tumor syndromes. *RET* proto-oncogene mutation has been reported in both sporadic and familial cases (4).

Cases of NF1-associated MTC or C-cell hyperplasia are rarely associated with other endocrine tumors or with MEN2 clinical findings. In these patients, mutations were detected in the *NF1* gene but no mutations were detected in the *RET* gene (5,6,7,8,9,10,11,12).

To our knowledge, germline mutations in both *NF1* and *RET* proto-oncogene have been reported only in one patient with thyroid C-cell hyperplasia, but no simultaneous mutation of these two genes in MTC has been reported (12).

In this article, we present a 15-year-old male patient diagnosed with both NF1 and MTC, and also had mutations in both *NF1* and *RET* genes, and will discuss the effectiveness of vandetanib therapy in MTC.

Case Report

A 15-year-old boy was admitted to a hospital with progressively increasing midline neck swelling for two months. Physical examination revealed a firm and mobile 3×2 cm swelling on the left side of the neck, multiple lymph nodes, multiple café-au-lait macules, and inguinal and axillary freckling. Lisch nodule was detected in the eye examination. Cranial magnetic resonance imaging showed focal areas of signal intensity and bilateral optic glioma. Family history revealed that his brother and mother had similar findings and also that his mother had a diagnosis of neurofibromas. Cervical ultrasonography and computed tomography showed a heterogeneous mass lesion in the left thyroid lobe and multiple lymphadenopathy. Total blood count and biochemical analysis were within normal range. Thyroid-stimulating hormone, free triiodothyronine and free thyroxine levels were 21.038 µIU/mL (0.35-5.5 µIU/mL), 6.28 pmol/L (8-22 pmol/L) and 14.76 pmol/L (0.83-1.43 ng/dL), respectively. After the cervical nodes were resected, pathological investigation demonstrated metastasis of MTC, and the patient was referred to the department of pediatric surgery. Preoperative calcitonin and carcinoembryonic antigen (CEA) levels were 2000 pg/mL (0-8.4 pg/mL) and 116.52 ng/mL (0-2.5 ng/mL), respectively. The patient was admitted to our department after total thyroidectomy and radical neck dissection. He was investigated for MEN syndrome. Serum parathyroid hormone, serum gastrin, 24-hour urinary catecolamine and metanephrine levels were within normal range.

Histopathologic examination showed MTC with presence of perineural and lymphovascular invasion (Figure 1A-1F). Due to the residual thyroid tissue and bilateral pathological lymph nodes detected on Tc99m-pertechnetate thyroid scintigraphy and positron emission tomography, the patient was re-operated but excision was incomplete. The patient's stage was T2N1aM0 (stage 3) according to the tumor node metastasis system proposed by the "American Joint Committee on Cancer" (13). Molecular testing revealed a heterozygous mutation in *NF1* gene [IVS38-2A>G (c.5610-2A>G) both in our patient and his brother. There was no NF1-related mutation in his mother. In addition, homozygous *RET* proto-oncogene mutation [c.2671T>G (p.S891A) (p.Ser891Ala)] was found in the patient with a heterozygous mutation in his mother, father and brother (Figure 2). As a result of incomplete removal of lymph nodes and remaining thyroid tissue, serum calcitonin level was 1563 pg/mL and serum CEA level was 57.28 ng/mL. Vandetanib treatment was initiated at a dose of 300 mg/day. Serum calcitonin levels at the sixth, twelfth and twenty-fourth months of treatment were 34.7 pg/mL, 4.4 pg/mL and 1.2 pg/mL, while CEA levels were 12 ng/mL, 3.2 ng/

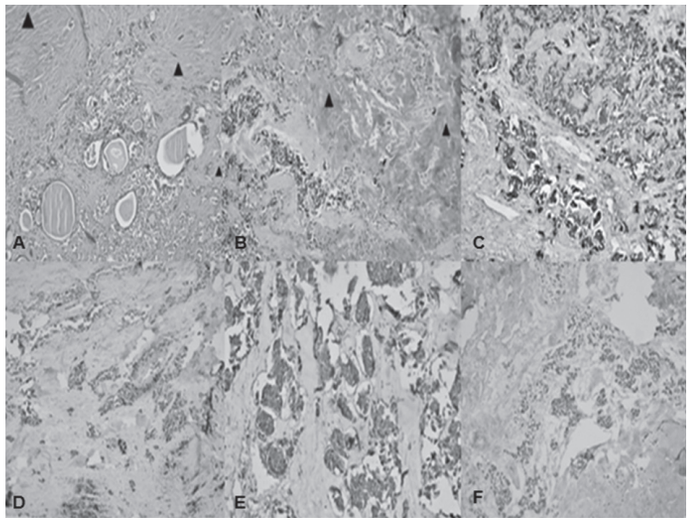

Figure 1. (A) Large areas of amyloid deposits (black triangle) can be seen around and between tumor cells and thyroid follicules (Hematoxylin and eosin x100), **(B)** Large deposits of amyloid (black triangle) in thyroid parenchyma (Congo Red x100), **(C)** Tumor cells showed positive immunoreactivity against monoclonal carcinoembryonic antigen (CEA) antibodies (CEA x200), **(D)** Tumor cells showed positive immunoreactivity against monoclonal calcitonin antibodies (Calcitonine x100), **(E)** Tumor cells showed positive immunoreactivity against monoclonal chromogranin antibodies (Chromogranin x200), **(F)** Tumor cells showed positive immunoreactivity against monoclonal TTF-1 antibodies (TTF-1 x100)

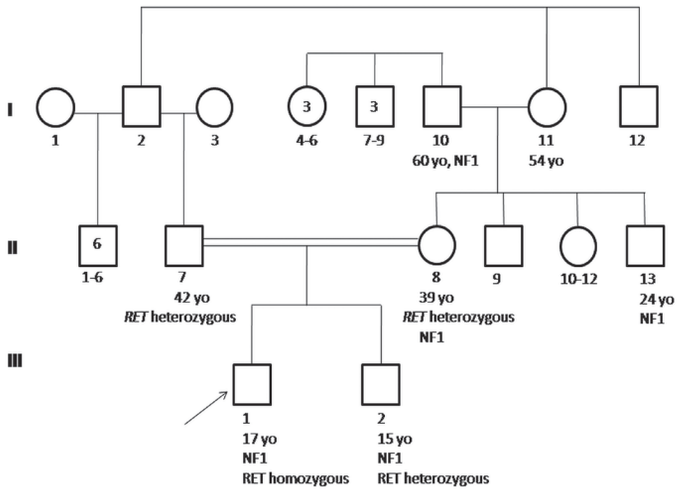

Figure 2. The pedigree of the family with *NF1* and *RET* mutations; the arrow (III-1) indicates the proband

mL and 1.1 ng/mL, respectively. The patient has been on vandetanib treatment for 32 months and no residual tissue and lymphadenopathy were detected in the neck tomography taken at the 30th month of the treatment. No side effects were observed during vandetanib treatment in our patient in this period.

Discussion

Besides leukemia, somatic *NF1* mutations have been reported in various cancers occurring in many different regions such as breast, colorectum, urothelium, lung, ovary, skin and nerve tissues (14). In addition, pheochromocytoma, parathyroid carcinoma, somatostatin producing neuroendocrine tumors, duodenal carcinoid tumors, and thyroid papillary carcinoma are reported endocrine neoplasms in patients with NF1. In these patients, it can be assumed that *NF1* mutations predispose to the development of endocrine tumors by affecting the growth and differentiation of parafollicular C cells, parathyroid cells and other cells from which different endocrine tumors develop.

To our knowledge, MTC has not been reported in patients with NF1 mutation. In these patients, the cause of this association is unclear because no germ-line mutation in the *RET* gene could be demonstrated. Mutations in both the *NF1* and *RET* genes have been described to date only in one case with thyroid C cell hyperplasia (12). Our case is remarkable since it is the first case of MTC in which both *NF1* gene and *RET* proto-oncogen mutation were identified simultaneously.

MTC is a rare tumor originating from the parafollicular or C-cells of the thyroid gland. MTC is sporadic in 75% of patients and usually occurs in the fourth to sixth decade of life. Less commonly, hereditary MTCs are found in MEN2A or MEN2B or as a part of familial MTC (15). *RET* proto-oncogene mutation is detected in almost all hereditary cases and in more than 40% of sporadic cases (15).

In our patient, homozygous mutation was detected in codon 891 in the *RET* gene. The p.S891A mutation was first described by Hofstra et al (16) in 1997 and associated with MEN 2A and MTC. Less than 5% of all MTC patients reported to date have RET mutationorrected as p.S891A and this mutation is reported to be heterozygous because *RET* oncogene, acts dominantly as is usual in oncogenes (17). Giacché et al (18) analyzed 251 relatives of individuals with 28 p.S891A mutations and reported that 108 had asymptomatic carriage and 64 had undergone thyroidectomy. As a result of histological examination, they reported that the mean age of patients with C-cell hyperplasia, micro-MTC and MTC was 30.2 ± 13.7, 37.9 ± 10.3 and 55.0 ± 14.7, respectively, and that malignancy development increased with age in individuals carrying the p.S891A mutation. As a result of the ItaMEN study, in which the germline RET mutations of 250 families with hereditary MTC were evaluated, the p.S891A mutation was present in 9.2% and was lower than other European studies (19). Also Schulte et al (20) stated that they found p.S891A mutation in 5% of patients followed up for MEN 2A. According to our knowledge there is only one study about the frequency of p.S891A mutation in Turkish patients (21). In this study 12 different RET oncogen mutations were detected in 32 of 155 patients who were diagnosed with isolated MTC or as part of MEN2, and p.S891A mutation was reported in two patients (6%). In this case the mutation was homozygous and, to our knowledge, it was not reported previously. The p.S891A mutation poses a moderate risk for MTC development according to the American Thyroid Association (ATA). The recommended ATA approach in individuals with moderate-risk *RET* mutations is to follow annual calcitonin and perform a total thyroidectomy when high values are detected (22). The mothers, fathers and siblings of our patient with heterozygous p.S891A mutations did not have any symptoms, pathological examination or laboratory findings in favor of cancer. Since the mother and father carry a moderately risky mutation according to ATA criteria and MTC risk will increase in later years, prophylactic thyroidectomy was recommended but they did not accept. Similarly, the family preferred long-term follow-up instead of total thyroidectomy for the other child with a heterozygous p.S891A mutation.

The *NF1* gene encodes neurofibromin, a GTPase-activating protein that negatively regulates the Ras/mitogen-activated protein kinase (MAPK) signalling pathway (23). Loss-of-

function mutations in *NF1* lead to uncontrollable activation of kinase and tumorigenesis. Also, the *RET* protooncogene encodes a receptor tyrosine kinase that mediates extracellular neurotropic signaling to intracellular transduction pathways including the MAPK/ERK pathway (24). We think that these two diseases occurred coincidentally, because MTC in our patient does not have a common etiological pathway with the NF1, according to the evidence concerning both the *NF1* gene and the *RET* oncogene.

Our patient was investigated for MEN2 due to *RET* proto oncogene mutation and MTC. In the family history, we learned that there were no patients with thyroid disease and therefore operated. *RET* proto-oncogene mutation analysis of the parents revealed that they were carriers of germ-line S891A mutation. On three-generation pedigree analysis no family member with cancer, including MTC, was reported. Although we could not perform the molecular testing of the *RET* gene for the rest of the family, since they lived in different cities, we believe the maternal grandmother and paternal grandfather to be carriers because they were siblings and the case had a homozygous mutation. The case was thought to be familial MTC although there was no clinical or laboratory finding in any of the heterozygotes, despite the mutation in the family; and currently follow-up was performed without prophylactic thyroidectomy.

In the follow-up of our patient, it was thought that vandetanib treatment would be appropriate, since residual tissue was still present after the second operation. Vandetanib is an orally available tyrosine kinase inhibitor that targets vascular endothelial growth factor dependent tumor angiogenesis and epidermal growth factor receptor, *RET* and *RET* dependent tumor cell proliferation (25). Several studies have evaluated the efficacy of vandetanib in the treatment of advanced MTC. In the ZETA trial, 331 patients with 5% local advanced stage and 95% metastatic MTC were randomized to vandetanib and placebo. At the end of the study, it was determined that the median survival of 19.3 months in the placebo group and the median 30.5 months in the vandetanib group were progression-free survival and a significant difference was found between the two groups (26). In a meta-analysis, 300 mg of vandetanib treatment was demonstrated to have a better objective response than 150 mg of vandetanib treatment (27). When compared to 150 mg and 300 mg vandetanib treatments, Hu et al (28) showed that administration of 300 mg increased overall response rate.

The efficacy of vandetanib in childhood and adolescence was investigated in 16 patients aged 5-18 years with locally advanced or metastatic MEN2B-associated MTC. In this study, the dose of vandetanib was 100 mg/m^2. M918T *RET* germline mutation was present in 15 patients and 7 of them (47%) had a partial response (29). Kraft et al (30) reported that the duration of vandetanib therapy was 6.1 (0.1-9.7+) years in children treated with vandetanib, which lasted a median of 7.4 years and that progression-free survival was 6.7 years. Our patient has been receiving vandetanib for two years and serum calcitonin and CEA levels gradually decreased and reached the normal reference range. Since the dose we administer is higher than the dose in other pediatric studies and our patient is 15 years old, we think that the 300 mg/day dose stated in adult studies may have contributed to our good response. Also, considering that vandetanib suppresses *RET* oncogene and *RET* oncogene dependent cell proliferation, we hypothesize that the high dose we applied may be more effective due to the homozygous mutation in our patient.

Conclusion

In conclusion, it should be kept in mind that different endocrinological tumors may rarely develop with NF1 and the patients should be carefully evaluated in this regard. Furthermore, we believe that vandetanib dose for children, especially for older or adolescent children, with MTC may be the same as in adults but this needs to be supported by further pediatric studies with larger sample size.

Ethics

Informed Consent: Written informed consent was obtained from the patient's parents for publication of this case report and the accompanying images.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices- Concept - Design - Data Collection or Processing - Analysis or Interpretation - Literature Search - Writing: Begümhan Demir Gündoğan, Fatih Sağcan, Sevcan Tuğ Bozdoğan, Yüksel Balcı, Ferah Tuncel Daloğlu, Elvan Çağlar Çıtak.

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