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Molecular Case Studies

RESEARCH REPORT

Identification of a secondary *RET* mutation in a pediatric patient with relapsed acute myeloid leukemia leads to the diagnosis and treatment of asymptomatic metastatic medullary thyroid cancer in a parent: a case for sequencing the germline

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Abstract The incorporation of tumor-normal genomic testing into oncology can identify somatic mutations that inform therapeutic measures but also germline variants associated with unsuspected cancer predisposition. We describe a case in which a *RET* variant was identified in a 3-yr-old male with relapsed leukemia. Sanger sequencing revealed the patient's father and three siblings carried the same variant, associated with multiple endocrine neoplasia 2A (MEN2A). Evaluation of the father led to the diagnosis and treatment of metastatic medullary thyroid carcinoma. Detection of *RET* mutations in families with hereditary MTC allows for genetic risk stratification and disease surveillance to reduce morbidity and mortality.

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INTRODUCTION

The recognition of cancer as a genetic disease has prompted the implementation of next-generation sequencing (NGS) in clinical practice by pediatric oncologists. Advances in genomic technology have allowed for the interrogation of all known protein-coding sequences in the human genome though whole-exome sequencing (WES), which can precisely identify cancer driving alterations through analyses of tumor-normal samples. Although the intent of performing WES is to identify targetable mutations, clarify diagnoses, or provide risk stratification for patients, it can also identify pathogenic germline mutations not related to the test indication, referred to as secondary findings. The American College of Medical

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Genetics and Genomics (ACMG) policy for clinical sequencing is to return pathogenic variants from a select set of genes associated with highly penetrant genetic disorders, including cancer predisposition and other treatable inherited diseases (Green et al. 2013; Kalia et al. 2017). Such findings may have important clinical significance for the patient and family members, including the identification of cancer risk variants, carrier status for recessive conditions, and other inheritable diseases.

RET (*rearranged during transfection*) is one such gene recommended for return by the ACMG. *RET* is a proto-oncogene composed of 20 exons located on Chromosome 10 (10q11.2) and encodes a transmembrane receptor tyrosine kinase important in cell growth and differentiation. Heterozygous mutations in *RET* are associated with familial medullary thyroid cancer (FMTC) and multiple endocrine neoplasia type 2A (MEN 2A) and type 2B (MEN 2B) (Mukherjee and Zakalik 2011). MEN 2A is characterized by medullary thyroid carcinoma (MTC) in 95% of cases, hyperparathyroidism in up to 30% of cases, and pheochromocytoma in up to 50% of cases (Eng et al. 1996). Based on well-documented genotype–phenotype relationships, guidelines have been established as to when to initiate surveillance and prophylactic thyroidectomy (Wells et al. 2015). Survival is greatly improved when MTC is detected and treated early in the course of the disease. Additionally, symptomatic or metastatic MTC caused by *RET*-activating mutations are targetable by FDA-approved multityrosine kinase inhibitors including sorafenib, vandetanib, cabozantinib, and lenvatinib, as well as more specific and presumably less toxic *RET* inhibitors now in clinical trials such as LOXO-292 (NCT03157128) and BLU-667 (NCT03037385) (Drilon et al. 2018).

Tumor-normal WES can provide an important benefit to patients and their families to detect highly penetrant mutations in cancer predisposition genes, especially in cases with an unremarkable family history. Here we describe the identification of a germline *RET V804M* variant by WES from a pediatric patient with relapsed acute myeloid leukemia (AML) that led to the diagnosis and treatment of advanced MTC in a parent and medical surveillance for affected siblings.

RESULTS

Clinical Presentation

A 13-mo-old male was diagnosed in early 2015 with myelomonocytic, MLL-rearranged AML with bone marrow involvement and chloromas of the mediastinum and kidney. He was treated with a standard chemotherapy regimen and achieved remission at the end of induction but experienced an isolated CNS relapse 6 mo after completing therapy. MRI of the brain revealed a right cerebral hemisphere lesion, which was successfully resected. The patient was then referred to CUMC for a bone marrow transplant. To identify and explore clinically actionable somatic mutations in the patient's AML, written consent for cancer WES testing was obtained from the patient's mother. The consent discussion detailed the risks and benefits of testing, including the potential disclosure of secondary findings, defined as "germline disease-causing mutations unrelated to the condition for which sequencing was being performed" (Oberg et al. 2016).

Genomic Analyses

Following informed consent, a formalin-fixed, paraffin-embedded sample of the excised right parietal brain myeloid sarcoma and a peripheral blood sample were submitted for tumor-normal WES. Testing revealed a potentially targetable somatic *KRAS* variant (NM_033360.2, c.183A>C, p. Q61H) that may inform future therapy should the patient experience a second relapse, as well as a heterozygous germline *RET* variant (NM_020975.4,

Table 1. Variant table

Gene	Chromosome	HGVS DNA reference	HGVS protein reference	Variant type	Predicted effect (substitution, deletion, etc.)	dbSNP/ dbVar ID	Genotype (heterozygous/ homozygous)	Coverage
RET	10	c.2410G>A	p.V804M	SNV	Substitution	rs79658334	Heterozygous	Coverage 7/16
RET	10	c.2832C>G	p.I944M	SNV	Substitution		Heterozygous	Coverage 120/297
KRAS	12	c.183A>C	p.Q61H	SNV	Substitution	rs17851045	Heterozygous	Coverage 56/105

c.2410G>A, p. V804M) confirmed by Sanger sequencing (Table 1; Fig. 1). The *V804M* variant, located in exon 14 of *RET,* has been described in patients with FMTC or MEN 2A and is classified as moderate risk (ATA-MOD) for MTC by the American Thyroid Association (ATA) (Wells et al. 2015). A second heterozygous *RET* variant observed (NM_020975.4, c.2832C>G, p.I944M) has not been previously reported in association with FMTC, MEN 2A, or MEN 2B and was classified as a variant of unknown significance (Table 1). The *I944M* variant is predicted to be damaging by SIFT, neutral by Provean, and damaging by MetaLR. It is not reported in the ExAC database.

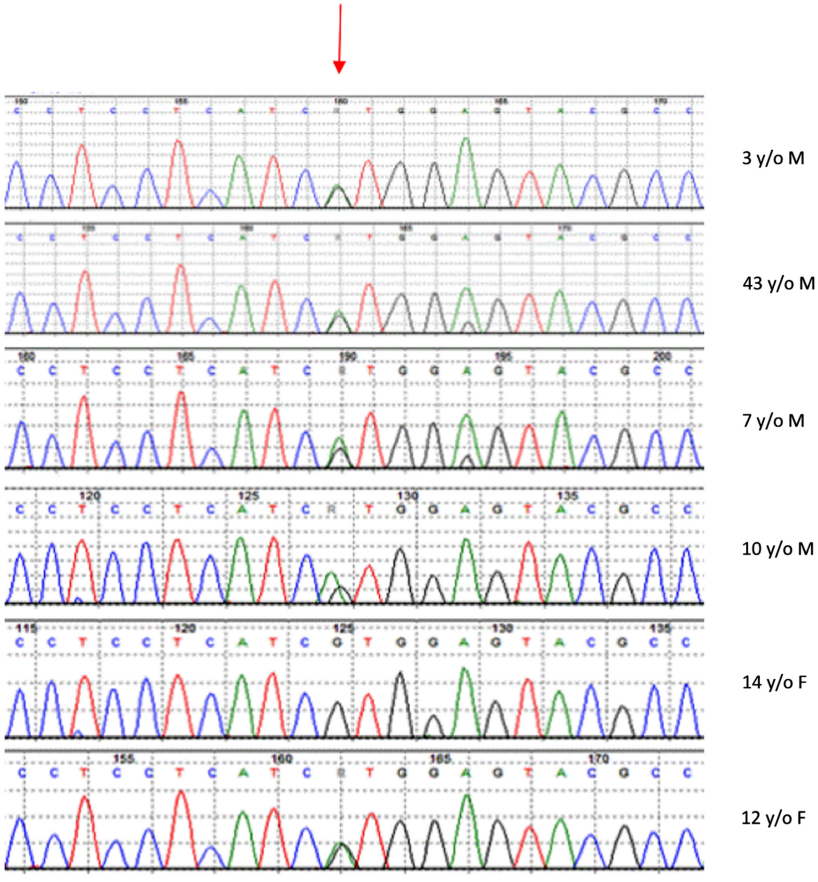

Figure 1. Sequencing traces.

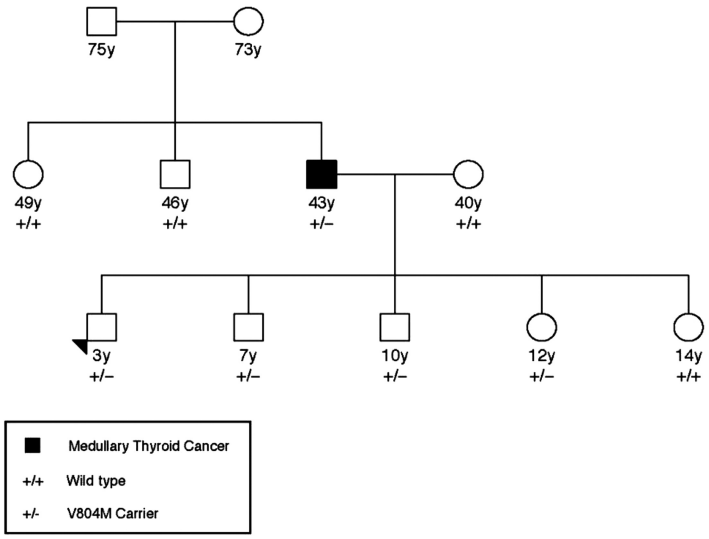

Figure 2. Case pedigree. Age at the time of sequencing is reported below each symbol.

Treatment Outcomes

The family was referred for genetic counseling, and Sanger sequencing was performed on peripheral blood from the patient's parents and four siblings identified the *V804M* and *I944M* variants in his 43-yr-old father, two brothers (ages 7 and 10 yr), and 12-yr-old sister. Subsequent testing of his 40-yr-old mother, 14-yr-old sister, and paternal aunt and uncle was negative. As of this writing, the paternal grandparents have not initiated testing. (See Fig. 2.)

Evaluation of the father revealed an enlarged thyroid, elevated CEA of 13.7 (normal up to 4.7 ng/mL), and calcitonin levels of 307.0 (normal up to 7.5 pg/mL). Thyroid sonogram confirmed the presence of a left-sided nodule; a preliminary biopsy was positive for malignant cells. The patient underwent a total thyroidectomy, bilateral central lymph node dissection, left lateral neck dissection, and autotransplantation of the left lower parathyroid gland. Pathology demonstrated a unifocal nodule consistent with a Grade II, MTC measuring 1.7 cm in longest diameter, with focal extrathyroid extension and no vascular invasion. Six of 30 ipsilateral nodes examined had tumor involvement, including one cervical level III node; he was thus staged as IVA (pT3, pN1b, M0). At 3 mo postsurgery, calcitonin and CEA levels were normal. It should be noted that prior to this diagnosis, the father felt entirely well, with no significant medical issues and a recent unremarkable comprehensive physical exam. Paternal family history was negative for endocrine tumors.

Initial screenings of our patient and two of his siblings were negative. Mildly elevated calcitonin levels were found in the 10-yr-old brother and a total thyroidectomy is planned. Future follow-up for family members carrying the *RET* mutation will proceed according to the 2015 ATA screening guidelines for MTC, pheochromocytoma, and hyperparathyroidism (Wells et al. 2015).

DISCUSSION

We describe the discovery of a germline *V804M RET* mutation through cancer WES performed on a pediatric patient with relapsed AML. Cascade genetic testing of the family resulted in the diagnosis and treatment of metastatic MTC in the asymptomatic father and surveillance for four mutation-positive children. Studies of individuals with negative family

history of endocrine diseases have identified disease-causing *RET* germline mutations in 6%–9.5% of these patients (Wiench et al. 2001; Elisei et al. 2007).

Phenotypic expression of mutations at codon 804 is highly variable, even within the same family (Feldman et al. 2000; Frohnauer and Decker 2000; Gibelin et al. 2004). The median age of onset in patients with codon 804 mutations is 44 yr (range: 7.2–77.0 yr) (Gimm et al. 2004). There are reports of individuals with *V804M* diagnosed with MTC at age 5 yr and fatal metastatic MTC at age 12 yr, whereas other individuals with the same variant have no clinical evidence of MTC at age 86 yr (Marquard and Eng 1999 Sep 27 [Updated 2015 Jun 25]). The incomplete penetrance of codon 804 mutations supports a previously proposed model in which individuals heterozygous for weakly transforming *RET* mutations require a second germline or somatic mutation in *RET* to clinically express the disease (Lesueur et al. 2005). The functional significance of the second heterozygous germline *RET* variant (I944M) found in *cis* with the V804M mutation is unknown and clinical significance could not be determined. Additionally, a causal link between AML and germline *RET* mutations cannot be ruled out yet.

Advances in high-throughput sequencing allows for genomic interrogation of tumors and offers promising avenues for the clinical management of patients. Germline comparison can help identify tumor-specific variants but can also reveal mutations associated with previously unsuspected heritable cancer risk. To date, cancer precision medicine programs have generally not performed comprehensive WES sequencing because it requires germline sequencing for analysis and therefore has the potential to reveal secondary findings. An informed consent discussion that adequately addresses this possibility and allows ample time for families to be appropriately counseled may require additional resources, and centers must be prepared to provide education and sufficient access to genetic counseling posttesting.

Recently, the incidence of germline mutations in cancer susceptibility genes detected by WES in childhood malignancies has been reported as ranging from 8.5% to 14%, highlighting the importance of germline assessment to pediatric precision oncology (Knapke et al. 2012; Zhang et al. 2015; Oberg et al. 2016; Parsons et al. 2016). In this case, germline analysis provided potentially lifesaving information (Parsons et al. 2014) for the father of our patient, including a tractable therapeutic target, and a health maintenance plan for the patient and three of his siblings. Our experience illustrates the need for comprehensive consenting practices and timely genetic counseling in order to inform families of inherited cancer risk and to guide surveillance strategies.

METHODS

Written informed consent for clinical cancer WES was obtained after the risks and benefits had been explained to the family, which included discussion of the potential disclosure of secondary findings. The clinical consent requires patients to opt in or opt out of the return of ACMG secondary findings recommended by ACMG (Green et al. 2013), which family described here chose to be informed of. WES, including variant calling and annotation, was performed in a CAP-accredited and CLIA-certified laboratory at Columbia University Medical Center (CUMC) following previously described methodology (Oberg et al. 2016).

ADDITIONAL INFORMATION

Data Deposition and Access

The variant was submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and can be found under accession number SCV000882830.1. Raw sequencing data was deposited to cBioPortal (https://www.cbioportal.org/) and the accession number is pending.

Ethics Statement

IRB approval for case reports is not required at Columbia University Medical Center. The family was consented for whole-exome and transcriptome sequencing per Columbia University IRB standard operating procedures.

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Author Contributions

J.L.G.B., W.K.C., C.K., J.H.K., P.S., and C.L.G. provided medical care to the patients. S.J.H., A.S., and M.M.M. analyzed the data and performed genomic analyses. D.M.P. and J.A.O. wrote the paper. All authors commented on the manuscript.

Competing Interest Statement

The authors have declared no competing interest.

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