NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

Genetic Testing: It's a Relative Question

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Part I – The Diagnosis and Biological Basis of the Disease

Tom glanced at the words on the page as they began to blur. He noticed that he had been feeling more tired than usual and had recently lost some weight. At 43, he thought his new workout regimen was working and the stress of work was weighing on him. Now the words "result" and "interpretation," seemingly innocuous concepts before today, suddenly became overwhelming. A diagnosis of MEN2A, that was all he could recall. Having left the doctor's office over an hour ago, Tom was glad that Dr. Sawyer had provided him a print-out of the results from his blood test. Tom carefully re-read each word on the report.

Test Ordered: *RET* Gene Sequencing

RET Result: V804M mutation detected

RET Interpretation: Within the *RET* gene, a G to A sequence change was detected at nucleotide position 2410 in exon 14 (c.2410G>A). This mutation is heterozygous in this individual resulting in a RET protein change from valine (V) to methionine (M) at position 804 (V804M). This mutation is often associated with familial medullary thyroid carcinoma (FMTC) and, in rare cases, causative for multiple endocrine neoplasia type 2 (MEN2A). With variable penetrance and expressivity, the phenotype of V804M greatly differs among individuals and even among members of the same family.

RET Comment: Genetic counseling is recommended as molecular results should consider the individual's clinical and family history for interpretation. Specific testing is recommended for appropriate at-risk family members to rule out the possibility of hereditary cancer for this individual and family members.

Questions

1. Tom has multiple endocrine neoplasia type 2 (MEN2A) due to a mutation in the *RET* gene, which can affect the endocrine system. What does it mean to have a heterozygous mutation? What glands comprise the endocrine system? (2 pts)

2. MEN2A exhibits autosomal dominant inheritance. What does this mean and why is it a concern in the clinic? (2 pts)

3. What type of mutation (silent/missense/nonsense) has occurred at nucleotide 2410 of exon 14 (V804M)? What are the chemical and physical differences between valine and methionine? Considering the structure/function relationship, what is one possible biological impact of this mutation with regard to protein folding? (2 pts)

4. Researchers who want to know more about a specific gene or mutation can access a wealth of information from bioinformatic databases. Once such resource, GenBank, is a catalog of information maintained by the National Institute of Health. GenBank consolidates information on genetic sequences and mutations observed within patient populations, also known as clinical variants. When a portion of a patient population with a disease shares a mutation at a specific base pair of a gene it is known as a single nucleotide polymorphism (SNP).
   - Visit GenBank at <https://www.ncbi.nlm.nih.gov/genbank>.
   - From the drop-down menu at the top of the page select "All Databases."
   - In the search box, type in "RET" and click "Search."
   - The results include a variety of current publications, gene datasets, proteins, and genome assemblies. Focus on the "Genetics" category and click on "ClinVar."
     ClinVar is a database that aggregates information about genomic variation and its relationship to human health.
   - Briefly scroll through the search results to observe the variety of records that mention "RET."
   - To learn more about Tom's mutations, at the top of the page search box, type in "RET[gene] V804M" and click search.

   a. There are multiple clinical variants listed for V804M. Notice that various conditions such as MEN2 are listed. The column labeled "Clinical Significance" lists mutations as pathogenic, likely pathogenic, or uncertain significance. What do you think is meant by this? (1 pt)

   - Return to the search results: <https://www.ncbi.nlm.nih.gov/search/all/?term=RET>.
   - Under the "Genetics" heading, click on "dbSNP."
     The dbSNP database contains human single nucleotide polymorphisms for common variations and clinical mutations.

   b. How many single nucleotide polymorphisms (SNPs) are annotated for the *RET* gene? (1 pt)

   - Return to the search results: <https://www.ncbi.nlm.nih.gov/search/all/?term=RET>.
   - Under the "Genetics" heading, click on "MedGen." (MedGen organizes information related to human medical genetics, such as attributes of conditions with a genetic contribution.)
   - Locate the heading "Multiple endocrine neoplasia, type 2a."
   - Click on the hyperlink for MEN2A (MedGen UID: 9958).
   - This will bring you to a summary of MEN2A, extracted from *GeneReviews*.
     GeneReviews is "an international point-of-care resource for busy clinicians, provides clinically relevant and medically actionable information for inherited conditions in a standardized journal-style format, covering

diagnosis, management, and genetic counseling for patients and their families. Each chapter in GeneReviews is written by one or more experts on the specific condition or disease and goes through a rigorous editing and peer review process before being published online."

- The "MedGen" section includes a wealth of information regarding the disease characteristics, differential diagnosis, clinical management, genetic counseling, and molecular genetics, among others.

c. Read about the disease characteristics of MEN2. MEN2 has three subtypes, all involved with a high risk for development of what cancer type? What additional phenotypes are observed with patients with MEN2A? (2 pts)

d. When does cancer typically occur in patients with MEN2A? (1 pt)

e. What is the gene location, chromosomal loci, for the *RET* gene associated with MEN2A? (1 pt)

- Return to the search results: <https://www.ncbi.nlm.nih.gov/search/all/?term=RET>.
- Under the "Genetics" heading, select "OMIM" (OMIM, *n.d.*).
  OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily.
- With the OMIM drop-down selected, within the search bar clear "RET," type "MEN2A AND V804M" and hit enter.
- Click on the highlighted link to OMIM: 164761.

5. The Online Mendelian Inheritance in Man (OMIM) contains information on gene summaries, genetic tests, and medical literature. In the cancer field, select genes can be classified as proto-oncogenes, oncogenes, or tumor-suppressor genes.

   a. For OMIM: 164761, the relevant entry for Tom's mutation, what role (classification of gene) does *RET* normally have? (1 pt)

   b. The "Gene-Phenotype Relationships" table lists the cytogenetic location, phenotypes, and inheritance patterns for selected genes. Notice the code that is listed under the "inheritance" column. What does AD represent and what does this mean in the context of heritability? (1 pt)

   c. Scroll to the "TEXT Description" heading. The *RET* gene is described as encoding a transmembrane receptor and member of the tyrosine protein kinase family of proteins. Explain how tyrosine kinases activate signaling proteins. (2 pts)

d. Understanding the role of *RET* in cell signaling, explain how the V804M mutation may contribute to Tom developing cancer? (2 pts)

f. The OMIM also contains brief summaries of various experiments which have enhanced our understanding of the biological activity of genes and their variants. Recalling that Tom's mutation is V804M, we are interested in the clinical variation among patients with this mutation. From the OMIM 164761 page, perform a search ("Ctrl + F") for "V804M." Based on the Lesueur (2005) study, what explanation is offered for the "wide clinical variability associated with germline mutations at codon 804"? (2 pts)

g. How does this support or refute the explanation that multiple mutations or genetic "hits" are necessary to develop cancer? (1 pt)

6. Physicians and scientists often need a quick reference format to access information on the diagnosis and treatment of genetic disorders. GeneReviews provides this resource in the form of book chapters on various conditions. Access GeneReviews (Adam, Ardinger, Pagon, & Wallace, 2018) (https://www.ncbi.nlm.nih.gov/books/NBK1116/) and search for MEN2. Click the link for MEN type 2. Based on a diagnosis of MEN2, when does medullary carcinoma of the thyroid (MTC) and familial medullary carcinoma of the thyroid (FMTC) typically manifest in patients? What clinical management or treatment is suggested for MTC? (2 pts)

Part II – Genetic Counseling

A few weeks later Tom was recovering from his thyroidectomy. During his follow-up he had also scheduled an appointment with a genetic counselor, Ms. Phillips.

"Tom, I know this has been a challenging time for you and your family. I wanted to talk with you about the genetics of your disease. MEN2 exhibits an autosomal dominant inheritance pattern so your family is something that we need to discuss," Ms. Phillips began.

"None of my siblings, my 38-year-old brother nor my 42-year-old sister have any history of thyroid cancer," Tom relayed. "My parents are relatively healthy and still living. My wife, Sue, is an only child. She has a family history of diabetes and heart disease. Her father died at 51 from a heart attack and her mother had a stroke at 63, but is still living. Sue and I are expecting our third child in a few months. We have two children, a four-year-old daughter and a six-year-old son. They are physically active and enjoying the soccer season."

As Ms. Phillips took notes she added, "The American Society of Clinical Oncology (ASCO) identifies MEN2 as a group 1 disorder. This means that molecular genetic testing of *RET* is suggested for first-degree relatives (parent, sibling, children of affected individuals) and children by the age of five years."

She went on to add, "As for your wife and unborn child, we should discuss that too."

Questions

1. Recalling that MEN2 exhibits autosomal dominant inheritance, draw a pedigree for Tom's family (including all grandparents, Tom's generations, and Tom's children). (4 pts)

2. Since none of Tom's relatives have a documented history of MEN2A, according to *GeneReviews* what is the probability that the mutation is *de novo* as opposed to inherited? (*Hint:* go to <https://www.ncbi.nlm.nih.gov/books/NBK1257/>, Eng (1999), and search the page for "*de novo*" variants, located under the genetic counseling subheading.) (1 pt)

3. Assuming that Tom's gametes harbor the mutation, what is the likelihood that one of Tom's children have inherited the pathogenic variant? (1 pt)

4. Prenatal testing can be a polarizing issue. More information on the various types of genetic testing is available from the Genetics Home Reference <https://ghr.nlm.nih.gov/primer/testing/uses>. Under what circumstances is testing during Sue's pregnancy warranted? What are additional considerations when testing is conducted during pregnancy? (3 pts)

5. What unique considerations are involved with genetic testing of children for disorders? Considering the ages of Tom's children, should he have them tested for the *RET* mutation? If so, should they be told of the rationale and results? (3 pts)

6. Molecular testing can involve select exon testing, single-gene testing, and multigene panels. If Tom decides to test his children, which approach is recommended? Why? (1 pt)

7. The American Thyroid Association (Alexander *et al.*, 2017, <https://doi.org/10.1089/thy.2016.0457>) recommends that families with V804M, in the absence of additional *RET* mutations, "may delay" prophylactic surgery past the age of five if they (children) have normal annual serum calcitonin and neck ultrasounds or have a "family history of less aggressive MTC" (Wells *et al.*, 2015, <https://doi.org/10.1089/thy.2014.0335>). The expression of MTC in patients with mutations at codon 804 ranges from early childhood fatality, middle-age onset of symptoms, to no detectable disease in the elderly. With the variety of phenotypes, if you were in Tom's position, what actions would you take with regards to testing of your children, your unborn child, and/or surgical interventions? (4 pts)