Foundations of Medicine

Practice Case Study for Foundations Session 164

Case Studies

Making connections between Genetics, Molecular Biology, & Biochemistry

With recent advances in the integration of various disciplines of molecular science and technological developments in genetic analysis, it is now possible to implement truly "personalized" medicine. The growing adoption of "Precision Medicine" involves the full understanding of a patient, including their own specific molecular pathology and disease etiology, which can help to establish an accurate diagnosis and to select an effective therapy.

NCBI has long had online resources for biologists to explore what is known about a biological molecule including its structure and function, but has recently developed clinically-focused resources enabling scientists and clinicians to integrate known molecular biological information with clinically-relevant genetic variations.

In Wednesday's Session:

- We discussed the state of clinical practice with regard to the application of Precision Medicine principles (examining a patient's specific molecular pathology).
- Together we explored a real-world case study and followed a workflow to discover the patients' molecular pathology for an undiagnosed/misdiagnosed problem.

Before Friday's Session:

- **This is a practice case study that you can solve!**
- **Your group also has it's own case study to solve by Friday!**

In Friday's Session:

- We will discuss each group's case and discover the underlying cause of pathology in these real patients.
- We'll compare what is happening at the molecular level in other patients that have seemingly related cases.

Jeff

Here's the patient's referral and the genetic test results for the molecular pathology work up.

Needs to be done and ready for presentation by Friday!

Thanks

MyClinicalService

Physician Referral Form

Patient Information	
Patient Name **JEFF**	Patient Barcode Sticker 
DOB, Medical Record Number (MRN)	

Requesting Provider	
Assigned Provider/Practice Name: Jane Ferreiro, MD / MyClinicalService	Specialty/Department: Internal Medicine
Address: 900 23rd St NW Washington, DC 20037	Phone: (202) 555-1212 Facsimile #: (202) 555-1212

Consultant Provider	
Provider's Name: to be assigned	Specialty/Department: Molecular Science/M1 Training
Address: 2300 I St NW, Suite 201 Washington, DC 20052	Phone: (202) 555-1212 Facsimile #: (202) 555-1212

Referral Information	
Authorization No:	Authorization Type:

Reason for Referral: **Evaluation of Hemochromatosis**

Diagnosis: **E83.119 – Hemochromatosis, unspecified, not elsewhere classified**

Clinical Notes: A 46 year old, caucasian male presented to the clinic demonstrating symptomology consistent with early stages of liver failure. Lab results indicated elevated liver enzymes with evidence of ketoacidosis, excess abdominal ascites, and splenomegaly. A preliminary diagnosis was made of cirrhosis with secondary complications due to diabetes caused by chronic alcohol consumption. Despite adherence to metformin therapy, dietary interventions, and abstinence of alcohol consumption, symptoms progressed.
The patient has requested a thorough re-evaluation of his case and insists on a genetic test for hemochromatosis. Initial review of the original lab results indicated a potentially missed set of important results - extremely elevated levels of Serum Iron (2300ug/dL) and Transferrin saturation (72%). Thus, the original diagnosis of alcohol-induced liver damage has been called into question.
As requested, a blood sample has been sent out for Hereditary Hemochromatosis - targeted variant analysis (genetic testing). The genetic test result report will be faxed to the Molecular Science/M1 Training program for evaluation.
Please consult with the patient and send a copy of the final report back to this office. Thanks

Procedures: Variant Interpretation – Molecular Impact Characterization

Visits Allowed: 3

Unit Type: V (VISIT)

Referral is Valid Until: 09/30/2018

Notes: Patient must arrive 30 minutes early, with a picture ID, Insurance card and have a copy of this referral. Please bring a list of medications the patient is taking with you to this appointment (including over the counter).

Please send the final report by Fax to: (202) 555-1212

Signature:

Ferreiro, Jane, MD on 08/29/2018 at 5:10 PM EDT

ClinGenLab
Clinical Testing Laboratory, Inc.

Clinical Testing Lab of Washington
2150 Pennsylvania Avenue NW
Washington, DC 20037
Phone: 202-555-1212

Specimen Number	Specimen Type	Control Number	Account Number	Account Phone Number	Route
	Peripheral Blood				

Patient Last Name: **Jeff**			Patient Barcode
Patient First Name	Patient Middle Name		
Patient SS#	Patient Phone	Total Volume	
Age (Y/M/D): **46 y.o.**	Date of Birth	Sex: **Male**	Fasting
Patient Address			Additional Information **Indication: Suspected Hemochromatosis** **Family History: No known family history** **Ethnicity: Western European Caucasian**

Date and Time Collected	Date Entered	Date and Time Reported	Physician Name	NPI	Physician ID
			Jane Ferreiro, MD		

Tests Ordered
Hereditary Hemochromatosis Panel

General Comments
Please send a copy of the final report to the Molecular Science/M1 Training office via Fax at (202) 555-1212

Clinical test results for Hereditary hemochromatosis

6 conditions tested:

- Hereditary hemochromatosis (type 1)
- Hemochromatosis type 2A
- Hemochromatosis type 2B
- Hemochromatosis type 3
- Hemochromatosis type 4
- Juvenile hemochromatosis

GENE	TEST RESULTS	EXPLANATION
HAMP	Negative	No known pathogenic variant detected
HFE	Pathogenic p.Cys282Tyr p.Cys282Tyr	**This result confirms the diagnosis of or predisposition for Hereditary hemochromatosis** (type 1). This result should be interpreted in the context of clinical presentation and results of other laboratory tests (e.g., serum transferrin-iron saturation and serum ferritin). A PCR/sequencing study has detected two copies of the Cys282Tyr (HFE g.10633G>A, c.845G>A, p.Cys282Tyr) variation. The Cys282Tyr mutation is a G to A change at nucleotide position 10633 in the HFE gene, 845 in the primary HFE transcript and results in a change from cysteine to tyrosine at amino acid position 282. In addition, this individual's result has important implications for other family members. Clinical and laboratory evaluations should be considered for at risk individuals. Genetic counseling is recommended for at risk individuals.
HFE2	Negative	No known pathogenic variant detected
SLC40A1	Negative	No known pathogenic variant detected
TFR2	Negative	No known pathogenic variant detected

DISCLAIMER:
Test results should be interpreted in context of clinical findings, family history, and other laboratory data. Misinterpretation of results may occur if the information provided is inaccurate or incomplete. Rare polymorphisms exist that could lead to false negative or positive results. If results obtained do not match the clinical findings, additional testing should be considered.

Researching the Referral

For more information on Jeff (*who is a real person!*), you are welcome to:
» read about his case in a Washington Post Medical Mystery article (https://bit.ly/3238vQP)
» watch a KATU News story video with him in it (https://bit.ly/2Q2F5Np)

1. To learn more about the preliminary diagnosis, **go to the NCBI website** (**https://www.ncbi.nlm.nih.gov** or "google" NCBI to find the homepage) and **search NCBI's MedGen database with:**

   `"Hereditary Hemochromatosis"[ExactTitle]`

   In the "Term Hierarchy" section you can see more specific sub-types of Hereditary Hemochromatosis. Five major forms are displayed. Click the names of the diseases to open the MedGen records to read about each sub-type. Identify the gene or genes associated with each subtype.

   WHAT IS/ARE THE MAJOR DIFFERENCES IN THE SUB-TYPES OF HEREDITARY HEMOCHROMATOSIS?

Understanding the Genetic Test Results

2. **Look at the provided Genetic Test Results** and determine:

   WHAT IS THE SPECIFIC GENE AND VARIATION IDENTIFIED IN JEFF?
   (Read the test results, sometimes it is really helpful!)

   WHAT DOES THE GENETIC TEST RESULT ASSERT FOR JEFF'S DIAGNOSIS?

3. To validate what is asserted by this clinical testing laboratory, **search NCBI's ClinVar database with:**

   `HFE Cys282Tyr`

   WHAT DOES CLINVAR SAY IN THE "INTERPRETATION" FIELD ABOUT THIS GENE AND VARIATION?

   Scroll down and look in the "Submitted interpretations and evidence" section,
   WHAT DO YOU CONCLUDE ABOUT THE IMPACT OF THIS GENE AND VARIATION ON THE DEVELOPMENT OF THE DIAGNOSED DISEASE?

INFORMATION ABOUT THIS GENE FROM HUMAN-CURATED SOURCES:

4. On the MedGen record, **click the link for the gene** identified as having a variant in Jeff.
   WHAT DOES THIS GENE NORMALLY DO?

5. From the Gene record, **scroll down to the "General gene information">"Gene Ontology" section** to learn more about the protein produced from this gene. This section displays terms for where this gene product is likely to be found within a cell (Component), what processes it is often involved in (Process), and what it does (Function).

   WHAT TYPE(S) OF PROCESS(ES) IS/ARE THIS PROTEIN NORMALLY INVOLVED WITH?
   DOES THIS MAKE SENSE BASED ON THE SUMMARY OF THE GENE THAT YOU JUST FOUND?

   WHAT SPECIFIC FUNCTION(S) DOES THIS PROTEIN HAVE?
   DOES THIS MAKE SENSE BASED ON THE SUMMARY OF THE GENE THAT YOU JUST FOUND?

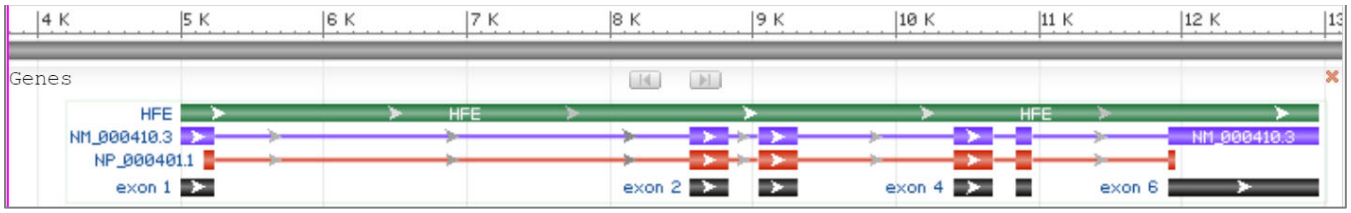
   IN WHICH COMPONENT(S) (SUB-CELLULAR LOCATION) IS THIS PROTEIN NORMALLY FOUND?

6. **Scroll down to the "Expression" section** to see in which tissues this gene is expressed.
   IN WHICH TISSUES HAS THIS GENE BEEN FOUND TO BE EXPRESSED?

   DO ANY OF THESE TISSUES CORRELATE WITH WHAT MAY BE MALFUNCTIONING IN JEFF?

INFORMATION ABOUT THIS GENE DETERMINED FROM SEQUENCE-BASED SOURCES:

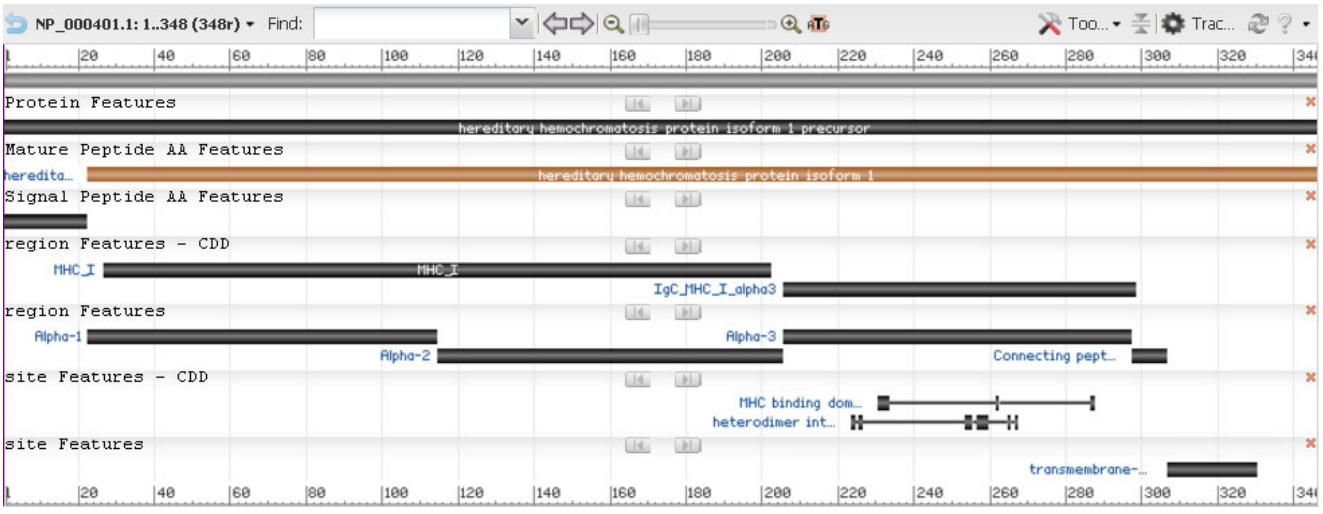
7. On the right-hand side of the Gene record, **click the "RefSeqGene" link** to see the "Graphic" view of the gene structure defined on the chromosome on a RefSeqGene nucleotide page.



WHERE IS JEFF'S GENETIC VARIANT LOCATED IN THIS GENE AND IN THE MRNA?
(On the picture above or on your screen – draw or visualize a vertical line at the variant's position.
You can type in the variant's gene position, from the genetic test result, into the "Find" box to automatically zoom in!)

BASED ON THE POSITION OF THE VARIANT IN THE GENE, WHAT IS THE MOST LIKELY MECHANISM FOR IMPACTING THE FINAL GENE PRODUCT? *(alter gene expression, influence transcript processing, or change encoded protein sequence)*

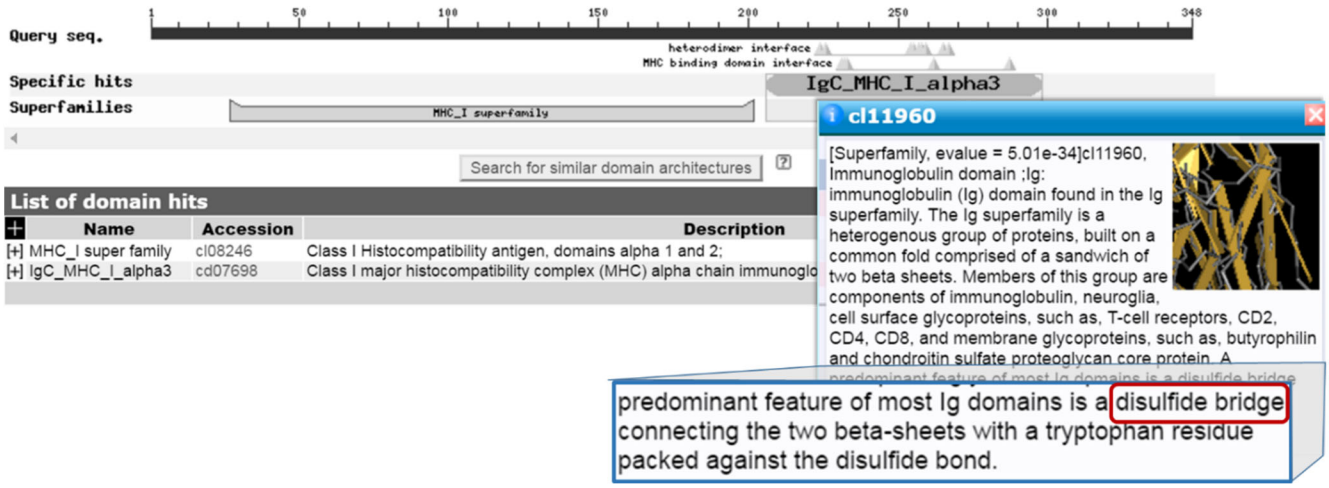
8. On the right-hand side of the RefSeqGene page, **can click the "Protein" link** or go back to the Gene record and **click the "RefSeq Proteins" link. Click "Graphics"** to see a graphical view of the annotated regions curated on the protein sequence. The information shown in in these "tracks" of this view can help you to learn more about this protein.



BASED ON THE TYPE (AMINO ACID CHANGE) AND/OR POSITION OF THE VARIANT IN THE PROTEIN, CAN YOU PREDICT WHAT MIGHT BE THE MECHANISM FOR IMPACTING THE FINAL GENE PRODUCT?

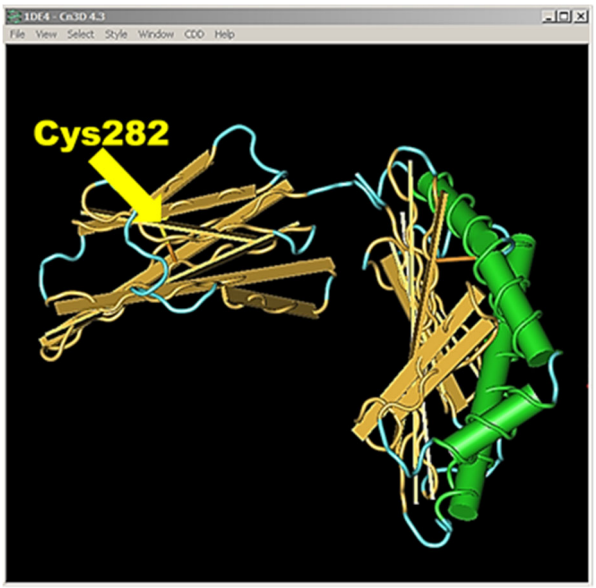
9. On the right-hand side of the page, **click "Identify Conserved Domains"** to get a better picture of the main functional domains for this protein. Then **click the domain bar** in the graphic which contains the genetic variant to learn more about this particular domain.

IN WHICH DOMAIN IS JEFF'S GENETIC VARIANT LOCATED?



BASED ON THE DESCRIPTION OF THIS DOMAIN, WHAT DO YOU PREDICT MAY BE THE IMPACT OF THE GENETIC VARIATION ON THE PROTEIN?

10. From either the Gene or Protein record, you can **click a link to 3D Structure** to visualize experimentally-determined molecular structures for this protein. In the 3D structure you can see precisely the locations of the amino acids affected by the genetic variations.



HINT: *De Almeida, SF, and M De Sousa. "The Unfolded Protein Response Haemochromatosis." Journal of Cellular and Molecular Medicine. PMC. Web. 7 Sept. 2016.* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3822533/

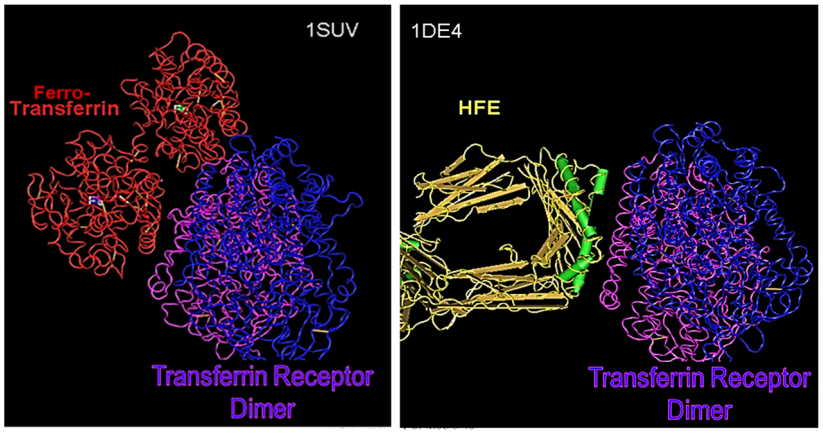
To make things easier for you right now…..**here's a picture of the 3D crystal structure** for the HFE Protein (PDB accession: 1DE4) in NCBI's Cn3D Viewer.

The Cys282 position is shown in a **yellow** and participates in a disulfide bond (with another cysteine side chain, **orange stick**) holding together two beta sheets in an Ig Fold.

WHAT WOULD THE GENETIC VARIATION CYS282TYR DO TO THIS STRUCTURE?

WHAT MIGHT BE THE IMPACT ON THE ABILITY OF THIS PROTEIN TO FUNCTION?

11. Now, to understand the role of the HFE protein in biology, let's look at two other 3D Structures that are related to this protein's function and purpose (PDB accessions 1SUV – Transferrin Receptor complexed with Ferro-Transferrin & 1DE4 – Transferrin Receptor complexed with the HFE protein).



In both structures, the Transferrin Receptor is shown in **purple** & **blue**. On the left in **red**, you see how it complexes with Transferrin (ferro-transferrin means it is carrying its Iron ion payload). On the right in **yellow**, **orange** & **green** (secondary structures – which are also shown in the picture of #9), you see how the Transferrin Receptor complexes with a homodimer of the HFE protein.

From an earlier question (**HINT:** see #4), back to the summary of what the HFE protein normally does….

WHAT IS HFE'S ROLE WITH REGARD TO THE TRANSFERRIN RECEPTOR?

BASED ON THE SUMMARY OF HFE'S FUNCTION/PURPOSE (ABOVE), HOW DO YOU THINK HFE "REGULATES THE INTERACTION OF THE TRANSFERRIN RECEPTOR WITH TRANSFERRIN"?

WHAT WOULD HAPPEN TO THE HFE PROTEIN'S STRUCTURE, FUNCTION AND ROLE IN THIS INTERACTION IF IT CONTAINED THE CYS282TYR VARIANT? (**HINT:** see #9)

12. **NOW, LET'S TIE ALL OF THIS TOGETHER!**

WHAT MIGHT DYSREGULATION OF IRON (FE ION) TRANSPORT AND STORAGE DO TO A CELL?

From an earlier question (**HINT:** see #5), **IN WHICH GENERAL CELL-TYPES WAS THIS GENE EXPRESSED?**

IF THESE CELLS ARE NOT FUNCTIONING AT FULL CAPACITY, COULD THIS HELP TO EXPLAIN THE MOLECULAR PATHOLOGY CAUSING JEFF'S DISEASE?

SUMMARY QUESTIONS – These are the types of questions I'd ask….
as might your Clinical Geneticist during rounds.

Who is Jeff? What is his story?  *(see the referral form)*
What was the preliminary diagnosis and the rationale for it? *(see the referral form & NCBI's MedGen database)*
What did the genetic test find and how does this relate to the preliminary diagnosis? *(see the genetic test result form & NCBI's ClinVar database)*
What is the implicated/affected gene and what is its normal function? *(NCBI's Gene database should help!)*
Where in the gene and gene product is the patient's genetic variant located? *(Where in the gene? In what part of the mRNA? Where in the protein? In what functional part of the protein?)*
What is the molecular impact of the genetic variant on the gene product? *(What do you think the variant ended up doing to the protein structurally?)*
What *do you think might be* the functional impact of the variant on the gene product and in the patient? *(What impact do you think the variant had on the function of the protein? How might this relate to the patient's symptoms?)*