HOW YOU NEED TO PREPARE!

- Work with your group on your assigned case study.
- Figure out what is going on with your patient at the molecular level.
- Be prepared to share your patient and his/her molecular pathology with the class!

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 Thursday’s Session:

- We will discuss the state of clinical practice with regard to the application of precision medicine principles.
- Together we will explore a real-world case study and follow a workflow to discover the patients’ molecular pathology for an undiagnosed/misdiagnosed problem.
- You will then be given a practice case study to solve, and we will go over the case and how to present your findings in preparation for…..

Before Friday’s Session:

- Your group will be assigned your own case study to explore and discover what is happening in your patient on the molecular level. You will be guided to prepare a summary that you can share with the class.

In Friday’s Session:

- Each group’s case will be presented to the class so that you can see additional examples of molecular pathology in real patients - and see diversity of molecular pathology even in patients with the same disease or who have pathogenic genetic variants in the same gene.

Facilitator:  
Rana Morris, PhD - an NCBI Customer Experience team member and Team Lead for Educational Programs (Courses/Workshops, Webinars, Educational Materials). Since 2002, she has provided user support and training, as well as working with supervisors and development teams to improve NCBI resources based on user-centered design principles. Her doctoral, post-doctoral and research fellowship work integrated disciplines of computational and experimental biochemistry, molecular and cellular biology and genetics, and has included diagnostic development, drug design and coordination of genetics/genomics components of clinical trials.
Foundations of Medicine: FDN164 & FDN167

Case Session:
Case-based Genetics, Molecular Biology and Biochemistry
Integrated Review

August 30, 10am-12pm and August 31, 1:30-3:30pm
(both sessions meet in Ross 101)
**Session Objectives**

**FDN164** (Part 1…..Thursday)

1. Recognize molecular biology and genetic factors that are relevant to underlying disease processes
2. Identify and apply on-line information databases toward explanation of molecular biology and genetics of disease processes
3. Discuss the dysfunctional molecular biology and genetics associated with a diagnosed disease
4. Apply principles of basic sciences to clinical scenarios

**FDN167** (Part 2…..Friday)

1. Recognize significant details in patient’s case relevant to underlying disease processes
2. Identify any additional information required and where/how to get it
3. Present details pertinent to their specific case and discover / discuss important factors in related cases down to the molecular level
4. Apply principles of basic sciences to studying clinical scenarios

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**This is what happened yesterday and what’s going to happen today**

**In Yesterday’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 learned a workflow to discover the patients’ molecular pathology for an undiagnosed/misdiagnosed problem.
- You were given a practice case study to solve, and we went over the case and how to present your findings in preparation for.....

**Before Today’s Session:**
- Your Group was assigned your own case study to explore and discover what is happening in your patient on the molecular level. You were guided to prepare a summary that you will share with the class.

**In Today’s Session:**
- We’ll go over each case - so that you can see additional examples of molecular pathology in real patients - and see diversity of molecular pathology even in patients with the same disease or who have pathogenic genetic variants in the same gene.
Today…

• You get to present what you found out about your cases to the rest of the class! We’ll go over them (I’ll help when needed).
  
  As I mentioned yesterday, I will ask for volunteers first….  
  but if no one volunteers – I’ll start picking on Groups.

• You get to see different diseases and how they are impacted by events on the molecular level (molecular pathology)….

• You may also get to notice a few patterns.

“**This is a lot of stuff and the exam is on Tuesday!”**

• Enjoy the case stories and what Groups were able to discover.

• Don’t worry about the details, as you will not be tested on the details of each case or disease (by me).

• Look at the big pictures…..I’ll help you focus on these.

**YES, WE’LL POST THE “ANSWERS”!**

**Cases – Real People**
NCBI’s Information Hubs & Some Databases

We’ll start with these people.....

Alexei  Bo  James & Raven
This is the true story of Alexei Nikolaevich, son of Tsar Nicholas II and Tsarina Alexandra of Russia. He suffered serious bleeding issues (hematomas & bruising, prolonged bleeding and blood loss, and long painful recoveries). It is now known that hemophilia was inherited from a de novo mutation in Queen Victoria of England. The exact genetic variant was identified in skeletal remains of Alexei found in an unmarked grave in 2008.

**What was the preliminary diagnosis & the rationale?**

Hemophilia – due to a personal and family history of severe bleeding episodes.

**What did the genetic test find and how does this relate to the preliminary diagnosis?**

Test Result = F9: g.15338A>G, c.278-3A>G

He has one copy of the F9 gene with a variant creating a new mRNA splice site -2pb down from the “normal” one, and supports the diagnosis of Hemophilia B.

**What is the implicated/affected gene and what is its normal function?**

F9: This gene encodes vitamin K-dependent coagulation factor IX that circulates in the blood as an inactive zymogen. This factor is converted to an active form by factor Xla, which excises the activation peptide and thus generates a heavy chain and a light chain held together by one or more disulfide bonds. The role of this activated factor IX in the blood coagulation cascade is to activate factor X to its active form through interactions with Ca+2 ions, membrane phospholipids, and factor VIII. Alterations of this gene, including point mutations, insertions and deletions, cause factor IX deficiency, which is a recessive X-linked disorder, also called hemophilia B or Christmas disease.

**Where in the gene and gene product(s) is the patient’s genetic variant located?**

The variant is in the gene and mRNA - 2 bases before the splice junction for exon 2.

**What is the molecular impact of the genetic variant on the gene product?**

This variant creates a new splice site for exon 4, changing the coding sequence by a -2 base frameshift. The new amino acid sequence formed encodes 11 residues and ends in a termination codon, causing a severely truncated (short) protein.

**What do you think might be the functional impact of the variant on the gene product?**

The new truncated protein is missing most of it’s sequence/structure which includes most of it’s important functional domains, including the key Tryp_SPc protease function. So, the new protein can’t do it’s job in the blood clotting cascade to regulate clotting – and the patient continues to bleed.

**F9**

g.15338A>G

c.278-3A>G

**THIS CASE ILLUSTRATES how:**

1) X-linked disorders can severely impact boys
2) genetic variations in or around a splice-site can disrupt mRNA processing
3) splice-site alterations can impact encoded proteins
4) newly encoded stop codons can prevent the production of full-length & fully-functional proteins
5) the lack of a single functional important regulatory enzyme can disrupt an important system and cause severe health problems.
Introduce your patient!
An 8 month old boy bumped into a coffee table causing a profuse nosebleed which was the reason for an ER visit. Additional concerns about visible bruising on his knees and palms since he began crawling at 6 months were expressed by his mother. A family history of bleeding issues was suggested upon further questioning. An ER Physician-ordered lab test indicated a deficiency in Factor IX, so the patient was referred for further examination.

What was the preliminary diagnosis & the rationale?
Hemophilia – due to a personal and family history of severe bleeding episodes. Hemophilia B is suspected due to the result of a blood test (no Factor 9 activity).

What did the genetic test find and how does this relate to the preliminary diagnosis?
Test Result = F9: c.223C>T, p.Arg75Ter
He has one copy of the F9 gene with a variant that creates a premature stop codon in the coding sequence, and supports the diagnosis of Hemophilia B.

<table>
<thead>
<tr>
<th>What is the implicated/affected gene and what is its normal function?</th>
<th>F9: This gene encodes vitamin K-dependent coagulation factor IX that circulates in the blood as an inactive zymogen. This factor is converted to an active form by factor Xa, which excises the activation peptide and thus generates a heavy chain and a light chain held together by one or more disulfide bonds. The role of this activated factor IX in the blood coagulation cascade is to activate factor X to its active form through interactions with Ca+2 ions, membrane phospholipids, and factor VIII. Alterations of this gene, including point mutations, insertions and deletions, cause factor IX deficiency, which is a recessive X-linked disorder, also called hemophilia B or Christmas disease.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Where in the gene and gene product(s) is the patient’s genetic variant located?</td>
<td>The variant is in the gene, mRNA (exon 2), and very early in the sequence of the protein.</td>
</tr>
<tr>
<td>What is the molecular impact of the genetic variant on the gene product?</td>
<td>This variant creates a new stop codon, causing a severely truncated (short) protein.</td>
</tr>
<tr>
<td>What do you think might be the functional impact of the variant on the gene product?</td>
<td>The new truncated protein is missing most of its sequence/structure which includes most of its important functional domains, including the key Tryp_SPc protease function. So, the new protein can’t do its job in the blood clotting cascade to regulate clotting – and the patient continues to bleed.</td>
</tr>
</tbody>
</table>

**James - F9: g.11409C>T, c.223C>T, p.Arg75Ter**

**THIS CASE ILLUSTRATES how:**
1) X-linked disorders can severely impact boys
2) the genetic variant location can have different impact on the gene product and its function
3) due to the presence of a genetic variant, a change in the charge of an amino acid can impact its ability to bind a critical ion cofactor – may not affect, decrease, or “kill” the protein’s activity
4) not all genetic variants impact protein function and a person’s symptoms at the same level – “penetrance”
Introduce your patient!

25 year old female was referred for consultation after her son was diagnosed with Hemophilia B. A family history of bleeding issues was suggested (6 year old brother who died of a “brain bleed” who she suspects might have “had Hemophilia too”). Upon questioning, she mentioned that she required a blood transfusion after “normal childbirth” and has “always had really, really heavy periods”. She requested evaluation of potential carrier status for family planning purposes.

What was the preliminary diagnosis & the rationale?

Carrier of Hemophilia – due to a family history of severe bleeding episodes in males. Hemophilia B carrier is suspected due to the results of her son’s blood test (no Factor 9 activity).

What did the genetic test find and how does this relate to the preliminary diagnosis?

Test Result = F9: Arg75 (normal) and c.223C>T, p.Arg75Ter
She has one normal copy of the F9 gene and one copy with a variant creating a premature stop codon in the coding sequence, and supports the status of Hemophilia B carrier.

What is the implicated/affected gene and what is its normal function?

F9: This gene encodes vitamin K-dependent coagulation factor IX that circulates in the blood as an inactive zymogen. This factor is converted to an active form by factor Xla, which excises the activation peptide and thus generates a heavy chain and a light chain held together by one or more disulfide bonds. The role of this activated factor IX in the blood coagulation cascade is to activate factor X to its active form through interactions with Ca+2 ions, membrane phospholipids, and factor VIII. Alterations of this gene, including point mutations, insertions and deletions, cause factor IX deficiency, which is a recessive X-linked disorder, also called hemophilia B or Christmas disease.

What is the molecular impact of the genetic variant on the gene product?

One copy of the gene should be normal. While the other copy contains a variant that creates a new stop codon, causing a severely truncated (short) protein with no activity.

What in the gene and gene product(s) is the patient’s genetic variant located?

She has one normal copy of the F9 gene, mRNA and protein. She also has one copy with a variant in the gene, mRNA (exon 2), and very early in the sequence of the protein.

What do you think might be the functional impact of the variant on the gene product?

One normal copy of the F9 gene, thus should be able to mount a clotting response. This normal F9 protein would need to compensate for the variant F9, so the total F9 activity may be lower than it should be. Thus, under stressful situations (childbirth, car crash…etc.) she may bleed longer than expected. She should also be aware that any of her genetic, male children have a 50% chance of acquiring the bad F9 gene copy and thus Hemophilia B.

Raven - F9: g.11409C>T, c.223C>T, p.Arg75

g.11409C>T, c.223C>T, p.Arg75Ter

THIS CASE ILLUSTRATES how:

X-linked genetic disorders can also impact females.
- for family planning purposes: 
  male progeny have 50% chance to inherit a copy of the variant allele
- and also with her own health concerns: 
  “genetic variant carrier” status can also convey the potential for development of symptoms (“traits”), as decreased activity of a functional important regulatory enzyme can still cause health problems under certain situations.
Bo is a 10 year old boy who was adopted from China with no known family history. He has a personal history of episodes of prolonged bleeding which were concerning to the parents, but they hadn’t risen to the level of an ER visit. This time while preparing dinner he cut himself on the middle phalanx of left index finger was brought to the ER and treated with stitches – but continued to bleed. The ER Physician ordered a lab test which showed a moderate deficiency in Factor IX activity, thus was referred for further examination.

What was the preliminary diagnosis & the rationale?

Hemophilia – due to a personal history of bruising and bleeding episodes. Hemophilia B is suspected due to the result of a blood test (low Factor 9 activity).

What did the genetic test find and how does this relate to the preliminary diagnosis?

He has one copy of the F9 gene with a variant that causes a change in an amino acid in the protein, and supports the diagnosis of Hemophilia B.

What is the implicated/affected gene and what is its normal function?

F9: This gene encodes vitamin K-dependent coagulation factor IX that circulates in the blood as an inactive zymogen. This factor is converted to an active form by factor XIa, which excises the activation peptide and thus generates a heavy chain and a light chain held together by one or more disulfide bonds. The role of this activated factor IX in the blood coagulation cascade is to activate factor X to its active form through interactions with Ca2+ ions, membrane phospholipids, and factor VIII. Alterations of this gene, including point mutations, insertions and deletions, cause factor IX deficiency, which is a recessive X-linked disorder, also called hemophilia B or Christmas disease.

Where in the gene and gene product(s) is the patient’s genetic variant located?

The variant is in the gene, mRNA, and in the protein. This variation is located in a calcium ion binding site at the position of specific amino acid that participated in binding the calcium ion.

What is the molecular impact of the genetic variant on the gene product?

This variant changes an important negatively charged amino acid required for binding to a positively charged calcium ion - which is required for the protein’s activity.

What do you think might be the functional impact of the variant on the gene product?

If the protein is not able to strongly hold onto it’s required calcium ion, the activity of the protein will be reduced. So, the new protein won’t be doing an optimal job in the blood clotting cascade to regulate clotting – and the patient will take longer to clot.

Hemo sapiens: GRCh38.p12 (GCF_0000001405.38)  Chr X NC_000023.11: 139,816,672

F9

g.15392A>G

p.Asp110Gly

This case illustrates how:
1) X-linked disorders can severely impact boys
2) the genetic variant location can have different impact on the gene product and it’s function
3) due to the presence of a genetic variant, a change in the charge of an amino acid can impact its ability to bind a critical ion cofactor – may not affect, decrease, or “kill” the protein’s activity
4) not all genetic variants impact protein function and a person’s symptoms at the same level – “penetrance”
What have you noticed about this cluster of cases?

THIS CLUSTER ILLUSTRATES how variations in different portions of the gene can have varying impact the function of the protein & the disease symptoms. Also, it shows examples of the impact of an X-linked gene variant both on males & “carrier” females.
Self-Assessment:
*Take one minute to write down your thoughts*....

My initial ideas about this cluster of cases:
- Why did I think this?
- How confident was I?

What did I miss?
- Why did I miss it?
- How could I have thought about it differently?

Now, let’s learn about…..

David

Ashanti
Introduce your patient! This is the true story of David Vetter ("Boy in the Bubble"), although his specific genetic variant is not known. Due to a previous son who died of SCIDS at 7 months, David was placed in sterile-isolation at birth and diagnosed with SCIDs based on monitoring of CBC w/diff. He lived in a "bubble" at home or in the hospital his whole life, due to the lack of an exact match for a bone marrow transplantation. Eventually, as his condition began to seriously deteriorate, he received a transplant which inadvertently infected him with Epstein Barr Virus and died of Burkitt’s Lymphoma. Now, new gene therapy-based treatment protocols are being developed – so the exact gene/variant cause of the disorder could provide a new potential therapeutic option.

What was the preliminary diagnosis & the rationale? Severe Combined Immunodeficiency Syndrome (SCIDS) with low T- and B-cell numbers – due to a family history and the results of repeated blood tests.

What did the genetic test find and how does this relate to the preliminary diagnosis? Test Result = IL2RG: c.343T>C, p.Cys115Arg. He has one copy of the IL2RG gene with a variant that causes a change in an amino acid in the protein, and supports the diagnosis of X-linked SCIDS.

What is the implicated/affected gene and what is its normal function? IL2RG: The protein encoded by this gene is an important signaling component of many interleukin receptors, helping to coordinate the structure and the function of interleukin-2, -4, -7 and -21 complexes, and it thus referred to as the common gamma chain. Mutations in this gene cause X-linked severe combined immunodeficiency (XSCID), as well as X-linked combined immunodeficiency (XCID), a less severe immunodeficiency disorder.

Where in the gene and gene product(s) is the patient’s genetic variant located? The variant is in the only copy of the gene, mRNA, and in the protein. This variation replaces a cysteine (with an arginine) that is normally involved in structural stabilization by participating in the formation of a disulfide bond.

What is the molecular impact of the genetic variant on the gene product? Loss of the disulfide bond prevents proper folding of the IL2RG protein, which is recognized by the cell’s "unfolded protein response" and targets the protein for degradation.

What do you think might be the functional impact of the variant on the gene product? The degradation of the IL2RG protein prevents proper formation of a number of interleukin complexes, thus drastically disrupts the signaling and coordination of T-cells and B-cells – rendering virtually the entire immune system non-functional. This causes the patient to be dangerously susceptible to infections and eventually death.

THIS CASE ILLUSTRATES how:
1) X-linked disorders can severely impact boys
2) disruption of a disulfide, due to an encoded genetic variant, can prevent proper protein folding
3) mis-folded proteins can be detected and destroyed, killing their activity/function
4) the loss of a protein who coordinates the structure of protein complexes can disrupt the function of complexes that normally include that protein
5) the loss of a single complexing protein can disrupt multiple complexes, impacting an entire and important system – causing severe health problems.
Introduce your patient! This is the true story of Ashanti DeSilva (1st recipient of gene therapy), although her specific genetic variant is not known. Ashanti was diagnosed with SCIDS after 2 years of repeated infections and over 11 blood transfusions. At the time, a new enzyme assay identified an adenosine deaminase deficiency which led to treatment with regular injections of recombinant PEG-ADA. However, at the age of 4 she was selected for a new gene therapy trial with a goal of permanently replacing a “normal” ADA gene – which was pretty successful! Genetic testing to validate the diagnosis of SCID due to Adenosine Deaminase deficiency would now be required for qualifying for the procedure.

What was the preliminary diagnosis & the rationale?
Severe Combined Immunodeficiency Syndrome (SCIDS) due to Adenosine Deaminase Deficiency--due to a history of recurrent infections and the results of blood tests (no ADA activity).

What did the genetic test find and how does this relate to the preliminary diagnosis?
Test Result = ADA: c.646G>A, p.Gly216Arg | c.646 G>A, p.Gly216Arg She has two copies of the ADA gene with a variant that causes a change in an amino acid in the protein, and supports the diagnosis of SCIDS due to ADA deficiency.

What is the implicated/affected gene and what is its normal function?
ADA: This gene encodes an enzyme that catalyzes the hydrolysis of adenosine to inosine. Various mutations have been described for this gene and have been linked to human diseases. Deficiency in this enzyme causes a form of severe combined immunodeficiency disease (SCID), in which there is dysfunction of both B and T lymphocytes with impaired cellular immunity and decreased production of immunoglobulins, whereas elevated levels of this enzyme have been associated with congenital hemolytic anemia.

Where in the gene and gene product(s) is the patient’s genetic variant located?
She has two copies of the ADA gene, mRNA, and protein that contain the variant. This variation is located immediately next to a designated active site residue that is “important for catalysis”.

What is the molecular impact of the genetic variant on the gene product?
The variant is located in an intra-protein loop between two amino acids which coordinate the binding of the ADA’s substrate and participate in catalysis. A change of this residue (which has just an “H” as a side chain) with a large negatively charged amino acid (arginine) drastically disrupts this structure. This prevents the proper folding of the ADA’s active site.

What do you think might be the functional impact of the variant on the gene product?
She has two copies of the variant-containing ADA gene which creates a poorly folded, thus non-functional enzyme. Through a mechanism TO BE DISCUSSED BY DR. ELLIOTT IN YOUR NUCLEOTIDE METABOLISM SESSION, this causes toxic (apoptosis-inducing) levels of an adenosine metabolite to accumulate in white cells of the immune system, rendering the patient dangerously susceptible to infections and eventually death.

This case illustrates how:
1) due to the presence of a genetic variant, a change in the size and/or charge of an amino acid can impact the surrounding 3D structure of the protein
2) structural perturbation of a binding or active site can decrease or “kill” the protein’s activity
3) the loss of function of a single enzyme can disrupt an entire and important system – causing severe health problems
4) knowing the particular affected gene can enable targeting of a therapy to “fix it”
What have you noticed about this cluster of cases?

David

Ashanti

THIS CLUSTER ILLUSTRATES how disorders that seem to have similar symptoms may arise due to variations in different genes. This can have strong implications on molecular pathology and the selection of effective therapies.
Self-Assessment:
*Take one minute to write down your thoughts*....

My initial ideas about this cluster of cases:
- Why did I think this?
- How confident was I?

What did I miss?
- Why did I miss it?
- How could I have thought about it differently?

Next are some really interesting cases.....

Sam  Jill  Priscilla
This is the true, remarkable story of Sam Berns who was diagnosed at 22 months with Progeria due to phenotypic symptoms of stalled growth, hair loss, and other progressive aging issues. He became a vocal and supportive voice for children with genetic conditions. His mother (a physician) began a research career to understand the biology behind her son’s disease and is now a researcher and leading authority on Hutchinson-Guilford Progeria at Boston Children’s Hospital.

<table>
<thead>
<tr>
<th>What was the preliminary diagnosis &amp; the rationale?</th>
<th>Progeria – due to phenotypic features (stalling of growth and weight gain, hair loss, signs of premature aging)</th>
</tr>
</thead>
<tbody>
<tr>
<td>What did the genetic test find and how does this relate to the preliminary diagnosis?</td>
<td>Test Result = LMNA: c.1824C, p.Gly608</td>
</tr>
<tr>
<td>What is the implicated/affected gene and what is its normal function?</td>
<td>LMNA: The lamin family of proteins make up a matrix lining the inner nuclear membrane and are highly conserved in evolution. During mitosis, the lamina matrix is reversibly disassembled as the lamin proteins are phosphorylated. Lamin proteins are thought to be involved in nuclear stability, chromatin structure and gene expression. Mutations in this gene lead to several diseases: Emery-Dreifuss muscular dystrophy, familial partial lipodystrophy, limb girdle muscular dystrophy, dilated cardiomyopathy, Charcot-Marie-Tooth disease, and Hutchinson-Gilford progeria syndrome.</td>
</tr>
<tr>
<td>Where in the gene and gene product(s) is the patient’s genetic variant located?</td>
<td>He has one normal copy of the LMNA gene, mRNA and protein. He also has one copy with a variant in the gene and mRNA – which shifts the splicing donor site of exon 11 to exon 12’s acceptor site, thus the processed mRNA’s encoded protein sequence.</td>
</tr>
<tr>
<td>What is the molecular impact of the genetic variant on the gene product?</td>
<td>One copy of the gene is normal. The other copy contains a C to T change at nucleotide position 1824 in the LMNA gene, however this does not result in an altered encoded amino acid (=). Instead, it has been reported that the nucleotide variant impacts post-transcriptional processing of the mRNA transcript, inducing the use of a novel/cryptic splice donor site within exon 11 at position 1818. This is ligated directly to the reference splice acceptor site of exon 12, resulting in the deletion of encoded amino acid residues 607 to 656. The loss of this protein region has been shown to prevent full post-translational processing (proteolytic cleavage) of the protein.</td>
</tr>
<tr>
<td>What do you think might be the functional impact of the variant on the gene product?</td>
<td>He has one normal copy of the LMNA gene, however the variant copy of the LMNA protein has been reported to have a dominant negative effect – and over time increasingly disrupts the structural integrity of the nuclear membrane, chromatin structure, and normal gene expression pathways –leading to cell death - the hallmark of aging both in Progeria and in the normal aging process.</td>
</tr>
</tbody>
</table>

![LMNA Genomic Structure Diagram](image)

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**THIS CASE ILLUSTRATES how:**

1. the identification of a genetic variant still needs experimental validation to assign a specific molecular mechanism i.e. a variant with no predicted change of an amino acid could still impact be pathological and impact the function of the protein
2. post-translational processing is critical for the function of the protein which can disrupt an important system and cause severe health problems
3. advocacy and participation in medical research can positively impact the community and increase an understanding of the disease (leading to a cure).
Introduce your patient! This is the true story of Jill Viles, an inquisitive biology undergrad who had been diagnosed with a Congenital Muscular Dystrophy as a child and who noticed another symptom of lack of padding (“fat”) accumulation starting in her pre-teen years. She got herself genetically diagnosed by contacting an Italian research team, and subsequently contacted others (father & a Canadian athlete) to warn them of having the potentially dangerous lipodystrophic genetic condition.

What was the preliminary diagnosis & the rationale? Congenital Muscular Dystrophy – due phenotypic assessment. A lipodystrophy is also suspected due to phenotype.

What did the genetic test find and how does this relate to the preliminary diagnosis? Test Result = LMNA: Arg453Trp & Arg482Trp She has two LMNA gene variants: one allele with a “normal” arginine and another with a tryptophan at position 453 & one allele with a “normal” arginine and another with a tryptophan at position 482. (There is no indication of whether the two variants exist together in a single gene copy or if they are distributed amongst the two copies.) This supports the diagnosis of Emery-Dreifuss Muscular Dystrophy and also suggests a diagnosis of Familial Partial Lipodystrophy, type 2.

What is the implicated/affected gene and what is its normal function? LMNA: The lamin family of proteins make up a matrix lining the inner nuclear membrane and are highly conserved in evolution. During mitosis, the lamina matrix is reversibly disassembled as the lamin proteins are phosphorylated. Lamin proteins are thought to be involved in nuclear stability, chromatin structure and gene expression. Mutations in this gene lead to several diseases: Emery-Dreifuss muscular dystrophy, familial partial lipodystrophy, limb girdle muscular dystrophy, dilated cardiomyopathy, Charcot-Marie-Tooth disease, and Hutchinson-Gilford progeria syndrome.

Where in the gene and gene product(s) is the patient’s genetic variant located? Both variants exist in the LMNA gene, mRNA and protein. They are also both located in the LID domain within the C-terminal tail. The 3D structure shows that they are located on opposite externally-facing regions of this domain.

What is the molecular impact of the genetic variant on the gene product? The faces of this LTD Domain (Ig-fold) are reported to be involved in protein-protein interactions. The substitution of a negatively-charged amino-acid (arginine) with a hydrophobic amino acid (tryptophan) in a binding face will alter the physicochemistry of the surface, thus impact the protein’s interaction with its normal binding partners.

What do you think might be the functional impact of the variant on the gene product? This is a challenging one, since the exact mechanism has not yet been identified. The role of LMNA protein as a coordination center for protein-protein interactions and the identification of many transcription factors involved in inducing gene expression of proteins involved in cell-type differentiation – suggests that the two faces of the LID Domain interact with either muscle- or adipose-differentiation regulators, respectively.

Jill - LMNA
g.58841C, c.1357C, p.Arg453
g.58841C>T, c.1357C>T or p.Arg453Trp
g.59412C, c.1444C, p.Arg482
g.59412C>T, c.1444C>T or p.Arg482Trp

THIS CASE ILLUSTRATES how:
1) the persistence of a patient can help in pushing forth a diagnosis and in assisting others with potential health problems
2) different genetic variants in the same gene (even when close together in sequence and type) can have different impacts on the cellular physiology and patient’s phenotypic
3) we still don’t always know exactly how things work in cellular biology and pathology
**Introduce your patient!**

This is the true story of Priscilla Lopes-Schleip, an Olympic Bronze medalist sprinter who was contacted by a girl who warned that she might have a genetic disorder involving “fat wasting” or lack of “fat differentiation”. After years of harassment by competition drug doping panels due to her muscular physique and low body fat, she became interested in exploring alternative explanations. The diagnosis caused her to start early, successful treatment for pancreatitis and adopt a proactive, preventative regimen.

| What was the preliminary diagnosis & the rationale? | Familial Partial Lipodystrophy, type 2 – due phenotypic assessment (and probably a lipid panel laboratory test) |
| What did the genetic test find and how does this relate to the preliminary diagnosis? | Test Result =\text{LMNA: Arg}482\text{Ag482Trp} \\ She has one \text{LMNA} allele with a “normal” arginine and another with a tryptophan at position 482, supporting a diagnosis of Familial Partial Lipodystrophy, type 2. |

**What is the implicated/affected gene and what is its normal function?**

LMNA: The lamin family of proteins make up a matrix lining the inner nuclear membrane and are highly conserved in evolution. During mitosis, the lamina matrix is reversibly disassembled as the lamin proteins are phosphorylated. Lamin proteins are thought to be involved in nuclear stability, chromatin structure and gene expression. Mutations in this gene lead to several diseases: Emery-Dreifuss muscular dystrophy, familial partial lipodystrophy, limb girdle muscular dystrophy, dilated cardiomyopathy, Charcot-Marie-Tooth disease, and Hutchinson-Gilford progeria syndrome.

**Where in the gene and gene product(s) is the patient’s genetic variant located?**

The variant exists in the LMNA gene, mRNA and protein. It is located in the LTD domain within the C-terminal tail. The 3D structure shows that it protrudes from the surface of this domain.

**What is the molecular impact of the genetic variant on the gene product?**

The LTD Domain (Ig-fold) is reported to be involved in protein-protein interactions. The substitution of a negatively-charged amino-acid (arginine) with a hydrophobic amino acid (tryptophan) in a binding face will alter the physicochemistry of the surface, thus impact the protein’s interaction with its normal binding partners.

**What do you think might be the functional impact of the variant on the gene product?**

This is a challenging one, since the exact mechanism has not yet been identified. The role of LMNA protein as a coordination center for protein-protein interactions and the identification of many transcription factors involved in inducing gene expression of proteins involved in cell-type differentiation – suggests that the LTD Domain interacts with adipose-differentiation regulators and when disrupted causes apoptosis and loss of mature adipocytes.

**THIS CASE ILLUSTRATES how:**

1) It is important to follow “your gut” with regard to your own healthcare
2) Getting a diagnosis, may not provide information for a cure (may still be too early in the understanding of the biology), but could guide preventative, proactive measures to improve your current and future health.

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**Priscilla - LMNA**
g.59412C, c.1444C, p.Arg482
g.59412C>T, c.1444C>T or p.Arg482Trp
What have you noticed about this cluster of cases?

THIS CLUSTER ILLUSTRATES how vastly different-seeming disorders may arise due to different variations in the same gene. Due to their differences in location and type, they may have different molecular mechanisms/pathologies.
Self-Assessment:  
*Take one minute to write down your thoughts*....

My initial ideas about this cluster of cases:  
- Why did I think this?  
- How confident was I?

What did I miss?  
- Why did I miss it?  
- How could I have thought about it differently?

Review of *major* concepts in these sessions

- Why should I care about molecular science while preparing to be a physician?  
  *knowledge is more power to act and act more effectively!*

- How could understanding a patient’s specific molecular pathology help you as a doctor?  
  *aiding in diagnosis, implementation of proactive/preventative measures, therapeutic selection, communicating with the patient & patient’s family*

- What in the patient’s case record could start you thinking about ordering a genetic test?  
  *known genetic disorder with or without family history*

- Which databases are good places to start to find helpful disease/condition or biological information?  
  NCBI MedGen & NCBI Gene
NCBI Workshops | References & Resources

THE NATIONAL CENTER FOR BIOTECHNOLOGY INFORMATION, a.k.a. THE “NCBI”

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HelpDesk EMail Address: info@ncbi.nlm.nih.gov

RESOURCES MENTIONED IN TODAY’S PRESENTATION

CLINICAL RESEARCH

MedGen – Aggregated information about medical genetic conditions and phenotypes

NLM’s ClinicalTrials.gov – A registry and results database for clinical studies
Homepage: http://clinicaltrials.gov

Genetic Testing Registry (GTR) – NIH’s registry of genetic tests and laboratories

ClinVar – A collection of assertions about the relationships of genomic variations with human health

U.Washington’s GeneReviews – A resource of clinically relevant and medically actionable information for inherited conditions
Homepage: https://www.ncbi.nlm.nih.gov/books/NBK1116/

NLM’s Genetics Home Reference – Consumer-friendly information about genetic variations and health

JHU’s OMIM – A compendium of human genes and genetic phenotypes

NLM’s Medline Plus – Consumer information about diseases, conditions, and wellness issues
Homepage: http://www.nlm.nih.gov/medlineplus/

MOLECULAR ETIOLOGY RESEARCH

Gene – Aggregated information with links to genomic, expression, homolog, structure and function data

RefSeq – A project to create a comprehensive, integrated, non-redundant, well-annotated reference set of annotated sequences for genomes, chromosomes, transcripts and proteins.
RefSeq Project Homepage: https://www.ncbi.nlm.nih.gov/refseq/
RefSeqGene Homepage: https://www.ncbi.nlm.nih.gov/refseq/rsg/

Conserved Domains Database (CDD) – A database of protein domain information with sequence fingerprints

Structure – A database of 3D macromolecular structures and complexes from the Protein Database (PDB)