An Overview of Limb-Girdle Muscular Dystrophy

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Our Goal.

- Our goal is to give a general overview of Limb-Girdle Muscular Dystrophy.

- This talk is a summary of basic ideas.

- This talk will give a fair amount of information and background but in a simple and understandable way.

- The basic ideas are really pretty straightforward and we can get a pretty good sense of what it's all about in just a few minutes.

- Scientific articles are full of very complex language. As much as possible, this talk is in plain English.
Knowledge Translation.

- This talk is an example of knowledge translation (KT).
- This is an important new tool in medical research.
- KT involves making a two way bridge of understanding between researchers, doctors and patients.
- Complex messages are lost if they are not easily understood. Our goal is to present information in a way that is understandable to all interested parties.
  - Patients can better understand research findings and judge information pertaining to them.
  - Doctors and researchers can better understand patients and their needs.
What is Limb-Girdle Muscular Dystrophy?  

- **Limb-Girdle Muscular Dystrophy** (LGMD) is a group of genetic, progressive disorders primarily affecting voluntary muscles.

- Many different mutations have been linked to LGMD.
  - These mutations affect the proteins necessary for proper muscle function.
  - The exact type of LGMD and its symptoms will depend on the **exact** protein involved.

- LGMD primarily affects the proximal muscles, those around the hips and shoulders — called the pelvic and shoulder girdles, also known as the **limb girdles**.
Common Presentation of Weakness.

Proximal means located toward the center of the body.

Muscles of the pelvis (the pelvic girdle)

Muscles of the shoulders (the scapular girdle)

LGMD Overview.

- **Onset:** Symptoms of LGMD can begin in childhood, adolescence, young adulthood or even later in life.
  - When LGMD begins in childhood, the progression is *usually* faster and the disorder more disabling.
  - When the disorder begins in adolescence or adulthood, it’s generally not as severe and progresses more slowly.
- Males and females are affected equally.
- Over time (usually many years), the person with LGMD loses muscle mass and strength. Eventually, a wheelchair may be needed. Cardiac and respiration problems may also eventually develop in some cases.
**LGMD Forms.**

- LGMD is not one disorder. As we will see, there are many different proteins in muscle. Each form of LGMD affects one of these proteins.
- Each of these proteins is made by a gene, when the gene is mutated the protein produced is defective and the muscle doesn’t work correctly.
- By 2005, there have been 18 forms of LGMD described and 13 corresponding specific gene mutations – protein abnormalities discovered.
- As research continues, more types and more gene mutations will likely be discovered.
LGMD Forms.

- In the past, each form was named as a type of LGMD, for example: LGMD1A, LGMD1B, etc..
- As each type is linked to a specific protein defect, the “new” name usually reflects this.
  - For example, LGMD involving the protein dysferlin is now often called LGMD2B dysferlin deficiency or dysferlinopathy [DIS-fur-lin-OPP-path-ee] – a pathology of the dysferlin protein.
- In spite of these advances, many patients still do not have a specific type identified and have to accept a general diagnosis of a Limb Girdle protein disorder.
### LGMD Proteins and Locations.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Type</th>
<th>Chrm</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calpain-3</td>
<td>LGMD2A</td>
<td># 15</td>
<td>30% of cases, Calpainopathy</td>
</tr>
<tr>
<td>Dysferlin</td>
<td>LGMD2B</td>
<td># 2</td>
<td>30% of cases, Dysferlinopathy</td>
</tr>
<tr>
<td>Sarcoglycan Proteins:</td>
<td></td>
<td></td>
<td>[SAR-CO-gly-can] Combined, the 4 sarcoglycan types make up 30% of LGMD cases, of these, alpha is the most common, delta the rarest.</td>
</tr>
<tr>
<td>- α (Alpha)</td>
<td>LGMD2D</td>
<td># 17</td>
<td>Alpha-sarcoglycanopathy</td>
</tr>
<tr>
<td>- γ (Gamma)</td>
<td>LGMD2C</td>
<td># 13</td>
<td>Gamma-sarcoglycanopathy</td>
</tr>
<tr>
<td>- β (Beta)</td>
<td>LGMD2E</td>
<td># 4</td>
<td>Beta-sarcoglycanopathy</td>
</tr>
<tr>
<td>- δ (Delta)</td>
<td>LGMD2F</td>
<td># 5</td>
<td>Delta-sarcoglycanopathy</td>
</tr>
</tbody>
</table>
LGMD Proteins and Locations.

- The other identified forms of LGMD combined make up the remaining 10% of cases currently diagnosed.

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<th>Type</th>
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<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telethonin</td>
<td>LGMD2G</td>
<td># 17</td>
<td>Only in Brazil. Telethoninopathy</td>
</tr>
<tr>
<td>TRIM32</td>
<td>LGMD2H</td>
<td># 9</td>
<td>Only found in Manitoba, “Manitoba Hutterite Dystrophy”</td>
</tr>
<tr>
<td>FKRP (Fukutin Related)</td>
<td>LGMD2I</td>
<td># 19</td>
<td></td>
</tr>
<tr>
<td>Titin</td>
<td>LGMD2J</td>
<td># 2</td>
<td>Largest protein discovered: one chain of 26926 amino acids.</td>
</tr>
<tr>
<td>?</td>
<td>LGMD2K</td>
<td># 9</td>
<td></td>
</tr>
<tr>
<td>?</td>
<td>LGMD2F</td>
<td># 5</td>
<td></td>
</tr>
</tbody>
</table>
# LGMD Proteins and Locations.

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<th>Protein</th>
<th>Type</th>
<th>Chrm</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myotilin</td>
<td>LGMD1A</td>
<td># 5</td>
<td>Myotilinopathy</td>
</tr>
<tr>
<td>Lamin A/C</td>
<td>LGMD1B</td>
<td># 1</td>
<td>Laminopathy</td>
</tr>
<tr>
<td>Caveolin-3</td>
<td>LGMD1C</td>
<td># 3</td>
<td>Caveolinopathy</td>
</tr>
<tr>
<td>?</td>
<td>LGMD1D</td>
<td># 7</td>
<td></td>
</tr>
<tr>
<td>?</td>
<td>LGMD1E</td>
<td># 6</td>
<td></td>
</tr>
<tr>
<td>?</td>
<td>LGMD1F</td>
<td># 7</td>
<td></td>
</tr>
<tr>
<td>?</td>
<td>LGMD1G</td>
<td># 4</td>
<td></td>
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</table>
LGMD Genes.

- The different genes discovered to cause the various forms of LGMD are on different chromosomes – they are not all in one section of the genetic code.

- In addition, the different forms have different patterns of inheritance. These will be described later in the talk.

- Due to the wide variety of types and ages of onset, it is difficult to say how common these disorders are in the population.
The cause of LGMD is **genetic**.

We will present some information on the **basic** genetic code and then discuss the problems that cause LGMD.

The **internet** contains a great deal of background information on all of these aspects.

Again, it is the **basic ideas** that are important here, not the complex details.
Basic DNA.

- Our genetic information is carried as two long strands of chemicals – they join together like a long zipper.
  
  Chemicals on each strand join in the middle >

- The two strands twist to form a helix of “DNA.”

- DNA: deoxyribonucleic acid.
  [dee-OX-see-rye-bow-noo-Clay-ick]

- Only FOUR different chemicals are used to make this message – think of each tooth in the zipper as one of these four chemicals.

- The genetic message is the sequence (order) of the chemicals in the zipper.
The 4 chemicals are called “bases” (or nucleotides) $A = \text{adenine}$, $T = \text{thymine}$, $C = \text{cytosine}$, $G = \text{guanine}$

Part of the complexity comes from the sheer sizes involved:

- One piece of DNA can have millions of bases (chemical teeth) in the zipper.
  - The largest single message yet found, the gene involved in causing Duchenne MD, has 2,220,223 bases on each side.
DNA and Its Base Pairs.

- On the left, we see the DNA backbone of one strand, shown in red, yellow and grey (it is mostly made out of sugar and phosphate molecules).

- On the right, we can see five bases attached in sequence (C G A A T)
Certain Bases Always Pair Up.

- Due to their chemical properties, certain bases on one strand pair up with certain bases on the other strand:
  - A always pairs with T and T always pairs with A
  - C always pairs with G and G always pairs with C.

- This pairing makes it easy: once Doctors know the chemicals on one side of the zipper, they will know exactly what is on the opposite side of the zipper.
Genetic Code – Codons.

- The 4 letters in the DNA code – A T C and G – make up 3 letter “words” (called codons) that spell out the genetic messages.

  Examples:

  G G G  G G C  A G T

- There are 64 different combinations possible.

- The entire genetic code is made up of series of these 64 codons, presented in different combinations to form “sentences” – these are what we commonly refer to when we talk about genes.

- Each gene is a “sentence” of code spelling out the formula for one or more proteins in the body.
DNA Forms Chromosomes.

- Each very long helix of DNA is tightly packed to form one chromosome.
- We inherit 23 from Dad & 23 from Mom. Each set of 23 contains about 25,000 genes with about 3.2 billion base pairs.
- The chromosomes (Chrome-muh-soams) are found in the center of the cell – the “nucleus.”

From: www.alzheimers.org/.../IMAGES/HIGH/Dna_high.jpg
National Institute on Aging, National Institutes of Health
DNA Summary.

- DNA is made up of two long strings of sugar (deoxyribose) and phosphate links (molecules) that form the outside backbone of each strand.
- The four base chemicals (also called nucleotides) attach along the inside of the backbone strands.
- Bases on opposite strands bond to each other in the middle, zipping the strands together into a helix.
- Sequences of bases form our 25 to 30,000 genes.
- DNA forms chromosomes: we get 23 from each parent, these are then copied for the rest of our lives.
- Chemicals in the cell can unzip and re-zip the helix as the genetic messages are needed by the cells.
Basic RNA.

- RNA stands for ribonucleic acid. [rye-bow-noo-Clay-ick]
- RNA is similar to DNA, except:
  - RNA has the same bases as DNA, except one – instead of \( T = \text{thymine} \) there is a \( U = \text{uracil} \).
  - In RNA, the chemical backbone of the strand has a different type of sugar – it has a ribose instead of a deoxyribose.
  - RNA is usually found as just a single strand – it usually does not form into a two stranded helix.
  - Many kinds of RNA have been identified, some are well understood, others, not at all.
The sequences on the DNA are **not** directly used to make proteins.

DNA is in the center of the cell (the **nucleus**) and it never leaves.

Proteins are made in the body of the cell.

RNA acts as a **messenger**, making a copy of the sequence needed from the DNA and carrying it out of the cell’s center into the body of the cell where it is used to make protein.

Here is an overall summary:

- DNA - **transcription** --> RNA - **translation** --> Protein.
DNA to RNA transcription has four basic steps:

- Step 1: The 2 strands of DNA unwind and unzip.
- Step 2: Primary RNA “sees” a start sign (“promoter”) on the DNA strand and it joins on, copying the sequence of the four bases – the reading frame – until it hits a stop signal.
- Step 3: This primary RNA carries a complete copy of the base sequence of the DNA – it is now edited into messenger RNA (mRNA).
- Step 4: The final mRNA moves into the body of the cell and acts as a template for protein synthesis.
The two unwrapped DNA strands are shown in blue. The top strip – called “sense” (coding) DNA – is not used in the copying (“transcription”) process.

The second blue strip is the template strand of DNA used to make the copy (this is the opposite side of the sense DNA strip, so it is called “antisense” (non-coding) DNA).

This yellow strip is the newly formed primary RNA strand. Notice it comes out as an exact copy of the top DNA strip (but with U for T).
Genetic Messages.

- Sections of base sequence are read as 3 letter words – **codons**, to form “sentences” – the gene’s message.

- In the next few slides, I am going to “cheat” a bit and use some common 3 letter English words to illustrate how the triplet genetic code makes sense and how mutations create problems.

- Remember, in “real life” there are just 4 letters in the genetic alphabet – **A T G** and **C** and the three letter codons they form don’t make much sense to us (but they **do** make sense to the protein factory in the cell).
The Sequence Contains the Message.

- The DNA sequence of bases contains the messages needed by the cell but not all of the DNA is used:
  - It is estimated that only about 3 percent of the DNA consists of coding sequences used to make proteins – it is not clear what the rest does, but there is a growing appreciation that it probably plays important roles.

- Recall, the whole DNA sequence in the gene is initially **transcribed** into primary RNA:
  - Primary RNA is then **edited**, some parts are kept (the actual **coding sequences called exons**) and the other parts (**untranslated regions called introns**) are removed from the final mRNA message.
The Sequence is Edited.

- An illustration:
  - Sequence: exons↓  introns↓
    dek|THEdkeOLDuteCATyjiWASkhyFAT|ert
  - The sequence is now edited – the introns are removed to yield the final mRNA message:
    |THE OLD CAT WAS FAT|
  - This message is now translated into a protein.

- In the illustration above, the start and the end of the reading frame is shown as | |.
- The sequence of exons between these bookends is the critical message used to make a protein.
Introns Play A Role?

- **Intron code** – untranslated regions (UTR) – are **not** kept in the mRNA & are not used to make proteins.
- Until recently, this untranslated intron code was thought to play no role and was called “**junk DNA**.”
- The **majority** of DNA (97%) is made up of intron code.
- Recently, it has been shown that some of this material plays a **crucial role** in switching on and off genes.
- Another clue to the importance of introns is found in diseases, e. g., in Myotonic Dystrophy, the genetic defect is in an untranslated section of the intron code.
- Overall, the role of this untranslated code and how it operates is **not well understood** yet.
Summary From DNA to mRNA.

- To put a genetic message into action:
  - The DNA helix unzips into one sense strand (not used) and one template, antisense strand.
  - An RNA strand forms by moving along the template DNA strand and adding new bases corresponding to the sequence it finds.
  - When done, the 2 DNA strands zip back together.
  - The RNA is edited and introns are removed before it moves into the body of the cell. Differences in editing allow one gene to make several different mRNAs and thus make several different proteins.
- Proteins are made according to the sequences of code carried by the mRNAs that move into the cell.
Protein is Made.

- The normal operation of proteins is critical to the function of every system in the body.
- Estimates are there are >100,000 proteins in humans.
- Each edited mRNA sequence spells out a protein.
- Proteins are made up out of chains of chemicals called amino acids (AA).
- Proteins are made by “factories” in the cell (ribosomes) that “read” the mRNA base sequence.
- Ribosomes assemble the different amino acids into the new chain according to the mRNA sequence.
- There are 64 three “letter” codons: 61 represent different amino acids, 3 stand for stop signs.
Translation.

- There is some overlap as 61 codons specify just 20 different amino acids.
- As the mRNA is read, the code tells the factory what amino acid to add next in the new protein chain: this is called translation.
- Proteins usually contain from tens to a few thousand amino acids.
- The largest protein yet found is Titin, a muscle protein with 26926 amino acids strung together in one long single chain.
One or more chains of AAs are used to form a protein. In a complex series of steps, the cell gathers the strand(s) into a final **folded, 3-D shape**.

This shape is critical to the protein’s ability to function.

It does not take much of a mistake to cause trouble.

Mutations in DNA leading to altered protein function are the usual culprit in most genetic disorders.

Example: a diagram of calpain protein.

http://www.umaine.edu/bmmb/biochemistry.htm
The major muscle proteins fit together like the gears in a watch, in what is known as the dystroglycan protein complex.

http://genomebiology.com/2001/2/4/reviews/3006/figure/F3
DNA carries the genetic code in sequences of chemicals that form genetic “messages.”

A message is read and used as a template to make a unique sequence of amino acids.

Amino acid sequences form into proteins.

Proteins form into complex structures that are the basis of living matter.

The code is not always final, some modifications can be made along the way that alter the final product and how it will function.

To devise genetic treatments, doctors will have to understand this whole process in great detail.
Most of the body’s cells carry a complete copy of all of an individual’s DNA.

As each cell is formed, the DNA in the parent cell is copied and put into the daughter cell:

- DNA is carried on as cells die and are replaced.
- This replenishment process goes on at an extremely high rate in many types of cells:
  - Examples: in skin, in the reproductive system and in the gastrointestinal tract.

This process is extremely reliable and there are mechanisms to check for accuracy but only one mistake in code reproduction can be disastrous.
A sequence of code is like a phone number:

- The code (number) represents some other target:
  - 911: represents the police, fire and ambulance
  - 411: represents directory assistance

Just as a phone number leads us to our target, DNA specifies the cell’s targets – the proteins that the cell produces and that are so critical to life.

- We can see that a difference in just one letter can change the meaning significantly:
  - If we use 411 instead of 911 we get the wrong target.
Common Mutations.

- Changes in the normal sequence are called **mutations**.
- Mutations **usually** affect the structure of proteins, altering their function & usually leading to disorders.
- Example: A mutation that adds or deletes even one letter can shift the triplet reading frame and garble the message – a **frameshift mutation**:
  - Using our example: [THE OLD CAT WAS FAT]
    - If we delete the T, the frame shifts right, giving:
      - [HEO LDC ATW ASF AT]
- The protein factory in the cell makes the protein spelled out by this odd message and the protein does not work the way it should, leading to a disorder.
Frameshift: A Severe Mutation.

- Because a frameshift mutation usually garbles the message so badly, it is commonly a very devastating type of mutation with severe consequences.

- The protein produced is often misshaped and cannot function or interact with other proteins (think of the example of the gears in a clock needing to all work together).

- This usually leads to serious physical symptoms.

- Found in some forms of LGMD.
Nonsense Mutation.

- Recall that the RNA will continue to copy the DNA code until it sees a stop signal.
- Some mutations can create an “accidental” stop sign in the middle of the message and the RNA stops copying too soon.
- This type of mutation is called a nonsense mutation. The result is that only part of the correct message is made and, in turn, only part of the protein is made:
  - From our example: \textbf{THE OLD CAT WAS FAT}\textbf{\textbackslash\textbackslash}  
    The nonsense mutation product code looks like:  
    \textbf{\textbackslash\textbackslash} \textbf{THE OLD CAT}\textbf{\textbackslash\textbackslash}  
- Found in some forms of LGMD.
Missense Mutations.

- Mutations can sometimes change the DNA code to create a different amino acid at a specific location.
- These changes often alter the way the protein works.
- This type of mutation is called a **missense mutation**. The new message is readable but slightly changed:
  - From our example: \textbf{THE OLD CAT WAS FAT}
    - The missense mutation product code looks like: \textbf{THE OLD FAT WAS FAT}
  - The effect of this type of mutation can vary in severity depending upon the protein in question.
  - Found in some forms of LGMD.
Autosomes.

- Our 46 chromosomes carry the DNA code.
- The 46 chromosomes are found in 22 pairs called autosomes and one pair of sex chromosomes.
- Different genes are located on different chromosomes.
- Each pair of autosomes holds two copies of a given gene, one inherited from Dad, the other from Mom.
- The various forms of LGMD involve different mutations in different genes on different chromosomes.
- [The composition of pair # 23 determines sex: boys have an XY; X from Mom, Y from Dad and girls have an XX; one X from Mom and one X from Dad.]
Autosomal Recessive Inheritance.

- Most forms of LGMD are inherited as **autosomal recessive** conditions; this means that **two copies** of the gene with the genetic mutation (inherited from **both** mom and dad) are necessary for LGMD to occur in a person (if a person only inherits one copy, they are a carrier and do not suffer symptoms but they can pass this defective gene onto their children).

- In a disorder that is genetically recessive, for each pregnancy, if both parents carry the mutation, there is a **25%** chance of a child inheriting two copies of the mutation and having LGMD, a **25%** chance they will be OK and a **50%** chance of becoming a carrier.
Autosomal Recessive Forms.

- Recessive forms are the common type accounting for >90% of cases. Usually have childhood or teen-age onset. Genes responsible for types 2B to 2F have been shown to be a part of the sarcoglycan protein complex, a key part of the dystroglycan complex and critical to muscle health. Clinical descriptions of the recessive LGMD forms range from severe progressive muscular dystrophy to mild late-onset forms.

Some forms of LGMD are inherited as **autosomal dominant** conditions; this means that only one copy of the gene with the genetic mutation (inherited from either mom or dad) is necessary for LGMD to occur in a person (the other gene copy is usually healthy but it is “overridden” by the dominant copy with the mutation and cannot help out).

In a disorder that is genetically dominant, for each pregnancy, a parent with the defect has a **50%** chance of transmitting the mutation to a child – in other words, each child has a 50% chance of inheriting the defect if a parent has the mutation.
Autosomal Dominant Forms.

- The dominant LGMDs usually show adult onset. In addition to muscle weakness, the creatine kinase (CK) blood levels are elevated (usually 4 - 10 times normal).

- LGMD1A, LGMD1B, LGMD1C, LGMD1D, LGMD1E, LGMD1F, LGMD1G.

- Limited genetic testing may be available for types; 1B and 2A to 2F, research continues on developing further genetic tests.
Spontaneous Mutations.

- **Spontaneous** (new) mutations, are not inherited from either mom or dad – occasionally, these mutations naturally occur in the DNA as cells divide.
  - They can occur in a body (somatic) cell, usually causing cancer (but they cannot be passed on).
  - They can occur in an egg or sperm (germ) cell, possibly causing a disorder in the person and they can be passed on to children.

- Depending upon the form of LGMD, a person with a spontaneous mutation in an egg or sperm cell will subsequently pass the mutation on to any children 25% to 50% of the time.
The DNA code in exons ends up being used as a template for making different proteins in the body.

Mutations in this DNA lead to altered protein function and are the usual culprits in most genetic disorders.

The various LG forms involve different mutations on different genes and one of many different proteins.

Different forms have different characteristics, for example, different ages of onset, affect muscles a bit differently, have different rates of progression, etc..

Discovery and research is progressing but it takes time to sort out such a complex group of disorders.
Some Helpful Webpages.

- http://www.chg.duke.edu/diseases/lgmd.html
- http://www.mdausa.org/disease/lgmd.cfm
- http://muscles.ca/