

An Overview of Facioscapulohumeral Muscular Dystrophy (FSHD)

Bill Tillier,
Muscular Dystrophy Canada
December 2005

Knowledge Translation.

- This talk is an example of **knowledge translation** (KT).
- KT is an important **new tool** in medical research.
- KT involves making a **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.
 - Health care providers and researchers can better understand patients and their needs.

A General Overview of FSHD.

- Our goal is to give a **general** overview of facioscapulohumeral muscular dystrophy (FSHD).
- 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 its 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.

What is FSHD?

- Facioscapulohumeral muscular dystrophy (FSHD).
[PHASE - she - oh - SKAP - pew - low - HUMOR - ill]
- FSHD is a complex **genetic disorder** that weakens and shrinks some of the muscles in the body:
 - The name outlines **some** of the muscles affected:
 - Facio = muscles around the **face**
 - Scapulo = muscles around the **scapula** (the shoulder)
 - Humeral = muscles around the **humerus** (upper arm)
 - However, **other muscles** are also affected.

- FSHD is the **third** most common muscular dystrophy overall (behind Duchenne and Myotonic). FSHD affects about 1 in 20,000 people worldwide.
- FSHD can affect **men or women**. People with FSHD can begin to show symptoms at any age, however, problems **usually** develop in the teens or **twenties**.
- FSHD does not shorten the **lifespan**. **Heart** problems are rare (<5%), **respiration** problems are seen in <10% of cases. Mental impairment and epilepsy occur in 40% of severe but rare childhood onset cases.
- FSHD is very **variable** and wide differences appear in the onset age, severity & pattern of affected muscles.

Two Types of FSHD.

- Two types of FSHD have been discovered:
 - Type 1A (**FSHD1A**):
 - The most common type, accounting for 90 to 95% of FSHD cases.
 - Researchers have a **good general** understanding of this type but many questions still remain.
 - Type 1B (**FSHD1B**): Accounting for about 5 to 10% of cases, this type appears to have a different genetic cause than type 1A. It is **not** well understood at present.

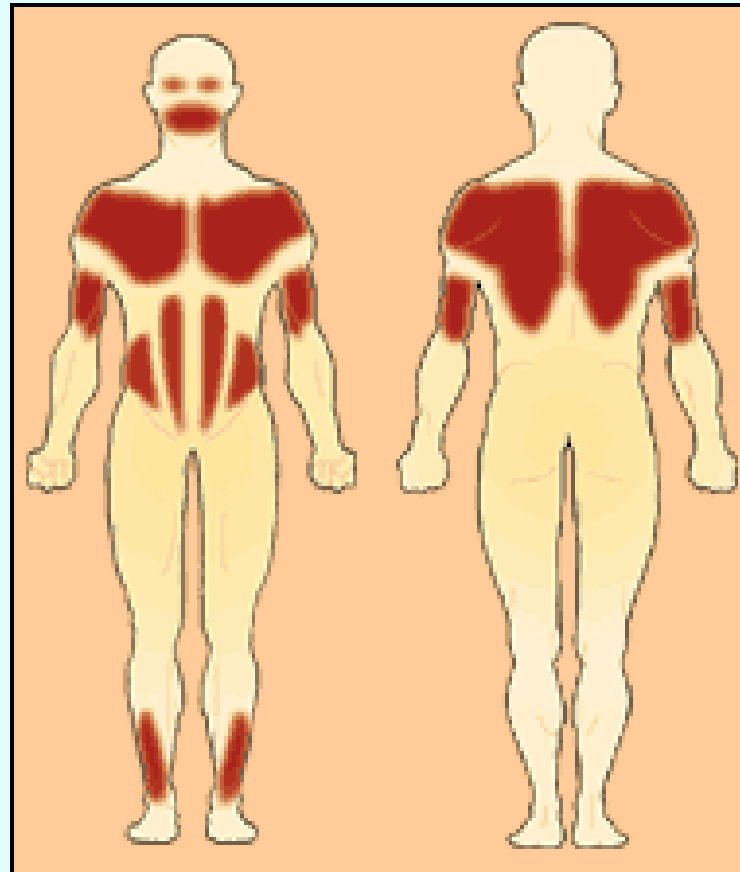
A Wide Range of Symptoms.

- Usually, FSHD starts with **weakness** in the face, shoulders and upper arms, followed by weakness in the abdomen, hips and lower legs.
- Major symptom: the **progressive weakening** and loss of skeletal muscles (**atrophy**) in the face (facio), shoulder area (scapulo) and upper arms (humeral).
 - Distinctive features are early weakness of the muscles of the **eye & mouth** (smile, pucker, whistle), with weakness in the shoulder muscles.
- FSHD symptoms may be so **mild** that adults may be unaware they have FSHD until a family member with more severe symptoms is diagnosed.

Overview of FSHD Symptoms.

- Shoulder, facial, and girdle (around the hips and waist) muscle weakness.
- Prominent facial weakness.
- Some weakness in and around the eyes.
- A recognizable shape and forward sloping of the shoulders.
- Different levels of weakness between the biceps / triceps (upper arms) and the deltoid (forearms).
- Different levels of weakness in the abdominal muscles
- Beevor's sign (a problem in the muscles of the abdomen).
- Initial leg weakness starting in the feet and quads.
- Uneven development (asymmetry) of muscle weakness.
- High-frequency hearing loss is common.
- About 50% of those with FSHD retain the ability to walk throughout their lives (others may require a wheelchair).

Common Presentation of Weakness.



Front

Back

From: http://www.chb-genomics.org/pat_fam-facio_dyst.php

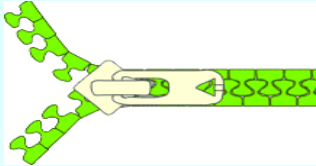
- FSHD is a genetic disease caused by a **mutation** of a section of the DNA near the end of chromosome # 4 (a deletion – some DNA is missing).
- The missing DNA isn't part of a gene, but it still somehow causes FSHD (it seems to affect other muscle related genes). This is a very **complex** problem – little is known about how this DNA works.
- FSHD is usually inherited in an **autosomal dominant** fashion (about 70% to 90% of the time). A son or daughter of an affected parent is at a 50% risk of inheriting the defect.

- In about 30% of cases, the mutation is **spontaneous** (the mutation was not passed on from the parents, it is a new mutation occurring during development).
- The size of the deletion is thought to be related to the **severity** of the symptoms (but the severity of FSHD in a parent does not always predict the severity in an affected child).
- For FSHD – 1 A, there is a **genetic test** for the mutation using a sample of blood. There is also a prenatal test. There is no test for type 1B yet.

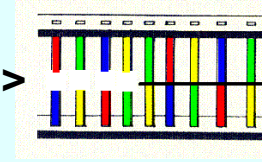
- The cause of FSHD is **genetic**.
- We will present some information on the **basic** genetic code and then discuss the problem that causes FSHD.
- The **internet** contains a great deal of background information on all of these aspects.
- Again, it is the **basic ideas** that are important here, not the complex details.

Basic DNA.

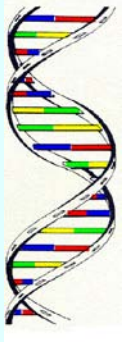
- Our genetic information is carried as two long strands of chemicals – they join together like a long zipper.



Chemicals on each strand join in the middle >



- The two strands twist to form a **helix** of “DNA.”
- DNA**: deoxyribonucleic acid.



[dee-**O**X-see-rye-bow-noo-**C**lay-ick]

Helix ↑

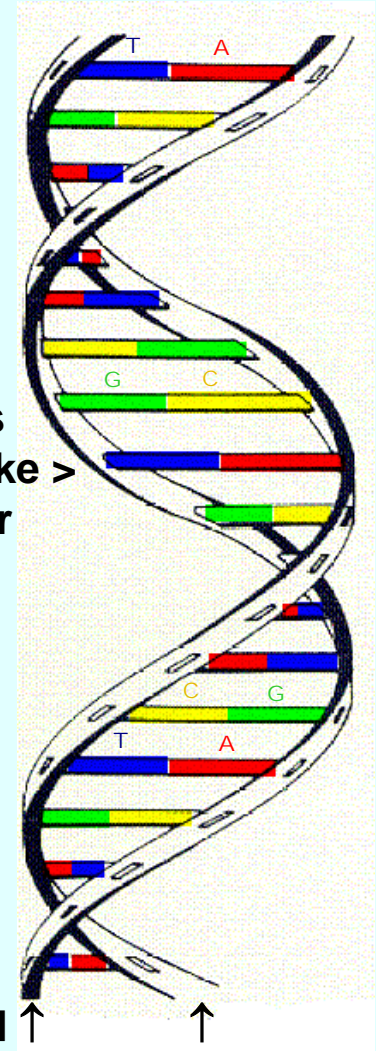
- Only **FOUR** different chemicals are used to make this message – think of each tooth in the zipper as one of these four chemicals.
- The genetic message is the **sequence** (order) of the chemicals in the zipper.

DNA Helix

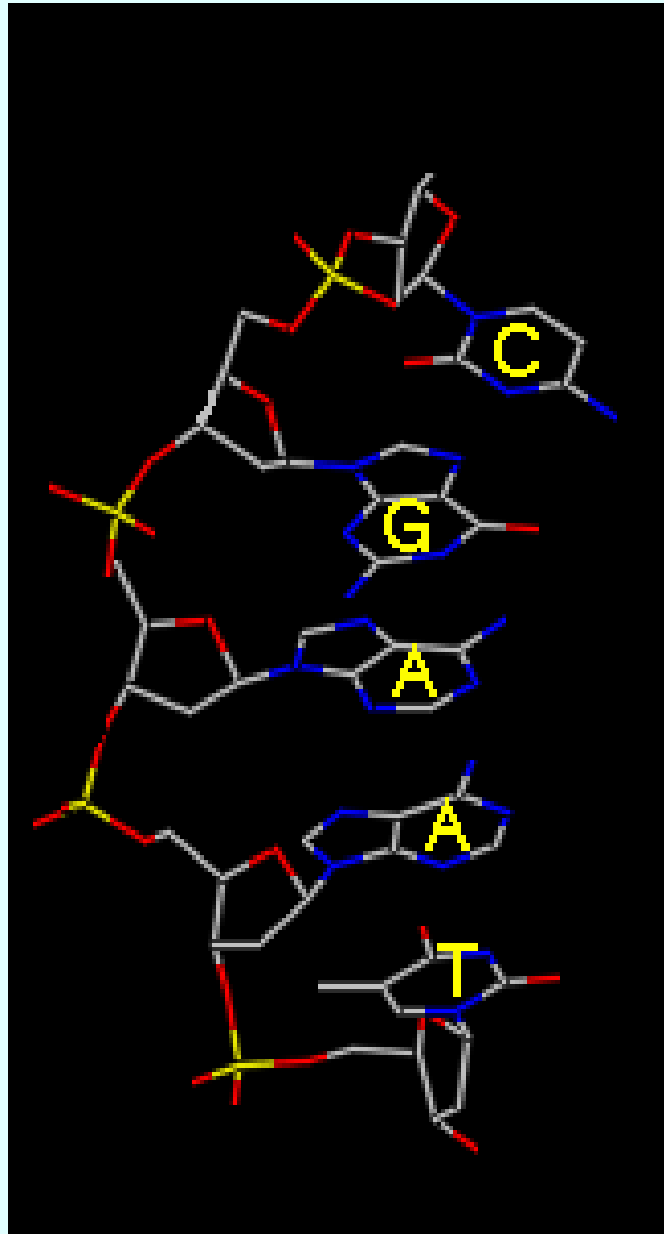
- The 4 chemicals are called “**bases**” (or **nucleotides**) **A** = adenine, **T** = thymine, **C** = cytosine, **G** = guanine
- Part of the **complexity** comes from the sheer sizes involved:
 - One piece of DNA can have **millions** of bases (chemical teeth) in the zipper.
 - The largest single message yet found, the gene involved in causing **Duchenne** MD, has 2,220,223 bases on each side.

Two bases
join to make >
1 base pair

Strand ↑ ↑
Backbones



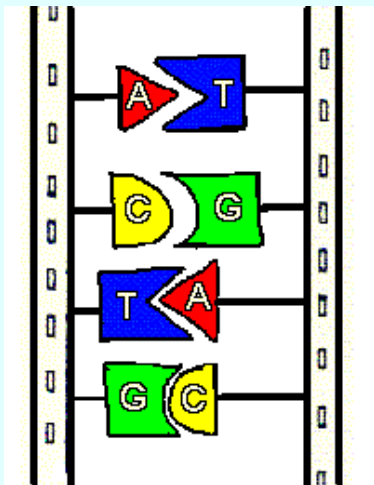
- On the left, we see the DNA **backbone** of one strand, shown in red, yellow and grey (it is mostly made out of sugar and phosphate molecules).



- 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.

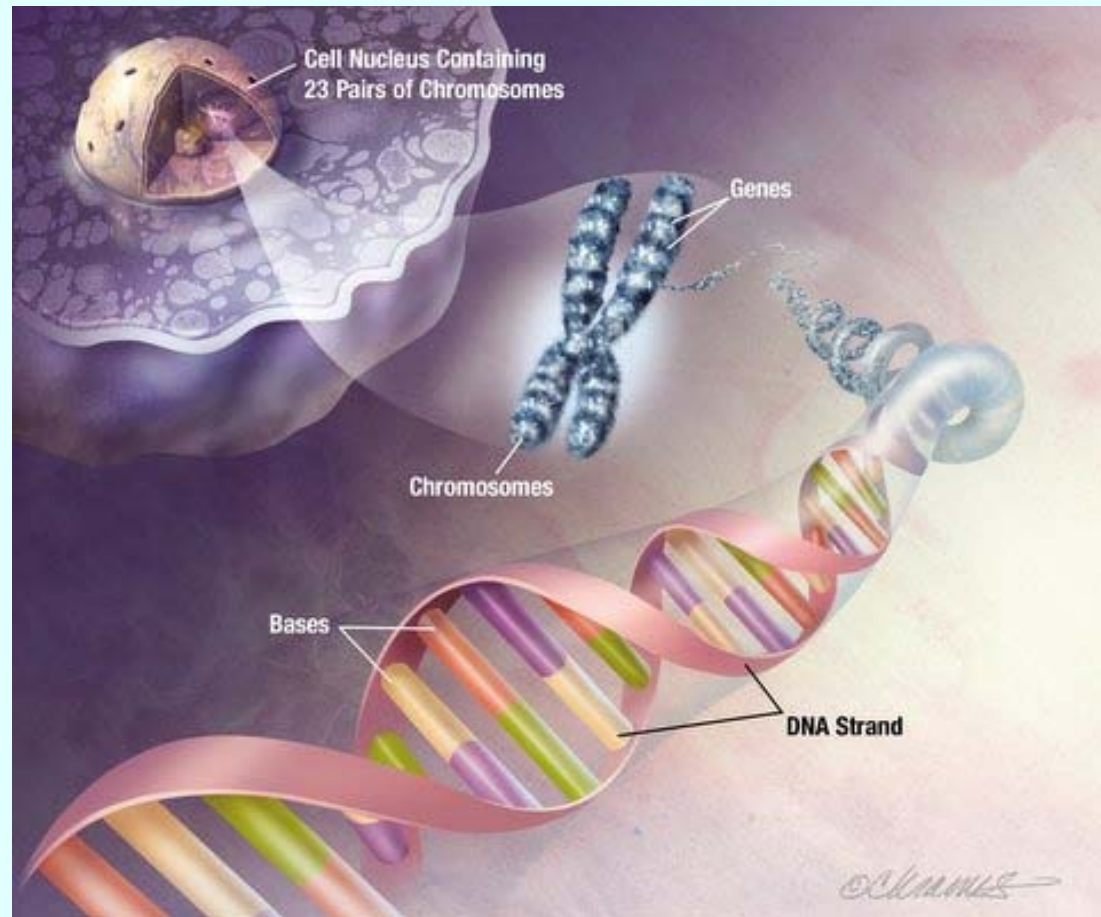
Examples:

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 a 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 = thymine** there is a **U = 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 Message Has To Be Copied: 1.

21

- 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:

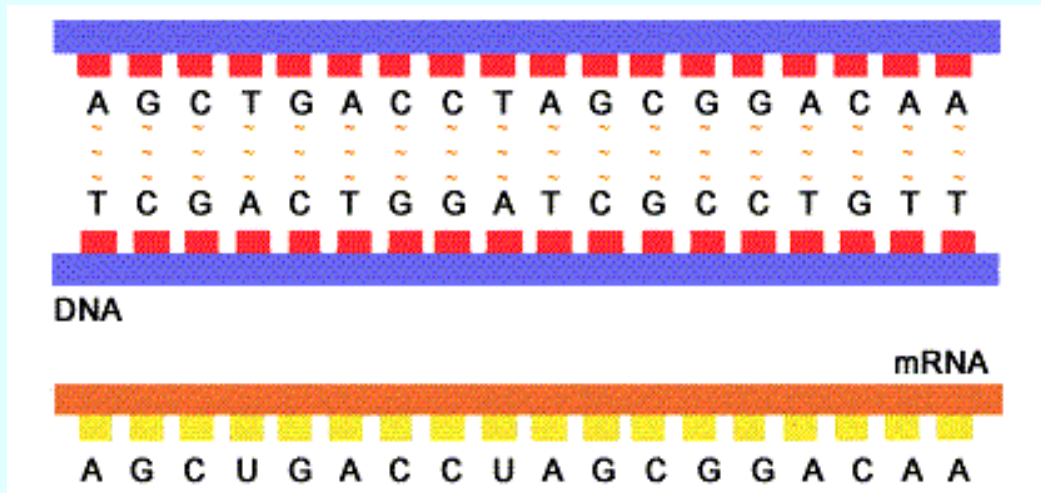
- **DNA - transcription--> RNA - translation-->Protein.**

The Message Has To Be Copied: 2.

- 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**.

Copying The Message.

- The two unwrapped DNA strands are shown in blue. The top strip – called “**sense**” (coding) DNA – is not used in the copying (“**transcription**”) process.



- 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**).

← 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).

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 **disorders**, e. g., in FSHD, 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.

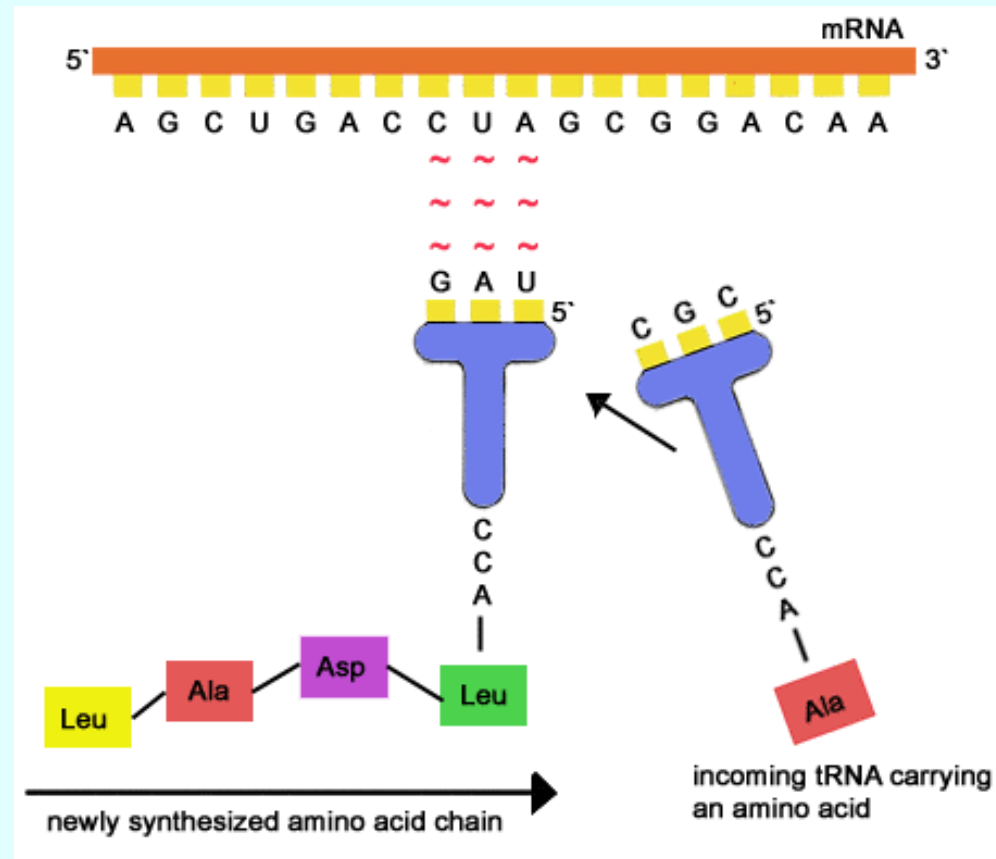
- To put a genetic message into action:
 - The DNA helix unzips into one sense strand (not used) and one template, antisense strand.
 - An RNA strand forms by moving along the template DNA strand and adding new bases corresponding to the sequence it finds.
 - When done, the 2 DNA strands zip back together.
 - 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.



- The **sequence** of amino acids defines the protein.

<http://library.thinkquest.org/C004535/media/translation.gif>

Finished Protein.

- 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 dystrophin protein.

http://imbs.massey.ac.nz/Staff_images/dystrophin-web1.gif Dr Andrew Sutherland-Smith

Synopsis.

- 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.

Code is Always Being Copied.

- 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.

Code is “Dumb” But Crucial.

- 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.
- An example is Duchenne muscular dystrophy.

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: |THE OLD CAT WAS FAT|
The nonsense mutation product code looks like:
|THE OLD CAT|

Base Pair Repeats.

- It is **normal** to see triplets or quadruplets – 3 or 4 bases together – repeated a number of times in a row, either within a gene or in the intronic code.
- When 3 chemicals are repeated, it's called a **trinucleotide** repeat:
Example: **CTG CTG CTG CTG CTG CTG CTG**
- There is normally some **variation** between different people, re: how many repeats are present and the number is **stable** over generations (children have roughly the same number as their parents).
- If the number of repeats is within “**normal**” **limits**, they do not cause problems.

Repeat Related Mutations.

- Sometimes a mutation can occur that **deletes** repeats or that creates **extra** repeats. If a section of repeats becomes too small or too large, it may lead to problems.
- In FSHD1A there is a genetic deletion and some repeats are lost. If the number of repeats becomes **too small** FSHD symptoms will appear. **Generally**, the fewer the repeats, the worse the FSHD1A symptoms.
- The section of repeats in FSHD1A is called **D4Z4**.

- This section is found on **chromosome #4** in DNA that does not code for a protein (an intronic section).
- People **normally** have 15 to >100 repeats in the D4Z4.
- People with mild FSHD1A may have from 12 to 15 repeats (borderline) or **fewer than 11** (definite FSHD)
[Tawil R., *Curr Neurol Neurosci Rep.* 2004 Jan; 4(1): 51-4.]

[In the medical research, these numbers vary a bit.]

Illustration.



Normal: 15 to more than 100 repeats.



FSHD1A: fewer than about 11 repeats.

Result: Normal proteins but too much of them.

How Does The Mutation Cause FSHD? 42

- It is not clear how this mutation causes the disorder, it is suspected that the missing repeats disrupt normal **translation** of one or more proteins (see slide 30).
- The D4Z4 section does not make a protein and it seems that the effects of the mutation are **indirect**:
 - Without the proper repeats, it is believed other genes near the D4Z4 section produce normal proteins (mainly enzymes) but are overactive and make too much. The **excess** somehow causes problems, leading to the symptoms.
(Gabellini et al *Cell*, 110(3), August 9, 2002, pp. 339-348.)
- There is a **genetic test** to detect the FSHD1A deletion.

Common Repeat Disorders Include:

- Fragile X syndrome: the most common cause of inherited mental retardation (IQ ~ 35-70).
 - Caused by extra **C G G** repeats in an exon on the X chromosome.
- Huntington disease is caused by extra **C A G** repeats in an exon in the Huntington gene on chromosome 4.
- Friedreich ataxia is a neurodegenerative disorder caused by extra **G A A** repeats, located in an untranslated region of code on chromosome 9.
- Myotonic dystrophy (DM1 and DM2) a common muscle disorder, involves an expansion of repeats on chrm. #19 (in DM1) or on chromosome #3 (in DM2).

- The location of the mutation for **FSHD1B** has not been found yet.
 - There is no genetic test for this type at this time.
- About 5% of individuals with the mutation that causes FSHD **do not develop any symptoms**, but can still pass on the mutation to their offspring.
- Some cases show **mosaicism**. This happens when a new mutation occurs in a single cell very early in development (the rest are OK). As this cell divides, its daughter cells carry the mutation. Thus, only some cells in the body have the mutation. The effect of FSHD mosaicism varies widely in each person.

- Our 46 chromosomes carry our 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.
- We are interested in the pair of # 4 chromosomes, this is where the FSHD1A mutation occurs.
- [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.]

- FSHD is an **autosomal dominant** condition; this means that if **one** of the two copies of the DNA carried on chromosome 4 has the mutation (inherited from **either** Mom or Dad) then FSHD will occur in a child (the DNA of the second chromosome of the pair is usually healthy but it is “overridden” by the dominant copy with the mutation and thus cannot help out).
- Because the disorder 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.

- In up to 30% of FSHD cases, the mutation is new.
- **Spontaneous** (new) mutations, are not inherited from either Mom or Dad – the mutation arises in one of the early cells that divide to form the embryo.
- In these cases, there is usually no prior family history of FSHD.
- A person with a spontaneous mutation in an egg or sperm cell will subsequently pass the mutation on to any children 50% of the time.

Summary: What We Need to Know.

- 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 DNA carried in the untranslated intron code is not used directly to make proteins.
 - It is not clear exactly what this DNA code does.
- The FSHD mutation is in a section of the intron code.
 - This mutation somehow disrupts normal gene function leading to the symptoms we see in FSHD.

- FSHD **usually** displays a fairly clear & unique pattern of muscle weakness (it's usually easier to diagnose).
- Genetic testing confirms about 95% of FSHD cases.
- There is no “treatment” for FSHD:
 - **Management** is focused on dealing with symptoms and learning to cope with specific weaknesses.
 - Braces are sometimes used for the ankles and occasionally surgery is used to help with the shoulder area to give more movement to the arms.

- Tawil, R. (2004). Facioscapulohumeral muscular dystrophy. *Current Neurology and Neuroscience Reports*, 4: 51-54.
- Tuplera, R. and Gabellini D. (2004). Molecular basis of facioscapulohumeral muscular dystrophy. *CMLS, Cell. Mol. Life Sci.* 61, 557–566.
- Gabellini, D. Green, M. R. and Tupler, R. (2002) Inappropriate Gene Activation in FSHD: A Repressor Complex Binds a Chromosomal Repeat Deleted in Dystrophic Muscle. *Cell*, 110: 339-348.