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CDG - Genetics

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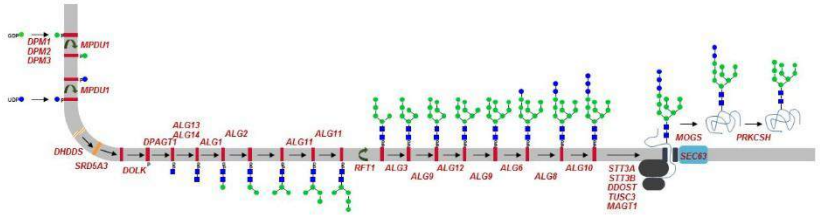
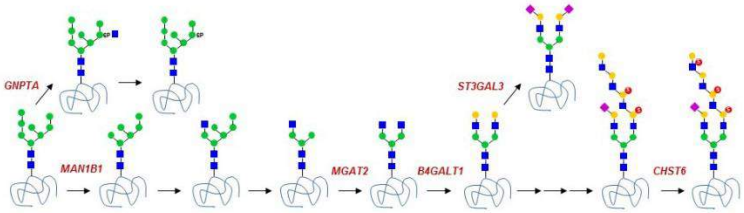
Why is glycosylation important?

- Glycosylation is by far the most complex form of protein and lipid modification in all domains of life

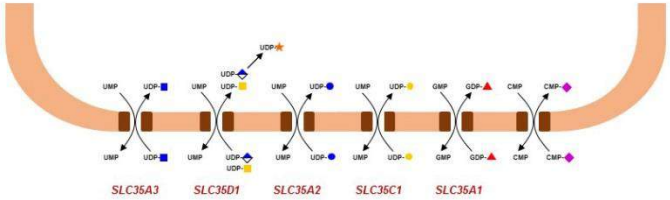
- The barrier of 100 genes defects with imparing glycosylation has just been passed:
http://www.physiol.uzh.ch/Glycosylation/Glyco_defects.pdf



ER N-glycosylation defects identified in CDG patients



Golgi glycosidase and glycosyltransferase defects



Golgi-localized nucleotide-activated sugar transporter defects

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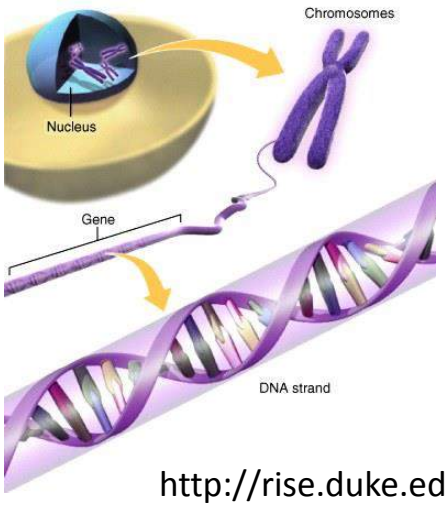
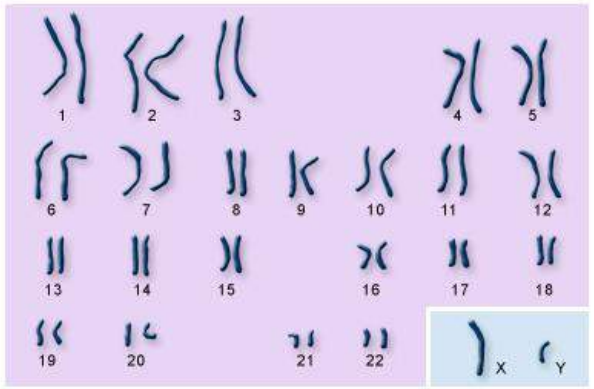
Passing on genetic information from parents to children



http://rise.duke.edu/



The 46 human chromosomes seen down the microscope
Hundreds of genes are located on each chromosome

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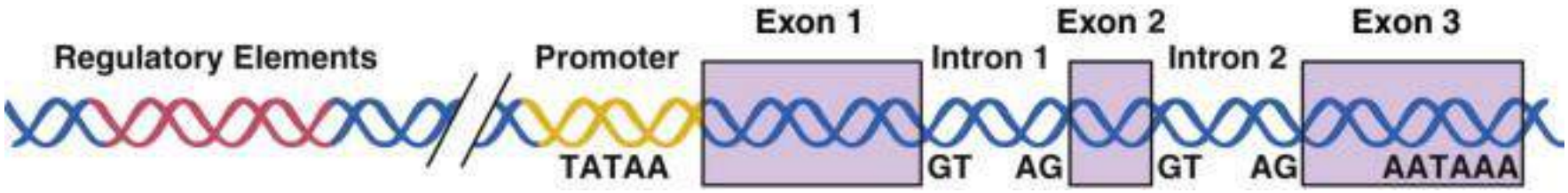
What is a gene?

- A gene is the basic physical and functional unit of heredity.
- Act as instructions to make molecules called proteins.
- Every person has two copies of each gene, one inherited from each parent.
- Alleles are forms of the same gene with small differences in their sequence of DNA bases.



Gene structure. The DNA sequences transcribed as RNA are collectively called the gene and include exons (expressed sequences) and introns (intervening sequences). Molecular and Cell Biology Ko, Tien C., Sabiston Textbook of Surgery, Chapter 3, 24-39; by Saunders, an imprint of Elsevier Inc.

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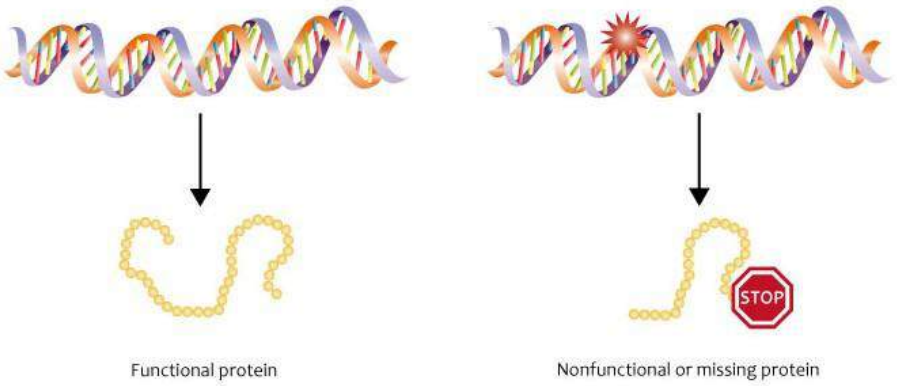
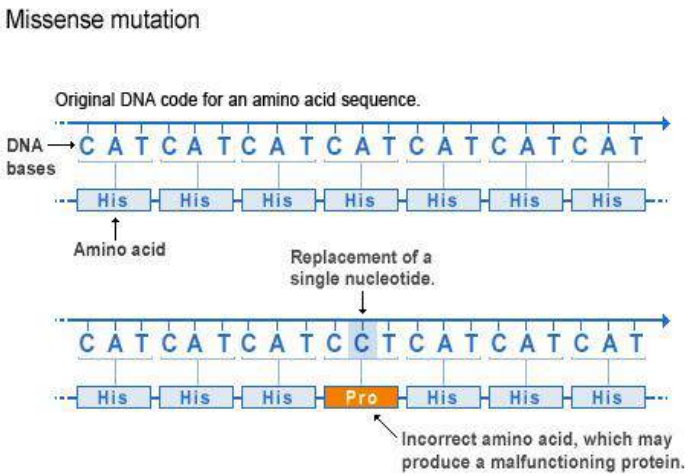
What is a mutation?

- Mutations are changes in the genetic sequence
- Main cause of diversity among organisms.
- Mutations are necessary for evolution.
- Can damage existing adaptations as well.



http://www.geneticseducation.nhs.uk/



A=T G≡C

T=A C≡G

http://www.geneticseducation.nhs.uk/

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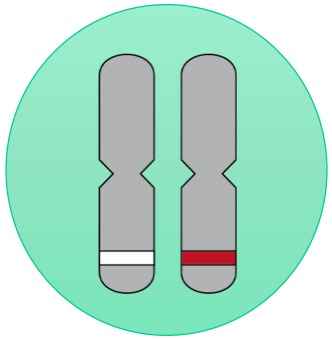
Inheritance in CDG

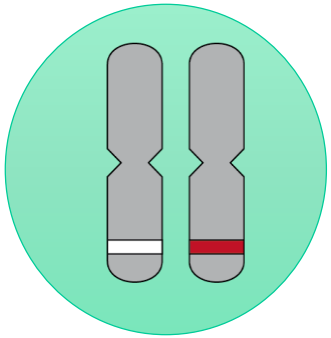
Autosomal recessive inheritance where both parents are carriers

As in PMM2- CDG and most CDG's

Parents

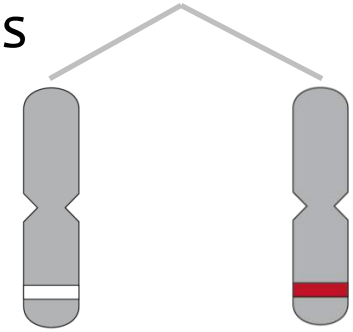


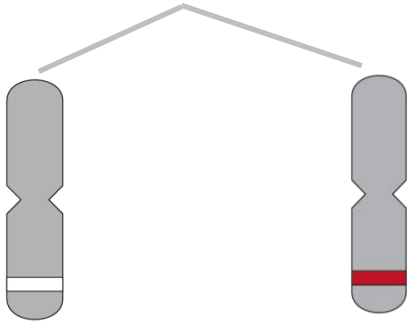
Carrier for the condition



Carrier for the condition

Sperm or eggs





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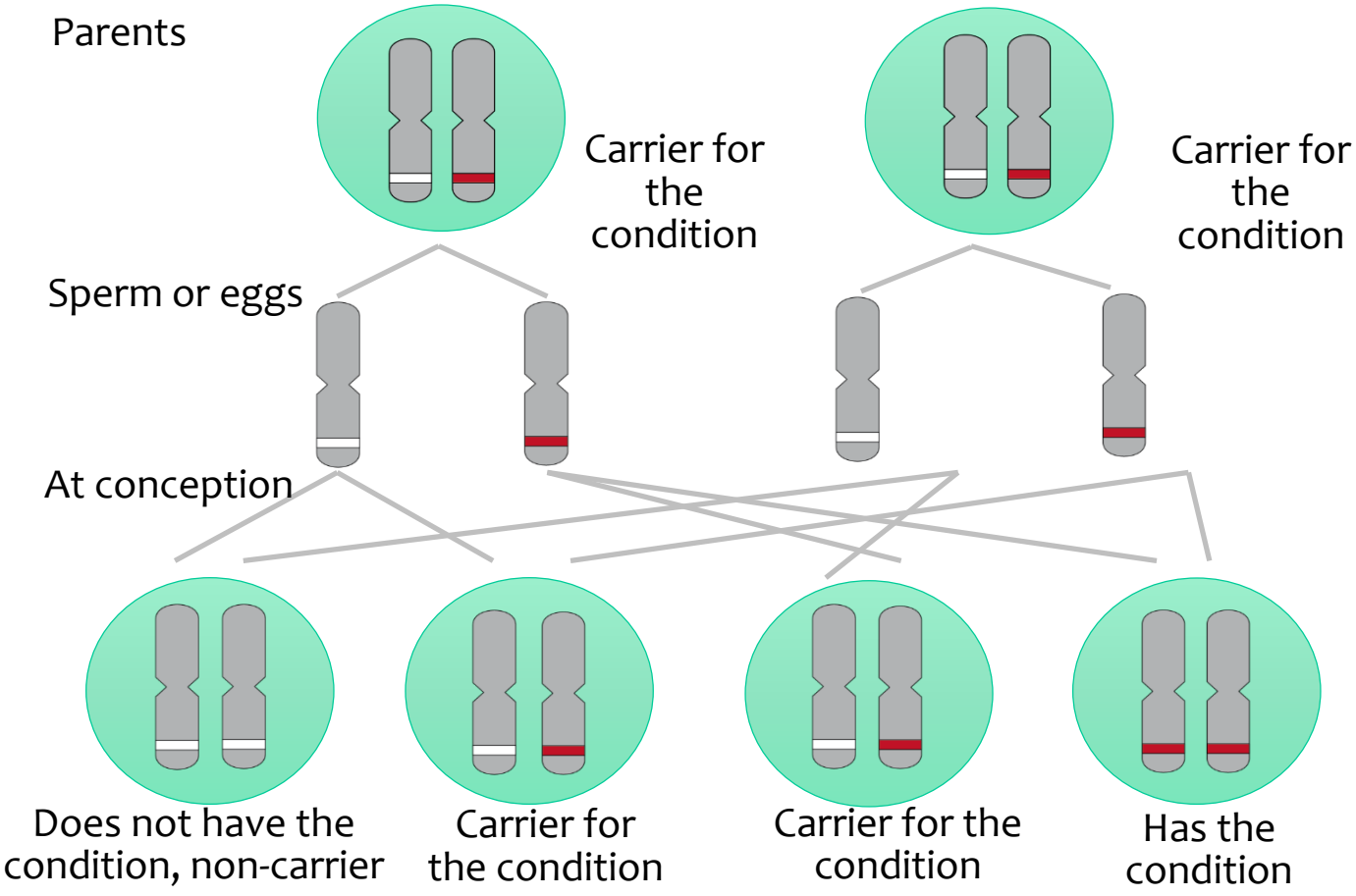
Inheritance in CDG

Autosomal recessive inheritance where both parents are carriers



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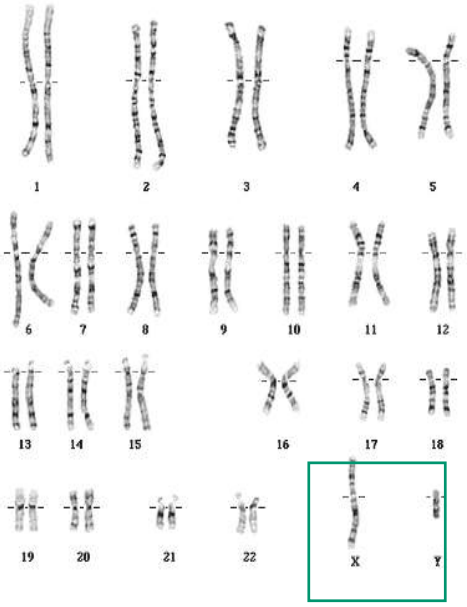
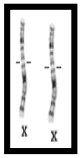
Inheritance in CDG
X- linked inheritance



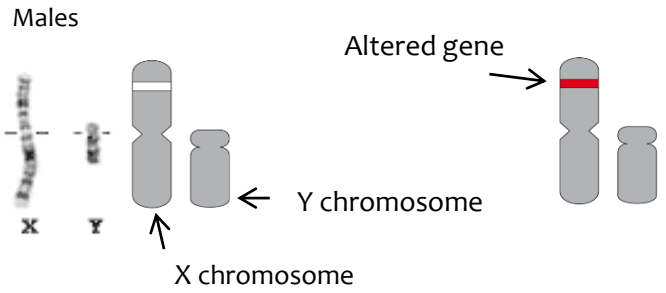
Sex chromosomes of a male

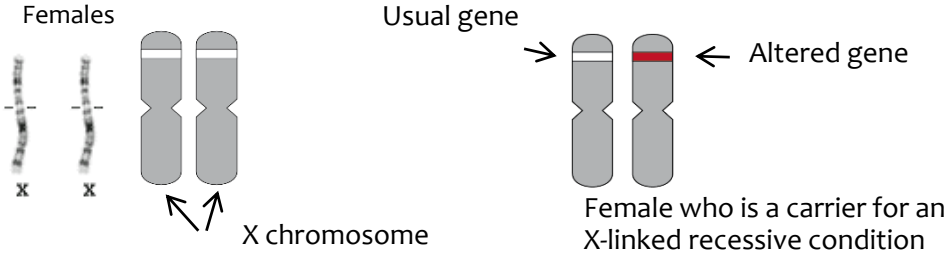


female

…Whereas a female has two X chromosomes.

There are clinical consequences of males having only one X chromosome. The majority of the genes on the X chromosome are present in only one copy .





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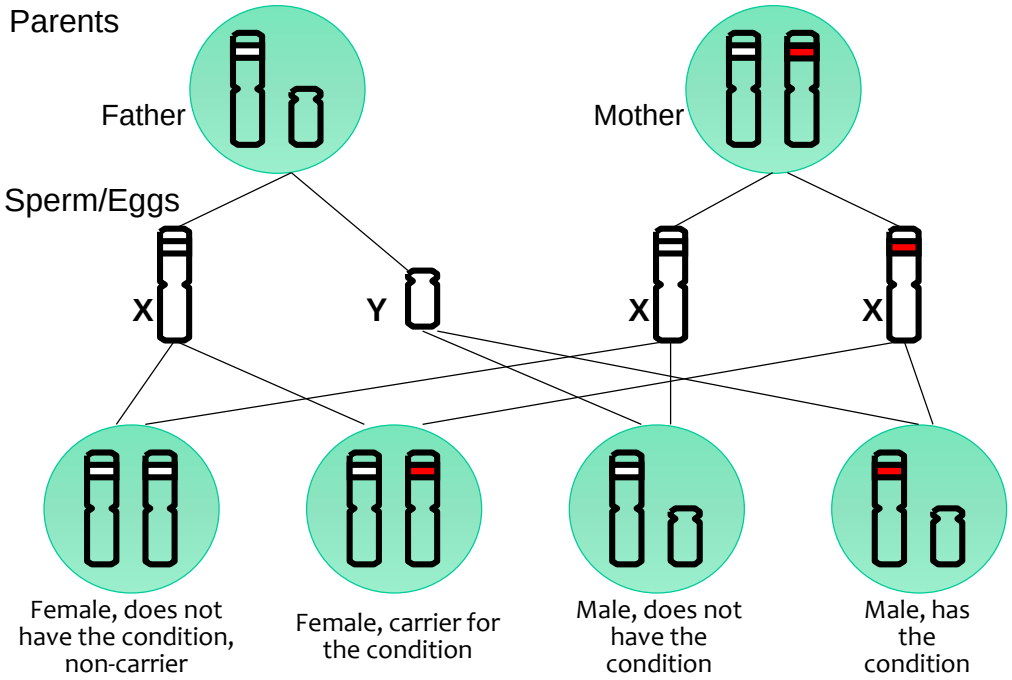
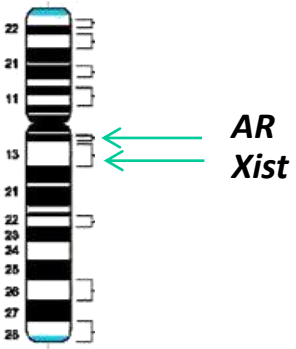
Inheritance in CDG
X- linked inheritance

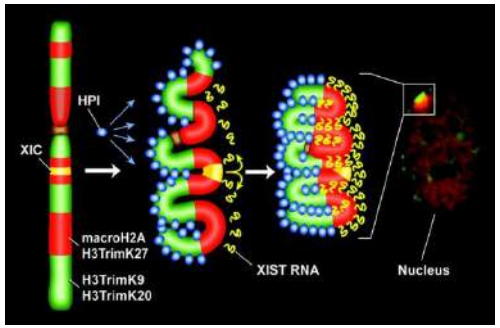
X-linked inheritance where the mother is a carrier for an X-linked recessive condition : SLC35A2- CDG



XIST gene
encoded at site of X inactivation center

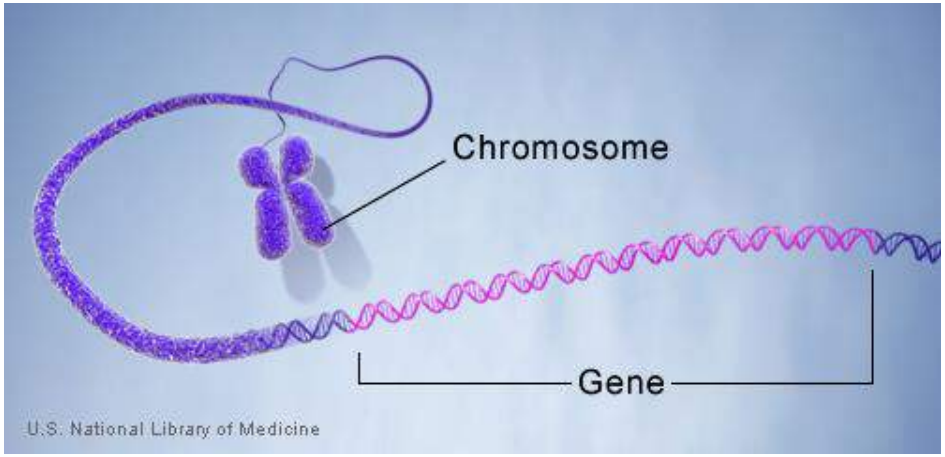


XIST RNA spread from X inactivation center



Genetic test?

- ✓ Medical test that identifies changes in chromosomes, genes, or proteins.
- ✓ The results of these tests can confirm or rule out suspected genetic conditions.



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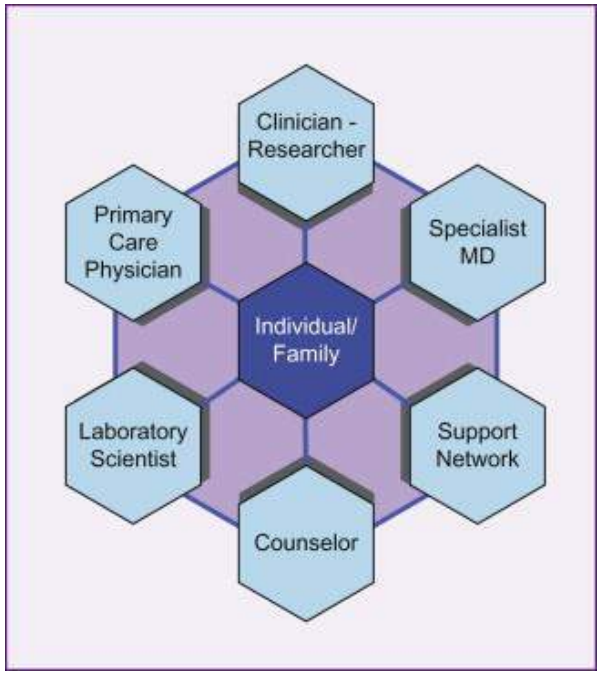
Molecular medicine team

The focus for molecular medicine is the individual and family.



DNA Genetic Testing
Molecular Medicine, Chapter 3, 81-115
Copyright © 2012 Copyright © 2012 Elsevier Inc.

- Depending on the type of DNA test other members of the molecular medicine team are needed.
- Ultimately, the primary care (family) physician will be the key professional
- Electronic health record (EHR) for data storage or a link to where data are stored.
- Important to allow all authorized team members to access EHR data and results and avoid unnecessary testing.

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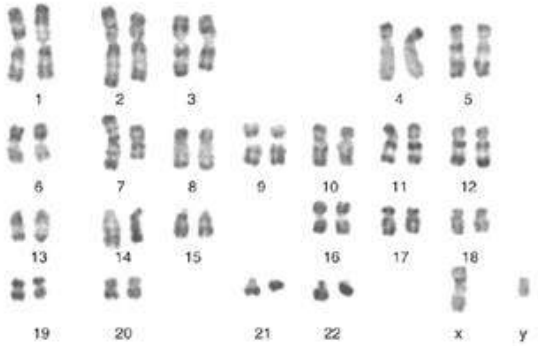
Types of genetic testing
From big to small detail…

✓KARYOTYPE

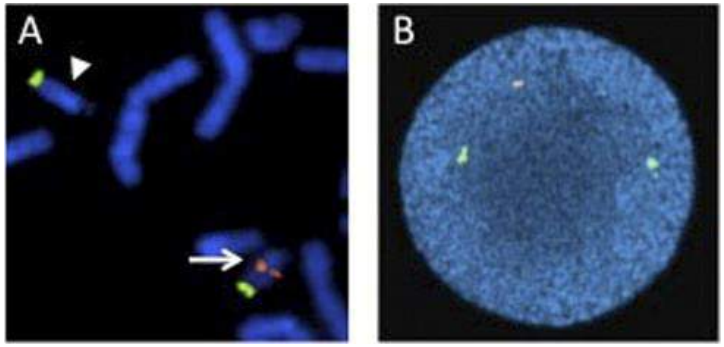
- A genetic test used to examine an individual's chromosome structure.



A karyotype of human chromosomes
© 2009 Nature Publishing Group Stamatoullas, A., *et al*. Conventional cytogenetics of nodular lymphocyte-predominant Hodgkin's lymphoma. *Leukemia* **21**, 2064–2067 (2009).

✓FISH (Fluorescence *In Situ* Hybridization)

- To assess for deletions/duplications for a suspected genetic syndrome, extra or missing chromosomes, and sex chromosomes
- A genetic test used to find small deletions or duplications in an individual's chromosomes.



Partial metaphase (A) and interphase (B) FISH images from a patient with the 22q11.2 (DiGeorge/Velocardiofacial) deletion syndrome.
Fluorescence In Situ Hybridization
Tsuchiya, Karen D., MD, Clinics in Laboratory Medicine, Volume 31, Issue 4, 525-542 Copyright © 2011 Elsevier Inc.

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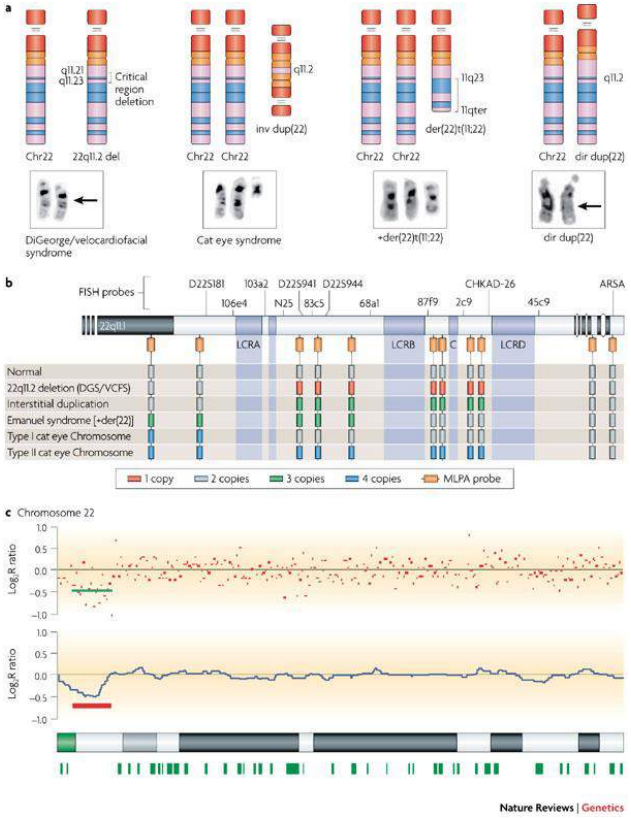
Types of genetic testing
From macro to micro…

✓CHROMOSOMAL MICROARRAY

A genetic test used to examine or quantify an individual's amount of genetic material; it can detect changes smaller than those found through FISH or karyotype



From microscopes to microarrays: dissecting recurrent chromosomal rearrangements Beverly S. Emanuel & Sulagna C. Saitta
Nature Reviews Genetics 8, 869-883 (November 2007)
doi:10.1038/nrg2136

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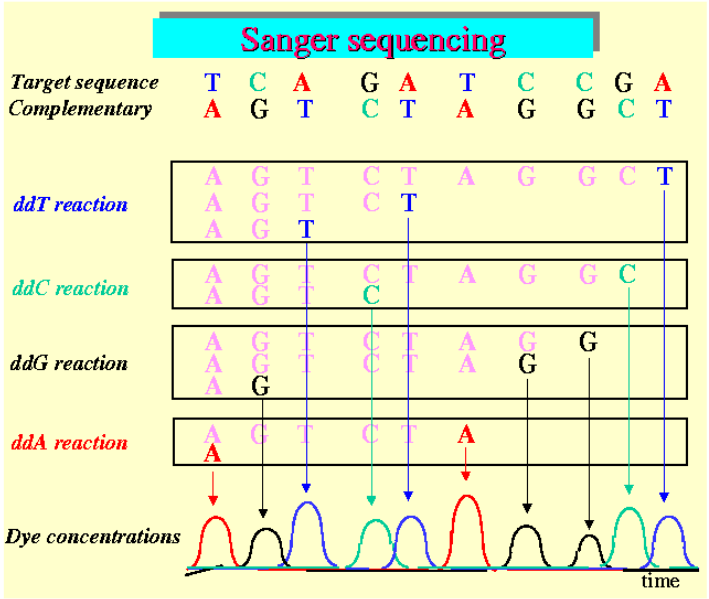
If I have a family history of CDG, should I have a genetic test? How is the CDG genetic test done?

Targeted mutation analysis - few specific genetic mutations within a single gene. Ex: known familial mutation, and/or e specific mutations common in certain ethnic groups for a specific disease



Single gene sequencing

- A genetic test used to examine a single gene's entire sequence at a high level of detail.
- Used for diagnostic purposes when a specific genetic syndrome is suspected

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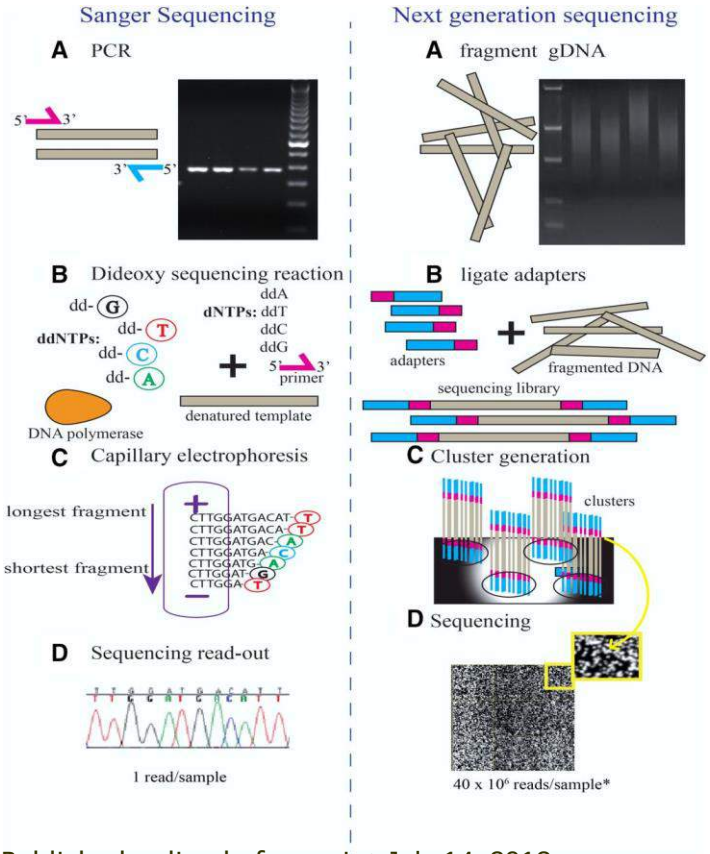
Types of genetic testing
From macro to micro…

- Targeted mutation analysis
- Single gene sequencing
- Multiple gene sequencing panel
- Whole exome sequencing
- Whole genome sequencing



Published online before print July 14, 2013, doi:10.1161/CIRCGENETICS.113.000085Circulation: Cardiovascular Genetics August 2013 vol. 6 no. 4 427-434

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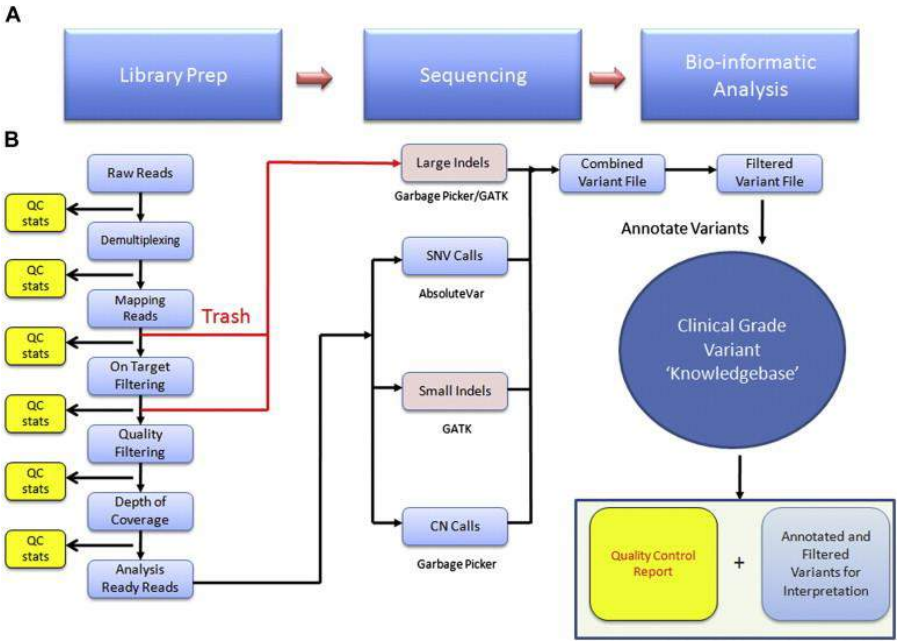
Types of genetic testing
From macro to micro…

<u>Whole exome sequencing</u>
All of the exons (i.e. coding regions) within all of the genes in an individual's genome.

<u>Whole genome sequencing</u>
Sequence an individual's entire genome (both coding and noncoding regions).
Individuals suspected of having an underlying genetic cause for their symptoms.



Understanding the limitations of next generation sequencing informatics, an approach to clinical pipeline validation using artificial data sets Daber, Robert, Cancer Genetics, Volume 206, Issue 12, 441-448 Copyright © 2014 Elsevier Inc

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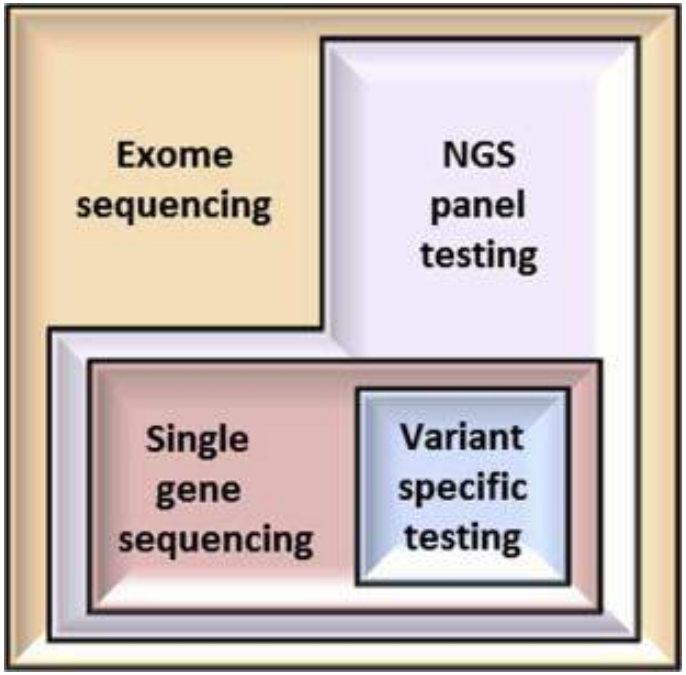
What have recent molecular biology strategies brought to CDG?



Gamut of Genetic Testing for Neonatal Care Ankala, Arunkanth, PhD, Clinics in Perinatology, Volume 42, Issue 2, 217-226
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- Variant specific testing interrogates known and highly frequent variant
- Single-gene sequencing interrogates the entire gene implicated with a disease.
- NGS panel testing involves sequencing of multiple genes associated with a specific disorder or phenotype.
- ES interrogates all of the genome regardless of the phenotype
  - Clinical presentation is atypical, complex or nonspecific
  - Disease specific panel not available.

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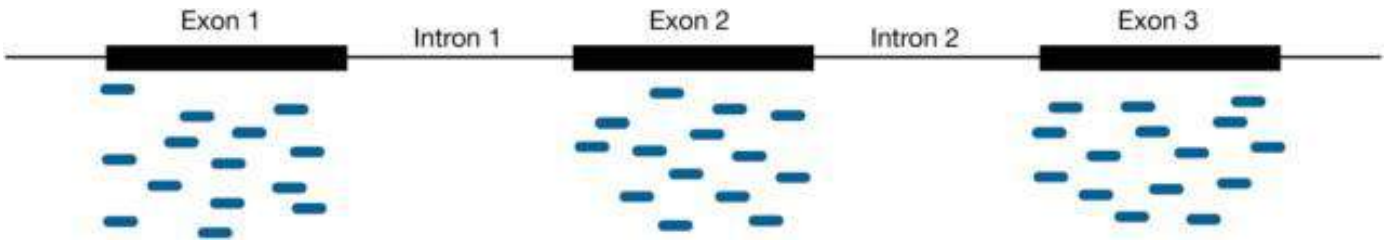
Whole exome sequencing – who is currently tested?

- Symptoms that cannot be diagnosed through continuous testing
- Multiple family members with similar symptoms/features without a specific diagnosis
- Multiple congenital anomalies/birth defects
- A history of multiple miscarriages and/or stillbirths
- An unexplained infant death
- General developmental delay/intellectual disability



Schematic illustrating gene exons targeted for whole-exome sequencing. Journal of Bone and Joint Surgery, Volume 95, Issue 23, e185(1)-e185(8) Copyright © 2013 The Journal of Bone and Joint Surgery, Inc

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What have recent molecular biology strategies brought to CDG?

- ✓ Exome and whole-genome sequencing
  - ✓ CDG-causing mutations in genes previously not associated with glycosylation
  - ✓ Broading CDG clinical scope even wider
  - ✓ Expanding our view on these complex pathways

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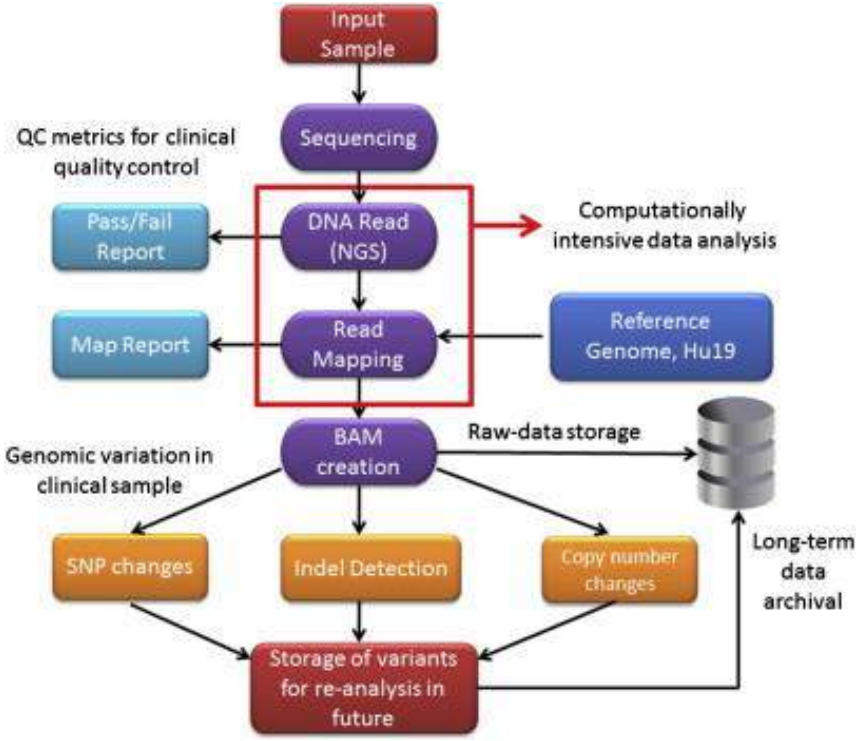
What do the results of genetic testing mean? How long does the genetic test take?

Whole exome sequencing

Whole genome sequencing

- Results in a large amount of data, more laborious.
- Some of which will be of uncertain significance; also can get results for other genetic diseases that are not related to the initial reason for testing.



Prototypical workflow in a clinical next generation sequencing laboratory.
Clinical Integration of Next-Generation Sequencing Technology
Gullapalli, R.R., MD, PhD, Clinics in Laboratory Medicine, Volume 32, Issue 4, 585-599
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Can I test my other children?

National regulations:
Portugal : Law n.º 12/2005 de 26 de Janeiro

Most bioethicists :

- Unless there is a clear benefit to the medical care of the child
- Genetic testing of asymptomatic children for a carrier state should be done only when the child is sufficiently old and mature, to decide whether to seek such testing.

- ASHG POSITION STATEMENT Points to Consider: Ethical, Legal, and Psychosocial Implications of Genetic Testing in Children and Adolescents The American Journal of Human Genetics 97, 6–21, July 2, 2015

"A Climb-on DNA Model" sourced under Creative Commons via Flickr from James Gentry.

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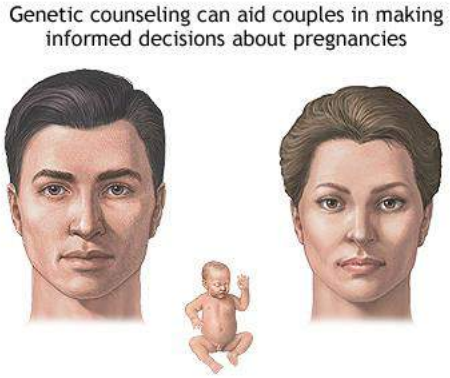
What is genetic counseling and why it is important?

- Comprehend the medical facts as the diagnosis, course of the disorder, and the available management.

Who should be tested?

- Understand the risk for recurrence for themselves and other family members.
- Choose the course of action that seems most appropriate
- Providing supportive counseling to families and making referrals to appropriate specialists, social services, and family and patient support groups.

http://learn.genetics.utah.edu/content/disorders/counselors/



https://www.nlm.nih.gov/medlineplus

Thank you

Portuguese Association Rare Metabolic Diseases

CDG

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