Inborn and Acquired Mutations

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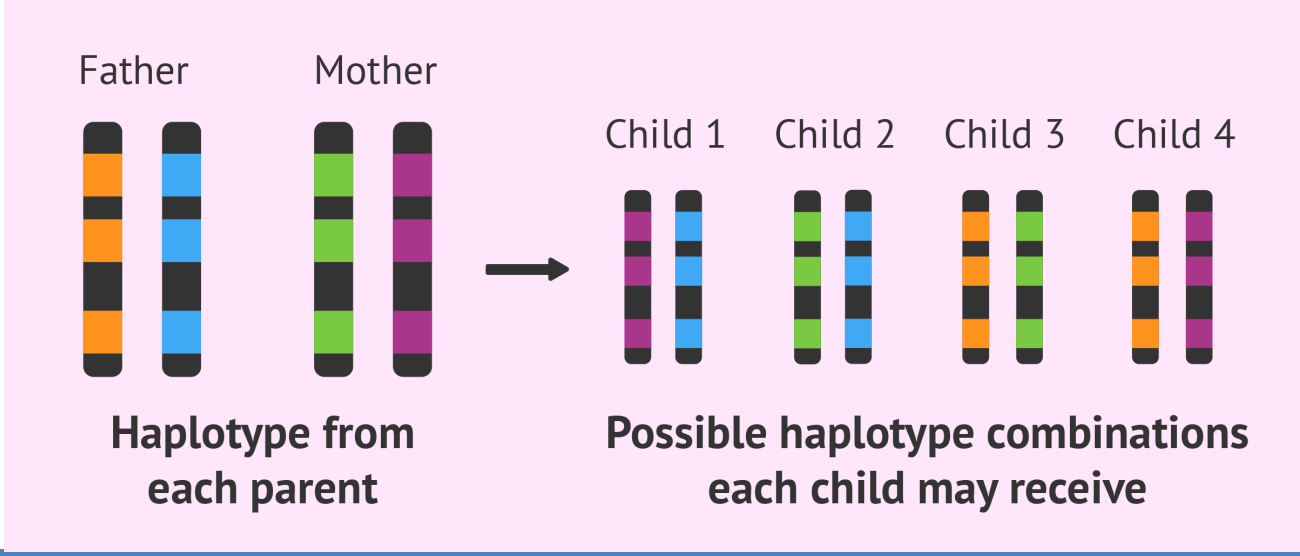
CLINICAL BIOMARKERS MODULE

Outline

- Genetic concepts for biomarkers
- Inherited variation and human disease
  - Newborn screening
  - Adult opt-in testing
- Acquired variation and human disease
  - Driver mutations in cancer
  - mtDNA heteroplasmy and disease
  - Infectious disease

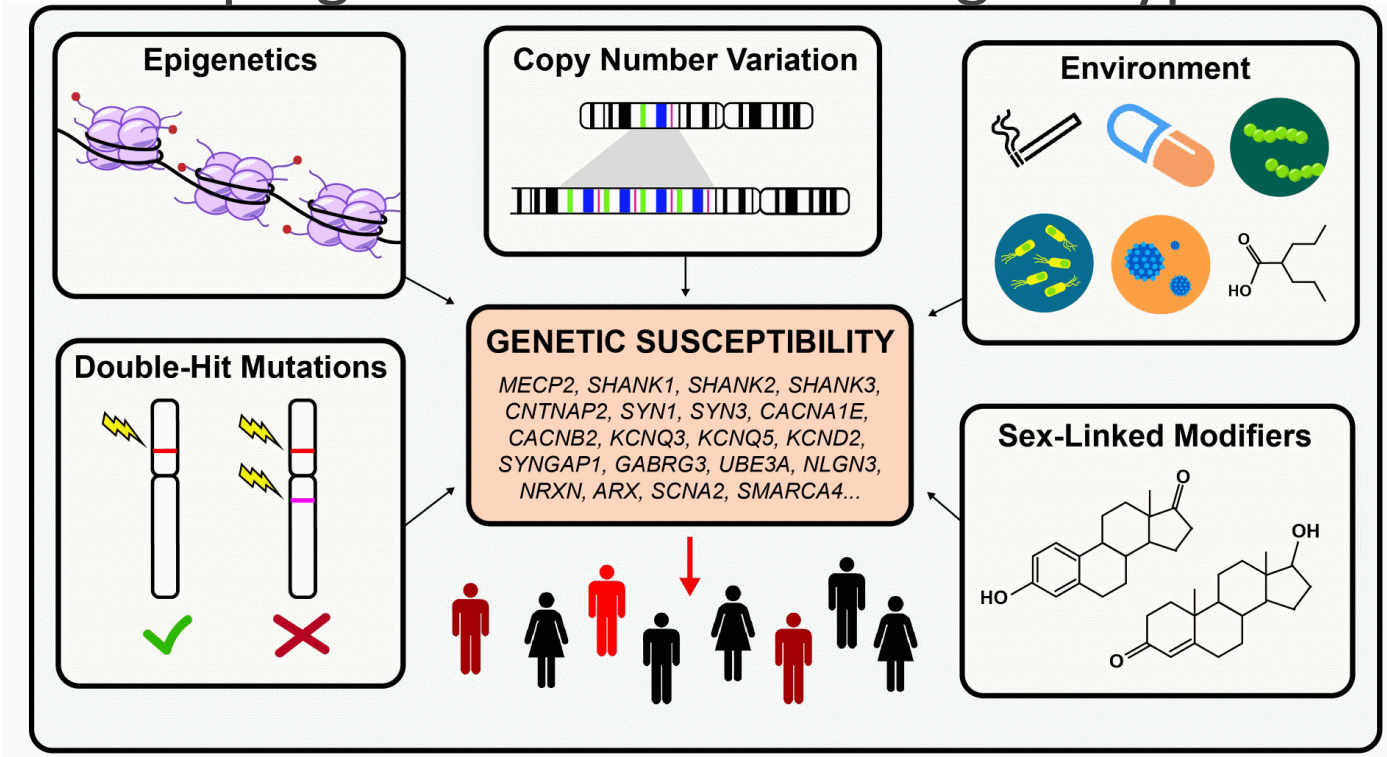
Fundamental Genetic Definitions

- An allele is a variant of a gene sequence.
- A person may be homozygous (2 copies of same allele) or heterozygous for each gene.
- Haplotype is a collection of variants inherited together.



https://www.invitra.com/en/savior-sibling/inheritance-of-hla-haplotypes/

- Penetrance: percentage of individuals with a genotype that exhibit its associated phenotype.
- Predisposition / Susceptibility: increased likelihood of developing a disease based on genotype



L Rylaarsdam et al. *Front. Cell. Neurosci*. (2019) 13: 385

GWAS odds ratios and p-values

- OR: association between genotype and being diseased via typical case-control study
- $OR = \frac{A/B}{X/Y}$ where

A = cases with disease genotype
B = controls with disease genotype
X = cases with WT genotype
Y = controls with WT genotype

- In general, an OR near 1 will result for non-associated loci. If OR is far from 1, it can be evaluated via χ^2 test to produce a p-value.
- $p < 5 * 10^{-8}$ often taken as "hits."

J Fadista et al. *Euro. J. Hum. Genet*. (2016) 24: 1202-1205.

What about polygenic traits?

- Missing heritability problem: GWAS-linked markers explain only a small fraction of phenotypic variation.
  - AI Young. *PLOS Genetics* (2019) 15: e1008222
- Familial aggregation: Does the disease cluster in families more than expected by chance alone?
  - AC Naj et al. *Meth. Mol. Biol.* (2012) 850: 119-150
- Epistasis: Multiple loci interact, mask each other, or modify each other's effects.
  - I Miko. *Nature Educ.* (2008) 1:197

Newborn screening

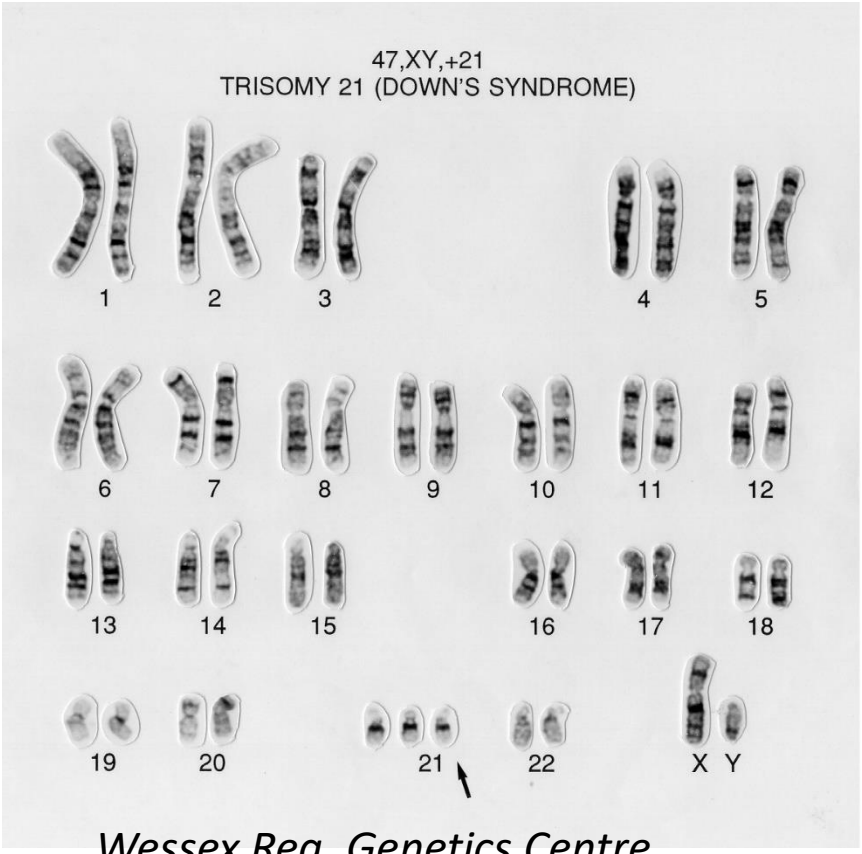
micahboygenius.wordpress.com

- Amniocentesis (15-16 weeks) / chorionic villus sampling (11 weeks)
  - karyotype for Trisomy, aneuploidy, Fragile X
  - Sequence variants: Sickle cell (HBB), cystic fibrosis (CFTR), muscular dystrophy (DMD sex-linked), Tay-Sachs (HEXA), Critical congenital heart disease

https://ghr.nlm.nih.gov/primer#newbornscreening

A type of aneuploidy

Down Syndrome: Trisomy 21

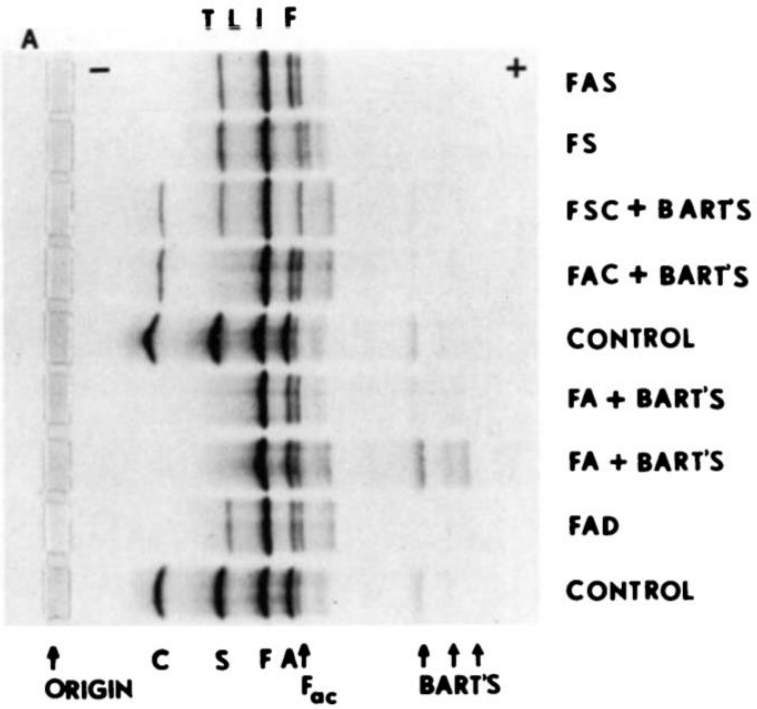


Wessex Reg. Genetics Centre

- Nondisjunction leads to three copies of chromosome 21.
- Maternal age linked.
- Screening via PAPP-A and HCG + ultrasound
- Chorionic villus or amniocentesis cells allow karyotype.

DS Newberger, *Am. Fam. Physician*. (2000) 62: 825-832.

Hemoglobin electrophoresis screens for sickle-cell allele



- Hemoglobin A is common adult type.
- F is fetal, falling off soon after birth.
- S is sickle, two copies of which cause disease.
- C causes anemia when homozygous.

F Galacteros et al. *Blood* (1980) 56: 1068-1071.

MS/MS-based screening for inborn errors of metabolism

- *Amino Acidopathies:*
  Phenylketonuria, Tyrosinemia, Maple Syrup Urine Disease, Homocystinuria, Hypermethioninemia, Citrullinemia
- *Organic Acidemias:*
  Glutaric aciduria, Propionic acidemia, Methylmalonic acidemia, Isovaleric acidemia
- Fatty Acid Oxidation and Ketogenesis Defects

PE Karam et al. *Clinical Biochem*. (2013) 46: 1787-1792.

PKU: Phenylketonuria

"In 1934, the mother of two intellectually impaired children approached [Asbjørn] Følling to ascertain whether the strange musty odour of her children's urine might be related to their intellectual impairment."

Required label (in USA) because aspartame sweetener contains Phe. Image by Emily Burke

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Why might adults choose genetic testing?

- Investigate family history of disease / explain family member's death
- Guide future medical management / motivate changes in diet and exercise
- Allay concerns about current family and potential children
- Relieve uncertainty regarding diagnosis

KE Erskine et al. *J. Genet. Couns*. (2015) 23: 849-859.

B2BU

BE HAPPY TO BE YOU

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- Direct-to-consumer genetic screening, sited at CPGR / Artisan Biomed in Cape Town
- Measures 700,000 SNPs via Infinium Global Screening Array (Illumina BeadChip)
- Seeks alleles associated with disease risk
- Offers ancestry evaluation to South Africans
- Enriches data for African genetic diversity

Huntington Disease (1983)

```
>HTT-201 ENSE00001251499 exon:protein_coding
GCTGCCGGGACGGGTCCAAGATGGACGGCCGCTCAGGTTCTGCTTTTACCTGCGGCCCAG
AGCCCCATTCATTGCCCCGGTGCTGAGCGGCGCCGCGAGTCGGCCCGAGGCCTCCGGGGA
CTGCCGTGCCGGGCGGGAGACCGCCATGGCGACCCTGGAAAAGCTGATGAAGGCCTTCGA
GTCCCTCAAGTCCTTCCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCA
GCAGCAGCAGCAGCAACAGCCGCCACCGCCGCCGCCGCCGCCGCCGCCTCCTCAGCTTCC
TCAGCCGCCGCCGCAGGCACAGCCGCTGCTGCCTCAGCCGCAGCCGCCCCCGCCGCCGCC
CCCGCCGCCACCCGGCCCGGCTGTGGCTGAGGAGCCGCTGCACCGACC
```

- CAG repeats <36 times for normal phenotype.
- Different tissues may show different repeat counts (a *mosaic*).

- Age of onset is 35-45 for most cases; after ~15 year progression, disease is fatal.
- *Knowing we have neither treatment nor cure, would you want to know your status?*

JM Lee et al. *Neurology* (2012) 78: 690-695.

"Our group, similar to other studies, showed a general predominance of females requesting testing, and we had more females present for testing than would be expected based on the general population. Multiple potential reasons for this predominance have been proposed, including that women are more invested in the reproductive decision-making process and the rearing of children, women are more willing to make difficult decisions and deal with the consequences of those choices, and women may be better able to cope with negative results."

RC Krukenberg et al. *J. Genet. Counsel*. (2013) 22: 643-653.

intended to prevent disease

Prophylactic mastectomy for people with BRCA mutations (1994)

- Predisposition to breast cancer and other cancers is associated with particular BRCA1 and BRCA2 alleles.
- Elective mastectomy can decrease odds of disease.
- "My chances of developing breast cancer have dropped from 87 per cent to under 5 per cent."

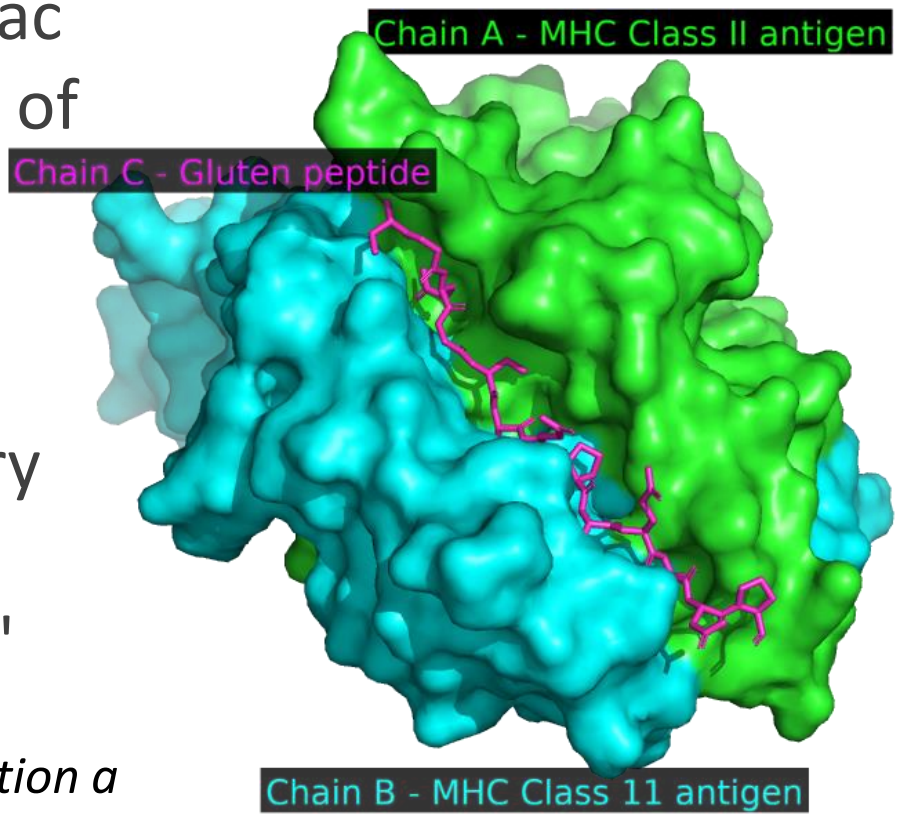
https://www.nytimes.com/2013/05/14/opinion/my-medical-choice.html Angelina Jolie by Gage Skidmore

JW Jakub et al. *JAMA Surgery* (2018) 153: 123-129.

Antigen presentation by HLA-DQ complex is key to celiac disease

"more than 90% of celiac patients carry a variant of DQ2, encoded by DQA1*05/DQB*02, whereas most of the remaining patients carry DQ8, encoded by DQA1*03/DQB1*0302"

haplotype



2NNA image by Kimberly Coetzer

Is wheat, barley, and rye consumption a trigger for celiac disease?

S. Tollefsen et al. *J. Clin. Invest*. (2006) 116: 2226-2236.

Intermission

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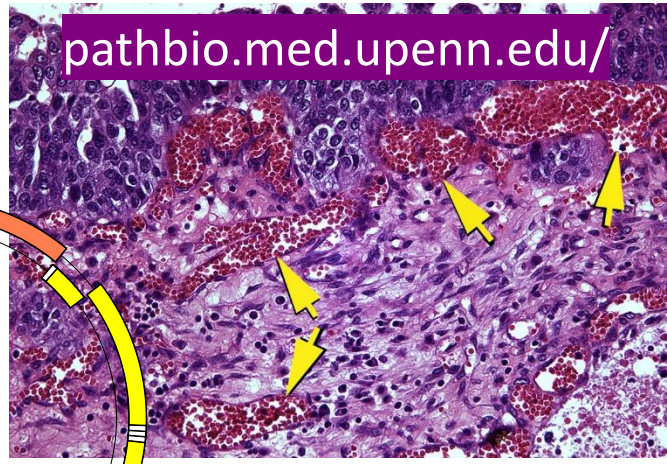
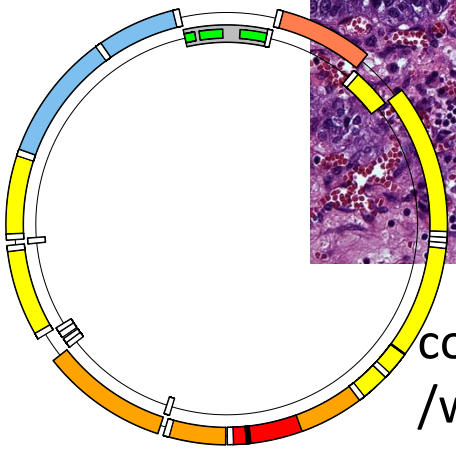
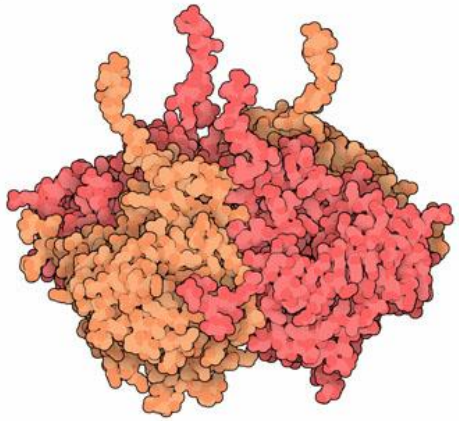
Diseases associated with acquired or sporadic mutation

- Cancer and tumor mutation acquisition
- Mitochondrial DNA, heteroplasmy, and neurodegenerative disease
- Infectious disease: genetic diversity of microbes and viruses; drug resistance





commons.wikimedia.org /wiki/User:Manudouz



Influenza neuraminidase
pdb101.rcsb.org/motm/113

Driver mutations speed cancer progression

- TP53: tumor suppressor that responds to diverse stresses, regulates cell cycle and apoptosis
- PIK3CA: oncogene and kinase associated with AKT and mTOR pathways
- KRAS: oncogene and GTPase with isoforms that misbehave in wrong tissues
- PTEN: tumor suppressor and phosphatase and repressor of AKT/PKB signaling
- ARID1A: helicase and chromatin remodeler

MH Bailey et al. *Cell* (2018) 173: 371-385.

Why do we sequence cancerous tumors?

- BRCA:
  - luminal A (ER+/HER2-)
  - luminal B (ER+/HER2+)
  - HER2E (ER-/HER2+)
  - basal-like (ER-/HER2-)

Estrogen receptor

Epidermal Growth Factor Receptor

- "triple negatives" are PR-, as well.

Progesterone receptor

No receptor, no hormone therapy.

- Colo-Rectal Cancers: hypermutated (16%)
  - microsatellite instability
  - hyper-methylation
  - MLH1 silencing

Part of DNA Mismatch Repair

Immunotherapy opportunity.

TCGA, *Nature* (2012) 490: 61-70

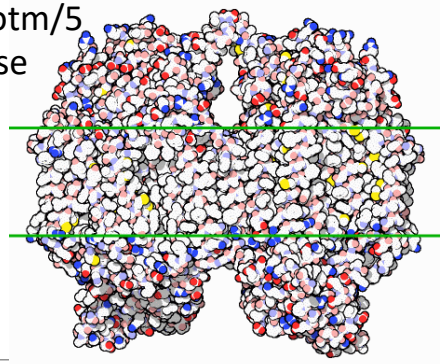
TCGA, *Nature* (2012) 487: 330-337

My Cancer Genome®

Genetically Informed Cancer Medicine

- My Cancer Genome is a precision cancer medicine knowledge resource for physicians, patients, caregivers and researchers.
- My Cancer Genome gives up-to-date information on what mutations make cancers grow and related therapeutic implications, including available clinical trials.
- My Cancer Genome is a one-stop tool that matches tumor mutations to therapies, making information accessible and convenient for busy clinicians.

pdb101.rcsb.org/motm/5
Cytochrome c oxidase



Mitochondrial DNA role and defining heteroplasmy

- mtDNA is only 16.6 kbp, but it encodes genes essential to oxidative phosphorylation.
- Failures of OXPHOS lead to production of reactive oxygen species (ROS).
- A cell may contain 1000 mitochondria, each with its own copy of the mtDNA genome.
- Heterogeneity of mtDNA in a cell is called heteroplasmy.

N Nissanka and CT Moraes, *FEBS Letters* (2018) 592: 728-742.

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Do mtDNA mutations lead to neurodegenerative disease?

- Over years, mtDNA replication introduces somatic mutations. Common deletions can reduce mitochondrial transcriptional activity.
- Brain regions such as *substantia nigra* are associated with mtDNA damage in aging.

Such as Parkinson's →

- Lewy body disease, Alzheimer's disease, and stroke have been associated with mitochondrial dysfunction.

The brain accounts for ~60% of glucose use by the whole body in the resting state.
ncbi.nlm.nih.gov/books/NBK22436/

MJ Keogh and PF Chinnery, *Biochmica et Biophysica Acta* (2015) 1847: 1401-1411.

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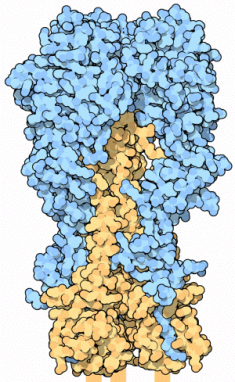
Why do we need a new influenza vaccine each year?

- Animal reservoirs contribute viruses that reassort with human ones, and viruses acquire mutations in human populations.
- Two representative influenza A (H3N2 and H1N1) and one influenza B must be selected.
- WHO chooses strains in February for Northern hemisphere and September for Southern hemisphere.



https://pdb101.rcsb.org/motm/76
Influenza hemagglutinin

C. Gerdil. *Vaccine* (2003) 21: 1776-1779.

Multiply drug resistant

Extensively drug resistant

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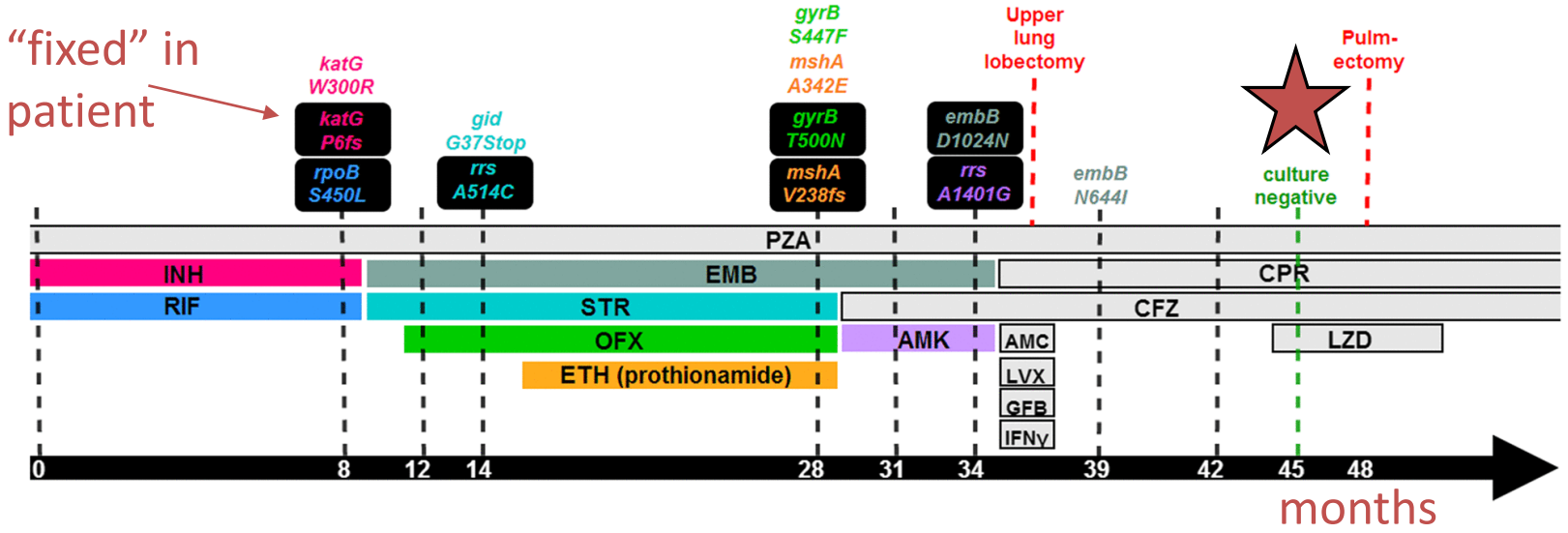
TB can become MDR or XDR through acquired mutation



INH=isoniazid STR=streptomycin AMK=amikacin

RIF=rifampicin OFX=ofloxacin CPR=capreomycin

PZA=pyrazinamide ETH=ethionamide LZD=linezolid

EMB=ethambutol CFZ=clofazimine

V. Eldholm et al. *Genome Biology* (2014) 15:490

Takeaway messages

- Generally, the alleles you carry do not *determine* your fate, though they may *predispose* you to particular diseases.
- You may have been born with a DNA variant or you may acquire it by a variety of means; the same goes for infectious agents.
- When we say every cell contains exactly the same DNA as every other cell, *we lie*!