GENETICS 2019
OUR DNA IS CHANGING

YOU CAN'T CHOOSE
YOUR GENES (YET)
BUT
TODAY YOU CAN CHOOSE
WHAT WE DISCUSS

May, 2019

William R Rooney MD
SCOR

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The Art & Science of Risk

AGENDA

Opportunities
Genetics
Challenges

1. **Important Concepts in Genetics**

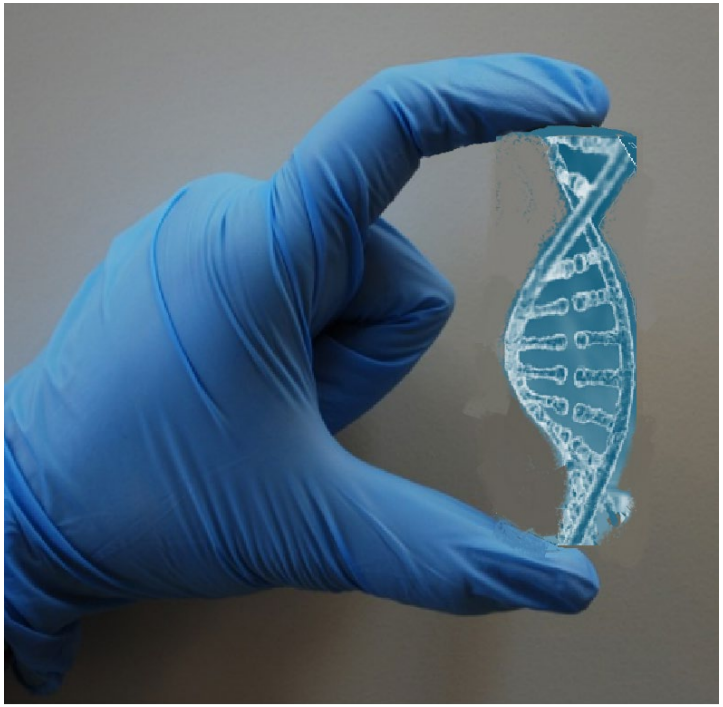
   Genetic Structure and Disease

2. **Direct-to-Consumer Genetic Testing**

   No need for clinicians.
   Just send in your DNA.

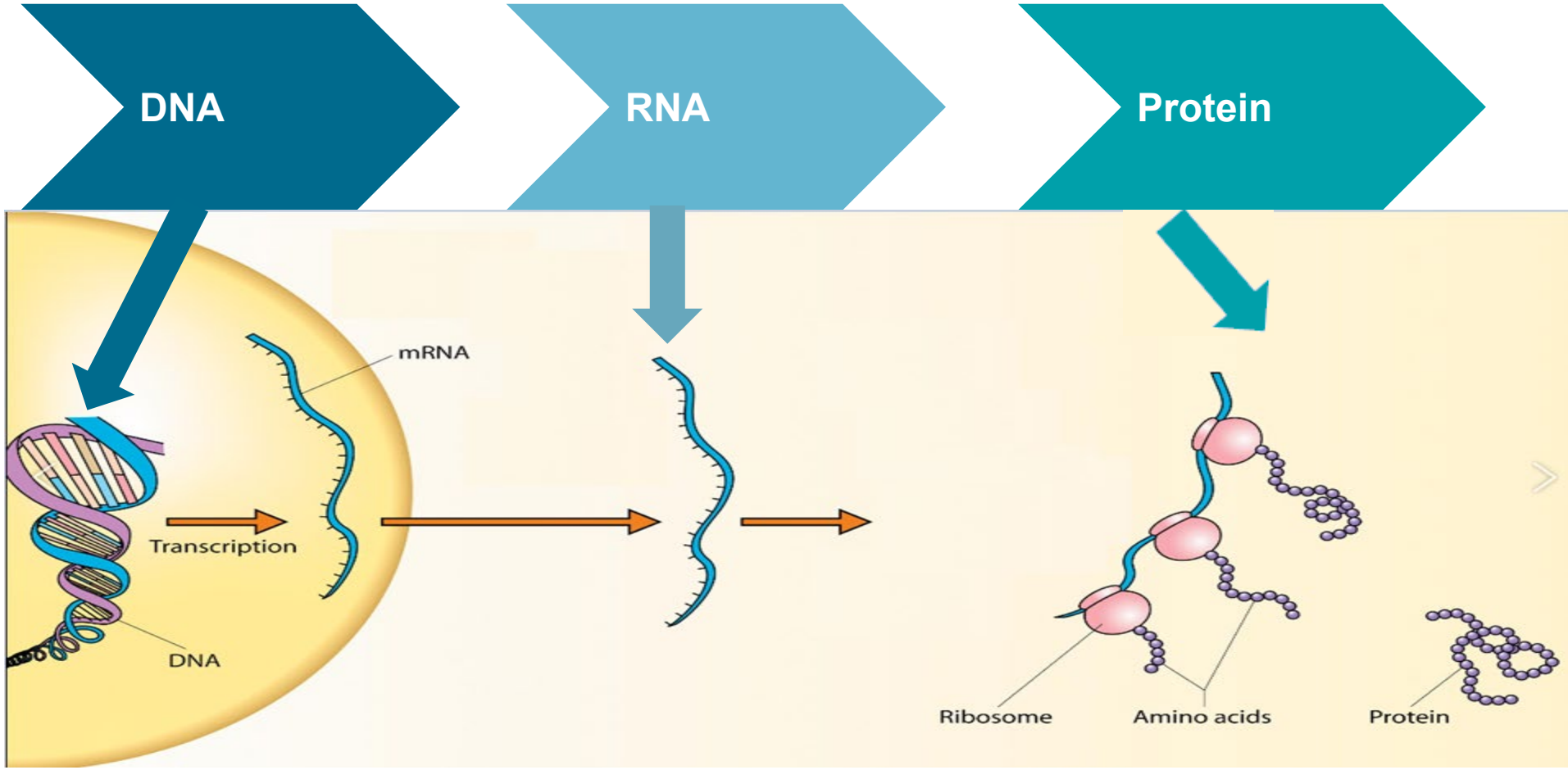
3. **Pick Your Presentation**

   You choose the topic



Important Genetic Concepts

The Central Dogma



- https://en.wikipedia.org/wiki/Protein Obtained 8/21/2018 using Bing's "free to modify, share, and use commercially" search engine

Sequence Matters

Polymer of sugar and phosphate groups

Nucleobases

- Adenine
- Thymine
- Cytosine
- Guanine

Deletions

Insertions

Large genomic rearrangements

Switches

Repeats

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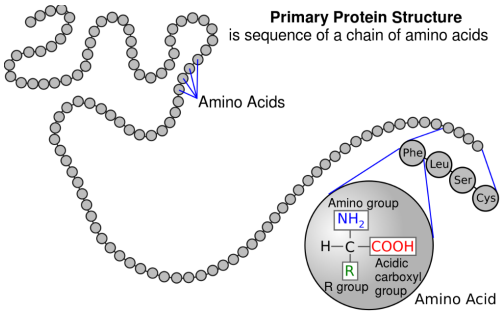
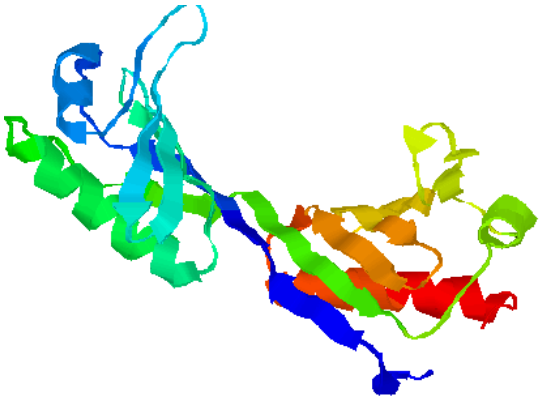
Important Concept:

"The DNA sequence dictates RNA sequence which dictates the structure of protein"





Abnormal mRNA

Abnormal Protein

Protein function

- Cell Structure
- Cell Function
- Cell Regulation

Examples of Protein function:

- Antibodies
- Enzymes
- Structural component
- Transport

Sequence Does Matter!

1) https://upload.wikimedia.org/wikipedia/commons/thumb/3/38/Protein_primary_structure.svg/1280px-Protein_primary_structure.svg.png Image downloaded 8/5/16 from Bing "free to modify, share, and use commercially
2) https://www.bing.com/images/search?q=protein+structure&view=detailv2&qft=+filterui%3alicense-L2_L3&adlt=strict&id=327BD63212E32BB7A857458B7357B1C1F3E7F31B&selectedIndex=11&ccid=Ft2uSk4j&simid=608047493385817479&thid=OIP.M16ddae4a4e23e52d43fc31b9b602833

Human Genome Project

1990

The NIH, the Dept. of Energy, and an international team launched the Human Genome Project.

- **The goal was to sequence the human genome.**

2003

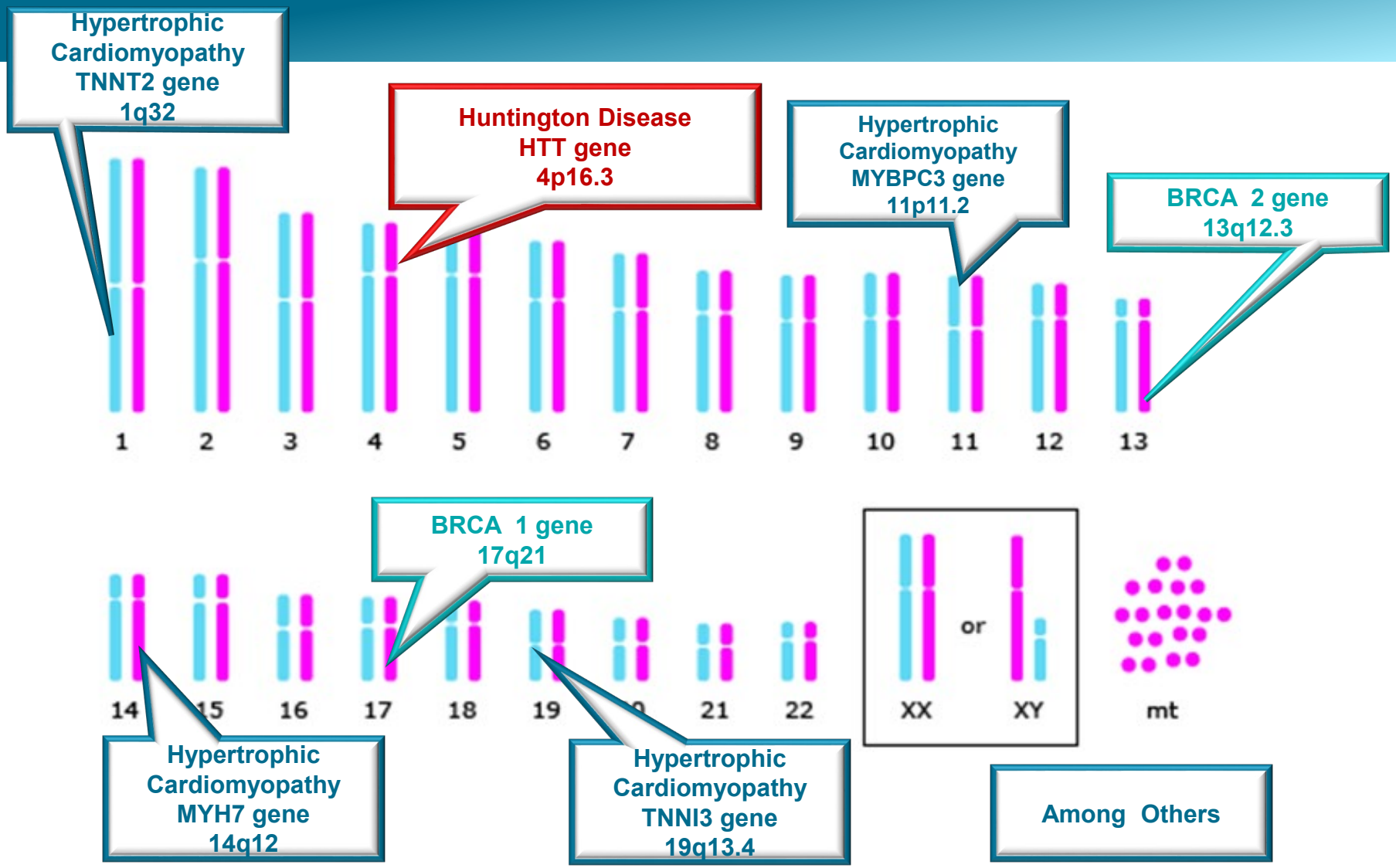
Researchers completed the project

Cost for sequencing a human genome

2004	~$28,800,000
2019	~$1,000

Topol, Eric Individualized Medicine from Prewomb to Tomb. Cell 157 March 27,2014

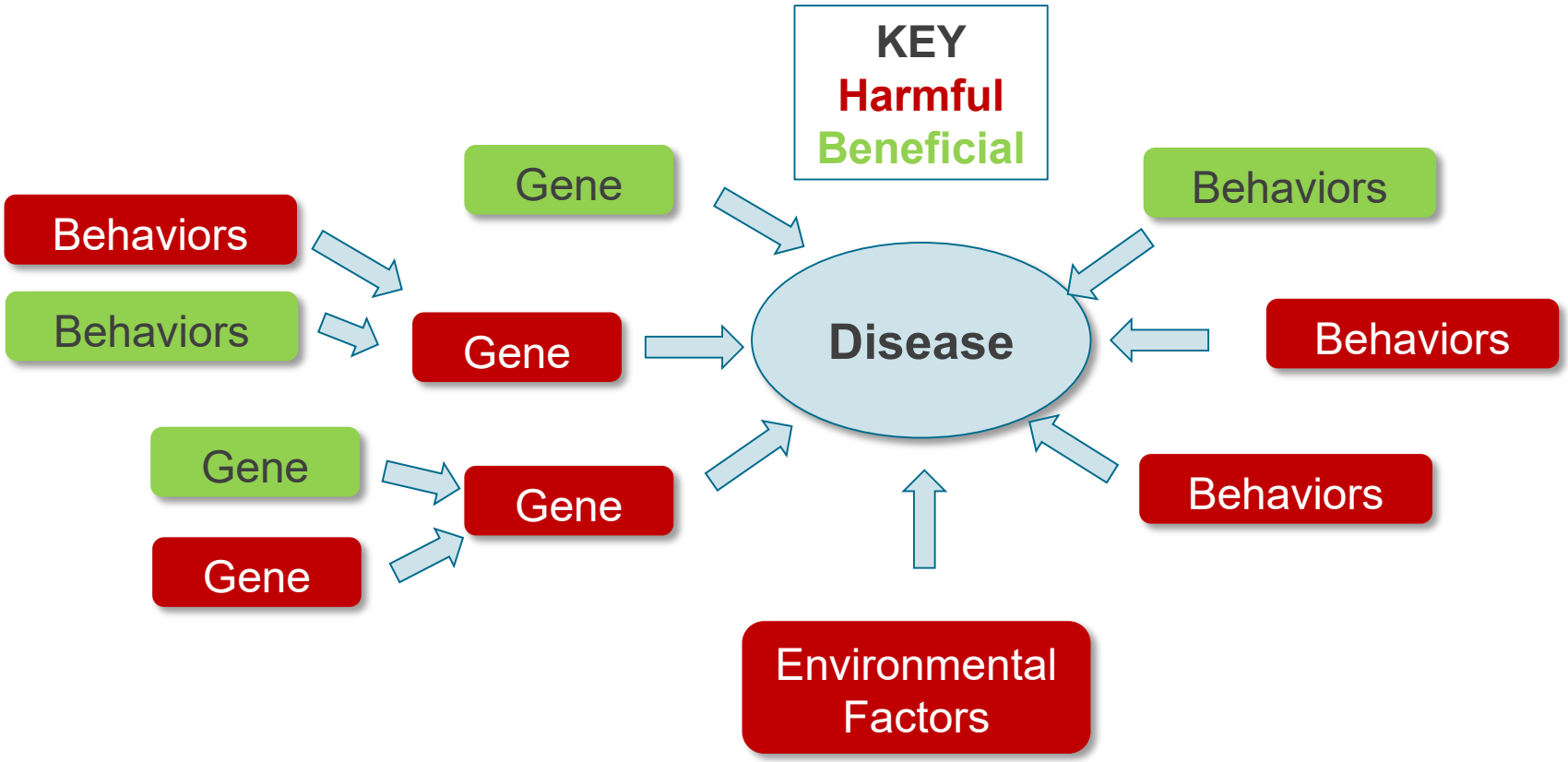
Examples of diseases with known gene locations



Concept

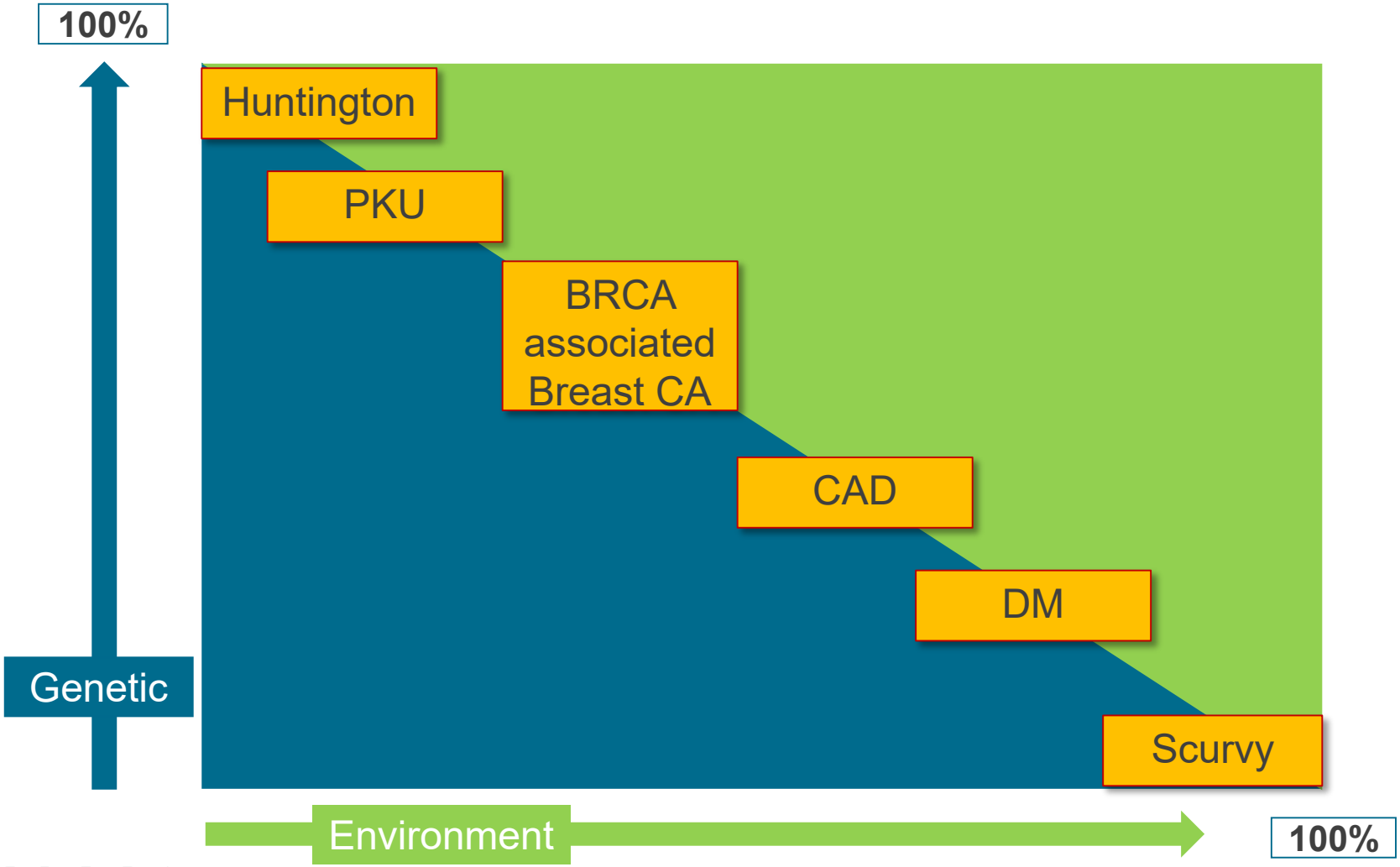
"Interaction of Many Factors"

Diseases can be a combo of one or more genes, one or more behaviors, and one or more environmental factors, with both good and harmful effects.



Concept

"Spectrum"



Concept:
Clinical Validity and Clinical Utility

Clinical Validity

The ability of a genetic test to predict a phenotype

✓ APC gene: Familial adenomatous polyposis	~ 100%
✓ MMR gene: Lynch syndrome	~ 80%
✓ APOE e4/e4: Alzheimer disease	~ 30%

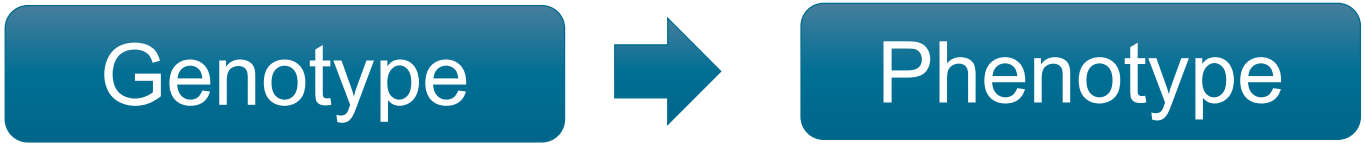
Clinical Utility

The impact of the genetic test on clinical care

✓ Huntington	Minimal
✓ Lynch syndrome:	Significant
✓ BRCA	Significant

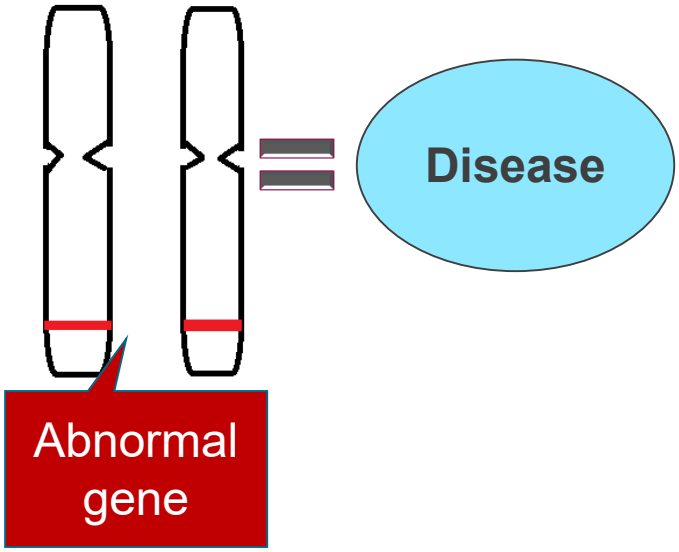
Concept:
"Finding a mutated gene doesn't necessarily help"

- Gene found → Disease happens
- Gene not found → Disease absent



- Ideally, this would be the case 100% of the time for predictive purposes. But….not so fast.

Important Concept:
"Multiple variables influence the impact of genetic expression"



Penetrance

- ❑ Full
- ❑ Variable

Variable expressivity

- ❑ Age
- ❑ Mutation type
- ❑ Genetic heterogeneity
- ❑ Environmental exposure
- ❑ Parent-of-origin effects (i.e. Prader-Willi Syndrome)
- ❑ Sex-limited expression (i.e. male pattern baldness)
- ❑ Anticipation
- ❑ Mosaicism

Testing

I want to have a healthy baby

Carrier testing
Prenatal testing
Preimplantation testing

I know my family member has a disease, I wonder if I have it too

Predictive and pre-symptomatic testing

I am not feeling well

Diagnostic testing

I am very curious

Average risk testing

Should I buy insurance….life?
Critical care?
Long term care?

Genetic Testing Statistics

- Genetic testing available for over 2,000 conditions
- Available in over 500 laboratories

The CDC

- Developed a Public Health Genomics website and classifies many genetic testing into 3 tiers. Tier 1 genomic applications are defined as "those having significant potential for positive impact on public health based on available evidence-based guidelines and recommendations". The first 3 conditions on the list (2 million Americans potentially have one of the three)
  - Hereditary Breast and Ovarian Cancer Syndrome
  - Lynch Syndrome
  - Familial hypercholesterolemia

The American College of Medical Genetics and Genomics (ACMG)

- Named 59 genes which when a genetic sequence is abnormal "would result in a high likelihood of severe disease that is preventable if identified before symptoms occur".

1) NIH statistics…page last updated 6/2018. https://report.nih.gov/nihfactsheets/ViewFactSheet.aspx?csid=43&key=G
2) https://www.cdc.gov/genomics/implementation/toolkit/tier1.htm

American College of Medical Genetics list of genes for which secondary findings should be disclosed

Disease category	Syndrome	Gene(s)
Cancer	Breast/ovarian cancer	*BRCA1*, *BRCA2*
	Li-Fraumeni syndrome, Peutz-Jeghers syndrome; Juvenile polyposis, PTEN hamartoma syndrome	*TP53*, *STK11*, *SMAD4**, *BMPR1A**, *PTEN*
	Lynch syndrome, familial adenomatous polyposis, MYH-associated polyposis	*MLH1*, *MSH2*, *MSH6*, *PMS2*, *APC*, *MUTYH*
	Von Hippel Lindau syndrome; retinoblastoma, tuberous sclerosis, Wilms tumor	*VHL*, *RB1*, *TSC1*, *TSC2*, *WT1*
	Multiple endocrine neoplasia 1 or 2; familial medullary thyroid cancer	*MEN1*, *RET*
	Hereditary paraganglionoma-pheochromocytoma syndrome, neurofibromatosis type 2	*SDHD*, *SDHAF2*, *SDHC*, *SDHB*, *NF2*
Cardiovascular disease	Hypertrophic or dilated cardiomyopathy	*MYBPC3*, *MYH7*, *TNNT2*, *TNNI3*, *TPM1*, *MYL3*, *ACTC1*, *PRKAG2*, *GLA*, *MYL2*, *LMNA*
	Catacholamenergic polymorphic ventricular tachycardia, arrhythmogenic right ventricular cardiomyopathy, Romano-Ward Long QT syndromes, Brugada syndrome	*RYR2*, *PKP2*, *DSP*, *DSC2*, *TMEM43*, *DSG2*, *KCNQ1*, *KCNH2*, *SCN5A*
	Familial hypercholesterolemia	*LDLR*, *APOB*, *PCSK9*
Connective tissue or vascular integrity	Ehlers Danlos syndrome	*COL3A1*
	Marfan syndrome, Loeys-Dietz syndrome, familial thoracic aortic aneurysms and dissections	*FBN1*, *TGFBR1*, *TGFBR2*, *SMAD3*, *ACTA2*, *MYH11*¶
Malignant hyperthermia sensitivity		*RYR1*, *CACNA1S*
Metabolism	Wilson disease (copper metabolism)	*ATP7B**
	Ornithine transcarbamylase deficiency (urea cycle)	*OTC**

This list includes genes identified by the American College of Medical Genetics and Genomics (ACMG) as clinically actionable when known pathogenic (and, in some cases, expected pathogenic) variants are identified by whole genome or exome sequencing. Refer to UpToDate topics on genetic counseling and secondary findings from genomic testing for further details.

ACMG: American College of Medical Genetics and Genomics.
* Added in the 2016 revision (ACMG 2.0).
¶ *MYLK* was removed in the 2016 revision due to lack of an effective confirmatory test or intervention to improve outcomes.

Prepared with data from:

1. *Green RC, Berg JS, Grody WW, et al. ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. Genet Med 2013; 15:565.*
2. *Kalia SS, Adelman K, Bale SJ, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): A policy statement of the American College of Medical Genetics and Genomics. Genet Med 2017; 19:249.*

Important Concept:
"There are many genetic testing techniques"

Biochemical testing

- Protein analysis/Enzyme assays/Analytes

DNA analysis

- Real-time PCR
  Small number of genes tested
- High-density DNA array testing
  Analyze for 1,000,000 gene variants
- Highly efficient DNA sequencing techniques
  (next generation sequencing)
  Entire human genome

Important Concept:
"There are many types of genetic testing"

Diagnostic testing	**Symptoms lead to testing**
Prenatal Testing	Offered to all pregnant women
Newborn Testing	Performed on all newborns (~4 million births per year)
Carrier Testing	Offered to most couples seeking prenatal care
Predictive and pre-symptomatic testing	? Number done annually Done mostly for family members with strong FH or after tumor testing
Direct to Consumer Testing	5 million total thus far— Trending higher
Preimplantation genetic Testing	Offered to most of the 78,000 babies born annually by IVF (7 million living in the United States)
Pharmacogenomic Testing	• The FDA mentions use of pharmacogenomic testing for 250 meds • Medicare, Medicaid and private insurance don't typically pay except in unusual conditions. • 23 and Me FDA approved now…but haven't offered the test yet.
Research Genetic Testing	

Direct-to-Consumer Genetic Testing

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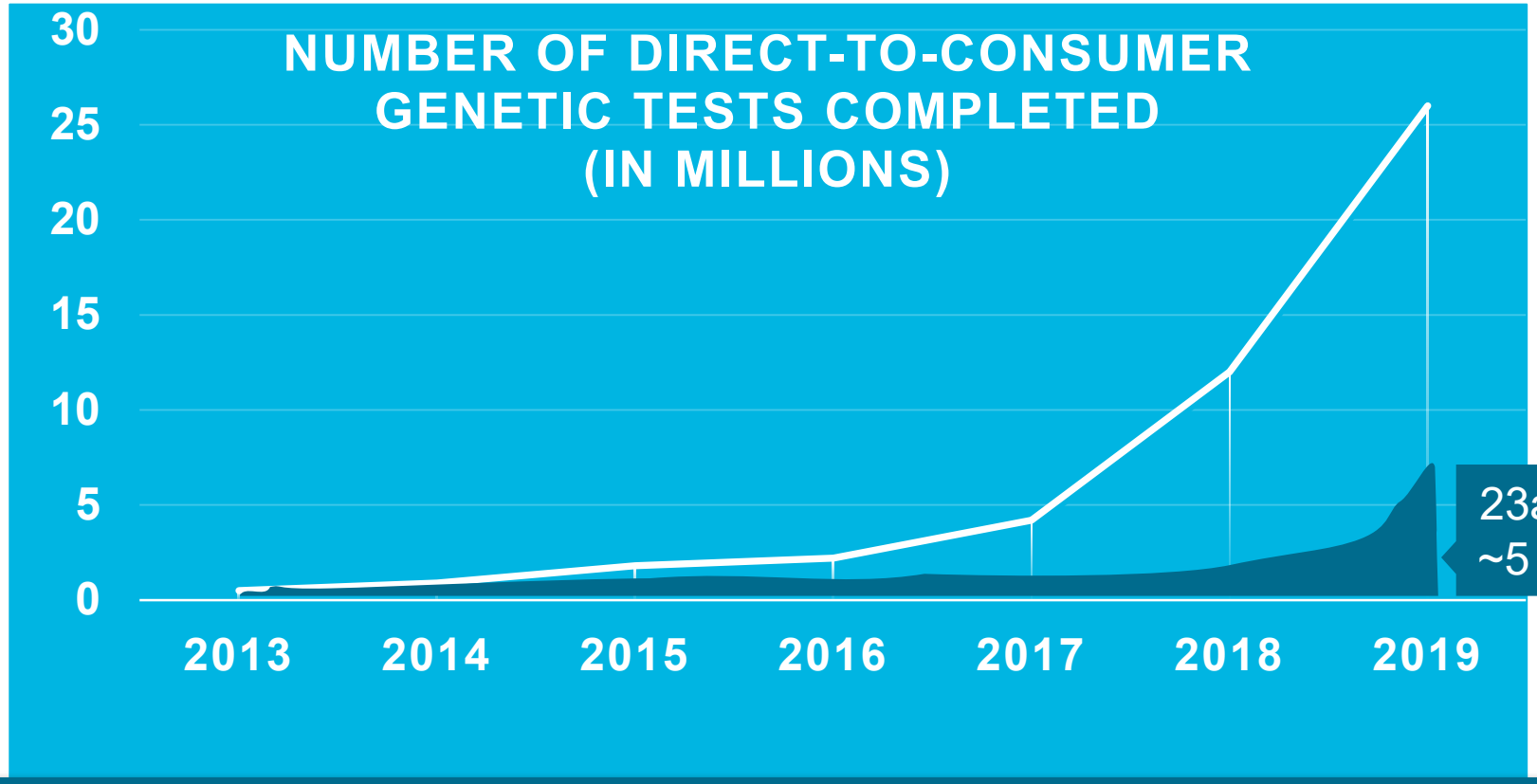
How many Direct to Consumer tests?

Ancestry and 23andMe



Multiple companies involved
(e.g. Family Tree DNA, My Heritage, Ancestry.com, 23andMe)

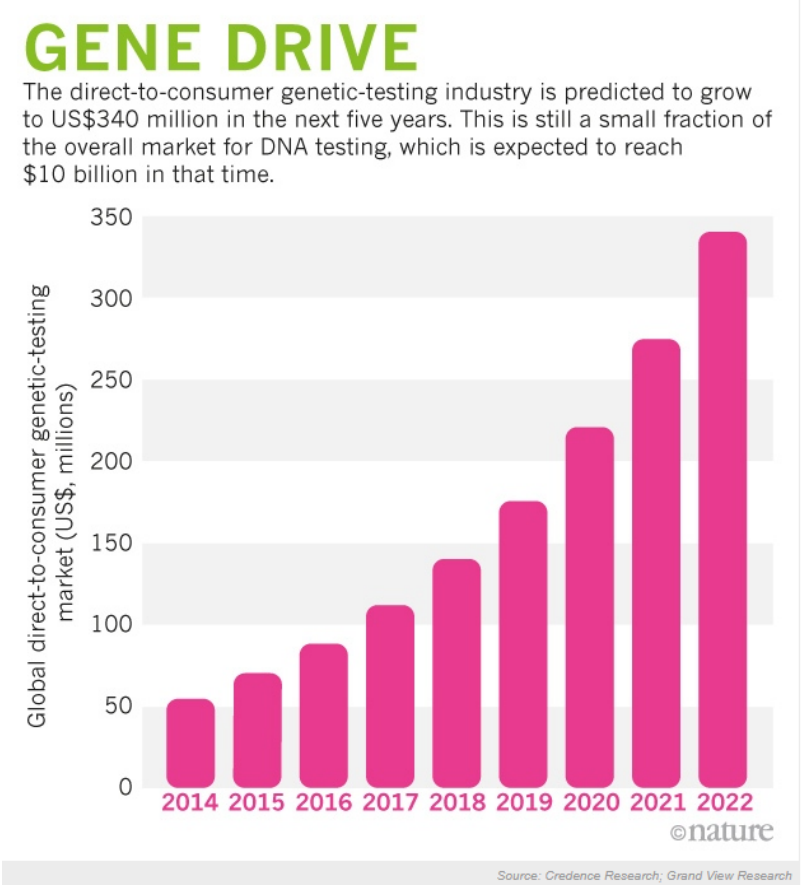
1) https://www.technologyreview.com/s/610233/2017-was-the-year-consumer-dna-testing-blew-up/

Direct-to-Consumer Genetic Testing
23andMe

As of October 2018

- **Does business in 50 countries**
- **Has >5 million customers**
- **Allows raw data to be downloaded for use with external tools for further DNA analysis (e.g. XcodeLife, Genetic Genie)**

Genotypes 640,000 SNP's using an Illumina Global Screening Array customized chip



Hayden, Erika 11 Oct 2017 The rise and fall and rise again of 23andMe.
https://www.nature.com/news/the-rise-and-fall-and-rise-again-of-23andme-1.22801

https://www.23andme.com/dna-reports-list/

As of January 2019 there were 44 reports

Examples

Bloom Syndrome	Agenesis of the Corpus Callosum with Peripheral Neuropathy
Autosomal Recessive Polycystic Kidney Disease	Niemann-Pick Disease Type A
Familial Mediterranean Fever	Limb-Girdle Muscular Dystrophy
Cystic Fibrosis	Glycogen Storage Disease Type 1a and 1b
Sickle Cell Anemia	Tay-Sachs Disease

23andMe
BRCA testing

23andMe tests for 3 variants

BRCA1 gene	BRCA1 gene	BRCA2 gene
• 185delAG	• 5382insC	• 6174delT

Most BRCA mutations (~80%) in Ashkenazi Jews will be detected. This disorder is common in Ashkenazi Jews (~1 in 40)

There are >1,000 variants in the BRCA1 and BRCA2 genes now known to increase cancer risk

Most BRCA mutations (~90%) in the general population will be missed with this test.

Test results–Example

Age-Related Macular Degeneration	Variant detected, not likely at increased risk >
Hereditary Hemochromatosis (HFE-Related)	Variant detected, not likely at increased risk >
Alpha-1 Antitrypsin Deficiency	Variants not detected >
BRCA1/BRCA2 (Selected Variants)	Variants not detected >
Celiac Disease	Variants not detected >
G6PD Deficiency	Variant not detected >
Hereditary Thrombophilia	Variants not detected >
Late-Onset Alzheimer's Disease	Variant not detected >
Parkinson's Disease	Variants not detected >

Test results–Example

Variants Detected | View All Tested Markers

Marker Tested	Genotype*	Additional Information
A69S Gene: ARMS2 Marker: rs10490924	G Typical copy from one of your parents T Variant copy from your other parent	Biological explanation Typical vs. variant DNA sequence(s) Percent of 23andMe customers with variant

Test Interpretation [5, 10, 11, 19, 23, 24, 25] | ClinVar

This report provides risk estimates for people of European descent. Estimates for other ethnicities are not currently available.

Test results–Example

Warnings and Limitations

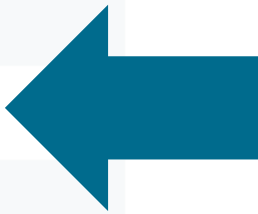
- This test does not cover all variants that could cause this condition.*
- This test does not diagnose any health conditions.
- Share results with your healthcare professional for any medical purposes.
- If you are concerned about your results, consult with a healthcare professional.

Likelihood ratios | Odds ratios

A "likelihood ratio" estimates how the test result affects the chances of a condition, compared to the chances of the condition prior to testing. In the table below, values greater than 1 mean that the chances of developing AMD are higher based on the test result. Values less than 1 mean that the chances are lower based on the test result. Values close to 1 mean that the chances of developing AMD have not changed significantly.

These values are calculated by 23andMe using data from Rivera et al. (2005).

Genotype	Likelihood ratio	95% confidence interval
No variants detected	0.23	0.17 - 0.30
One copy of Y402H variant	0.50	0.42 - 0.59
One copy of A69S variant	0.67	0.51 - 0.88
Two copies of Y402H variant	1.64	1.25 - 2.14
Two copies of A69S variant	1.99	1.18 - 3.38
One copy of Y402H and one copy of A69S variant	1.24	1.03 - 1.50
One copy of Y402H and two copies of A69S variant	4.12	2.60 - 6.53
Two copies of Y402H and one copy of A69S variant	4.49	3.18 - 6.33



Test results—Type 2 Diabetes

In 2019
23andMe started reporting on the genetic likelihood of developing Type 2 DM

23andMe developed the report based upon the FDA's guidelines for low-risk general wellness devices, products that promote a healthy lifestyle.

This report uses a polygenic risk score

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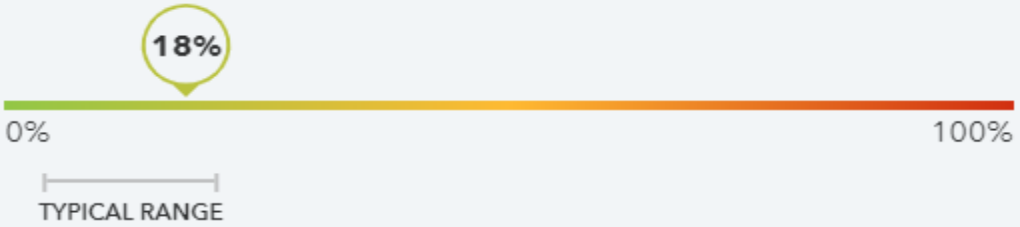
https://www.wired.com/story/23andmes-new-diabetes-test-has-experts-asking-who-its-for/ dated 3/10/19. accessed 3/25/2019

Test results—Type 2 Diabetes—Example

Bill, your genetics are associated with a **typical likelihood** of developing type 2 diabetes.



Your genetic likelihood falls in the range that is considered typical. But your overall likelihood also depends on factors like weight, diet, and exercise. This means it's important to maintain a healthy lifestyle.

This report **does not diagnose** type 2 diabetes. It also does not provide information about or diagnose other forms of diabetes.

The likelihood of developing type 2 diabetes also depends on **other factors**, including age, weight, ethnicity, and family history.

This report **does not account for every possible genetic variant** that could affect your likelihood of developing type 2 diabetes.

This report is based on a genetic model created **using data from 23andMe research participants** and has not been clinically validated.

23andMe
Summary slide

Advantages	Disadvantages
Identifies disease risk early	Misreading the test results
Identifies disease carrier conditions	Misunderstanding the completeness of the testing
Sparks interest in educating oneself	Misinterpreting the degree of reassurance in some cases which could lead to decreased surveillance
Relieves some anxiety when testing is negative	

Other options for individuals to get relatively inexpensive testing

Hybrid?

INVITAE

DIAGNOSTIC GENETIC TESTING can help identify

PROACTIVE GENETIC TESTING helps healthy

REPRODUCTIVE GENETIC TESTING helps make

- Simple, convenient, and affordable testing options
- Rapid answers in 10-21 calendar days, on average, for single-gene and panel tests and 6-8 weeks for exome tests
- Expert analysis for clear understanding
- Testing at no charge to clarify risks for your blood relatives if you test positive

Since its first test launched five years ago, Invitae has sequenced the genes of more than half a million patients.

Patients will be able to order genetic tests online through a clinician by this summer, George said. Nearly any test on Invitae's clinical menu will be available this way, making Invitae one of the first companies to offer wider access to clinical testing for an array of conditions and inherited health risks.

https://www.businessinsider.com/invitae-genetics-will-offer-personal-genetic-testing-23andme-2019-1 Jan 19, 2019
https://www.invitae.com/en/ Accessed 4/1/2019

Other options for individuals to get relatively inexpensive testing

INVITAE

- CARDIOLOGY
- METABOLIC DISORDERS AND NEWBORN SCREENING
- DERMATOLOGY
- NEPHROLOGY
- EXOME
- NEUROLOGY
- HEMATOLOGY
- OPHTHALMOLOGY
- HEREDITARY CANCER
- PEDIATRIC GENETICS
- IMMUNOLOGY

https://www.invitae.com/en/ Accessed 4/1/2019

Other options for individuals to get relatively inexpensive testing

HEREDITARY CANCER

INVITAE

GENES TESTED:

ALK	APC	ATM	AXIN2	BAP1	BARD1
BLM	BMPR1A	BRCA1	BRCA2	BRIP1	CASR
CDC73	CDH1	CDK4	CDKN1B	CDKN1C	CDKN2A
CEBPA	CHEK2	CTNNA1	DICER1	DIS3L2	EGFR
EPCAM	FH	FLCN	GATA2	GPC3	GREM1
HOXB13	HRAS	KIT	MAX	MEN1	MET
MITF	MLH1	MSH2	MSH3	MSH6	MUTYH
NBN	NF1	NF2	NTHL1	PALB2	PDGFRA
PHOX2B	PMS2	POLD1	POLE	POT1	PRKAR1A
PTCH1	PTEN	RAD50	RAD51C	RAD51D	RB1
RECQL4	RET	RUNX1	SDHA	SDHAF2	SDHB
SDHC	SDHD	SMAD4	SMARCA4	SMARCB1	SMARCE1
STK11	SUFU	TERC	TERT	TMEM127	TP53
TSC1	TSC2	VHL	WRN	WT1	

https://www.invitae.com/en/ Accessed 4/1/2019

Other options for individuals to get relatively inexpensive testing

INDIVIDUAL HEREDITARY CANCER CONDITIONS

INVITAE

Invitae Lynch Syndrome Panel

5 genes

Genetic testing for 5 genes associated with Lynch syndrome. This condition increases the risk for colorectal, ovarian, and uterine cancer.

ORDER

GENES TESTED:

EPCAM	MLH1	MSH2	MSH6	PMS2

Panel details and technical assay limitations

https://www.invitae.com/en/ Accessed 4/1/2019

Other options for individuals to get relatively inexpensive testing

INVITAE

PATIENT PAY

Genetic testing should be affordable and accessible to anyone who needs it. For panel and single-gene testing, Invitae offers a patient-pay price of $250 per clinical area to make testing affordable for more patients, including those who do not meet coverage policies for testing, those with high-deductible plans, and those not covered by insurance.

INSURANCE AND INSTITUTIONAL BILLING

The most we will ever bill an insurance company or institution is $1500 per clinical area for a panel or single-gene test. (For exome prices, please see the Exome Testing section below). In many cases the amount will be lower due to contracts between Invitae and the insurance company or institution. Our mission is to bring genetic information into mainstream medical practice—and our success depends on this type of responsible billing practice.

https://www.invitae.com/en/ Accessed 4/1/2019

DECISION POINT #1

Option 1:

There are some large research initiatives in the USA evaluating genetic diseases in the general public.

- What is being tested?
- What are the early results?

Option 2:

Cancer Specific Genetics.

Cancer involves mutations to our DNA?

- Hereditary
- Somatic (acquired)

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Important Concept:
Geisinger

Geisinger integrated health system

- >3 million patients
- Located in Eastern Pennsylvania and Southern New Jersey
- Physician led health system
- Pennsylvania and New Jersey
- 13 hospital campuses, 2 research centers, 1 medical school
- 600,000 member health plan

https://www.geisinger.org/mycode
And
https://www.healio.com/family-medicine/practice-management/news/online/%7B5a99b5b4-6040-47be-be24-82063b66f639%7D/ready-or-not-dna-screening-enters-primary-care?page=3

Geisinger

Geisinger initiatives

- MyCode Community Health Initiative (as of 4/10/2019)
  - 227,000 volunteer participants
  - 59 genes tested
  - Anticipated 2-4% of patients would receive abnormal genetic test results
  - 1,048 patient-participants have received results from the testing
  - Eventually they anticipate having all 3 million patients in their system tested
  - 2,500 participants will have pharmacogenomic testing
- Detect Research Study
- Dynamics of Childhood Obesity study

MyCode: How does the program work?
Geisinger

- Sign the consent form
- Donate blood
- Each sample is coded and stored for research
- About 2% of cases a genetic abnormality is found that is part of a higher risk genetic condition which can be treated or managed.
- In those cases the blood is analyzed in a certified clinical laboratory to confirm the finding.
- If abnormal patient and doctor are informed. Patient can speak to the doctor and the genetics team counselor.
- If pharmacogenomic testing shows a potential problem with the medications a pharmacist will notify the pt.

MyCode: Conditions prompting patient notification

Geisinger **1068 patients** as of 4/11/2019

Cardiovascular		Cancer				Other	
Familial hyper-cholesterolemia	125	Hereditary breast and ovarian cancer BRCA 1 BRCA 2	290 102 188	Lynch Syndrome	101	Hereditary Hemochromatosis	203
Arrhythmogenic R Ventricular Cardiomyopathy	82	Hereditary pheochromocytomas and paragangliomas	17	Multiple Endocrine Neoplasia type 1 and type 2	27	Malignant Hyperthermia	23
Cardiomyopathy	74	Li-Fraumeni Syndrome	9	Familial Adenomatous Polyposis	14	Marfan Syndrome	9
Inherited arrhythmias	65	PTEN Hamartoma Tumor Syndrome	5	Tuberous Sclerosis	4	Vascular Ehlers-Danlos	6
Familial Aortic Aneurysms and dissections	11					Fabry Disease	4

Catecholaminergic Polymorphic Ventricular Tachycardia
Retinoblastoma
Juvenile Polyposis

< 2

Neurofibromatosis Type 2
Hereditary Hemorrhagic telangiectasia
Von Hippel-Landau

DETECT Research Study: How does the program work?
Geisinger

- Goal: Evaluate liquid biopsies
- Enrollees:
  - Women ages 65-75 with no prior cancer
  - 10,000 targeted (7,600 enrolled as of 1/2019)
- What is tested:
  - Circulating DNA
  - 15 genes
  - 10 protein markers
- Abnormal results are followed by PET-CT scans and further testing.
- Geisinger in cooperation with Johns Hopkins physicians
- This is the only large health plan involved in this study

Dynamics of Childhood Obesity in Pennsylvania From Community to Epigenetics

Geisinger

- Observational Study
- Started in 2012
- Evaluate diet and exercise changes in DNA methylation
- 950 parent/child enrollees
- Monitor weight/height/DNA methylation via saliva over time.

Northshore University Health System (Chicago area) DNA10k initiative

- Enroll 10,000 patients in the study
- Evaluate for CAD, breast cancer and colorectal cancer
  - 30 genes involved in cancer
  - 30 genes involved in heart disease
  - 14 genes involved in medication response
- Announced the plan in 1/2019—starting enrollment in 4/2019

South Dakota based Sanford Health

- Enrolled 2,000 patients since 2018
- 3% of those tests have found reportable abnormalities

https://www.chicagotribune.com/business/ct-biz-northshore-genetic-testing-in-primary-care-20190116-story.html

Precision Medicine Initiative
National Institutes of Health (NIH)

- Goal: Enroll 1 million + volunteers in the U.S. to contribute their health data over many years to improve health outcomes
- Enrollees:
  - Enrollment now open
  - Program started in 2016
  - >18 years of age
- What is tested:
  - Not focused on a specific disease
- Enrollees have access to their results and can share them with their own physician

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Cancer and Genetics

How often is DNA altered (mutated) in cancer?

How many genes have mutations in a typical human cancer?

The average solid tumor has 33-66 genes with mutations which alter protein.

95% of those are single-base substitutions. The remainder are deletions/insertions

The number can vary:

~9 in childhood malignancies

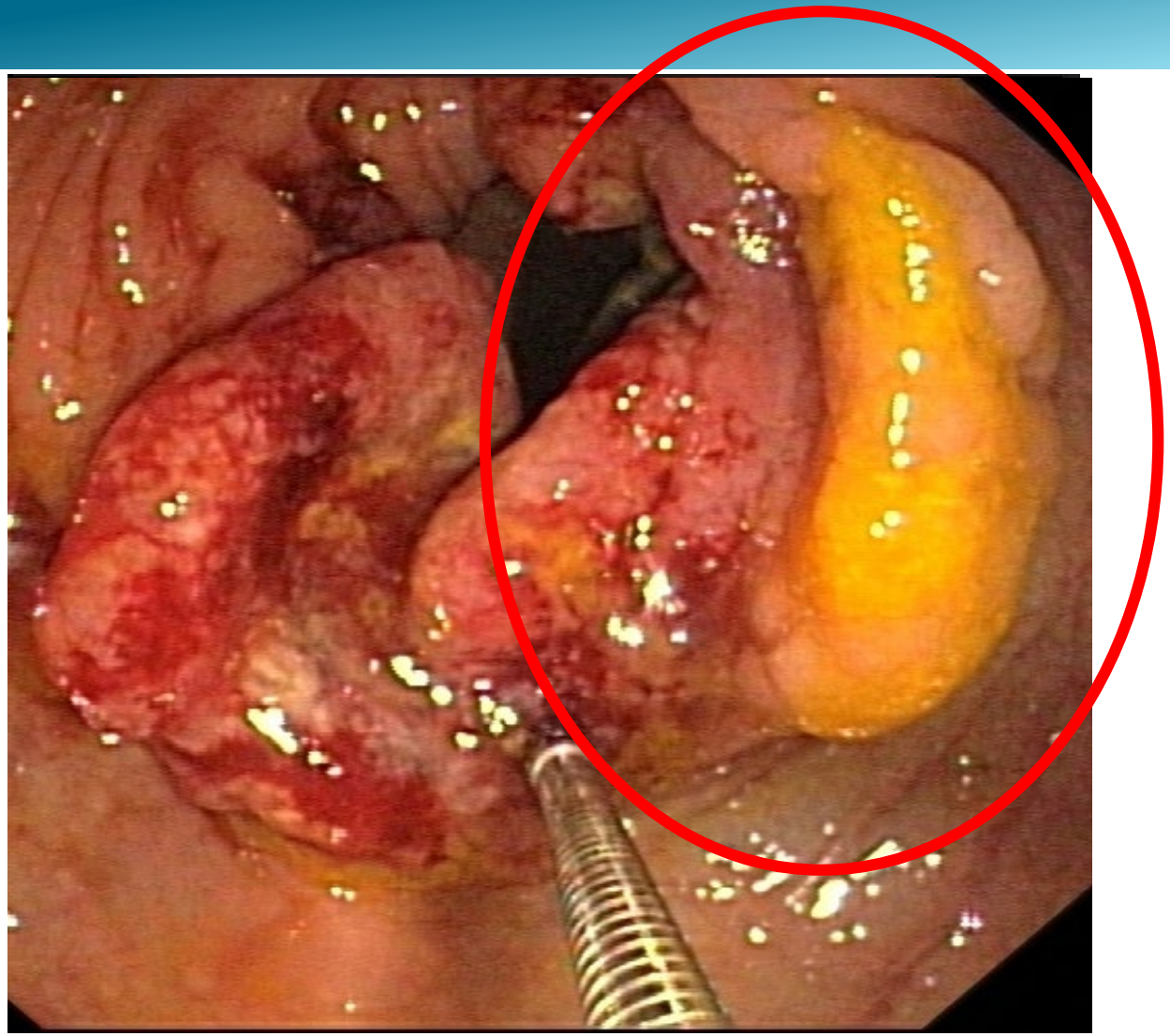
~33 in breast cancer

~66 in colon cancer

~163 in lung cancer

Vogelstein, Bert et al Cancer Genome Landscapes. Science. 29 March 2013 Vol 339

Cancer—Genetics—Example—Colon cancer



Cancer—Genetics—Example—Colon cancer

APC Gene Mutation

KRAS Gene Mutation

PIK3CA, SMAD4 and TP53 Gene Mutations

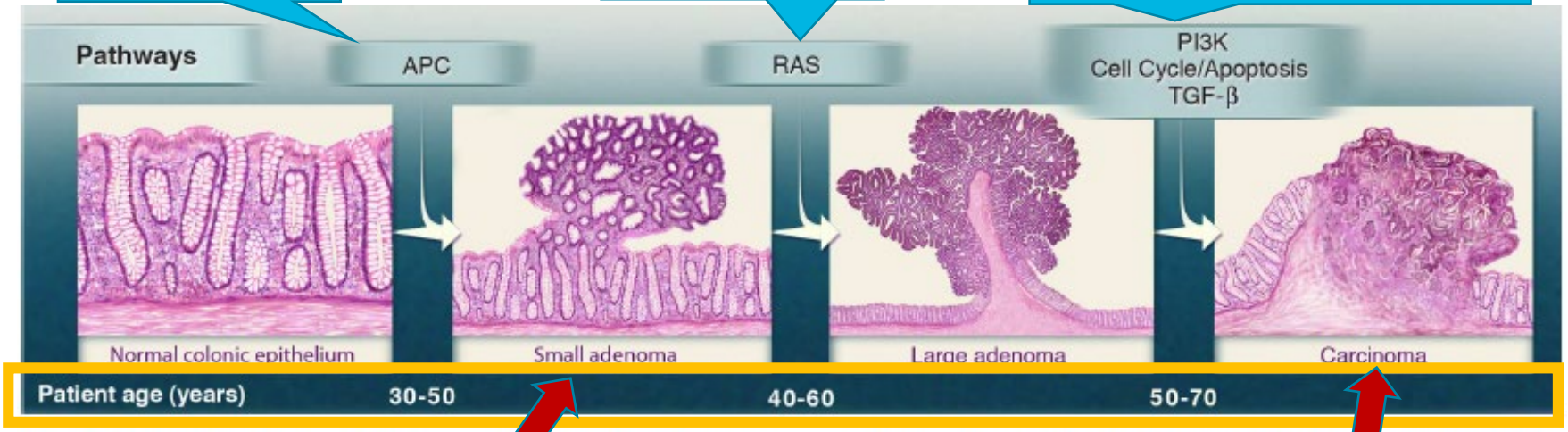
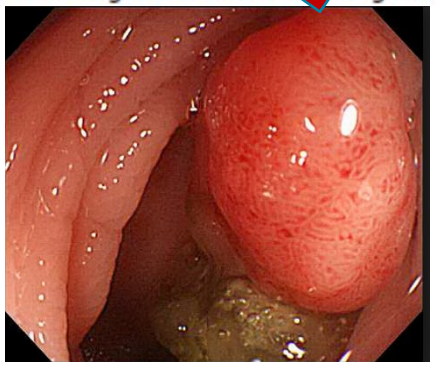


Fig. 2. Genetic alterations and the progress[illegible] of colorectal cancer. The major signaling pathways that drive tumorig[illegible]sis are shown at the transitions between each tumor stage. One of several[illegible]ver genes that encode components of these pathways can be altered in any individual tum[illegible] Patient age indicates the time intervals during which the driver genes are usu[illegible] mutated. Note that this model may not apply to all tumor types. TGF-β, transf[illegible]ing growth factor–β.



TIMING: Typically several years between each phase

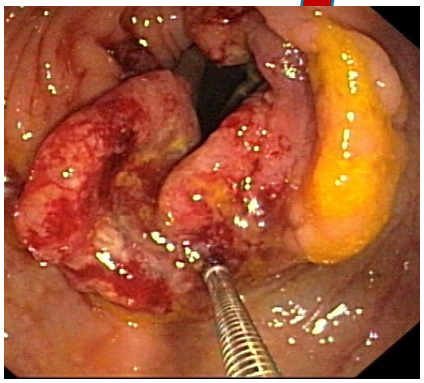


Figure as used in Vogelstein, Bert et al Cancer Genome Landscapes. Science. 29 March 2013 Vol 339

Cancer and Genetics

Germ line

Born with a genetic configuration which makes cancer development likely

- 5-10% of all cancers
- 50 different hereditary cancer syndromes

Somatic (acquired)

Develop a genetic modification after birth which causes cancer

- 90-95% of all cancers

Important Concept:

Genes Involved in Cancer

Oncogenes

A gene that has the potential to cause cancer

~84 known

TP 53 Gene
Keeps cells from growing and dividing too fast

Tumor Suppressor Genes

A gene that protects from getting cancer

~54 known

BRCA Genes
Repair damaged DNA (radiation or other environmental/chemical injuries)

Mismatch Repair Genes
Repairs DNA as its being reproduced

DECISION POINT # 2

Oh my, tough choices. I am glad I don't have to decide!!

Option 3:

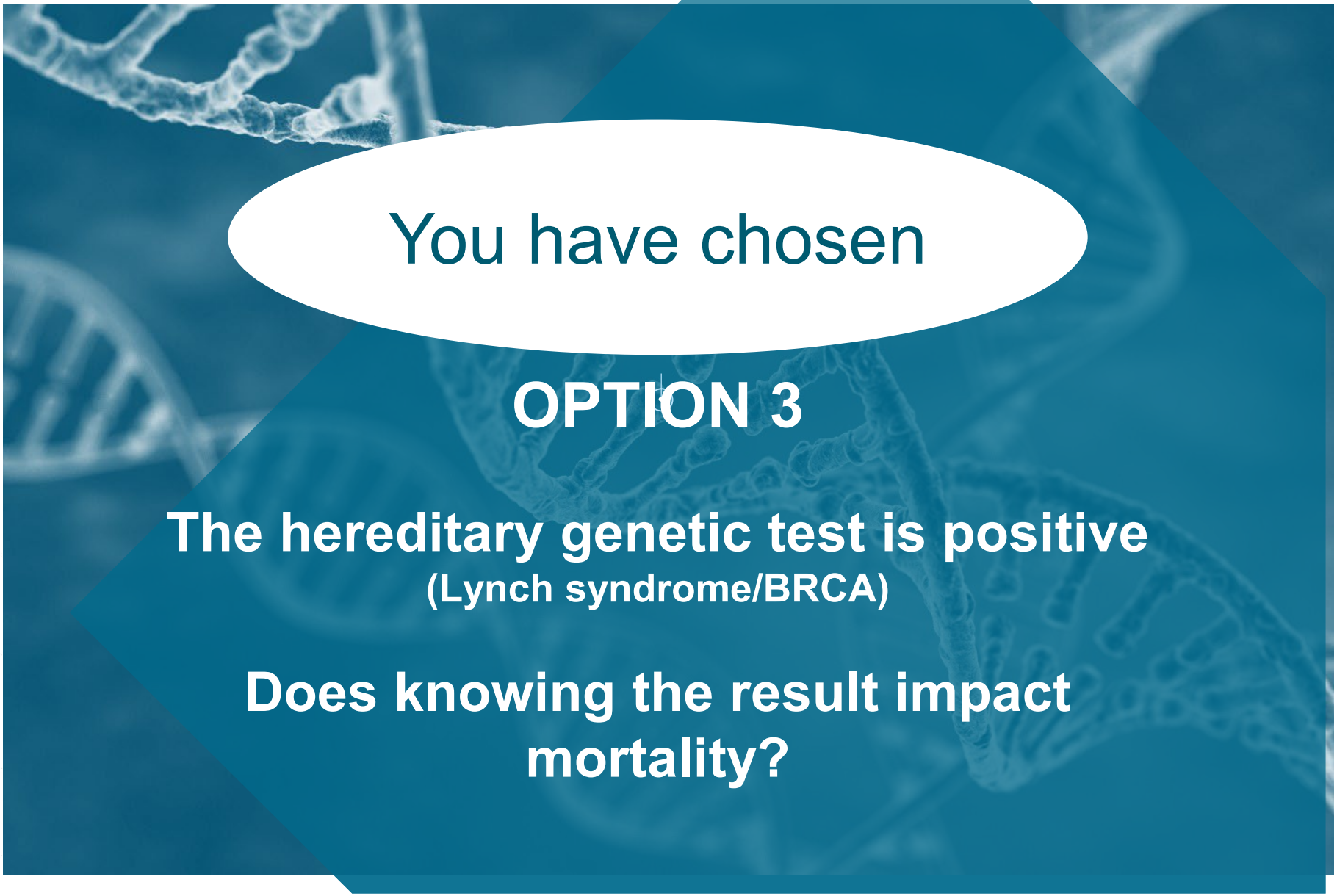
The hereditary genetic test is positive
(Lynch syndrome/BRCA)

- **Does knowing the result impact mortality?**

Option 4:

Liquid Biopsies What are they?

- **Are we getting close to seeing results of liquid biopsies in the records?**



You have chosen

OPTION 3

The hereditary genetic test is positive
(Lynch syndrome/BRCA)

Does knowing the result impact mortality?

Cancer Risk Increases with Lynch Syndrome

- Is one of the most common cancer susceptibility syndromes.

~1:370 people have this disorder.

- Is a result of a germline mutation in one of the DNA mismatch repair genes
- Predisposes an impacted individual to a high incidence of cancer.

~1.2 % people with it know they have it

Cancer Risk up to age 70 years old for Lynch syndrome patients

Colorectal	Up to ~80%
Endometrium	~60%
Stomach	~13%
Ovary	~24%
Many others	Smaller %

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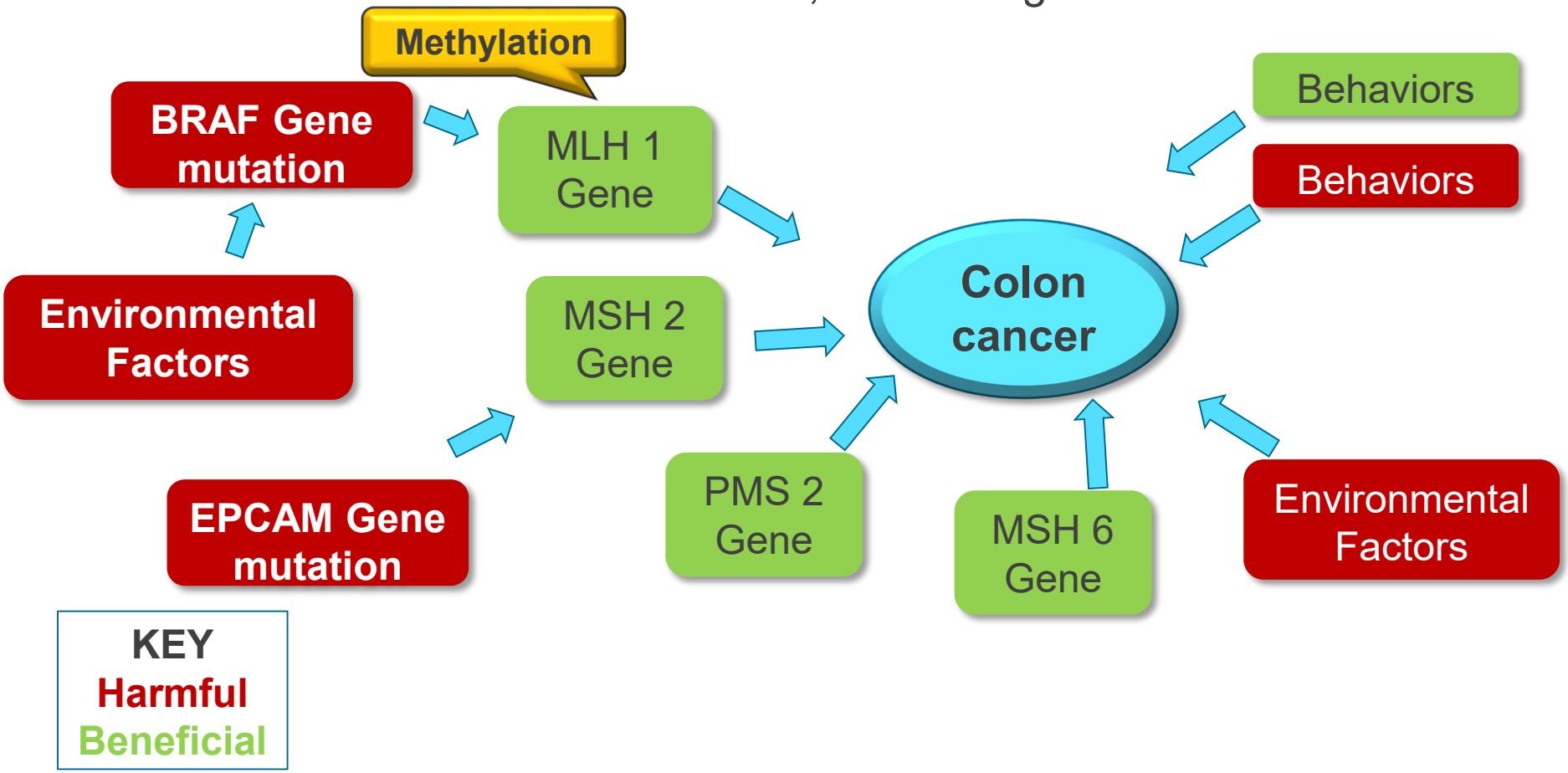
Source:
1) NCCN Guidelines Version 2.2016

Lynch Syndrome Cancer Genetics: It can be complicated

Diseases can be a combo of one or more genes, one or more behaviors, and one or more environmental factors, with both good and harmful effects.



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Lynch Syndrome: Prevention/Screening Mortality Results

A 2013 article by H. Jarvinen et al documents that:

Colon cancer screening helps!

Impact

- Decreases incidence of invasive colon cancer by 62%
- Decreases mortality by 65%

Adherence

- Colonoscopy 80%
- Endometrial cancer surveillance 63%
- Hysterectomy and BSO choice 19%

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Palomaki, Glenn et al EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. *Genetics in Medicine. Volume 11, Jan, 2009*

Cancer Risk Increases with BRCA 1 and 2 mutations

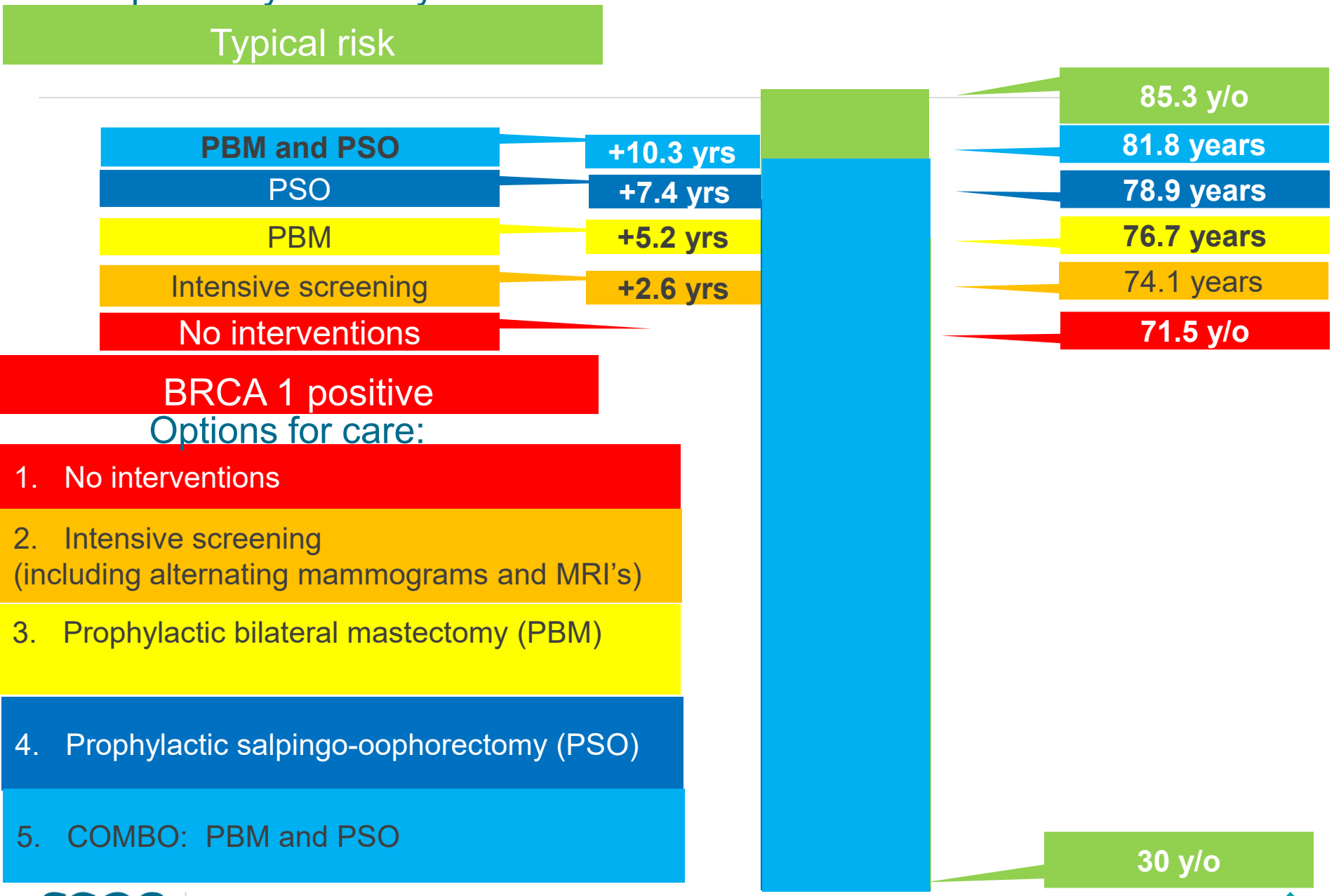
- BRCA genes code for proteins which repair damaged DNA
- Mutations in the BRCA gene predispose an individual to an increased risk of cancer
- Several interventions can improve mortality risk
  - Intensive screening
  - Hormonal medical therapy
  - Risk-reducing surgery

Can genetic testing lead to improved risk?

Cancer Risk up to age 70 years old for BRCA patients	
Breast (women)	45 to 70%
Ovary	15 to 40%
Prostate	14 to 19%
Many others	Smaller %

Source:
1) NCCN Guidelines Version 2.2016

Life Expectancy of a 30 year old woman



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1) Sigal BM et al. A simulation model to predict the impact of prophylactic surgery and screening on the life expectancy of BRCA1 and BRCA2 mutation carriers. Cancer Epidemiology. Biomarkers $ Prevention. 21 (7) July 2012

Other conditions:

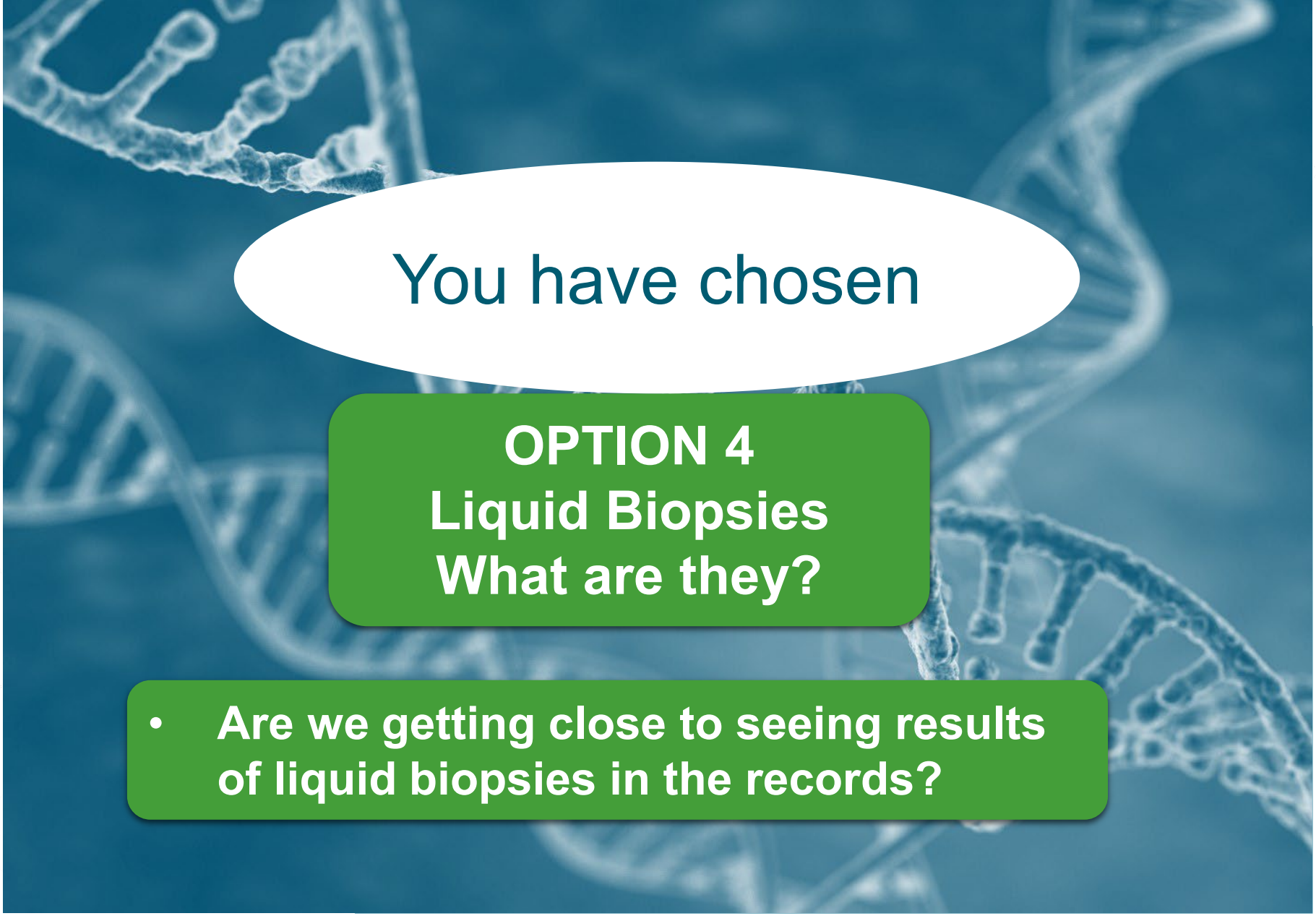
Hereditary Hemochromatosis

Malignant Hyperthermia

Early dx and great treatment compliance can led to mortality risk similar to that of the unaffected

All the other diseases listed in the American College of Medical Genetics and Genomics have treatments/strategies thought to favorably impact mortality

Palomaki, Glenn et al EGAPP supplementary evidence review: DNA testing strategies aimed at reducing morbidity and mortality from Lynch syndrome. *Genetics in Medicine. Volume 11, Jan, 2009*


You have chosen
OPTION 4
Liquid Biopsies
What are they?
• Are we getting close to seeing results of liquid biopsies in the records?

Liquid Biopsies

Able to find small amounts of DNA, RNA, and proteins in the circulating blood that can indicate disease

Sudden Unexplained Death	**Molecular Analysis of DNA for heritable heart disorders (NY—Erdman, 2013)**
Evaluating the unborn baby	**cffDNA—cell free fetal DNA (Bianchi)**
Evaluating cancer (detect, track treatment, check for recurrence)	**ctDNA—circulating tumor**

Liquid Biopsies
2017 study
Sudden arrhythmic death syndrome (SADS)

SADS definition:
Sudden death with negative autopsy and toxicological analysis

302 validated SADS cases

Median age: 24 years
Males: 65%

Test: 77 electrical and cardiomyopathy genes using American College of Medical Genetics guidelines

Results: Pathogenic or "likely pathogenic" variant found in 40 cases (13%)

1) Lahrouchi, N et al. Utility of Post-Mortem Genetic Testing in Cases of Sudden Arrhythmic Death Syndrome.
2) *J Am Coll Cardiol*. 2017;69(17): 2134

Liquid Biopsies---ctDNA

Science (Jan 18, 2018)

A blood test (CancerSEEK) which detects 8 common cancers described.

Ovary	Esophagus
Liver	Colon
Stomach	Lung
Pancreas	Breast

- **DNA analysis and protein analysis.**
- **Test detected cancer 70% of the time**

  Sensitivity: ranged from 69% to 98% for 5 cancer types with no screening tests currently available

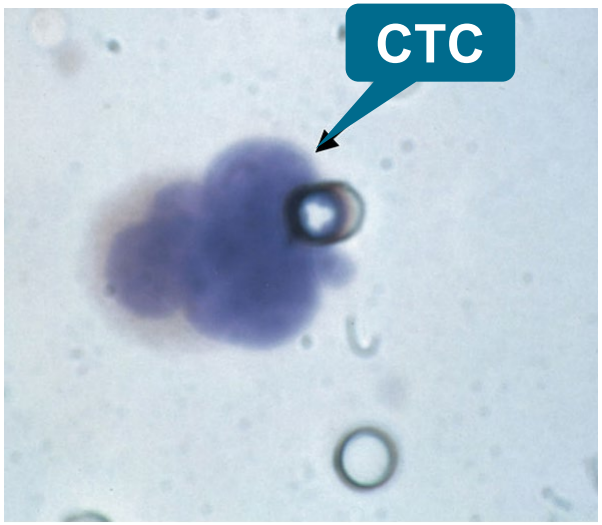
  Specificity: 99% (7 out of 812 healthy controls scored positive)

1) Cohen, J et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science. 10.1126/science.aar3247 (2018)

Example of studies on liquid biopsies and future use

December 2018
Researchers from MD Anderson/Northwestern/Other make the following points:

- **Metastatic breast cancer (MBC) prognosis varies by presence/amount of circulating tumor cells (CTCs)**
- **CTC counts might be important for treatment decisions. Future clinical trials needed.**



2,436 patients

- **Threshold < or > 5 CTC's per 7.5 ml**

Those with <5 CTC's had much better survival regardless of cancer characteristics (HER/ER status)

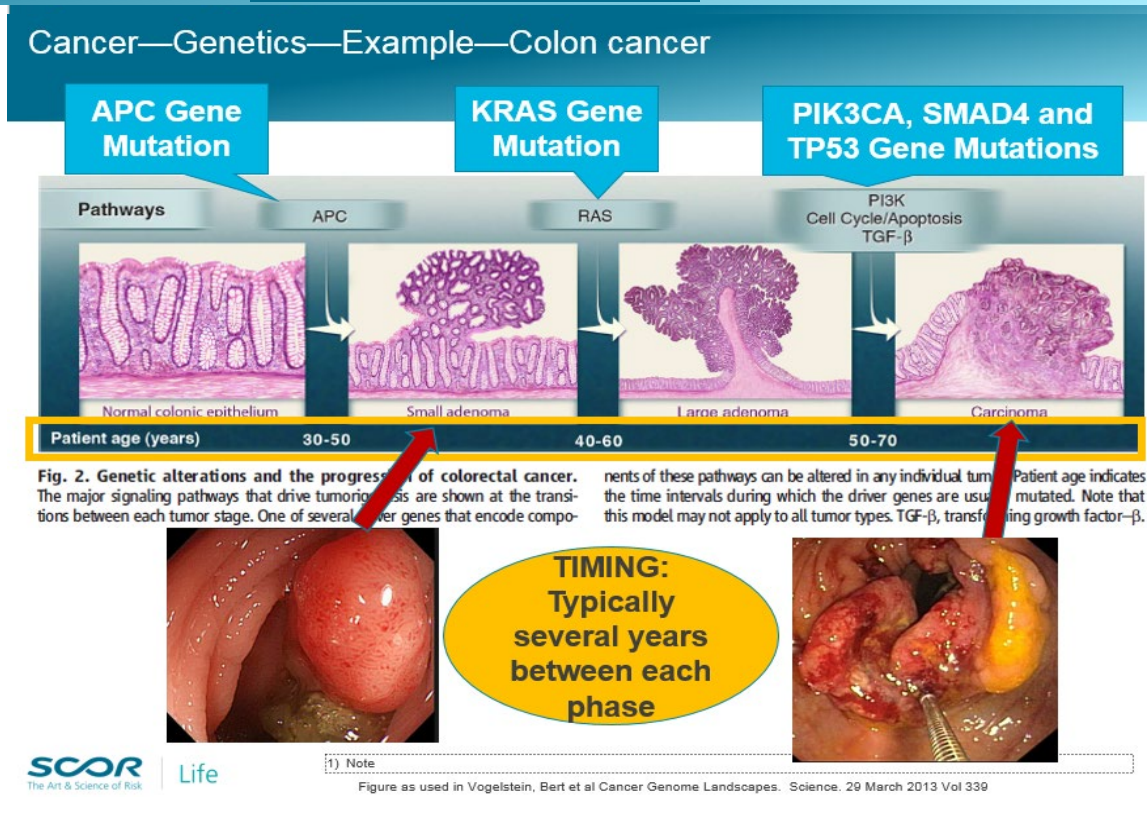
1) Cristofanilli, M et al. The clinical use of circulating tumor cells (CTC's) enumeration for staging of metastatic breast cancer (MBC): International expert consensus paper. Critical Reviews in Oncology/Hematology 1134 Dec 2018
2) https://commons.wikimedia.org/wiki/File:Figure_1,_Picture_of_a_circulating_tumor_cell,_arrow,_diagnosed_by_the_ISET_test..jpg Image obtained using Google's "labeled for reuse with modification" search engine

Testing for cancer---things might be chan

Remember this slide

Testing for cancer in the future might include:

- More use of pre-symptomatic predictive testing
- Cancer mutation testing
  - Hereditary cancer syndrome
  - Somatic mutations



DECISION POINT # 3

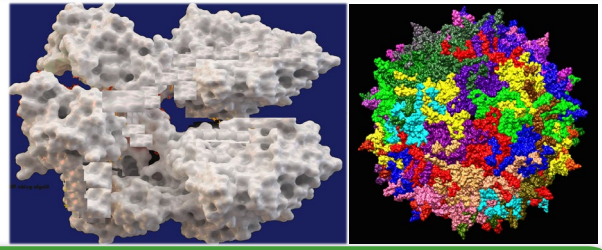
Option 5:

The PI obtained a genetic test. How accurate is it?

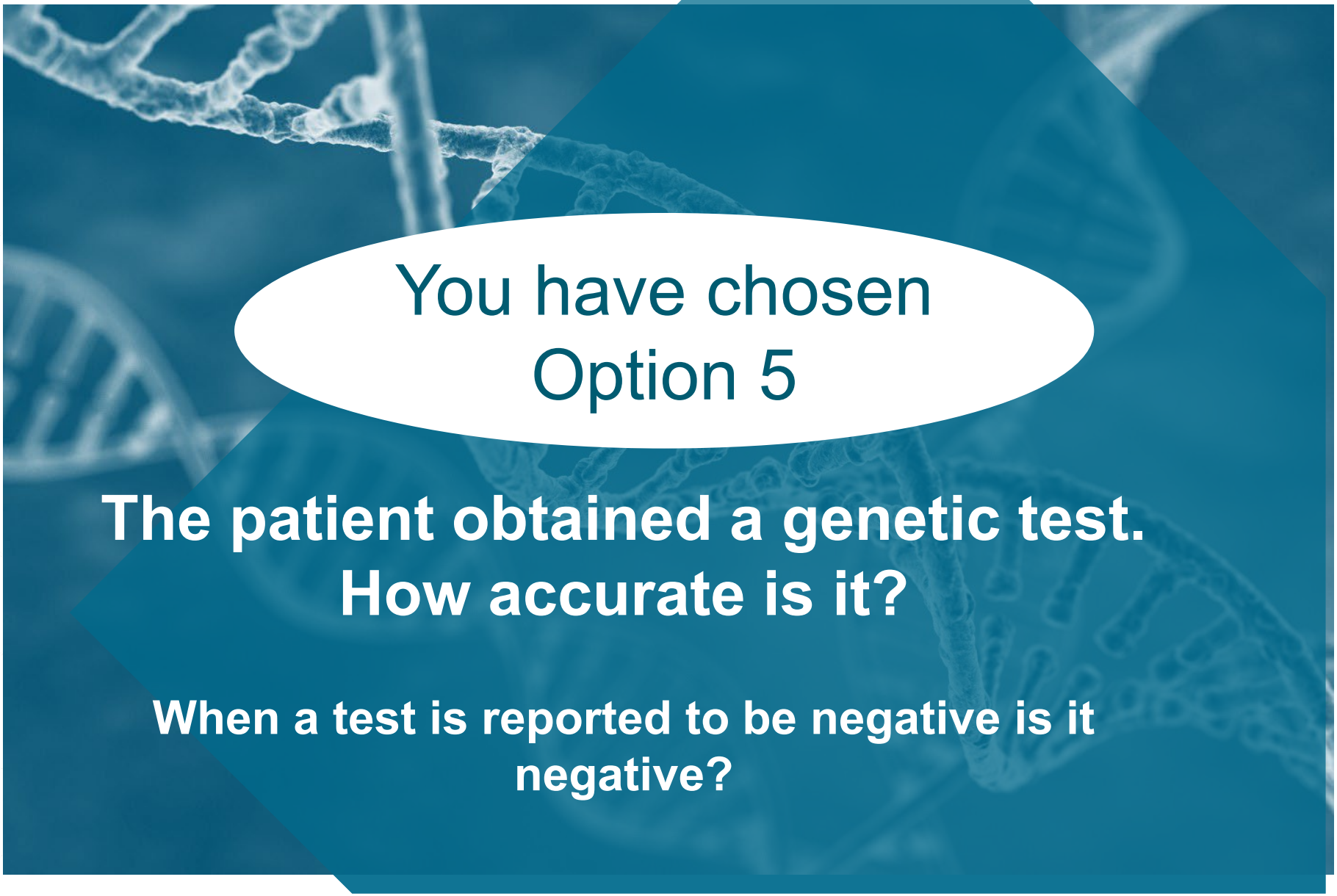
When a test is reported to be negative is it negative?

Option 6:

Gene Editing:



CRISPR and adeno-associated virus

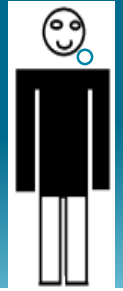


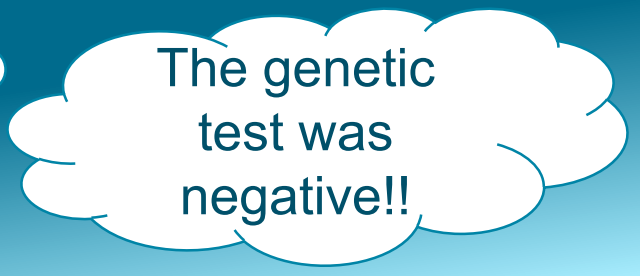
You have chosen Option 5

The patient obtained a genetic test. How accurate is it?

When a test is reported to be negative is it negative?

Genetic Testing: Characteristics of a good test





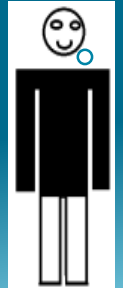
Characteristics of a good test	Genetic testing
100 % Sensitive 100% Specific	Varies
Inexpensive	No
Accurate and reproducible	Typically
Ordering person has a thorough understanding of the test	Varies
Tested individual can easily understand the results	Varies
Established testing protocol/process with little modification or changes anticipated	Not typically
Abnormalities point to one and only one disorder	Not typically
Always actionable	No
Always one test for each disorder	No
Results are always yes or no	No

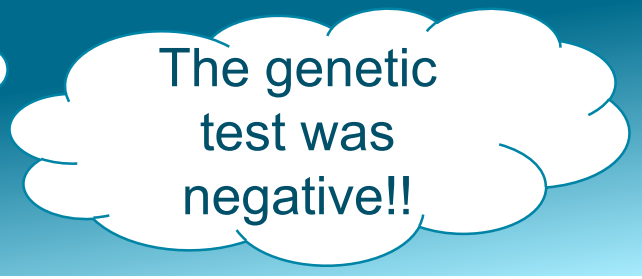
Genetic Test Results

Category	Characteristics
Pathogenic	Variant previously associated as cause of the disorder
Likely Pathogenic	Variant expected to cause the disorder
Variants of uncertain significance	Variant that might be causative
Likely benign	Variant probably not causative
Benign	Variant recognized as neutral

It is not uncommon for sequence variations to move from one category to another over time

Genetic Testing:
Characteristics of a good test





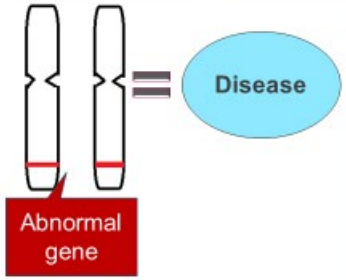
Some important questions:

1. How long ago was the test done?
2. Is there a known mutation in the family?
3. Do we have the test report or is it a verbal result? If verbal, did the patient get pre and post test genetic counseling?
4. Is this a diagnostic test or a pre-symptomatic test?

Genetic Testing:
Remember these slides?

A positive test doesn't mean the person will get the disorder

Important Concept:
"Multiple variables influence the impact of genetic expression"



Penetrance
- ❑ Full
- ❑ Variable

Variable expressivity
- ❑ Age
- ❑ Mutation type
- ❑ Genetic heterogeneity
- ❑ Environmental exposure
- ❑ Parent-of-origin effects (i.e. Prader-Willi Syndrome)
- ❑ Sex-limited expression (i.e. male pattern baldness)
- ❑ Anticipation
- ❑ Mosaicism

SCOR Life 14

Knowing exactly what test was done is important

Important Concept:
"There are many genetic testing techniques"

Biochemical testing
- Protein analysis/Enzyme assays/Analytes

DNA analysis
- Real-time PCR
  Small number of genes tested
- High-density DNA array testing
  Analyze for 1,000,000 gene variants
- Highly efficient DNA sequencing techniques (next generation sequencing)
  Entire human genome

SCOR Life 15

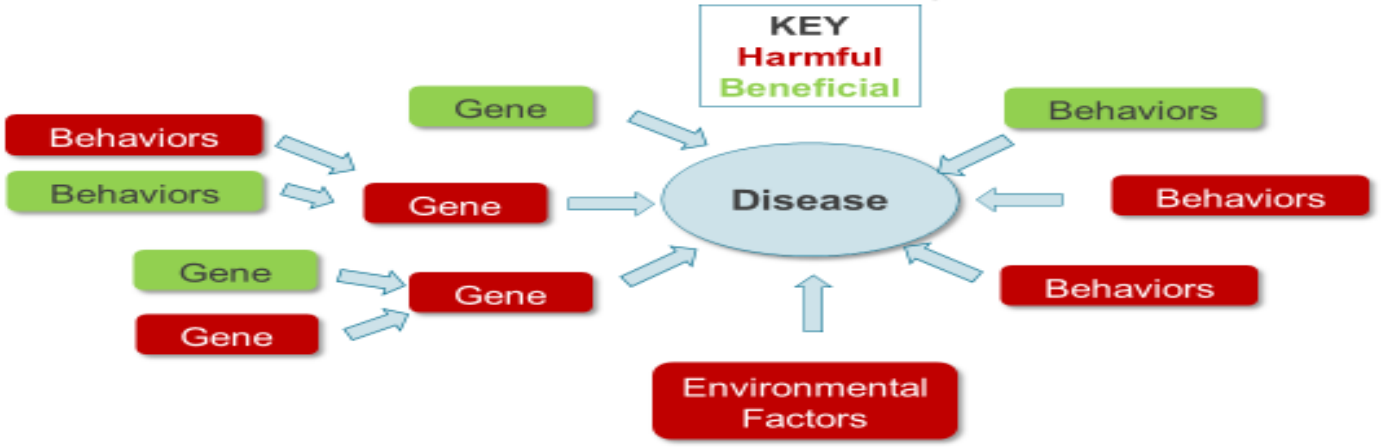
Remember this slide?

For many diseases there are multiple genes and environmental factors involved. Are all of these known?

Concept

"Interaction of Many Factors"

Diseases can be a combo of one or more genes, one or more behaviors, and one or more environmental factors, with both good and harmful effects.

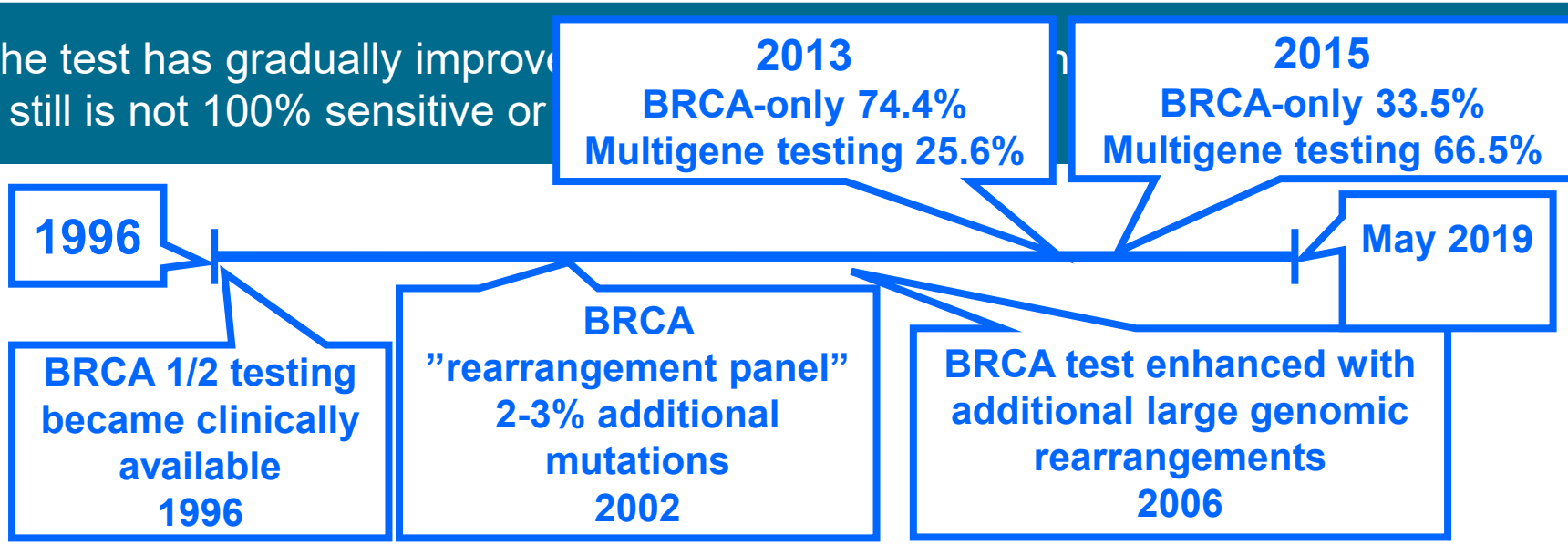


SCOR | Life

Testing: One important question is "How long ago was the test completed?"

Example: BRCA

- The test has gradually improve[illegible] e.
- It still is not 100% sensitive or [illegible]



4 types of clinical testing:

1) Full gene sequencing
2) Panel for the founder mutations common in Ashkenazi Jewish populations
3) Mutation specific assay
4) Large genomic re-arrangement testing

But remember that BRCA is not the only gene associated with hereditary cancer syndromes involving the breasts.
TP53, PTEN, PALB2, CHEK2, ATM, MSH6 and others

1) Breastcancer.org

Testing: Another question is “Are most genetic mutations involving the disease in question known?”

In Hypertrophic Cardiomyopathy for instance only about 50% of the time can a specific mutation be found in an impacted individual

Testing:
Another question to consider
"Is the disease mostly genetically influenced?"

Huntington's Disease is always associated with a genetic abnormality. This is not true for many other diseases.

Huntington's Disease involves only 1 gene. The genetic test is close to 100% sensitive and specific

Huntington's Disease involves only inheriting 1 copy of the abnormal gene

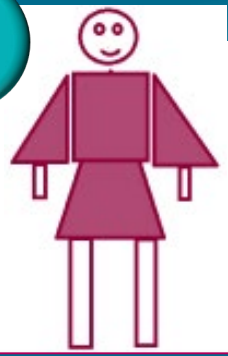
Huntington's Disease always develops when the gene is mutated

There is no effective treatment or cure for Huntington's disease.

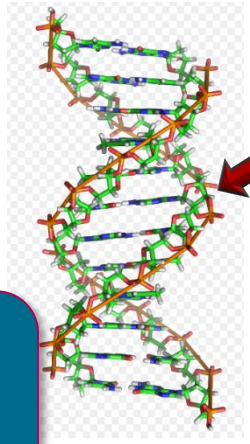
Only about 15% of people take the reliable test (available since 1993) when the disease is present in a parent.
This compares to 60% of people given the choice of BRCA testing

Genetic Testing—Scenarios 1 and 2

1



Family member who has Lynch Syndrome



Mutation found

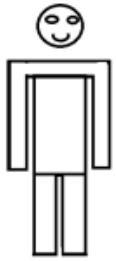
Test for that specific mutation in all family members

If negative this is a true negative genetic test

If positive, the individual has Lynch syndrome

This individual would typically be managed like those with Lynch syndrome

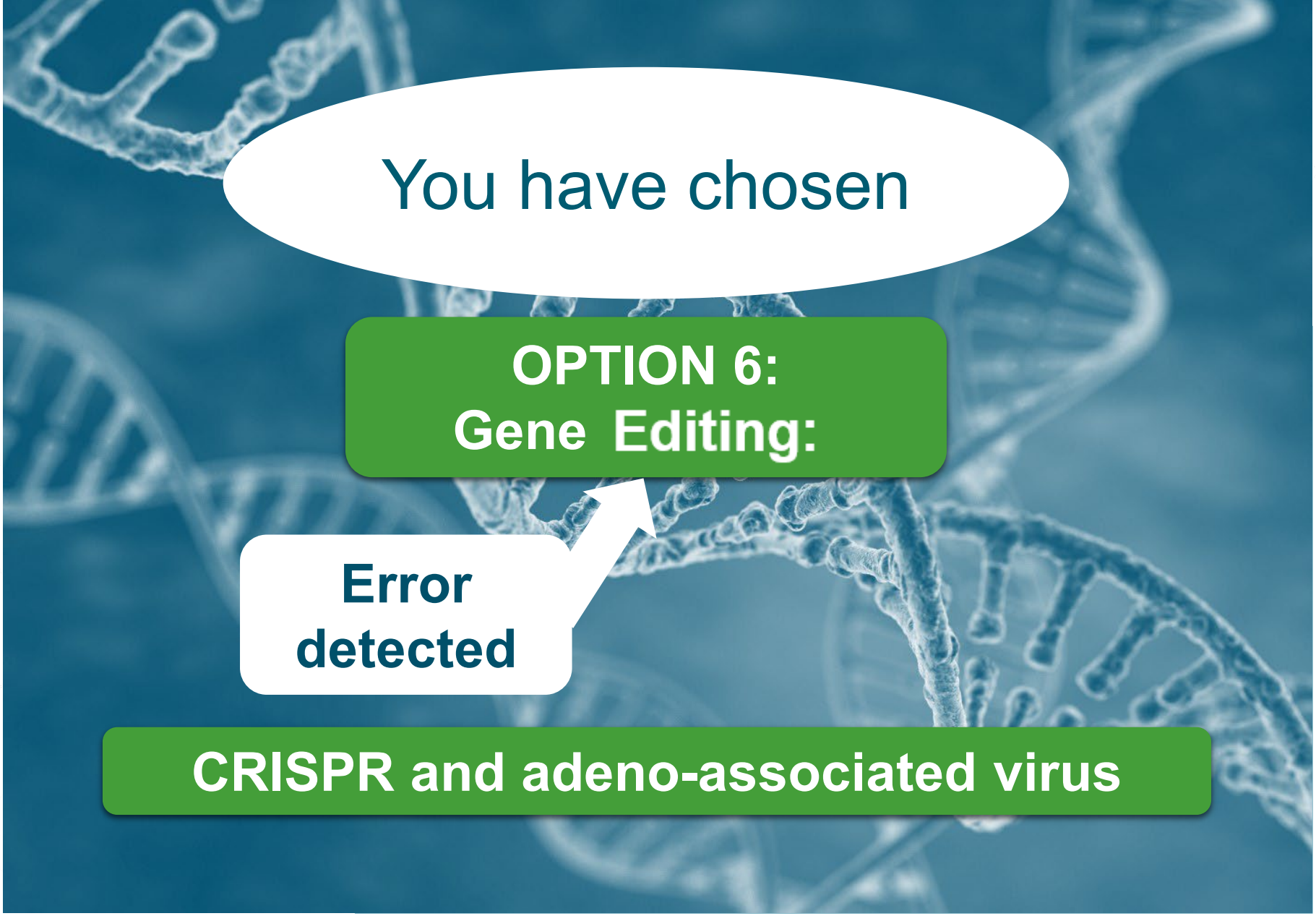
2



Increased chance for inconclusive results

- FH of Lynch-like tumors
- No personal hx. of cancer
- No specific mutation known in the family

If test is negative, is it:
Because that gene is not mutated in the family?
or
The mutation does exist in the family but the tested person doesn't have it?


You have chosen
OPTION 6:
Gene Editing:
Error detected
CRISPR and adeno-associated virus

Exciting New Technologies
Gene Therapy/Editing

Gene editing involves modifying the genetic sequence of DNA

2576 studies were identified using "Gene Modification" in the search engine of the ClinicalTrials.gov site

Gene Therapy/Editing with use of viral vectors

Viral vector characteristics

- Able to attach to and enter the target cell
- Successful transfer to the nucleus
- Lack of toxicity

Viral vectors:

- Retroviruses
- Lentiviral viruses
- Foamy viruses
- Adeno-Associated virus

Advantages of the virus

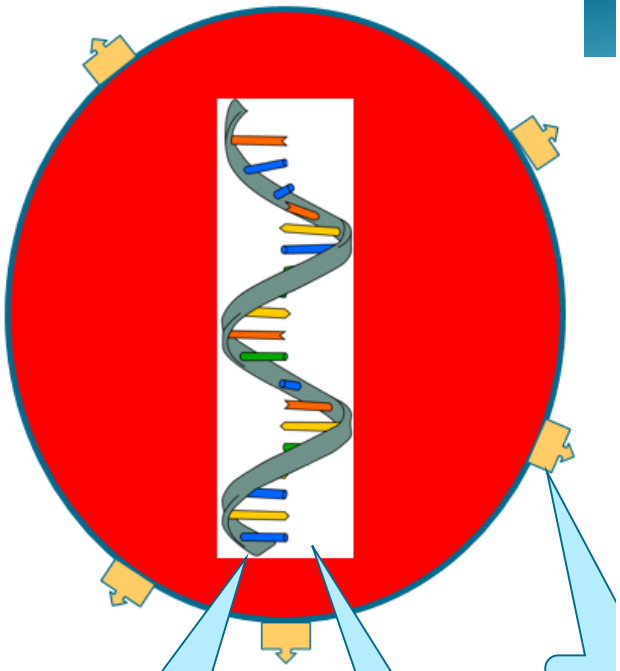
- Reliably (~100%) inserts genetic material into Chromosome 19 at a specific site
- Is non-pathogenic
- Requires a single injection

Disadvantages of the virus

- Carries only a small amount of genetic material
- Difficult to produce
- Expensive to produce

117 clinical trials thus far

Gene Therapy/Editing



Adeno-associated virus

DNA

Re

Human Cell

Autosomes, sex-chromosomes, mitochondrial genomes

The human genome is DNA c ized as 23 chromosomes including 22 autosomes (named 1-22), ar e sex chromosome (either X or Y). Humans are diploid, with eac matic cell consisting of two sets of 23 chromosomes, one paternally erited (blue) and one maternally inherited (pink). The Y chromosome is ssarily paternally inherited. The mitochondrial genome (mt) is ved solely from mitochondria in the ova and therefore exhibits exclusi atrilineal inheritance.

UpToDate®

The virus inserts genetic material at a specific site on Chromosome 19

Gene Therapy/Editing

Hemophilia A –Factor VIII clotting factor gene 80% of cases
Hemophilia B- Factor IX clotting factor gene 20% of cases

Hemophilia B treatment

Adeno-associated virus (AAV)

10 men injected with:

- **AAV with a bioengineered capsid**
- **Liver-specific promotor**
- **Factor IX Padua transgene**

Followed for 28-78 weeks

- **Bleeding rate decreased from 11.1 events/year to 0.4 events/year**
- **8/10 able to stop factor injections**
- **No significant side effects**

1) George, L.A. et al NEJM. 2017;377:2215-2227

Gene Therapy/Editing

The first FDA approved gene therapy for a genetic disease (not cancer).

- Hereditary Retinal Dystrophies:
  - Group of genetic retina disorders—Mutation in one of ~220 genes.
  - Biallelic RPE65 mutation is one of these
  - ~1,000 to 2,000 patients in the USA
- Luxturna was FDA approved in Dec, 2017

- **A normal copy of the RPE65 gene is injected into the eye.**
- **Adeno-associated virus (AAV) used.**
- 150 billion viral vector particles carrying the normal gene injected.

93% of patients had improved vision

Price tag reported to be ~$850K

1) Reference MedPage Today 1/3/2018

Gene Therapy/Editing—CAR-T

2 medications were FDA approved in 2017

Dramatic results in 3 recent studies!

1) 57% complete response in 28 patients with refractory B-cell lymphomas

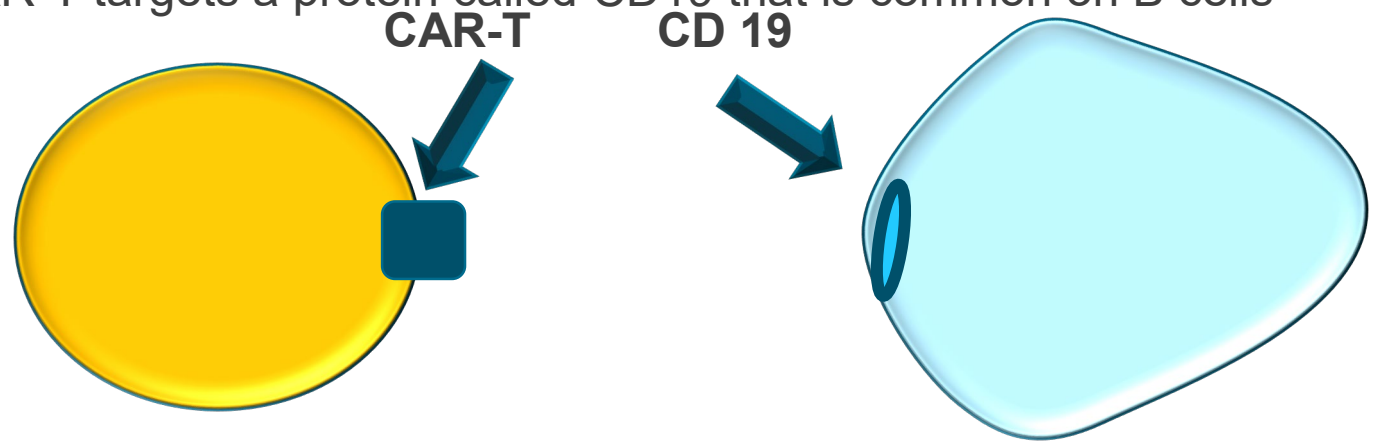
2) 54% complete response in 101 patients with refractory large B-cell lymphomas

2) 90% complete remission in 30 children with refractory acute lymphoblastic leukemia (ALL)

- Serious side effects and death have been associated with the tx.
  - Cytokine release syndrome occurs frequently (~50-90% of the time)
  - Fever, hypoxia, hypotension

Gene Therapy/Editing—CAR-T

- Killer T cells are part of the immune system
  - They seek out and destroy invading antigens.
  - They are very specialized and have specific targets
- **T cells removed from a cancer patient** (B-cell acute lymphoblastic leukemia and non-Hodgkin lymphoma).
- **Genetically engineer a specific T-cell receptor** (Chimeric T cell antigen receptor (CAR-T) that interacts with the cancer
  - These cells are synthetic molecules—they don't exist naturally
- **Administer these cells to the cancer patient**—the cells further multiply in the patient's body
- The CAR-T targets a protein called CD19 that is common on B cells



Gene Therapy/Editing—Sickle Cell Treatment

That is a lot of breaking news!!!

EVEN MORE BREAKING NEWS!!!!
March, 2017 Development

French investigators published in the NEJM the results of a cure for sickle-cell disease in a young boy

- **Stem cells were removed** from the bone marrow
- The **stem cells were genetically edited** to remove the single genetic mutation that causes sickle cell disease
- The **stem cells were then infused back**
- 2 years later **the patient has enough normal RBC's** to avoid the side effects of the disorder

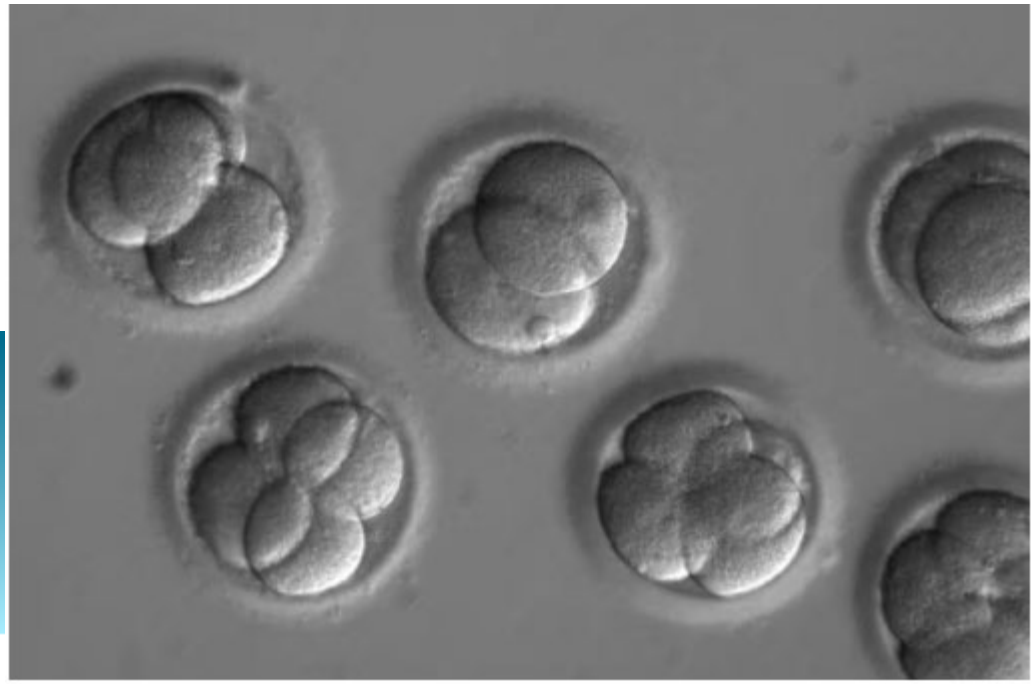
Current and Future Developments
CRISPR----Headlines

BREAKING NEWS!!!!
August 2017 Development

Human embryo genes edited at the University of Oregon

CRISPR-cas technology was used to alter the MYBPC3 mutation involved with hypertrophic cardiomyopathy.

CRISPR-Cas was Science as 2015 "Breakthrough of the Year"



Pictured: Individual blastomeres within early embryos two days after introducing the CRISPR gene editing system. A new study revealed that each new cell in the developing embryos was uniformly free of a disease-causing mutation. *Credit: OHSU*

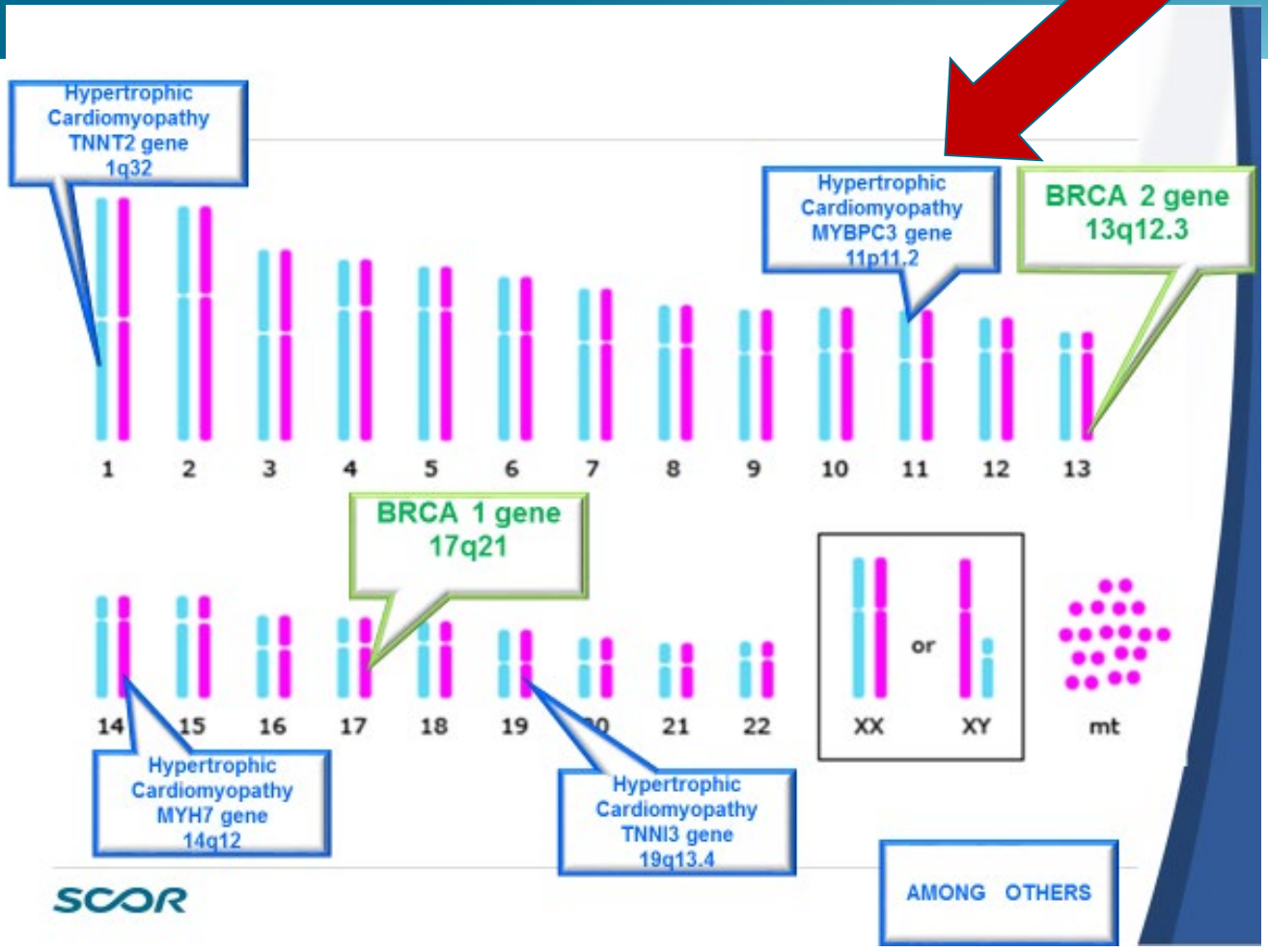
SCOR The Art & Science of Risk | Life

https://www.scientificamerican.com/article/embryo-gene-editing-experiment-reignites-ethical-debate/ Last accessed 8/2/2017

CRSPR-cas and HCM gene editing

MYBPC3 gene

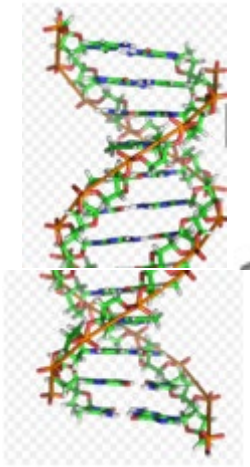
- **Accounts for 40% of all known HCM genetic defects**
- **Study took a male with a known 4 bp GAGT deletion in exon 16**

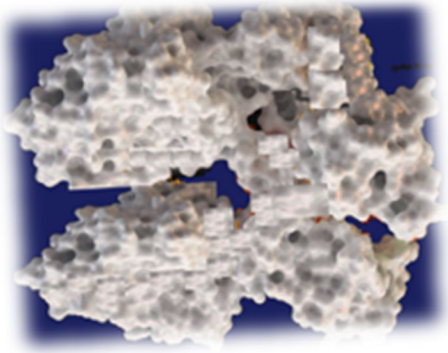


GAGT

1) Ma, H et al, Correction of a pathogenic gene mutation in human embryos. Nature. Published Online 2 August 2017

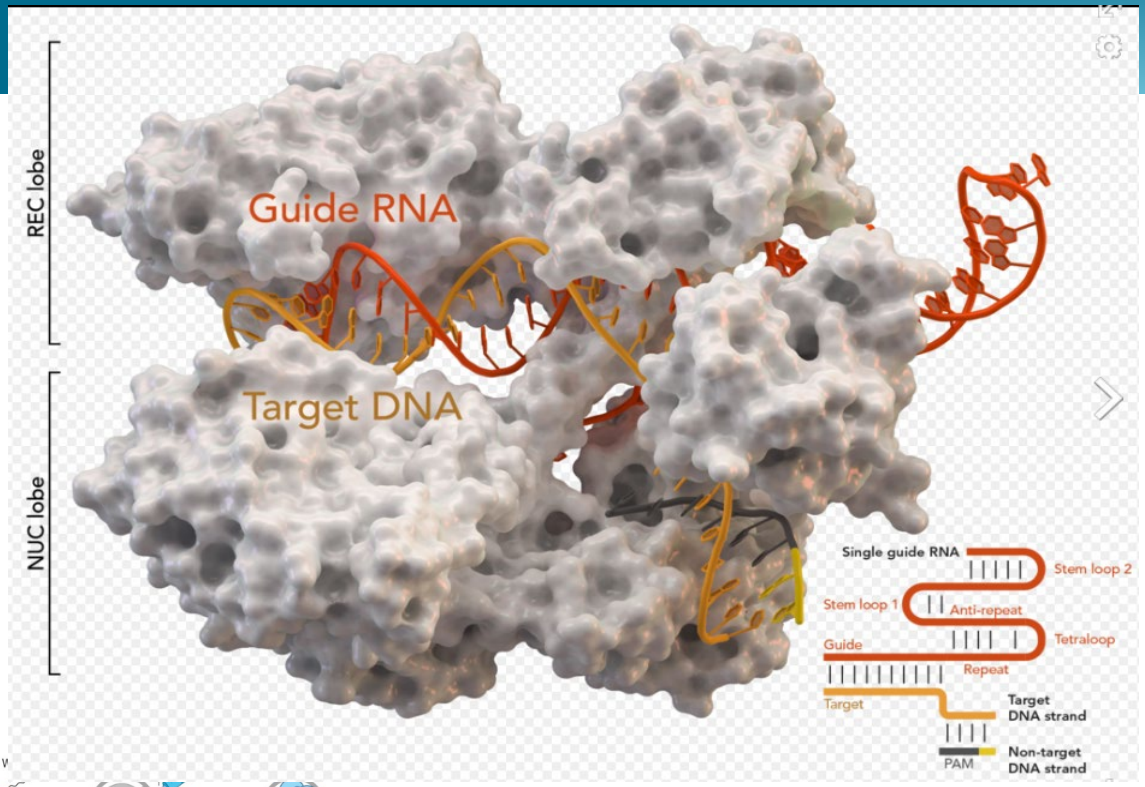
CRSPR-cas 9 functions much like scissors

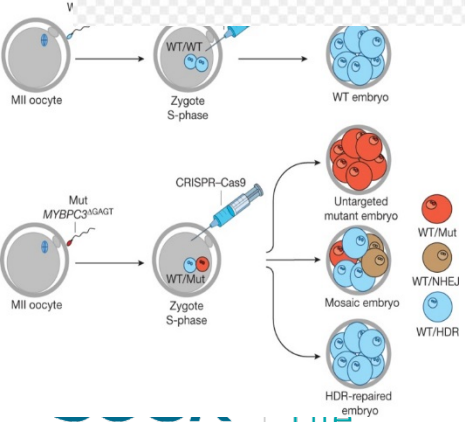




CRSPR-cas 9

CRSPR-cas and HCM gene editing





- The CRSPR-cas 9 unzips the DNA with the defect
- Both strands of the DNA are cut
- The DNA repairs itself using the homology-directed repair (HDR) type system using the non-mutant homologous chromosome

1) Ma, H et al, Correction of a pathogenic gene mutation in human embryos. Nature. Published Online 2 August 2017

The story of CRISPR

Clustered **R**egularly **I**nterspaced **S**hort **P**alindromic **R**epeats

RACECAR
MADAM

1987 — Japan, Yoshizumi Ishino
First to describe CRISPR

1993 — Netherlands, D Van Soolingen
Used CRISPR to type M tuberculosis

2005 — Spain, Francisco Mojica, Ruud Jansen
Coined the CRISPR Term in 2001 and proposed the theory that it was used in microbial immunity

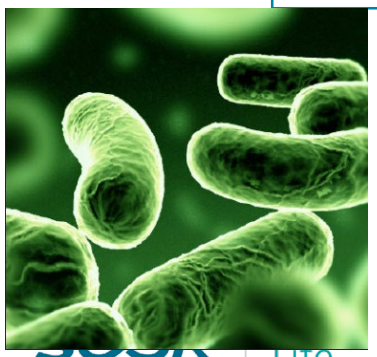
2012 — USA, Jennifer Doudna, Emmanuelle Charpentier
Developed a single-guide RNA which when combined to Cas9 could cut any DNA

2014 — USA, Feng Zhang, George Church
Used CRISPR to edit DNA in human cell cultures

2016 — 1st Clinical Trial started Cancer patients

2017 — Modified a human embryo

Late 2018



The Art & Science of Risk | Life

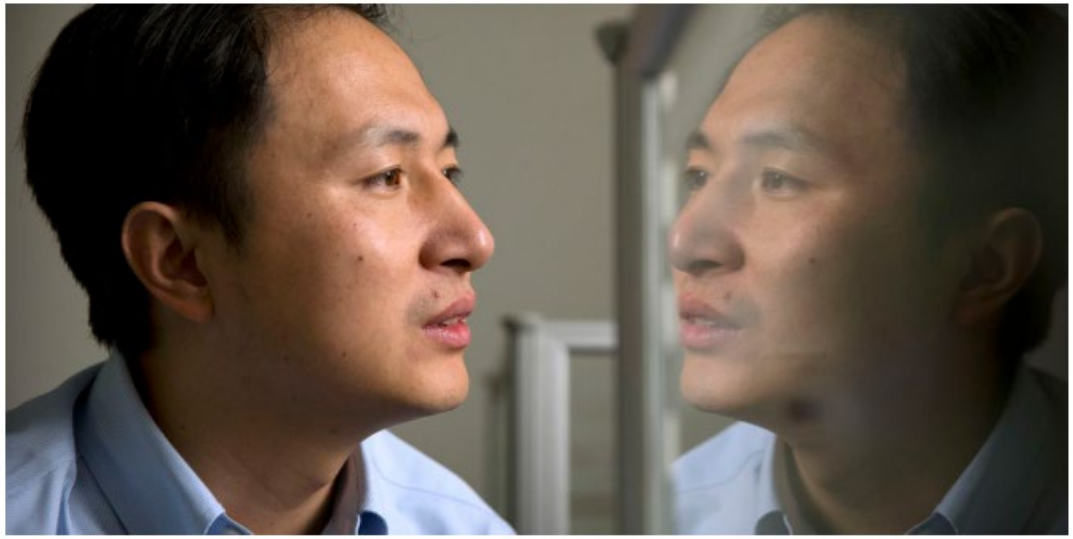
http://vedicsciences.net/design/bacteria5.jpg *Search using Bing's "free to modify, share, and use commercially" search engine.*

Current and Future Developments
CRISPR----Headlines

BREAKING NEWS!!!!
November 2018

FOX19 NOW FOX19NOW.com

Chinese researcher claims first gene-edited babies



Chinese researcher, He Jiankui Ph.D. of Shenzhen

Twin girls born!
Lulu and Nana

DNA altered by CRSPR technology.

Gene editing done to prevent HIV acquisition

http://www.fox19.com/2018/11/26/ap-exclusive-first-gene-edited-babies-claimed-china/

Current and Future Developments
January 2019

After the Storm — A Responsible Path for Genome Editing

George Q. Daley, M.D., Ph.D., Robin Lovell-Badge, Ph.D., and Julie Steffann, M.D., Ph.D.

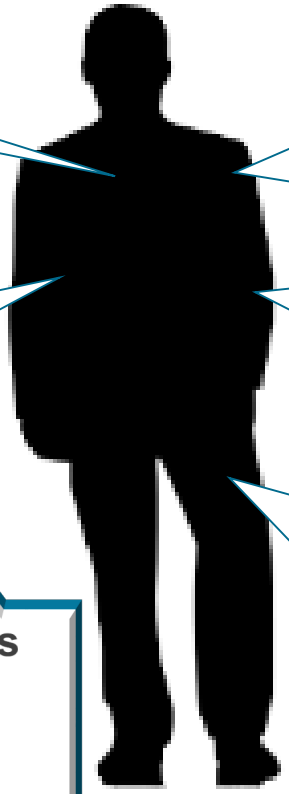
https://www.scientificamerican.com/article/embryo-gene-editing-experiment-reignites-ethical-debate/ Last accessed 8/2/2017

Natural history of HIV infection prior to ART

Viral Count 30,000 to 50,000 copies/mL

Average life expectancy 10 years

CD4 count drops 50/mm 3 per year



Opportunistic infections are common once CD4 count is <100.
Pneumocystis pneumonia ~50% chance in 1 year

Once the CD4 count drops to <50, the average life expectancy is 12-18 months

Pregnant women will infect their baby 15-30% of the time. (If prolonged breast feeding occurs that number increases to ~45%)

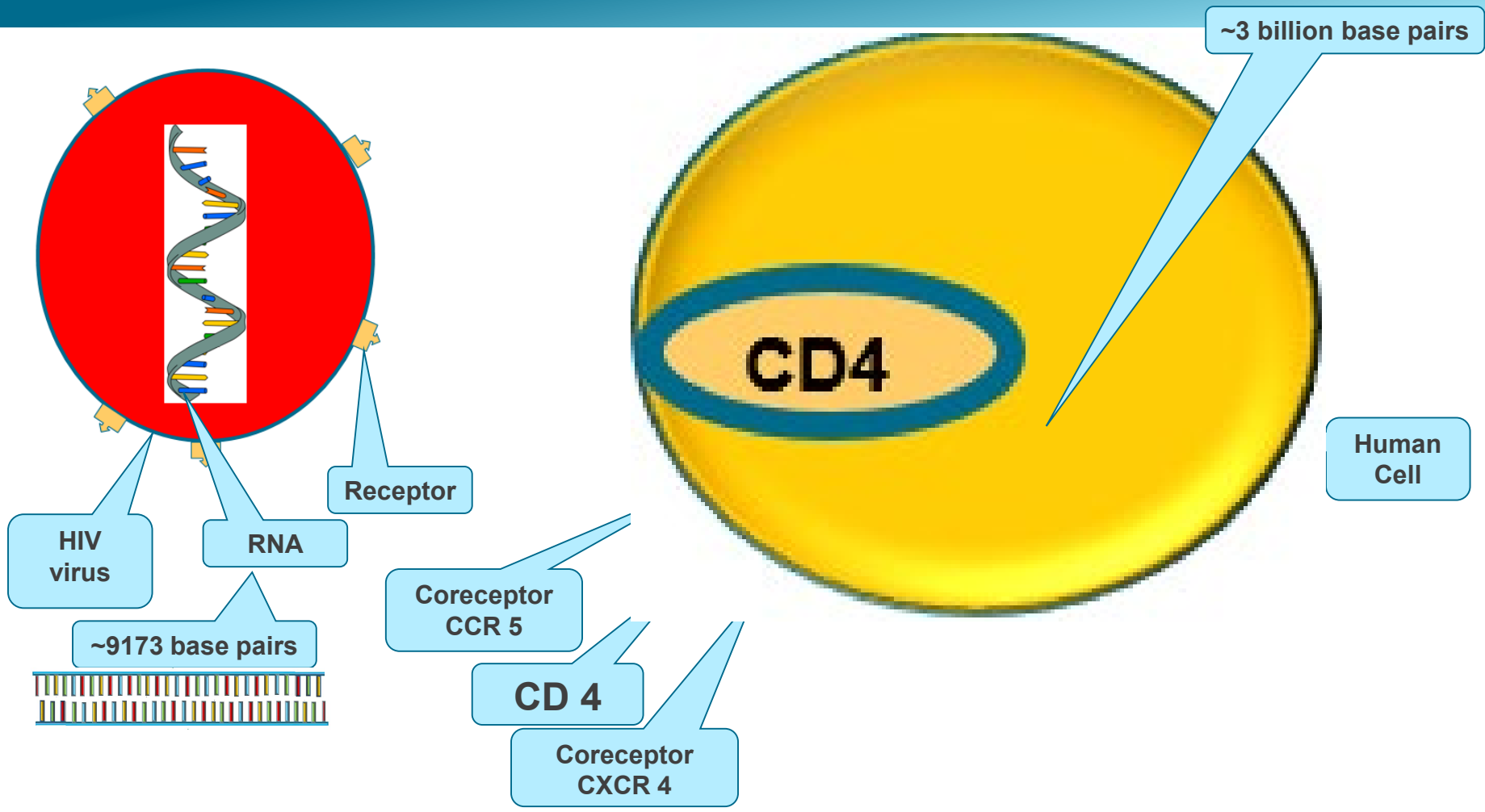
Long-term non-progressors

- A minority of HIV patients (~13%)
- Do not develop clinical progression
- Have stable CD4 counts
- Have low levels of viremia (<10,000 copies)

Elite controllers

- A minority of HIV patients (~1:200)
- Do not develop clinical progression
- Have stable CD4 counts
- Have undetectable viral counts

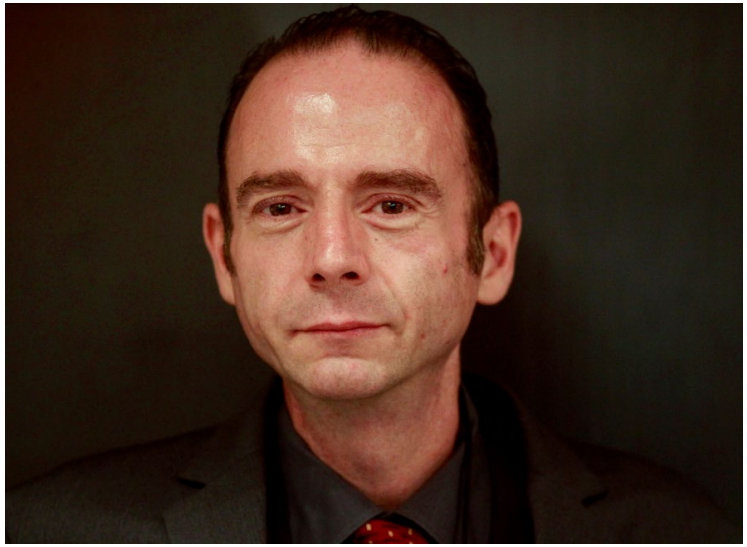
HIV--Invasion of the T Helper Cell



HIV and the Berlin Patient

Time For a Story

Timothy Ray Brown



https://62e528761d0685343e1c-f3d1b99a743ffa4142d9d7f1978d9686.ssl.cf2.rackcdn.com/files/67161/area14mp/image-20141214-6027-1q4lt2o.jpg Accessed with Bing's "free to share and use commercially" search engine. Last accessed 5/21/2018

Dr. Gero Hutter

http://www.cureaidsreport.org/interviews-q/#PrettyPhoto[426]/0/ Accessed 5/21/2018

Current work to develop a future “cure”

The Story Continues

Chemo was given but 7 months later the leukemia relapsed.

Mr Brown needed an allogenic stem cell transplantation

Although Dr. Hutter had never treated an HIV patient before he wondered if he might be able to cure the HIV.

Dr. Hutter searched for and then performed a transplantation from a donor who was homozygous for the CCR5 mutation.

Mr. Brown required a second transplant after another relapse.

He suffered from graft versus host disease

Once recovering from this second transplant he is now AML free.

Amazingly he also became the first person to be cured from HIV

The Berlin Patient

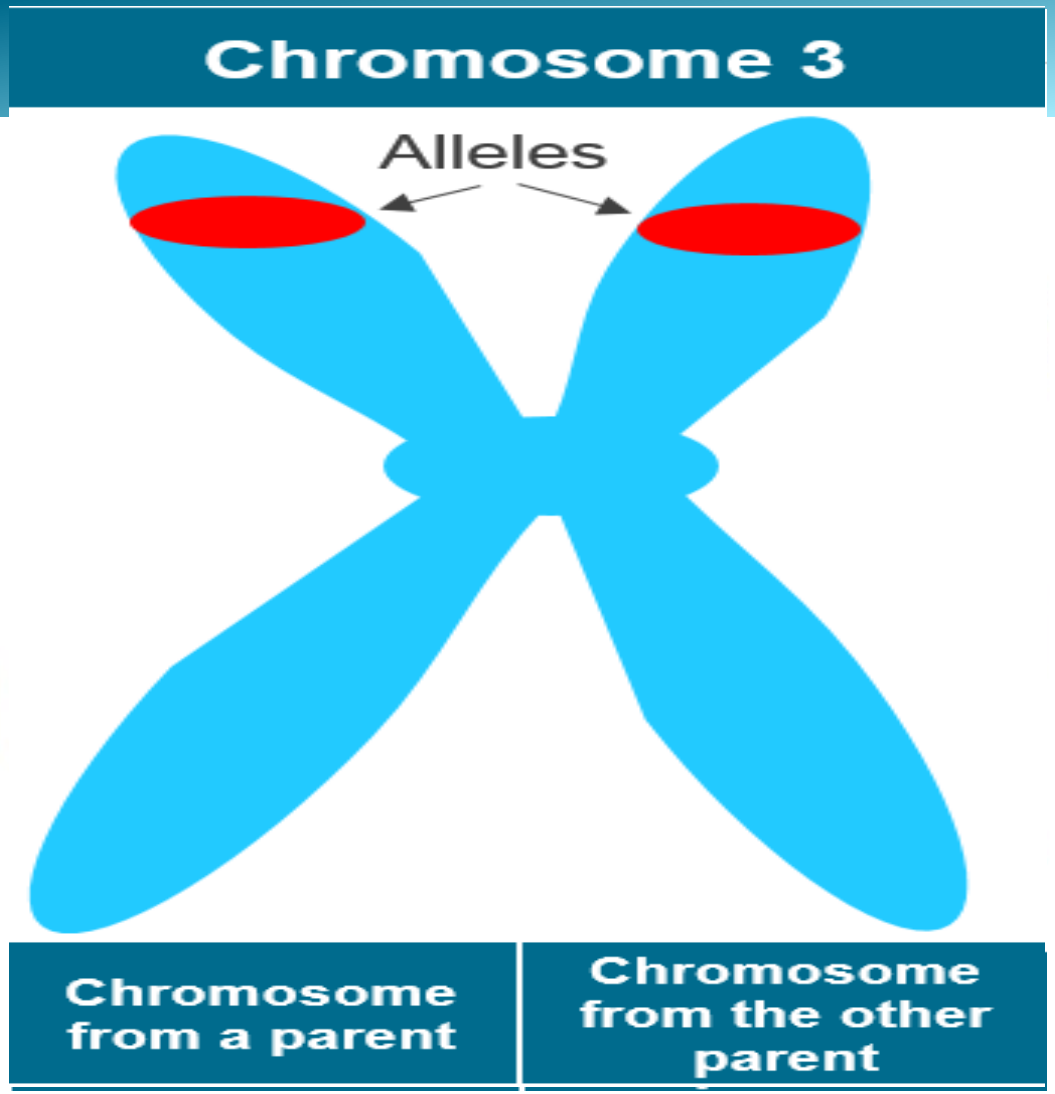
The protein in the CCR5 receptor is made from a gene in chromosome 3.

Some people have a deletion of 32 bp in the gene which makes the CCR5 protein receptor.

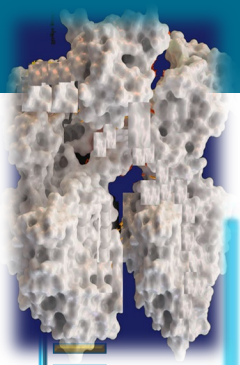
Without this functional receptor, the HIV virus has a harder time getting into the cell.

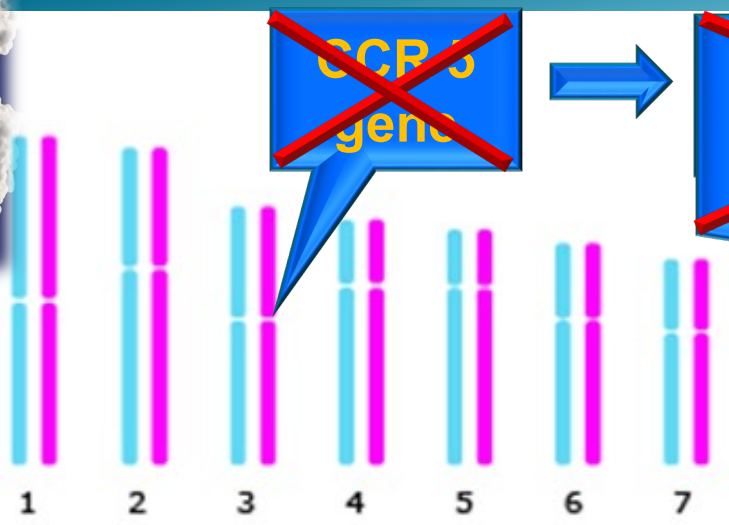
When the mutation involves one allele, it slows HIV progression.

When the mutation involves both alleles, it can prevent HIV disease or disease progression.

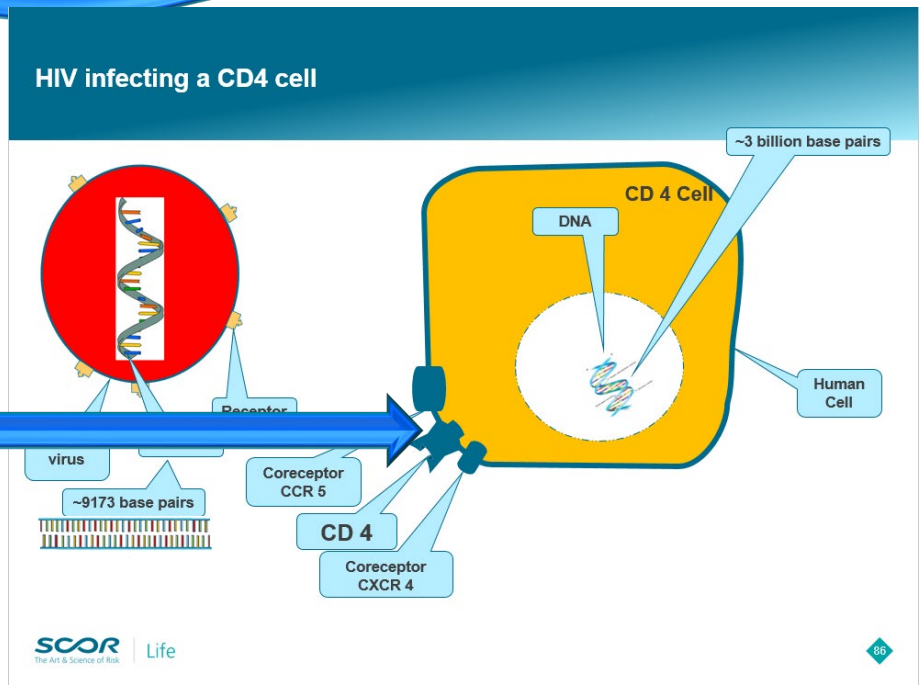


CRSPR-cas and HIV prevention gene editing



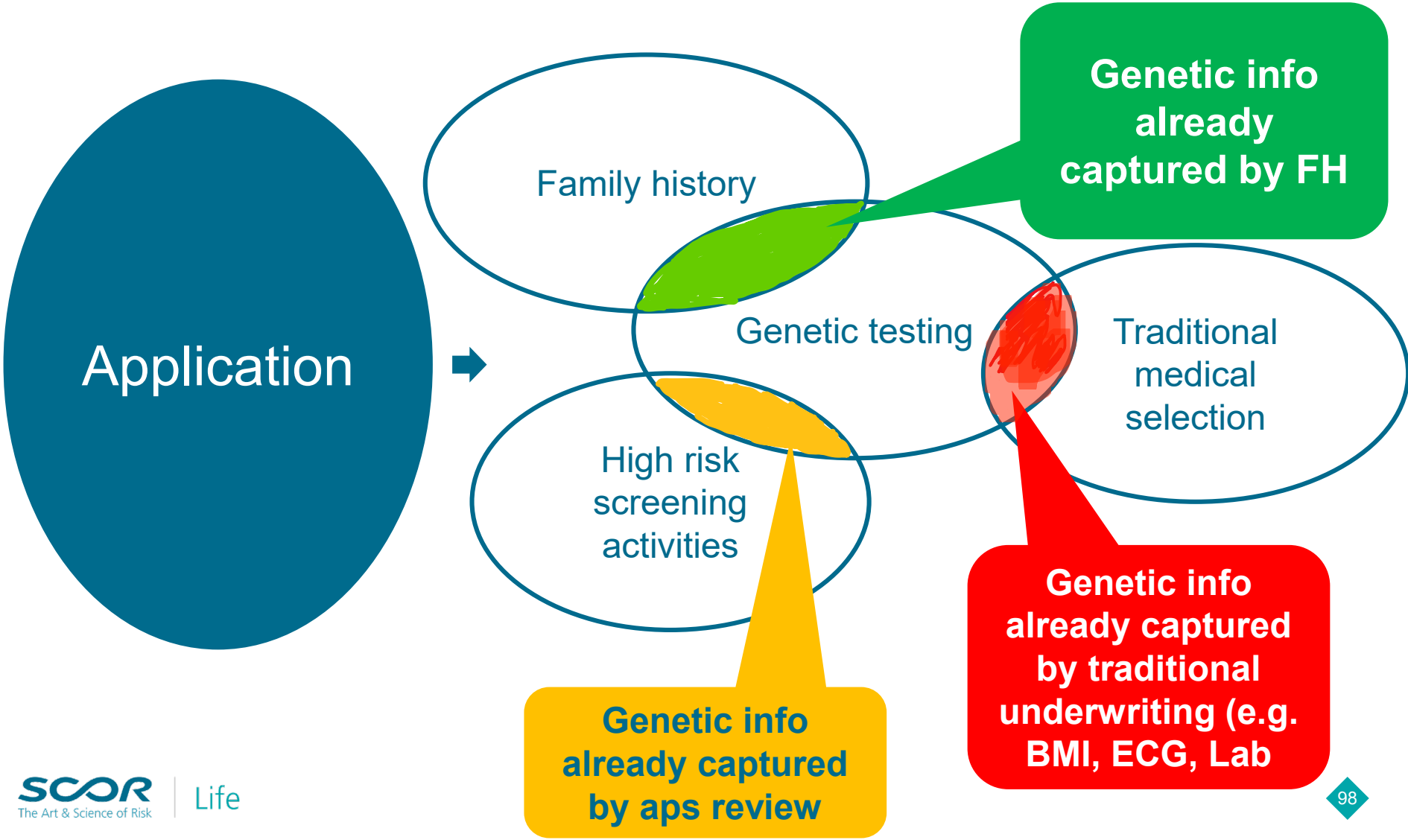


32 base pairs removed



1) h

Final thoughts regarding underwriting those with genetic testing



In Summary

DNA

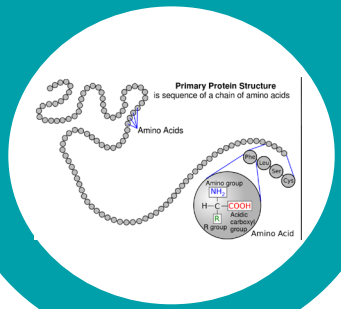
- Increased genetic testing
- Increased pre-symptomatic genetic testing
- Improved treatments

RNA

Significant increase in diagnostic and treatment opportunities

Protein

Work will continue



Questions or Comments?