Alphabet Soup: Genetics for the Practicing Allergist

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UC San Diego Medical Center

Rady Children's Hospital San Diego

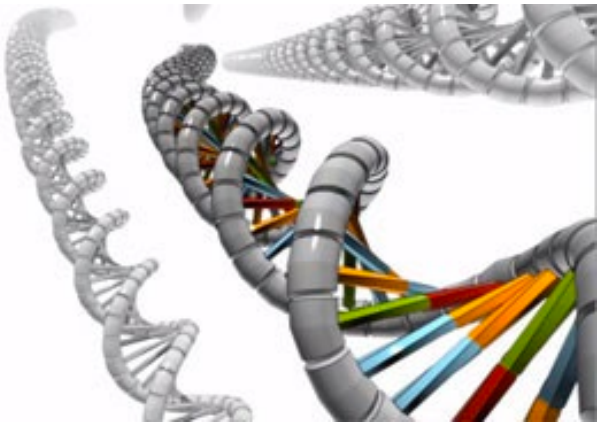
Learning Objectives

- *Upon completion of this learning activity, participants should be able to describe the different modalities of genetic testing currently available to patients.*

- *Upon completion of this learning activity, participants should be able to define variants of unknown significance and describe their relationship to clinical disease.*

Outline

- Overview of genes and the immune system
- Introduction to genetics
- “The pipeline”
- Interpretations
- What next?



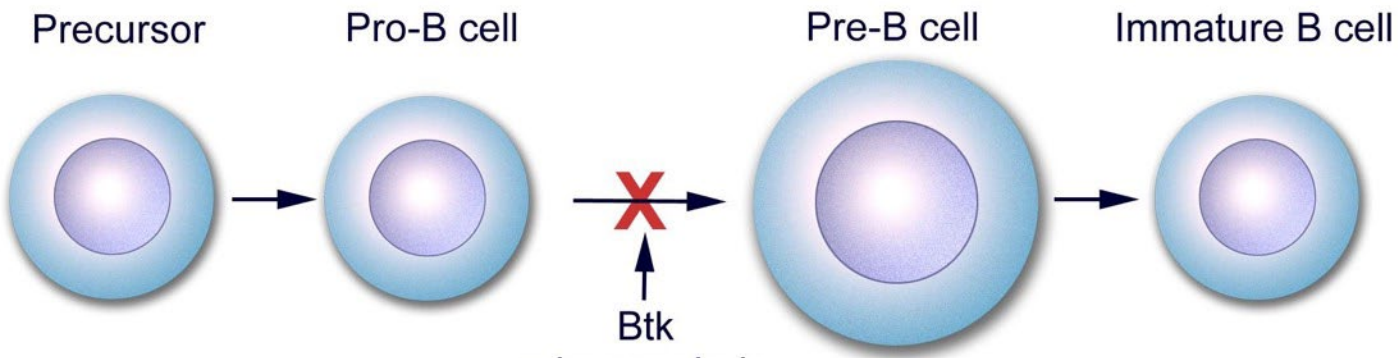
Partnering our understanding of genes and the immune system

Bruton's Agammaglobulinemia

- 1952: Colonel Ogden C. Bruton reported an 8 yo male with extreme susceptibility to infections.
  - Experiments showed a lack of IgG in the patient's serum.
  - Administering IgG controlled the life-threatening infections.

This was the first description of a PID and IgG replacement therapy

Bruton's Agammaglobulinemia

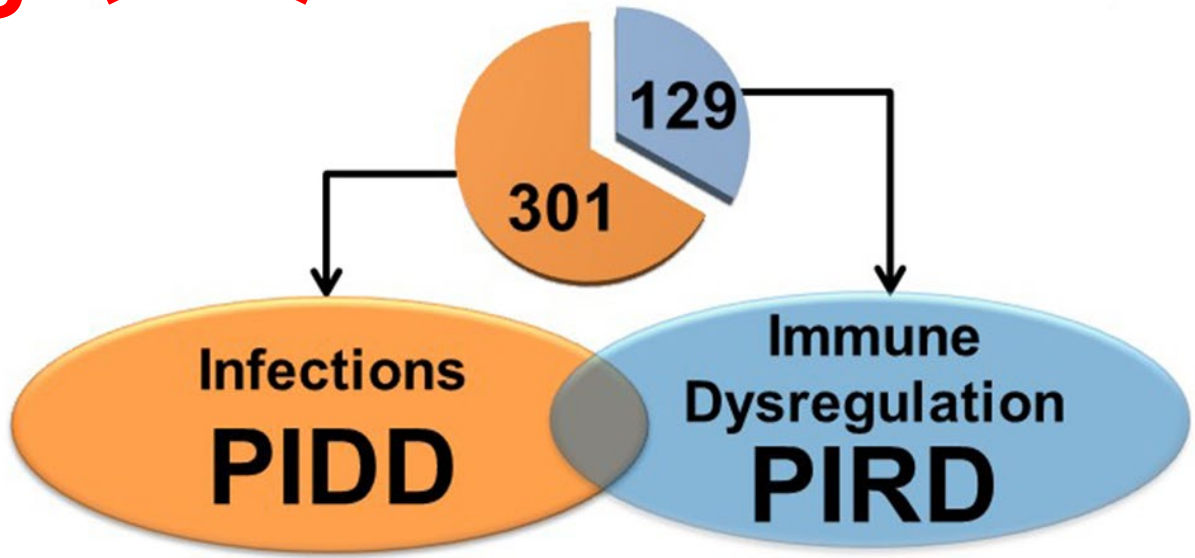


- Gene identified in early 1990s
- Treatment started without genetics

Conley. J Clin Invest. 2003; Tsukada et al. Cell. 1993; Vetrie et al. Nature.1993

>500

Inborn Errors of Immunity



- Tregopathies
- Autoinflammatory
- VEO-IBD
- ALPS-like
- Hyperinflammatory

Primary Immune Deficiency Disorders (PIDD)

- Infection-dominant pathology
- Therapies focused on infection treatment or prevention
- Example: SCID

Primary Immune Regulatory Disorders (PIRD)

- Immune-mediated pathology dominant
- Therapies focused on immune modulation
- Example: IPEX

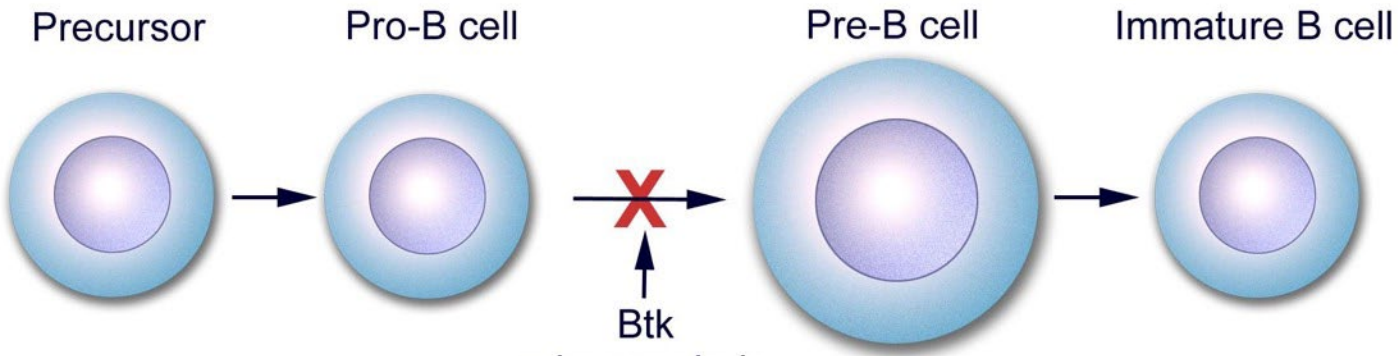
Tangye SG, et al. J Clin Immunol 2022l and pending update

Chan AY, Torgerson TR. Current Opinion in Allergy and Clinical Immunology. 2020

Genes and Immune Dysregulatory Disorders

- Some diseases have only 1 known gene
- Multiple genes can cause 1 disease
- New disease-causing genes are still being identified

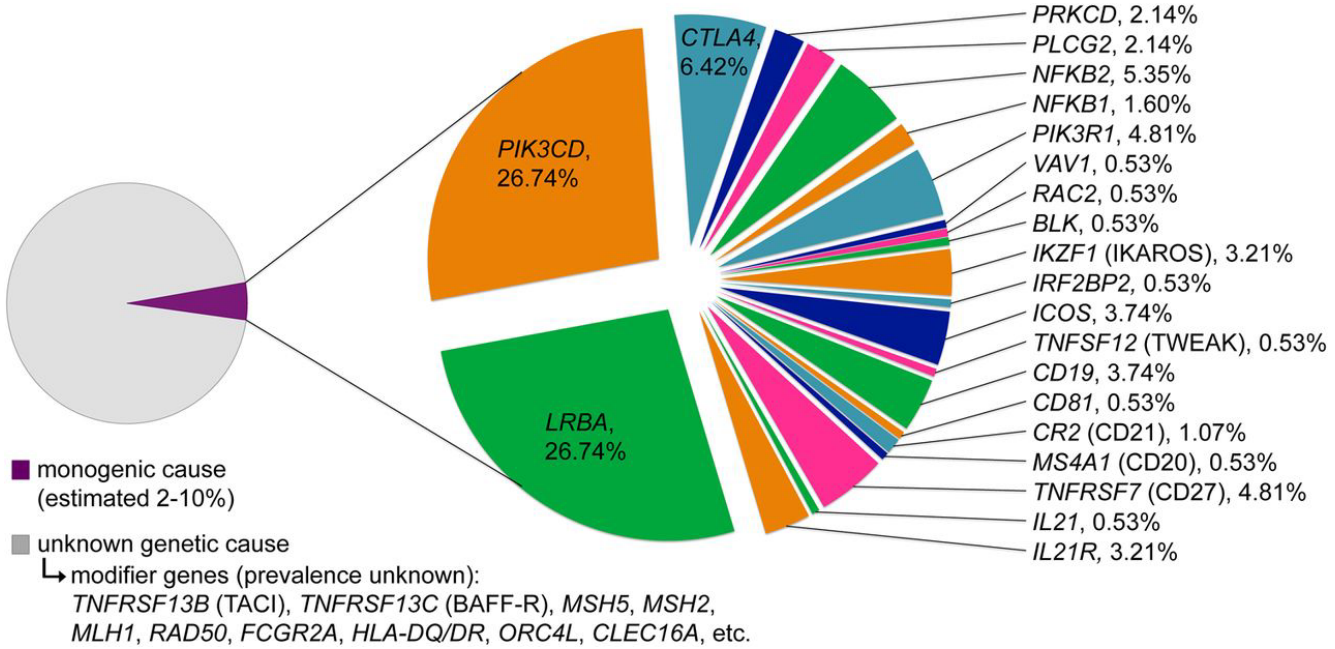
Bruton's Agammaglobulinemia



Conley. J Clin Invest. 2003; Tsukada et al. Cell. 1993; Vetrie et al. Nature.1993

Genes associated with CVID

Estimated share of each disease gene within the common variable immunodeficiency population based on published cases.



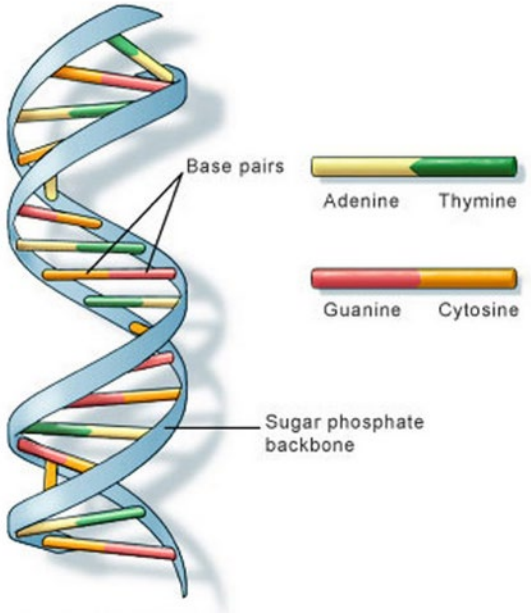
Delfien J A Bogaert et al. J Med Genet 2016;53:575-590

What is a gene?

What is DNA?

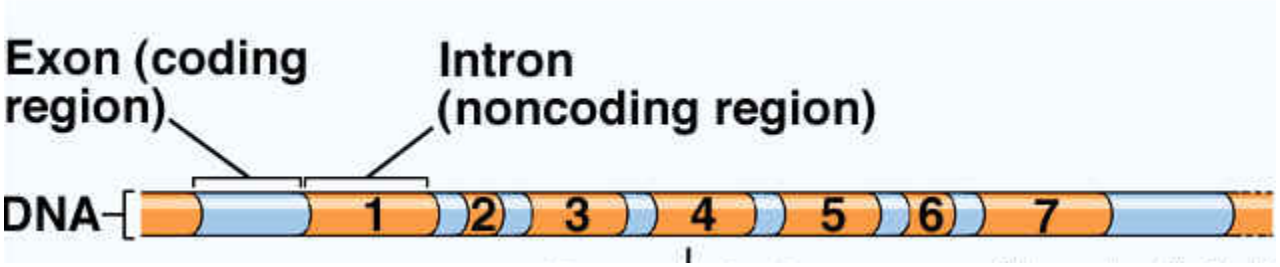
- The information in DNA is stored as a code made up of four chemical bases:
  - adenine (A)
  - guanine (G)
  - cytosine (C)
  - thymine (T)

- The organization of these 4 bases is most stable as the double helix



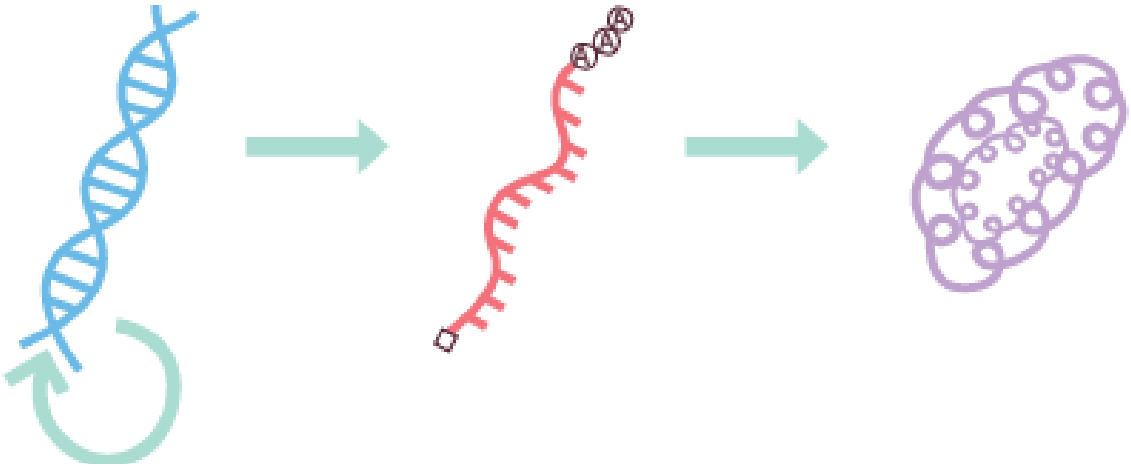
Genes are made of DNA

- The functional unit of inheritance
- Gene is made up of exons
- Exons are separated by introns



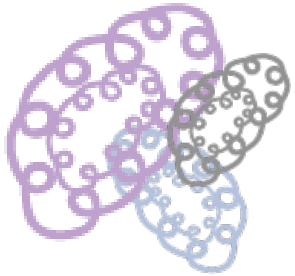
Flow of Genetic Information

DNA → RNA → PROTEIN



Flow of Genetic Information

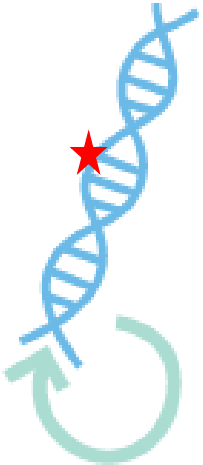
Protein interactions



PHENOTYPE

Genotype – Phenotype

DNA → PHENOTYPE



Genetic variants are spelling errors

- **Types** **SURFING**

  – Substitution – SERFING or SURGING
  – Insertion – SURFFING or SURFFERING
  – Deletion – SURF-NG or S---ING
  – Truncation – SURFI--

- These genetic variants may be anywhere in the gene

Slide courtesy of HM Hoffman

Other genetic changes

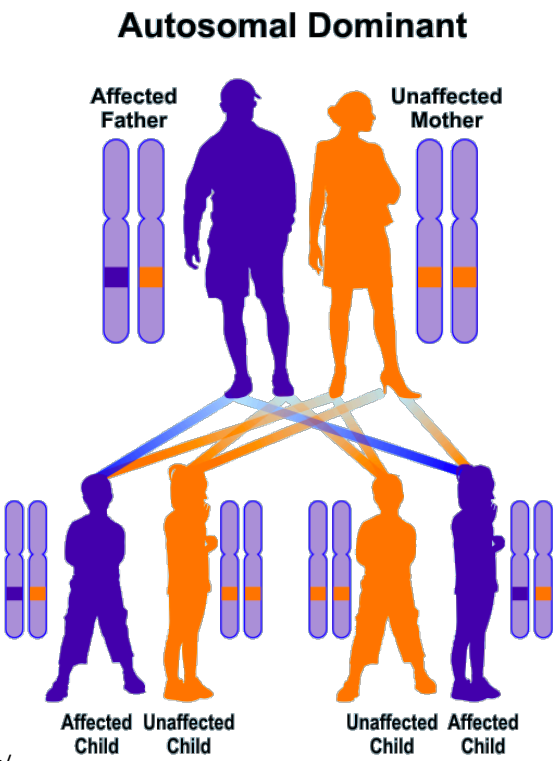
- Moderate sized insertions or deletions (1 or more exons)

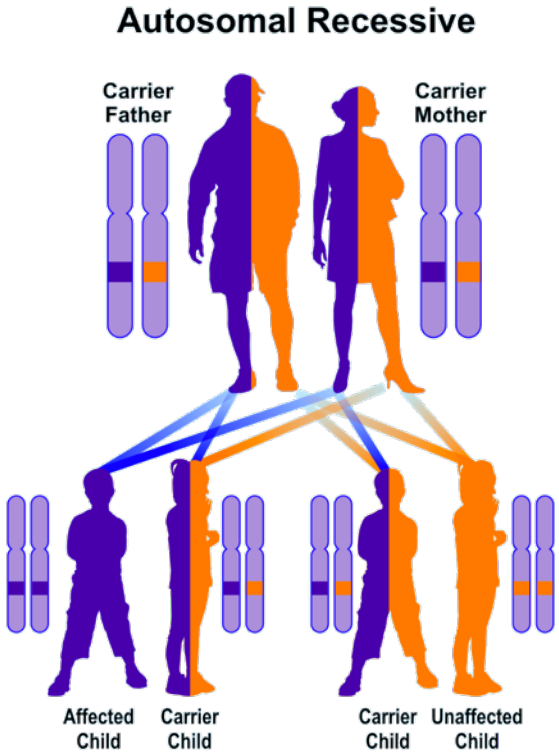
- Copy number variants
  - Gene duplications
  - Entire gene deletions

- Large Deletions/Duplications or entire chromosome number changes (monosomy or trisomy)

How can we inherit genetic changes?

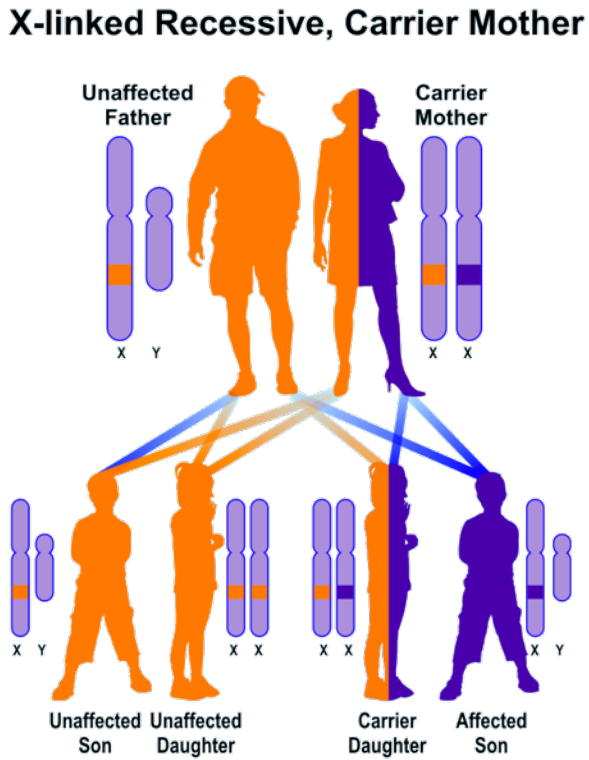
Inheritance Patterns

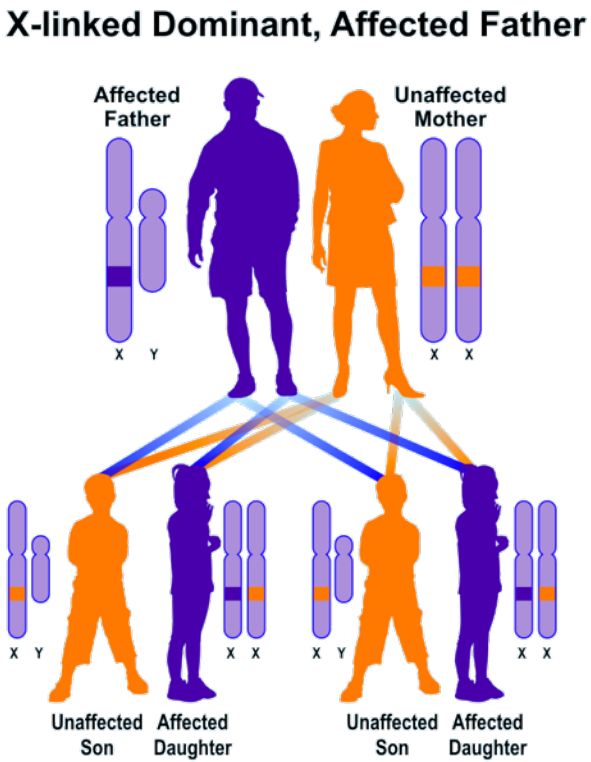




https://disorders.eyes.arizona.edu/

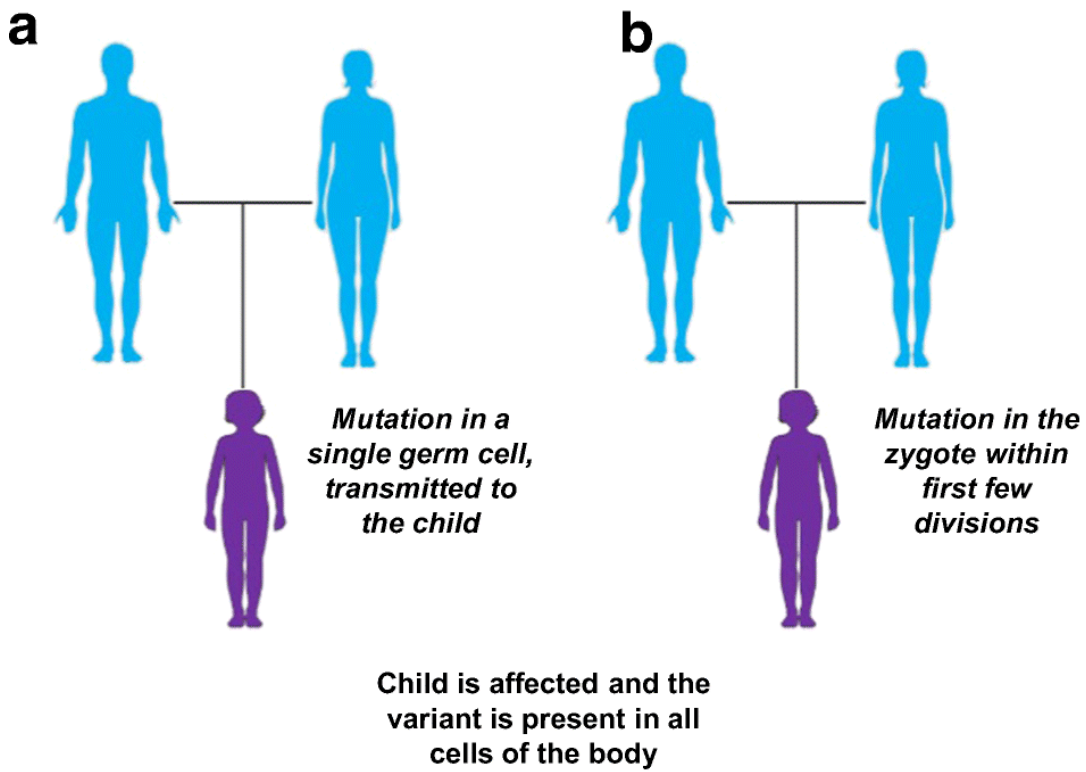
Inheritance Patterns





https://disorders.eyes.arizona.edu/

Sporadic or *de novo* variants



Aluri, J., Cooper, M.A. *J Clin Immunol* **41**, 718–728 (2021).

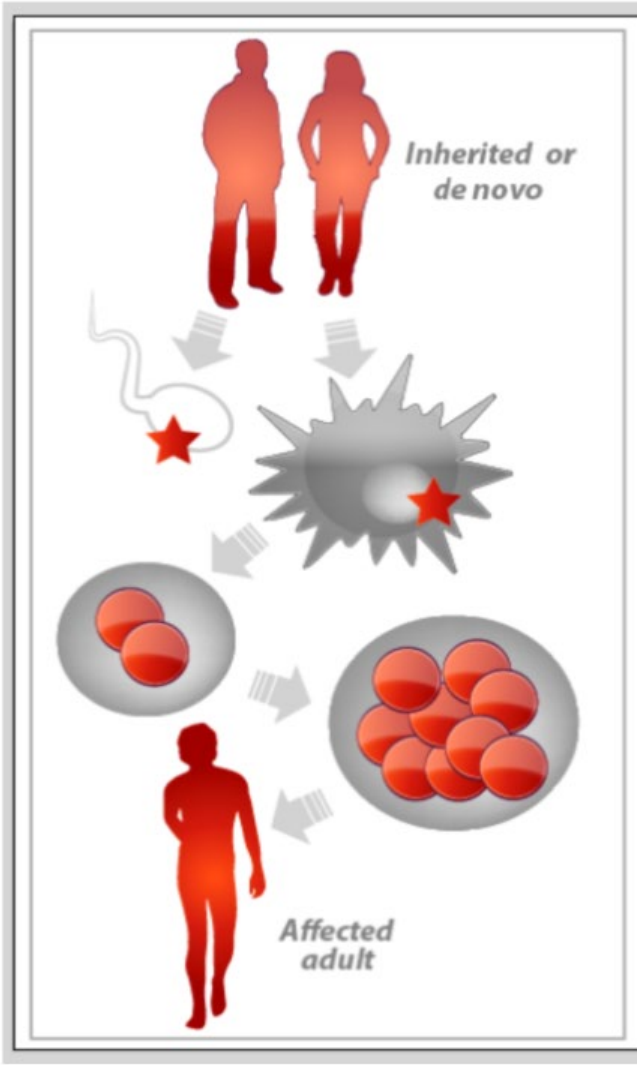
This happens in every person

Sometimes it leads to disease

Role of Genetic Testing

- Confirm diagnosis
- Define prognosis
- Treatment implications
- Family planning

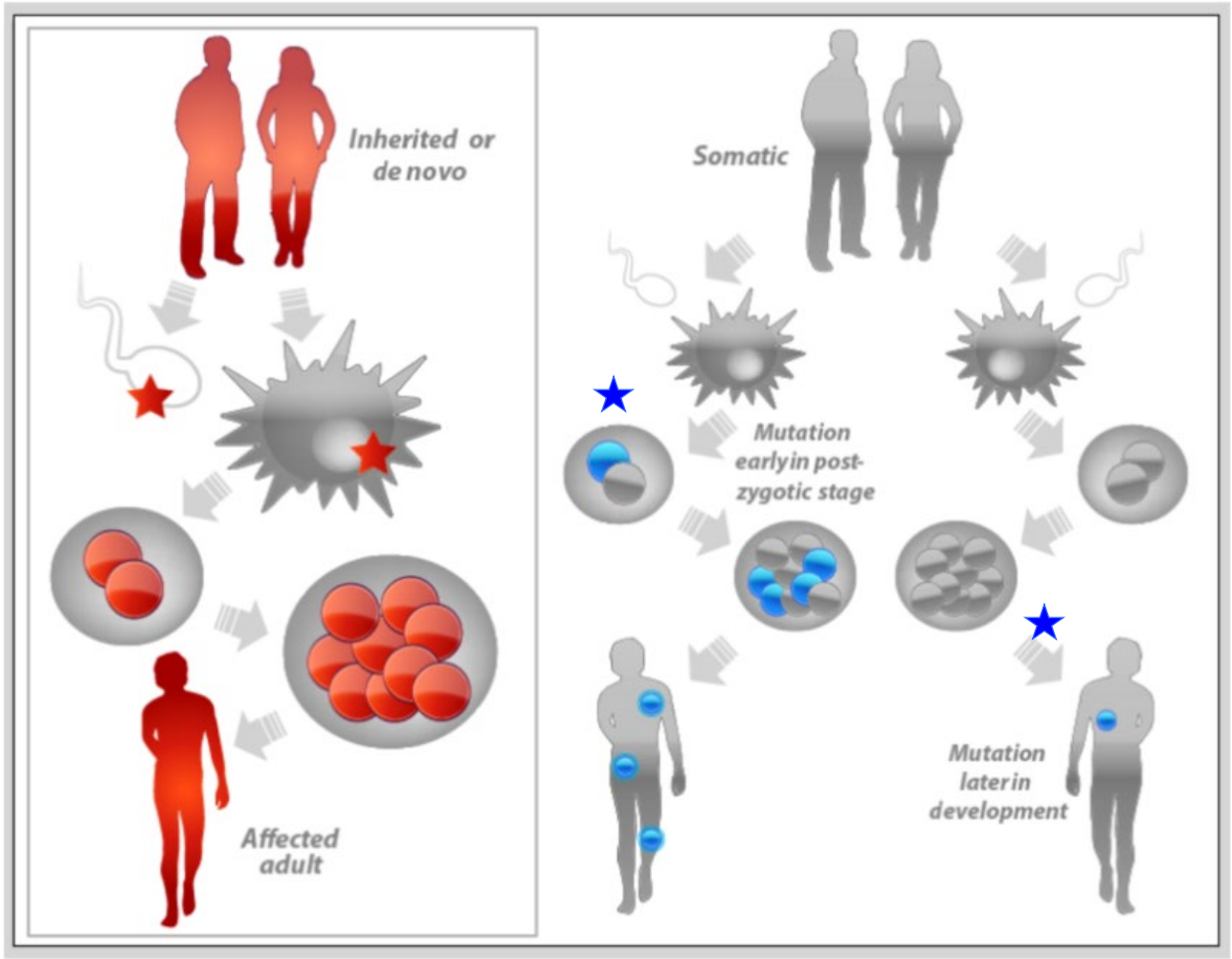
Is a genetic cause likely to be identified?



A family history may reveal the inheritance pattern, *but*:

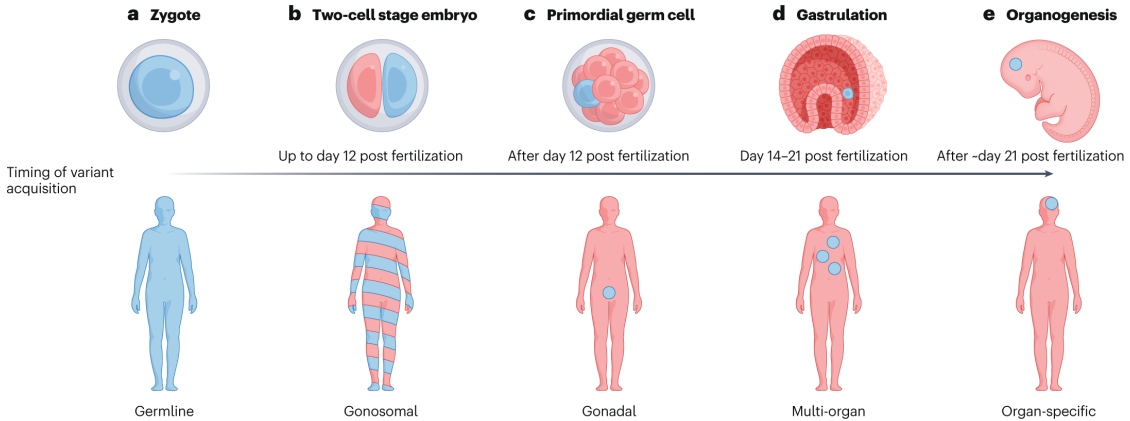
- *De novo* mutation
  - no other family member is affected (NOMID, TRAPS).
- Variable expressivity
  - Milder disease presenting later may be missed.
- Disease penetrance may vary,
  - not all mutation carriers are affected

Hoffman & Broderick, A&R 2016

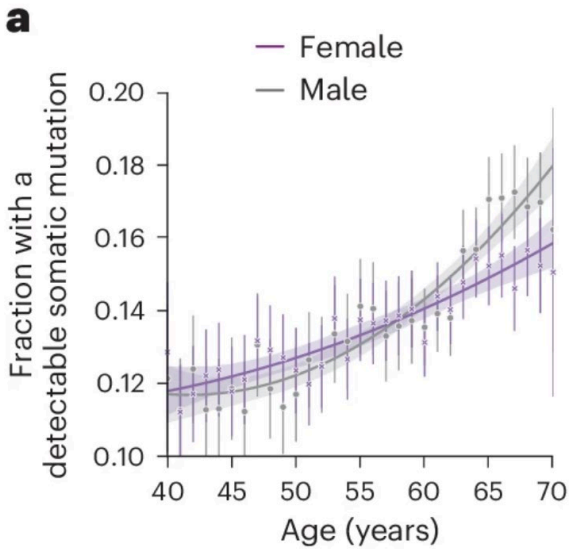


Hoffman, H. M. and Broderick, L. (2016), It just takes one: Somatic mosaicism in autoinflammatory disease. Arthritis & Rheumatology. doi:10.1002/art.39961

Embryonic mosaicism



Age associated mosaicism



Early onset disease

Late/adult onset disease

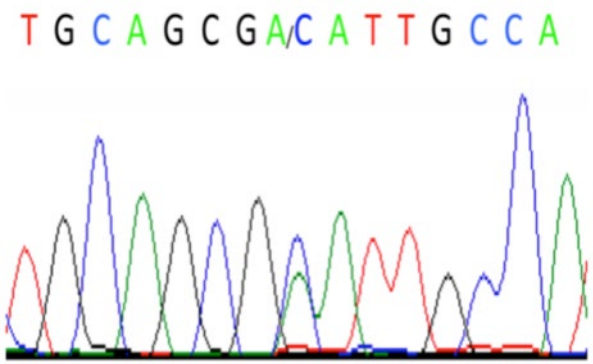
Waldvogel, S.M., Posey, J.E. & Goodell, M.A. *Nat Rev Genet* **25**, 698–714 (2024).

Bernstein, N., *et al. Nat Genet* **56**, 1147–1155 (2024).

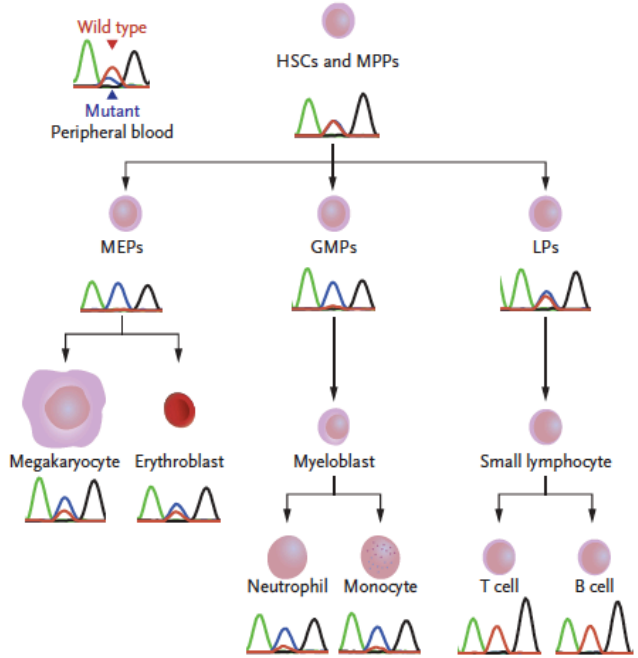
Somatic Mosaicism:

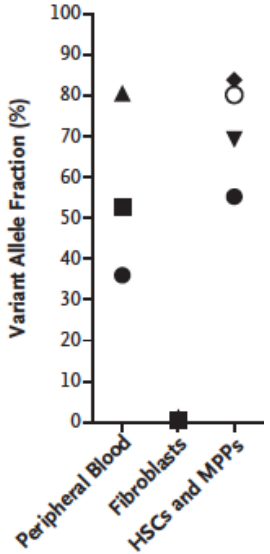
Two genetically distinct cell populations within an individual

- Clinical Implications
  - Difficult to detect in blood – sometimes only 4% of cells
  - Late onset phenotype – adults / parents of affected kids
  - Different phenotype due to expression
  - Difficult to predict inheritance
  - May be more common than thought



VEXAS: Vacuoles, E1 enzyme, X-linked, autoinflammatory, somatic syndrome





VEXAS occurred in 1 out of every 4,269 men older than age 50

- myeloid lineage-restricted *UBA1* somatic mutations -> anemia, thrombocytopenia

Beck, DB et al. NEJM. 2020
Beck DB, et al. *JAMA*. 2023

What kind of genetic test should I get?

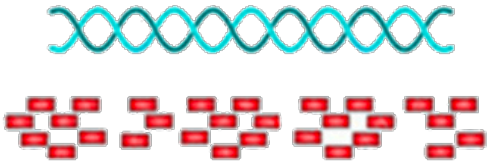
Types of genetic testing

- Chromosomal microarray
- Single gene
- Gene panels
- Whole exome
- Whole genome

Decision should be based on clinical history and other testing results

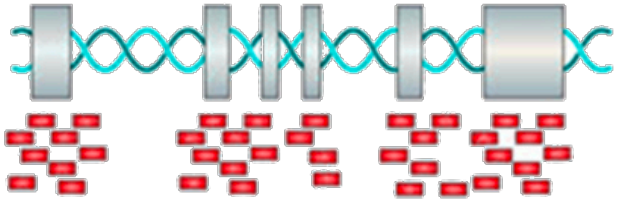
Whole Genome vs Exome vs Targeted Sequencing

Whole genome sequencing



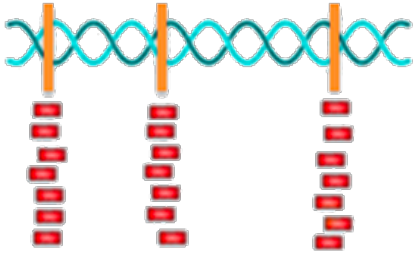
- Sequencing region : whole genome
- Sequencing Depth: >30X
- Covers everything – can identify all kinds of variants including SNPs, INDELs and SV.

Whole exome sequencing



- Sequencing region: whole exome
- Sequencing Depth : >50X ~ 100X
- Identify all kinds of variants including SNPs, INDELs and SV in coding region.
- Cost effective

Single gene or Panel

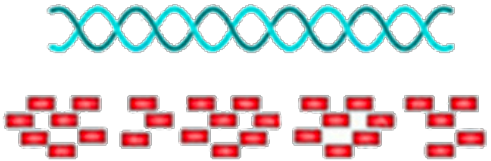


- Sequencing region: specific regions (could be customized)
- Sequencing Depth : >500X
- Identify all kinds of variants including SNPs, INDELs in specific regions
- Most Cost effective

Cortini et al 2017. Exploratory Research and Hypothesis in Medicine. 2. 57-62.

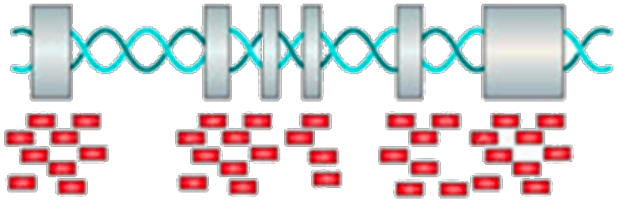
Whole Genome vs Exome vs Targeted Sequencing

Whole genome sequencing



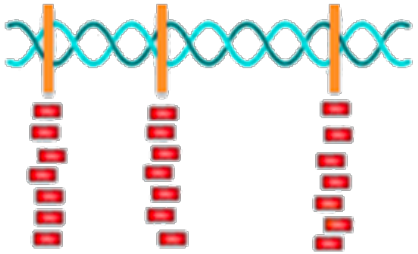
- Sequencing region : whole genome
- Sequencin… >30X
- Covers ev… can identify all kinds of variants including SNPs, INDELs and SV.

Whole exome sequencing



- Sequencing region: whole exome
- … variants including SNPs, INDELs and SV in coding region.
- Cost effective

Single gene or Panel



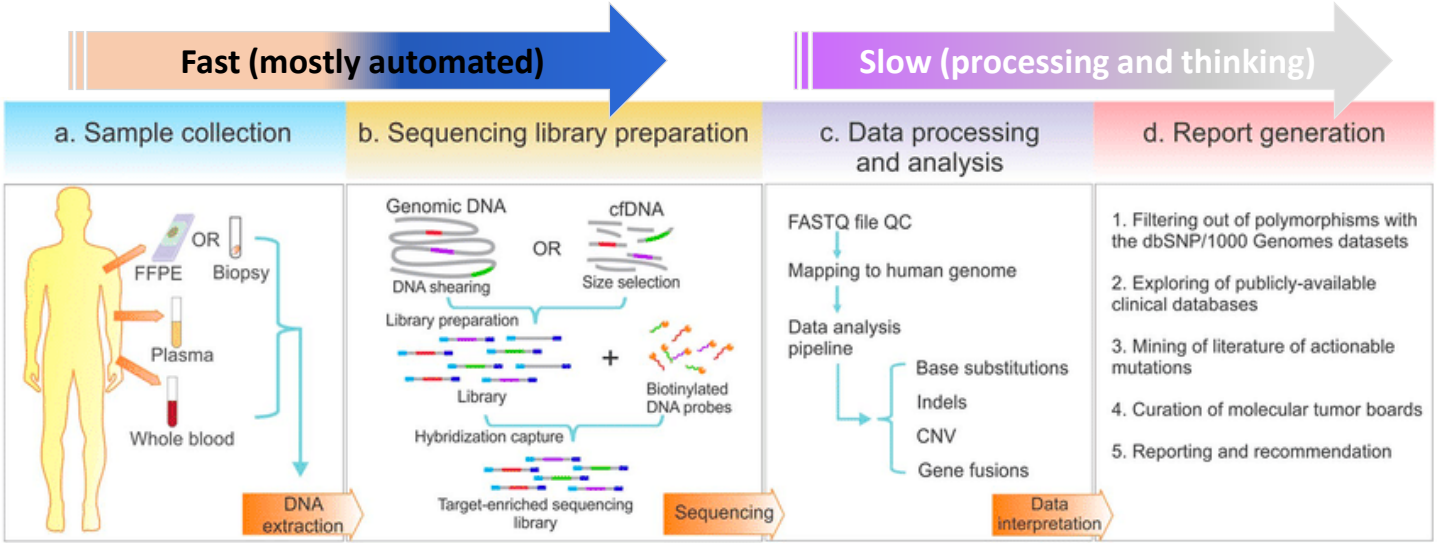
- Sequencing region: specific regions …e customized)
- …cing Depth :
- Identify all kinds of variants including SNPs, INDELs in specific regions
- Most Cost effective

All have ~20% success rate

Cortini et al 2017. Exploratory Research and Hypothesis in Medicine. 2. 57-62.

How did they generate this report (and what does it mean)?

The sequencing pipeline



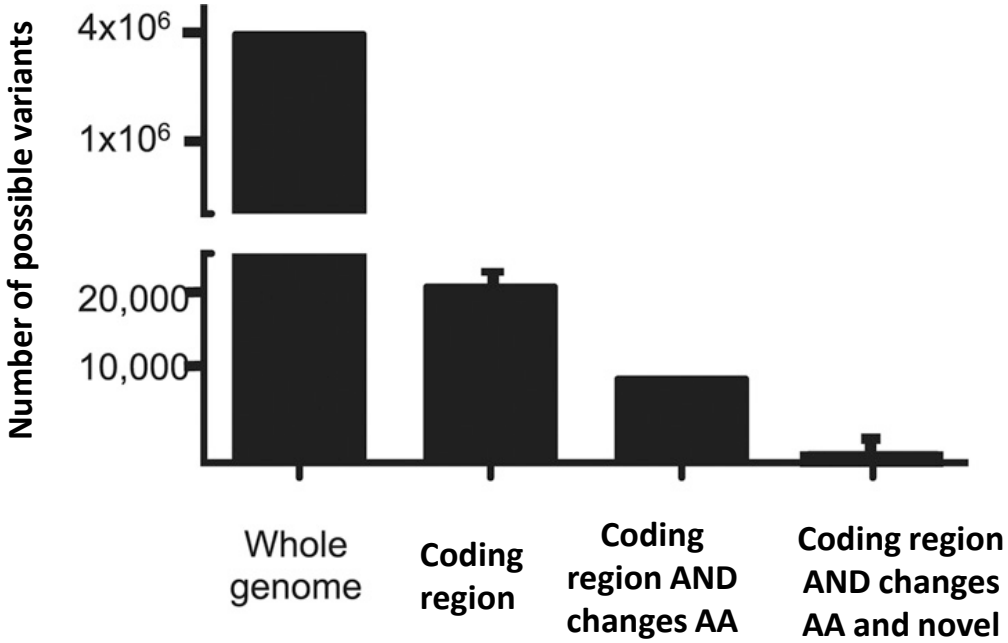
- Consent
- Insurance coverage
- Genetics consult
- Family discussion

Modified from Shu, et al. Scientific Reports. 7. 583. 10.1038/s41598-017-00520-1.

Interpretation

- Requires correlation between a positive finding and the clinical disease picture

Interpretation can be difficult



Platt et al. J Allergy Clin Immunol. 2014 Aug; 134(2): 262–268.

Clinical phenotype = candidate genotype: criteria*

- Family studies and population studies must indicate that the patient's candidate genotype is monogenic and does not occur in individuals without the clinical phenotype (complete penetrance).

- In-depth experimental and mechanistic studies must indicate that the genetic variant destroys or markedly impairs or alters the expression or function of the gene product (or two genetic variants in the case of compound heterozygosity).

- The causal relationship between the candidate genotype and the clinical phenotype must be established via a relevant cellular or animal phenotype.

*applies to single patient studies

Casanova JL, Conley ME, Seligman SJ, Abel L, Notarangelo LD. J Exp Med. 2014.

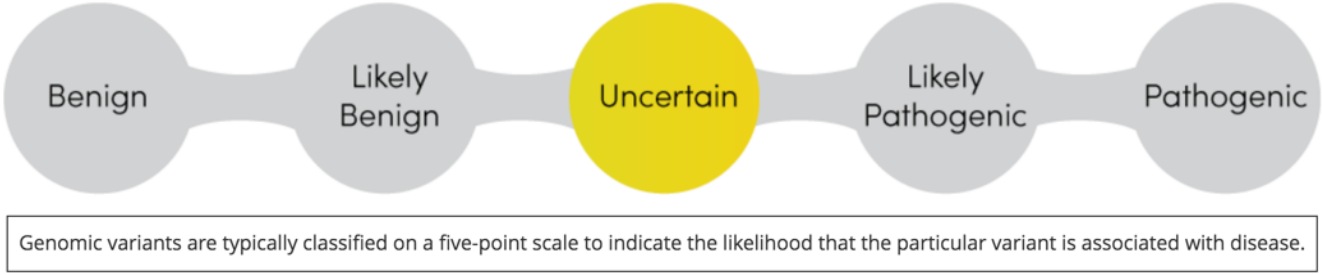
This means reports are ”living documents” and updated

Types of genetic variants

Category	Description
1	Findings relevant to the reason WGS was performed.
2	Clinically relevant variants for which treatment is or is not available.
3	Variants causing high risk for future mendelian diseases.
4	Carrier status that can impact reproductive life decisions.
5	Variants of variable risk for future diseases.
6	Variants of unknown significance.

Ayuso C. J Hum Gen. 2013

Variant classification



Genomic variants are typically classified on a five-point scale to indicate the likelihood that the particular variant is associated with disease.

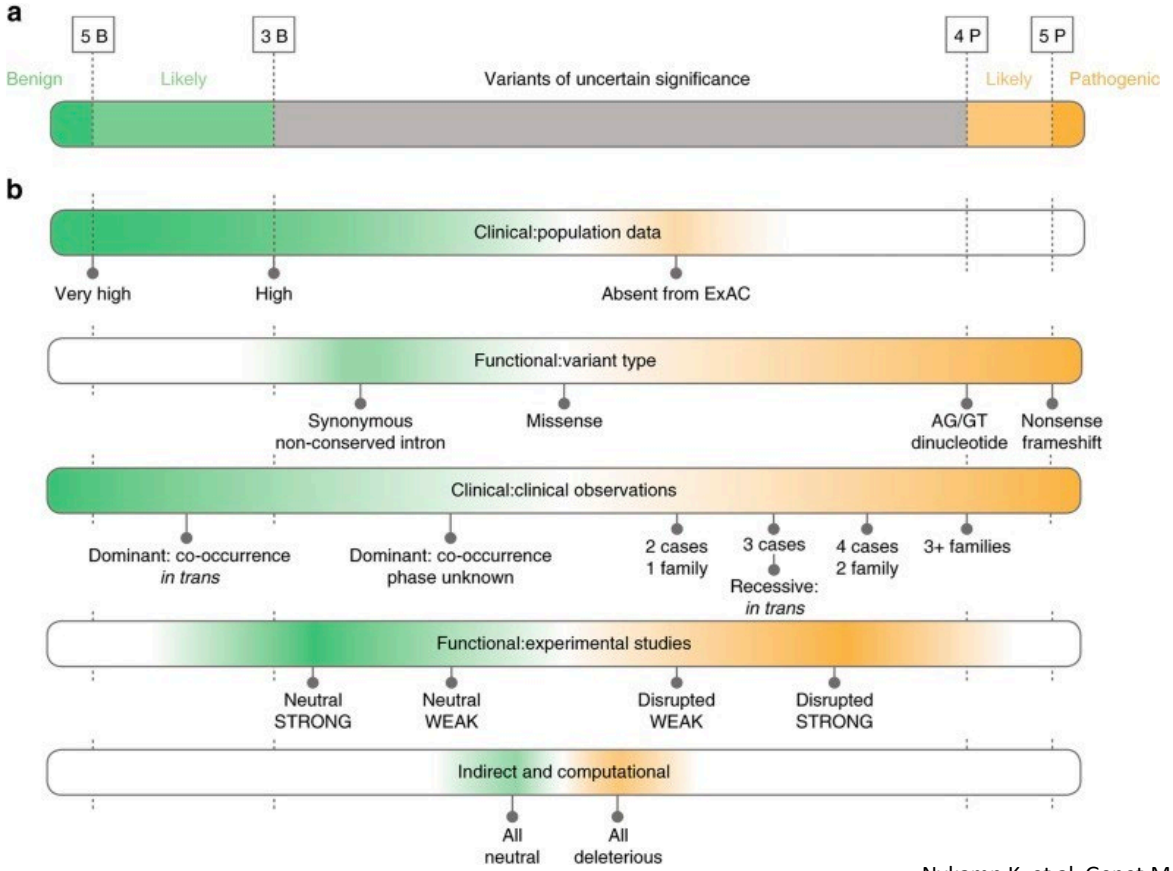
Commonly identified in gene panels, whole exome, and whole genome sequencing

TABLE VII. Prediction algorithm resources for variant interpretation

Resource	Web site
NMD prediction	
NMD Prediction Tool	https://nmdpredictions.shinyapps.io/shiny/
Splicing prediction	
FSPLICE	http://www.softberry.com/berry.phtml?topic=fsplice&group=programs&subgroup=gfind
GeneSplicer	http://www.cbcb.umd.edu/software/GeneSplicer/gene_spl.shtml
Human Splicing Finder	http://www.umd.be/HSF3/
MaxEntScan	http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html
MutPred Splice	http://www.mutdb.org/mutpredsplice/submit.htm
NetGene2	http://www.cbs.dtu.dk/services/NetGene2
NNSplice	http://www.fruitfly.org/seq_tools/splice.html
PESX	http://cubio.biology.columbia.edu/pesx/pesx/
SKIPPY	https://research.nhgri.nih.gov/skippy/index.shtml
Spliceman	http://fairbrother.biomed.brown.edu/spliceman/index.cgi
Missense prediction	
Align GVGD	http://agvgd.iarc.fr/agvgd_input.php
CADD	http://cadd.gs.washington.edu/
Condel	http://bg.upf.edu/fannsdb/help/condel.html
ConSurf	http://consurftest.tau.ac.il
DANN	https://cbcl.ics.uci.edu/public_data/DANN/
EA	http://mammoth.bcm.tmc.edu/uea/hEAt.html
Eigen	http://www.columbia.edu/~ii2135/eigen.html
FATHMM	http://fathmm.biocompute.org.uk/
GenoCanyon	http://genocanyon.med.yale.edu/GenoCanyon
GERP++	http://mendel.stanford.edu/SidowLab/downloads/gerp/
GWAVA	https://www.sanger.ac.uk/sanger/StatGen_Gwava
hEAt	http://mammoth.bcm.tmc.edu/uea/hEAt.html
integrated_fitCons	http://compgen.bscb.cornell.edu/fitCons/
LRT	http://www.genetics.wustl.edu/jflab/lrt_query.html
MAPP	http://mendel.stanford.edu/SidowLab/downloads/MAPP/index.html
M-CAP	http://bejerano.stanford.edu/mcap/
MetaLR	https://sites.google.com/site/jpopgen/dbNSFP
MetaSVM	https://sites.google.com/site/jpopgen/dbNSFP
MutationAssessor	http://mutationassessor.org/
MutationTaster	http://www.mutationtaster.org/
MutPred	http://mutpred1.mutdb.org/
nsSNPAnalyzer	http://snpanalyzer.uthsc.edu
PANTHER	http://www.pantherdb.org/tools/csnpScoreForm.jsp
phastCons100way	http://compgen.cshl.edu/phast/index.php
PhD-SNP	http://snps.biofold.org/phd-snp/phd-snp.html
phyloP100way	http://compgen.cshl.edu/phast/index.php
PolyPhen-2	http://genetics.bwh.harvard.edu/pph2/
PROVEAN	http://provean.jcvi.org/index.php
REVEL	https://sites.google.com/site/revelgenomics/about
SIFT	http://sift.bii.a-star.edu.sg/sift-bin/contact.pl
SiPhy	http://www.broadinstitute.org/mammals/2x/siphy_hg19/
SNPs&GO	http://snps-and-go.biocomp.unibo.it/snps-and-go
VEST3	http://karchinlab.org/apps/appVest.html
Other prediction tools	
Mutation Significance Cut-off	http://pec630.rockefeller.edu:8080/MSC/
Gene Damage Index	http://pec630.rockefeller.edu:8080/GDI/
gnomAD pLoF	http://gnomad.broadinstitute.org/

GWAVA, Genome-wide Annotation of Variants; *M-CAP*, Mendelian Clinically Applicable Pathogenicity; *NMD*, nonsense-mediated decay; *pLoF*, probability of loss of function intolerance.

Invitae and Sherloc



Nykamp K, et al. Genet Med. 2017.

Variants of Unknown Significance

- Variants of unknown significance have an uncertain relationship to disease.
- The effect of the specific genetic alteration on gene function is not known.
- There are insufficient genetic data to definitively confirm that the variant is associated with disease.

I don't feel comfortable explaining all this…

INVITAE

VUS RESULTS GUIDE

What your results mean for you

No significant genetic changes ("pathogenic variants" or "mutations") were found in your genetic test. However, your test did find a genetic change called a variant of uncertain significance (VUS) in one or more of the genes tested. When we see a genetic change, but are unsure of its impact on health, it is called a variant of uncertain significance.

Right now, there is not enough information about the VUS to know whether it causes disease or not. A VUS is a common type of result. We all have many genetic changes that do not cause medical problems. Most of the time, we later learn that a VUS is not related to disease risk.

Your risk for disease could still be influenced by a combination of unidentified genetic, personal, lifestyle and/or environmental factors. So, it's important to talk to your healthcare provider if you have questions about your risk.

Create a plan with your healthcare provider

These genetic test results should be shared with your healthcare providers. The chance for you to develop a disease is not determined by genetic test results alone. Your provider can help you make informed decisions about your healthcare.

What your results mean for your family

Testing family members for a VUS is usually not recommended. However, your report will note if testing your family members will help us learn more about your specific VUS.

Although your genetic test did not find a significant genetic change, your family members have their own unique genetic makeup. Genetic testing can help them understand their overall chance of developing a genetic disease.

We (and others) are here to help

Genetic counseling can help you clearly and accurately understand your results so it's important to talk to your genetic counselor or other healthcare provider about your test results. Invitae also has board-certified genetic counselors who are available to answer questions about your test results or your personal or family medical history.

Log in to your patient portal (invitae.com) to view your results, search for a local or Invitae genetic counselor, or join Invitae's Patient Insight Network (PIN), a community where you can connect with other patients and share your experience.

What does a typical report look like?

Summary

Gene name

One Pathogenic variant identified in MVK.

bp change aa change

Clinical Summary

- A Pathogenic variant, c.1129G>A (p.Val377Ile), was identified in MVK.
  - The MVK gene is associated with autosomal recessive mevalonate kinase deficiency which encompasses hyper-IgD syndrome (MedGen UID: 140768) and autosomal recessive mevalonic aciduria (MedGen UID: 368373). In addition, the MVK gene is associated with autosomal dominant porokeratosis (MedGen UID: 401352).

Clinical information

  - This individual is a heterozygous carrier for autosomal recessive MVK-related conditions. Please note that this specific variant has not been reported to confer risk for autosomal dominant MVK-related conditions. Therefore this Pathogenic variant may not be sufficient as an explanation for this individual's condition and/or family history. It is possible that a second Pathogenic variant is present in this individual but has not been detected by this assay, which includes sequence and deletion/duplication analysis of the MVK gene.
  - Mevalonate kinase deficiency is an inborn error of cholesterol biosynthesis with autoinflammatory symptoms that can present with a range in severity from the milder hyperimmunoglobulin D (hyper-IgD) syndrome to the severe mevalonic aciduria. Patients with hyper-IgD have a periodic fever syndrome characterized by attacks of idiopathic fever accompanied by chills, joint pain, headache, vomiting, diarrhea, and lymphadenopathy with leukocytosis. Attacks typically occur once every 1-2 months, with duration of up to a week. While most patients present in the first year of life, there is variability in expression and age of onset (PMID: 8190036). Mevalonic aciduria is characterized by varying degrees of developmental delays, hypotonia, dysmorphic features, cerebellar ataxia, cataracts, myopathy, and recurrent fevers with hepatomegaly, lymphadenopathy, joint pain, and rash (PMID: 8386351). While the earliest patients described typically had a severe infantile presentation, milder phenotypes have been reported (PMID: 12563048, 16835861). Clinical management guidelines for mevalonate kinase deficiency can be found at PMID: 26109736.

LIABILITIES

Complete Results

Does not report source!

How? Why?

Gene	Variant	Zygosity	Variant Classification
MVK	c.1129G>A (p.Val377Ile)	heterozygous	PATHOGENIC

The following genes were evaluated for sequence changes and exonic deletions/duplications:
ACP5, ADA, ADA2, ADAM17, ADAR, AICDA, BTK, CARD14, CD3G, CD40LG, COPA, CTLA4, CYBA, CYBB, DCLRE1C, DKC1, DOCK8, ELANE, FOXP3, G6PC3, ICOS, IFIH1, IL10, IL10RA, IL10RB, IL1RN, IL21, IL2RA, IL2RG, IL36RN, ITGB2, LIG4, LPIN2, LRBA, MEFV, MVK, NCF2, NCF4, NFAT5, NLRC4, NLRP12, NLRP3, NOD2, PIK3CD, PIK3R1, PLCG2, PSMB8, PSTPIP1, RAG1, RAG2, RBCK1, RNASEH2A, RNASEH2B, RNASEH2C, RTEL1, SAMHD1, SH2D1A, SH3BP2, SLC29A3, SLC37A4, STAT1, STAT3, STIM1, STXBP2, TMEM173, TNFRSF1A, TREX1, TRNT1, TTC7A, WAS, XIAP, ZAP70

Results are negative unless otherwise indicated

Benign and Likely Benign variants are not included in this report but are available upon request. An asterisk (*) indicates that this gene has a limitation. Please see the Limitations section for details.

Variant Details

Variant information

MVK, Exon 11, c.1129G>A (p.Val377Ile), heterozygous, PATHOGENIC

- This sequence change replaces valine with isoleucine at codon 377 of the MVK protein (p.Val377Ile). The valine residue is moderately conserved and there is a small physicochemical difference between valine and isoleucine.
- This variant is present in population databases (rs28934897, ExAC 0.2%).
- This variant has been reported as homozygous or compound heterozygous in numerous individuals and families affected with hyper IgD syndrome (HIDS), and is one of the most commonly reported variants found in HIDS patients (PMID: 12634869, 26977311, 15536479, 11313769, 10369261). Asymptomatic individuals homozygous or compound heterozygous for this variant have also been reported, suggesting this variant may be associated with a mild phenotype or reduced penetrance (PMID: 12634869, 26977311). ClinVar contains an entry for this variant (Variation ID: 11929).
- Experimental studies have shown that this missense change results in reduced MVK enzymatic activity (PMID: 10369261, 26977311).
- For these reasons, this variant has been classified as Pathogenic.

The usual result…

RESULT: UNCERTAIN

Variant(s) of Uncertain Significance identified.

GENE	VARIANT	ZYGOSITY	VARIANT CLASSIFICATION
CHD7	c.6341A>G (p.Tyr2114Cys)	heterozygous	Uncertain Significance
DDX58	c.2666T>C (p.Ile889Thr)	heterozygous	Uncertain Significance
STIM1	c.1754C>T (p.Ala585Val)	heterozygous	Uncertain Significance
TLR3	c.1660C>T (p.Pro554Ser)	heterozygous	Uncertain Significance

About this test

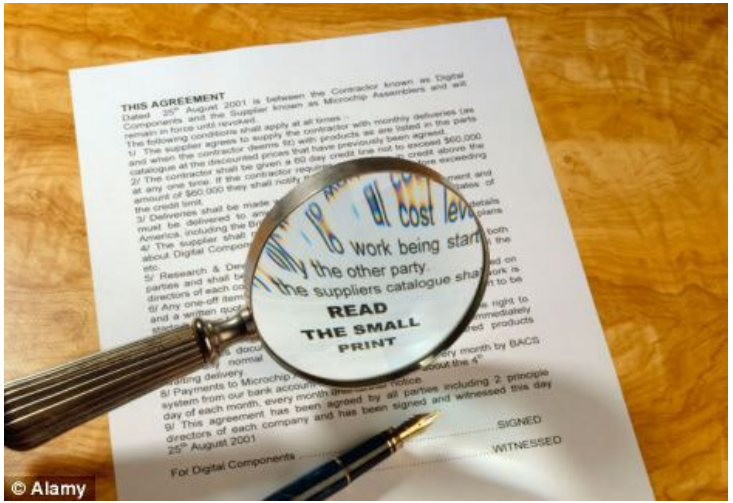
This diagnostic test evaluates 429 gene(s) for variants (genetic changes) that are associated with genetic disorders. Diagnostic genetic testing, when combined with family history and other medical results, may provide information to clarify individual risk, support a clinical diagnosis, and assist with the development of a personalized treatment and management strategy.

How can I look into the results further?

Finding your own evidence

- PubMed
  - Abstracts
  - New publications
- Scientific proceedings
- Phone a friend
- Reports may be updated as new information is generated

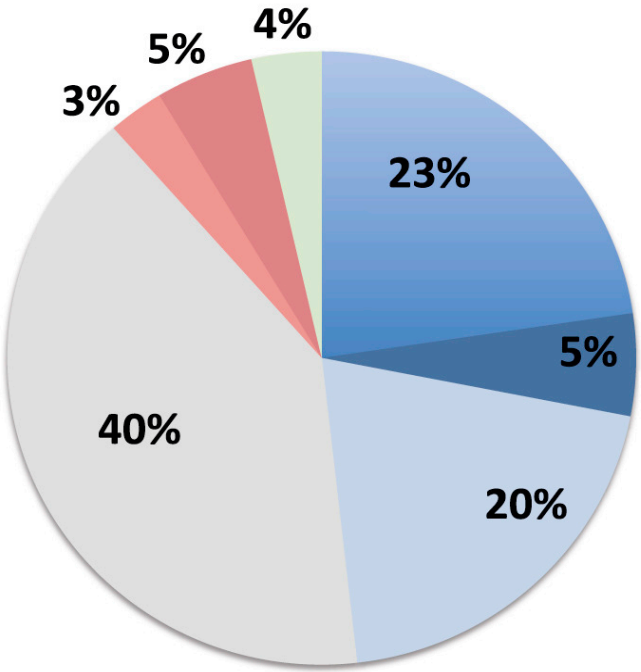
If there are so many genetic changes, why was my test negative (or didn't give the result I expected)?



The small print…and setting expectations

Immune genetics are complicated

n=278 families



- Known disease gene
- Two disease genes, mixed phenotype
- Finding in phenotypic relevant gene, inconclusive
- No relevant finding
- Potential novel disease gene
- Novel PIDD gene, confirmed
- Immunophenotypic expansion

Stray-Pedersen et al. JACI

Focus on Mendelian disease

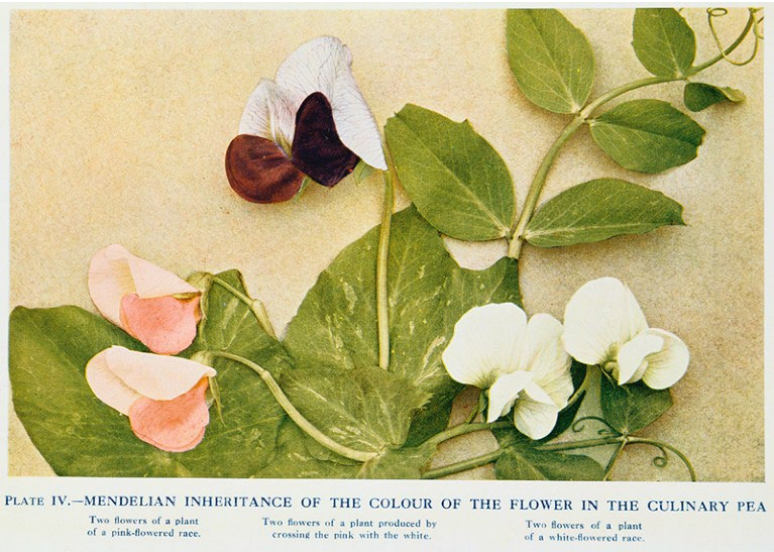
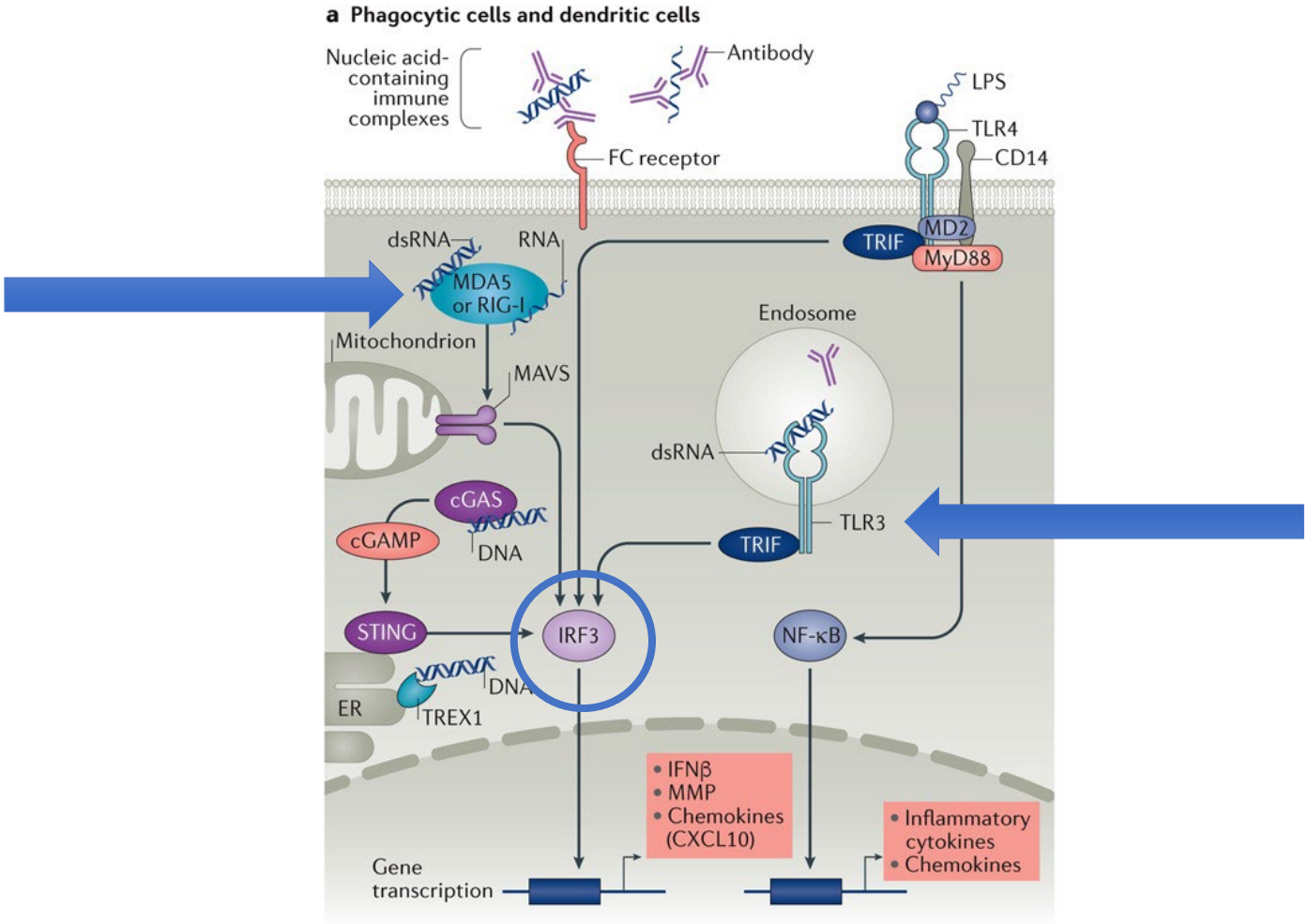


PLATE IV.—MENDELIAN INHERITANCE OF THE COLOUR OF THE FLOWER IN THE CULINARY PEA

Two flowers of a plant of a pink-flowered race. Two flowers of a plant produced by crossing the pink with the white. Two flowers of a plant of a white-flowered race.

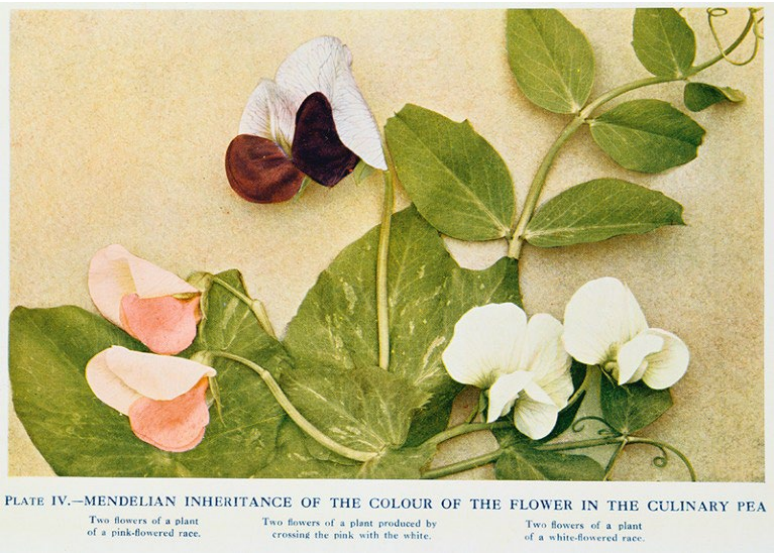
- Non-mendelian inheritance
  - Digenic
  - Oligogenic
  - “Burden of variants”

Nature **607**, 421-422 (2022)



Muskardin, T. L. M. & Niewold, T. B. (2018) *Nat. Rev. Rheumatol.* doi:10.1038/nrrheum.2018.31

Focus on Mendelian disease



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- Non-mendelian inheritance
  - Digenic
  - Oligogenic
  - “Burden of variants”
- Multifactorial
  - Contribution of environment
  - Epigenetic changes

Nature **607**, 421-422 (2022)

Mosaicism and malignancy

Additionally, it may not be possible to fully resolve certain details about variants, such as mosaicism, phasing, or mapping ambiguity. Unless explicitly guaranteed, sequence changes in the promoter, non-coding exons, and other non-coding regions are not covered by this assay. Please consult the test definition on our website for details regarding regions or types of variants that are covered or excluded for this test. This report reflects the analysis of an extracted genomic DNA sample. While this test is intended to reflect the analysis of extracted genomic DNA from a referred patient, in very rare cases the analyzed DNA may not represent that individual's constitutional genome, such as in the case of a circulating hematolymphoid neoplasm, bone marrow transplant, blood transfusion, chimerism, culture artifact or maternal cell contamination.

Sequence issues

- Was the region adequately covered?

- Introns
- Pseudogenes
- Repeats

Cause	International Union of Immunological Societies primary immunodeficiency disease gene(s)
Incomplete (<100%) exonic coverage by WES platforms at a minimum read depth of 10×	**A** *AIRE, AP3D1, ATP6AP1* **B** *BCL11B* **C** *C4A, C4B, CARMIL2, CD8A* **E** *ERCC6L2* **I** *IKBKG, IRAK1* **M** *MALT1* **N** *NCF1, NFAT5* **P** *PEPD, PRKDC* **R** *RBCK1, RMRP, RNU4ATAC* **S** *SLC29A3* **T** *TBX1, TPP2* **U** *UNC93B1, USP18*
Pathogenic intronic variants	*ATM, BTK, CYBB, DCLRE1C, DOCK8, GATA2, IL2RG, IKBKG, IRAK4, ITGB2, JAK3, LRBA, SKIV2L, UNC13D*
Pathogenic 5′-UTR variants	*RPSA*
Pathogenic 3′-UTR variants	*IL2RG, LAMTOR2*
Pathogenic polyadenylation signal variants	*FOXP3, WAS*

UTR, Untranslated region.

Chinn et al JACI 2019

Some exons are not covered

ATM: Sequencing analysis for exons 6, 24, 43 includes only cds +/- 10 bp. RANBP2: Deletion/duplication and sequencing analysis is not offered for exons 1-11, 15-29. IFNGR2: Sequencing analysis for exons 6 includes only cds +/- 10 bp. GFI1: Sequencing analysis for exons 6 includes only cds +/- 0 bp. SI: Deletion/duplication analysis is not offered for exon 7. PTPRC: Sequencing analysis is not offered for exons 3, 15. EFL1: Deletion/duplication and sequencing analysis is not offered for exons 7, 15. TOP2B: Deletion/duplication analysis is not offered for exon 5. AK2: Deletion/duplication and sequencing analysis is not offered for exon 6. CSF2RA: Deletion/duplication analysis is not offered for this gene. SLC9A3: Deletion/duplication analysis is not offered for exon 8. UNC93B1: Deletion/duplication analysis is not offered for exon 11. DUOX2: Deletion/duplication and sequencing analysis is not offered for exons 6-7. POLD1: Sequencing analysis for exons 22 includes only cds +/- 10 bp. STAT5B: Deletion/duplication and sequencing analysis is not offered for exons 6-8. ALPK1: Sequencing analysis for exons 8 includes only cds +/- 10 bp. KDM6A: Sequencing analysis for exons 18 includes only cds +/- 10 bp. CFH: Deletion/duplication analysis is not offered for exons 20, 22 and sequencing analysis is not offered for exons 15, 20, 22. SAR1B: Deletion/duplication analysis is not offered for exon 5. FANCL: Sequencing analysis for exons 4, 10 includes only cds +/- 10 bp. ADGRE2: Deletion/duplication analysis is not offered for exons 3, 6-9 and sequencing analysis is not offered for exons 6-9. Sequencing analysis for exons 17 includes only cds +/- 10 bp. CORO1A: Deletion/duplication and sequencing analysis is not offered for exon 11.

Not all VUS are reported

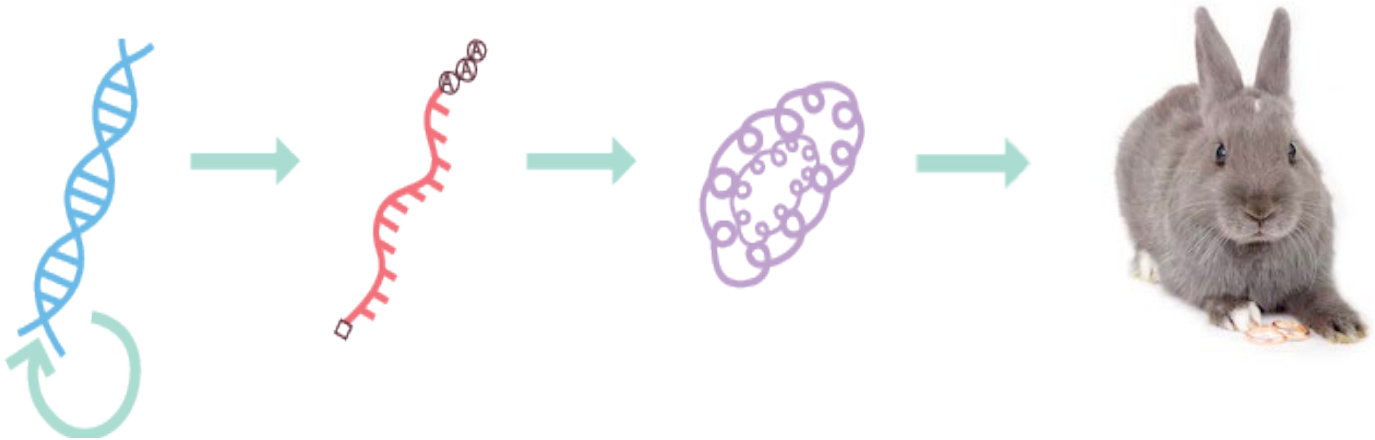
Clinical comments

- When a single Variant of Uncertain Significance is found in a requisitioned gene that is only associated with autosomal recessive condition(s), it may not be included in the report.

I *still* can’t find anything…

It’s not just in the sequence…

DNA → RNA → PROTEIN → PHENOTYPE



It’s not just in the sequence…

- DNA sequence is not the only determinant of phenotype
  - RNA Expression is regulated in several ways
    - Variable amounts (promoters, epigenetic, stability)
    - Different cells and tissue
    - Alternative splicing
    - Monoallelic expression
  - Protein expression and function is regulated
    - Variable amounts (stability and degradation)
    - Proteins are modified by a number of processes that affect function

Some regions of DNA do not code for protein but have other important functions

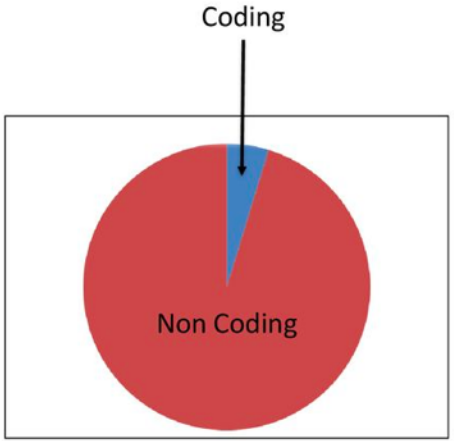
Non coding DNA

Not all DNA codes for a polypeptide to be made

May have another useful function

Non-coding sequences of DNA e.g. STRs

Another example: Promotor



Applications:

- The promoter as an example of non-coding DNA with a function

“I don’t want to do it.”

Implications of direct-to-consumer genetic testing

Advantages of DTC Testing

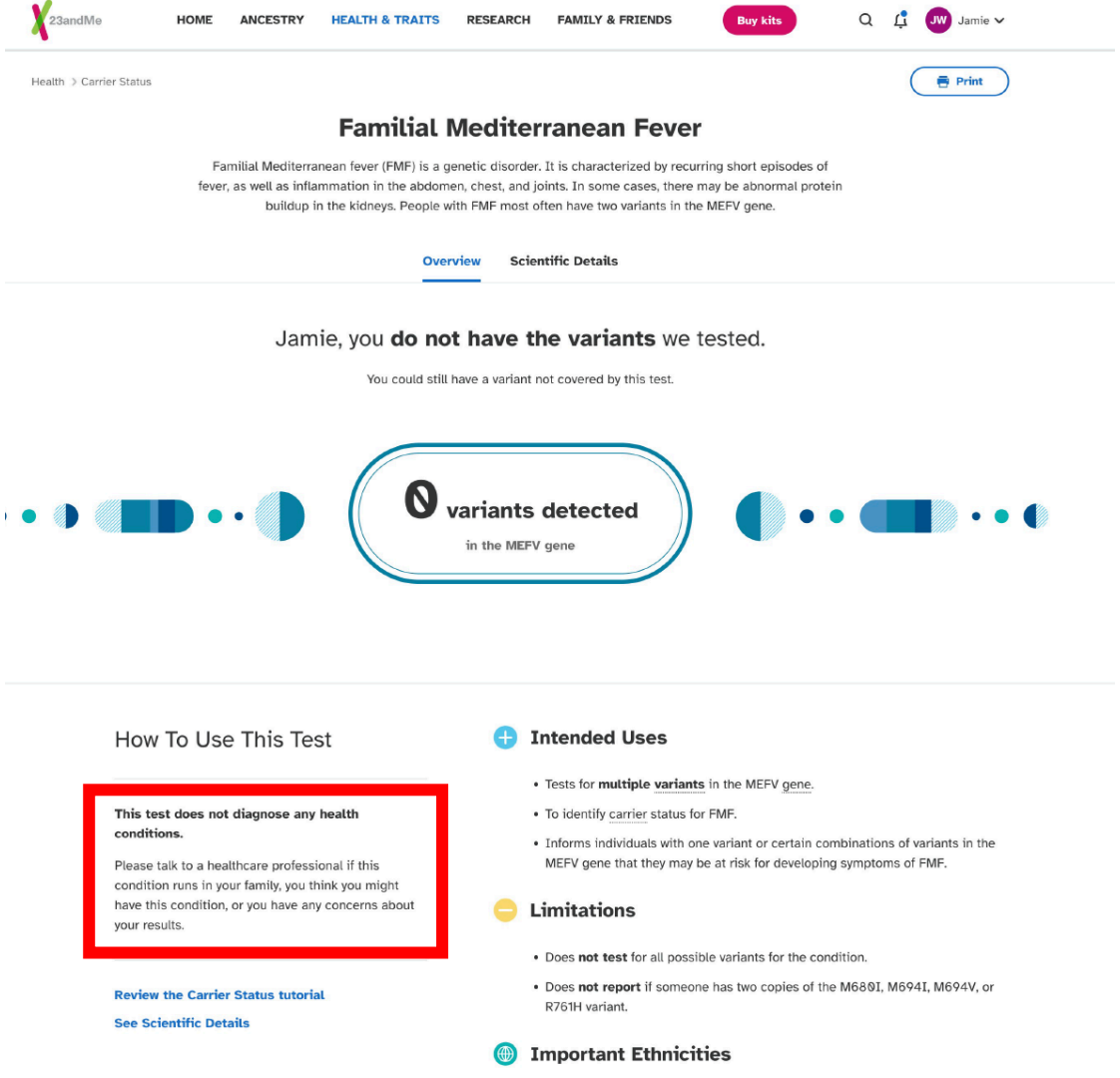
- Convenient
- Greater access to care
- No physician order or contact with healthcare system required
- Emphasis on prevention or early intervention
- Patient empowerment, education, and autonomy regarding health decisions
- Confidentiality of results
- Potential cost-savings compared to clinical testing

Disadvantages of DTC Testing

- Often limited diagnostic value or clinical actionability of results
- Lack of regulation
- Requires consumer self-interpretation of results
- Consumer may be misled by marketing strategies
- Issues with validity and reliability of laboratory methodology, i.e., false negative or positive results
- Risks of overtreatment or lack of intervention based on results

- *BRCA1* and *BRCA2*
- *APOE* e4 vs *APOE* e2

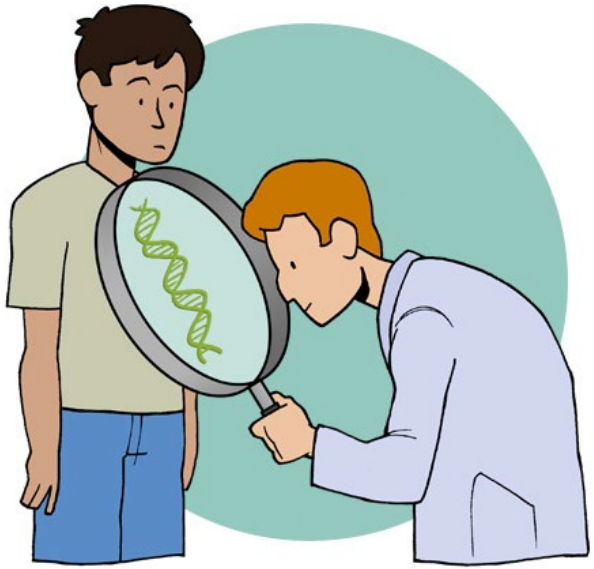
Stanczyk FZ, Mandelbaum RS, Lobo RA. F S Rep. 2022
Oh B. Genomics Inform. 2019



https://medical.23andme.com/wp-content/uploads/2022/09/Familial-Mediterranean-Fever-zero-variants.pdf

What do I do in the meantime?

Treat what you can.



"Good news.
Your cholesterol has stayed the same,
but the research findings have changed."

Implications of genetics studies

Risks and benefits
(beyond *simple* confidentiality)

Future effects

- No “take backs” – learning something you didn’t want to know
  - Emotional stress
  - Medical care

- Insurability
  - Genetic Information Nondiscrimination Act (GINA) 2008
  - GINA does not apply when
    - an employer has <15 employees
    - in the U.S. military, VA or Indian Health Service
    - For other forms of insurance (life, disability, long-term care)

New concerns

- What makes us, “us?”

- How / can we control how others interpret our genetic links to others?

So…should I get genetic testing for my patient?

"Look, pal, I just GAVE you my answer. What part of 'Maybe yes, maybe no' don't you understand?"

- Will it help get therapy?
- Does everyone understand the possible outcomes?
  - Patient
  - Family
  - Physician
- Use genetic counselors as a resource

Take Home Messages

- Genetics are only ONE PART of the clinical picture.
- Genetics have improved diagnosis and led to improved therapy,
  but is not always straightforward
- Genetic counseling is important.
- Follow up testing may still be needed to determine a conclusive diagnosis.
- Genetic testing should not be a pre-requisite confirmatory test to initiate or continue disorder-specific or supportive therapy where the clinical history and routine testing demonstrate a clear need.