

Genetic testing

A primer for non-geneticists

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DC T 07/29/2020



Center for Human Genetics & Genomics

Director: Aravinda Chakravarti

Advancing human genetics programs in research, education, and patient care.

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<https://med.nyu.edu/centers-programs/human-genetics-genomics/>

Educational programs, seminars,
Genetic Medicine Colloquium.

A mini-seminar in 3 parts

Part 1: The Basics

Part 2: Understanding genetic test reports and basic counseling

Part 3: Genomics and exome sequencing



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Learning objectives

Part 1: The Basics

1. Recognize general principles of when to consider a genetic disease.
2. Understand the available genetic testing methods, and the limitations of each.
3. Learn when and how to order genetic testing in your clinic, versus when to refer to clinical genetics/neurogenetics.

Disclosures

John Pappas: None

Gilad Evrony: None

Heather Lau

Consulting: Amicus, ASPA Therapeutics,
Genzyme/Sanofi, Prevail, Takeda/Shire,
Ultragenyx

Ellen Moran: None

When to consider a genetic syndrome or disease?

General for all age groups

1. Known hereditary disease in the family / multiple affected.
2. Rare clinical, lab or imaging findings.
3. Clinical, lab or imaging findings known to be associated with a genetic disease.

Common indications by age group (Not an exhaustive list, these are just examples)

Neonates

1. Encephalopathy or metabolic abnormalities
Hypotonia, lethargy, seizures, poor feeding
2. Congenital anomalies
Especially multiple malformations or unusual facial features
3. Abnormal growth
Especially overgrowth with no maternal diabetes or symmetric small for gestational age

When to consider a genetic syndrome or disease?

Common indications by age group – cont'd (Not an exhaustive list, these are just examples)

Children

1. Stagnation or regression of development, autism
Milestone delays, especially with malformations or unusual facial features
2. Neurological or neuromuscular disorders
Ataxia, weakness, seizures, progressive spasticity - *Further details in later slides*
3. Abnormal or asymmetric growth
Short stature, skeletal dysplasias, connective tissue, vascular malformations
4. Vision or hearing loss
5. Cardiomyopathies and arrhythmias
6. Hematologic or immunologic abnormality
7. Organ failure (e.g., kidney, liver)
8. Malignancy pointing to a familial cancer syndrome

When to consider a genetic syndrome or disease?

Common indications by age group – cont'd (Not an exhaustive list, these are just examples)

Adults

1. Intellectual disability and/or psychiatric disease
Especially individuals with malformations and/or positive family history.
2. Neurological or neuromuscular disorders especially with onset in young adulthood
Cognitive decline, regression, abnormal gait, ataxia, weakness, seizures
Further details in next slides
3. Familial cancer syndromes
4. Cardiac or vascular conditions
Dilated aorta and/or dissection, arrhythmias, cardiomyopathy

Neurogenetics - common indications for testing

Children

1. Developmental Delay/Intellectual disability/Autism
2. Developmental regression heralding neurodegeneration
Inborn Errors of Metab., Neuronal Ceroid Lipofuscinosis, Tay Sachs, Wilsons
3. Abnormal brain myelination
Leukodystrophies (Canavan, Krabbe); Hypomyelinating disorders
4. Brain malformations
Joubert syndrome, microcephaly, heterotopia
5. Epilepsy
Neonatal epileptic encephalopathies, as well as juvenile onset.
6. Hypotonia/Encephalopathy
7. Neuromuscular disorders
Anterior horn cell (Spinal muscular atrophy); Neuromuscular junction (Congenital Myasthenia); Myopathies/Muscular dystrophies
8. Inherited Ataxias and movement disorders
Cerebellar ataxias, dystonia, chorea, tics

Neurogenetics - common indications for testing

Adults

1. White matter disease
Atypical “Multiple Sclerosis” not responding to treatment may be a leukodystrophy or vascular leukoencephalopathy
2. Recurrent strokes, familial strokes
Fabry, CADASIL, CARASIL
3. Familial cognitive dementias, especially early onset
4. Movement disorders
Dystonia; Parkinsonism (especially early onset and familial);
Huntington/Chorea: requires co-consultation with psychiatry prior to testing
5. Ataxia and/or spasticity disorders
6. Neuropathy
Charcot Marie Tooth
7. Acute onset psychiatric condition
May herald late onset genetic disease (NPC, Late onset Tay Sachs)
8. Mitochondrial disorders (across lifespan with variable expressivity)

When to test yourself versus refer to genetics/neurogenetics?

Initiate genetic testing yourself if you:

1. Suspect a specific clinical diagnosis or relatively specific diagnostic category related to your specialty.
2. Understand the test and its limitations, and how to order the test.

Examples: Polycystic kidney disease, Long QT syndrome, Primary immunodeficiency, Marfan syndrome, etc.

When to test yourself versus refer to genetics/neurogenetics?

Refer to clinical genetics if:

1. The differential is broad.
2. You do not know which test to order.

Examples:

- Newborn with cleft palate, VSD and polydactyly → Microarray?
- Smith-Lemli Opitz → Biochemical test? Exome?
- Clinical genetics and neurogenetics can also help advise on testing by your clinic.

3. Advanced genetic counseling is needed.

Examples:

- The parents ask you about future pregnancy options.
- Exploring reasons for testing vs. not testing.

If you test, when to follow up with clinical genetics/neurogenetics

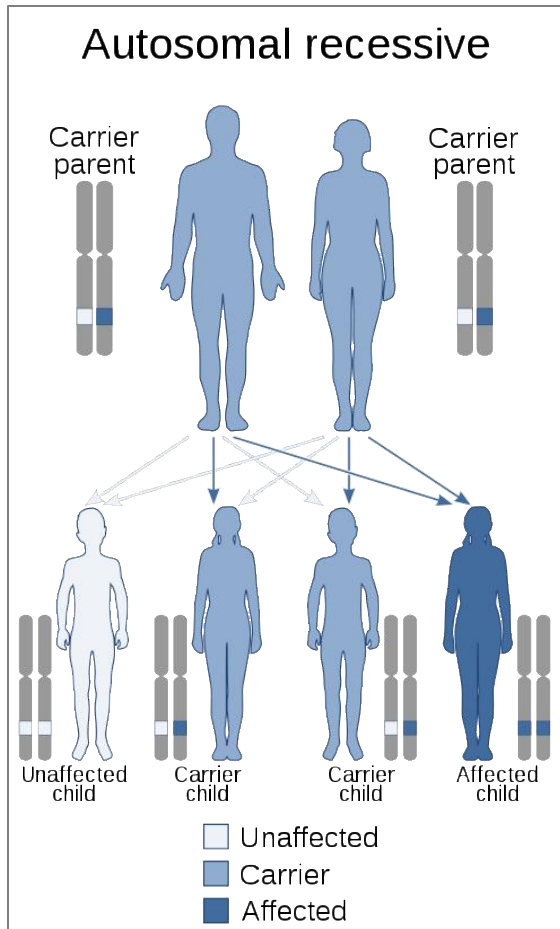
After positive genetic testing, refer to genetics for:

- Planning appropriate follow-up care for complex syndromes: For example, cardiology and endocrinology for Noonan syndrome; tumor surveillance for Beckwith-Wiedemann.
- Consultation regarding updated guidelines for the syndrome: ACMG and AAP publish clinical guidelines for many syndromes.
- Parental testing and reproductive counseling: Options for future pregnancies; pre-natal and pre-conception counseling.
- Communication of results to other family members: The patient desires to communicate results with other relatives.

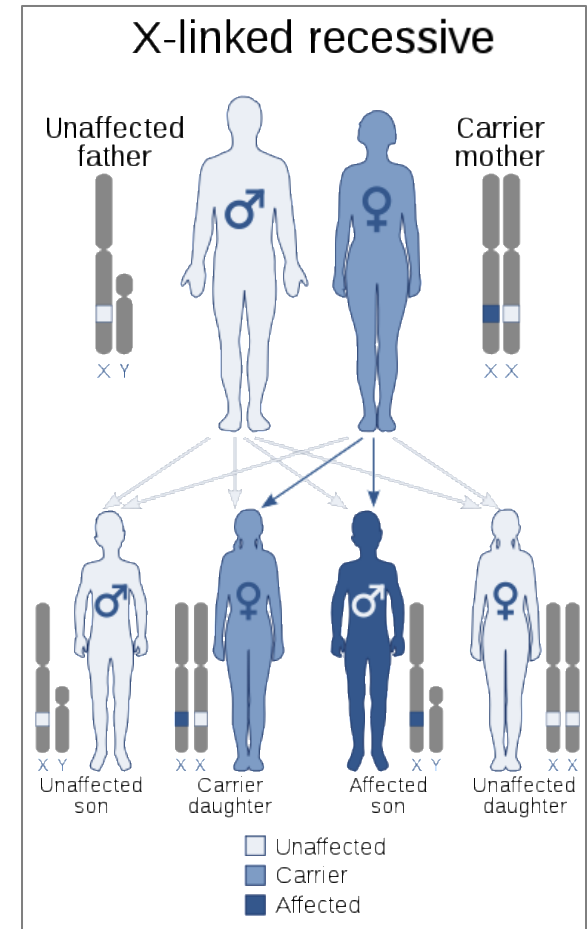
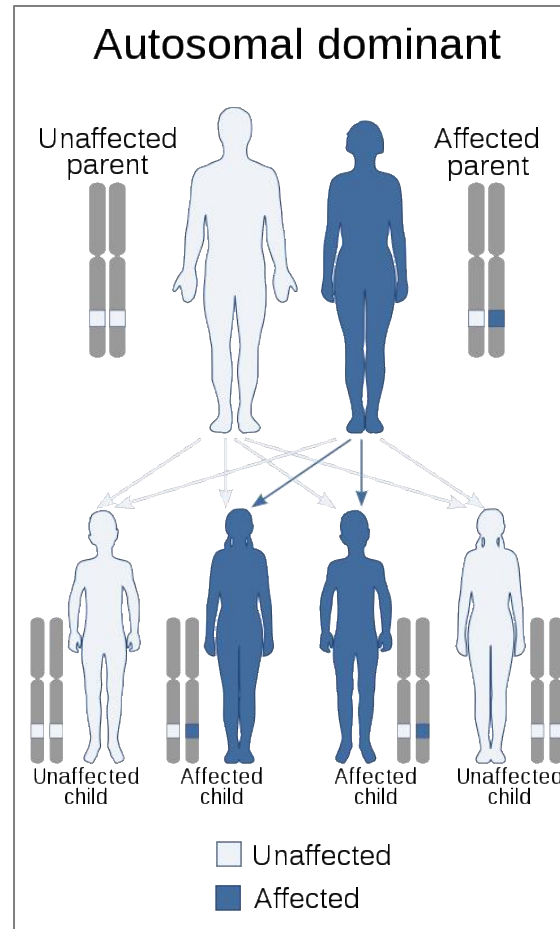
After negative genetic testing, refer to genetics for:

- Additional genetic testing: If you are unsure which or how to order them.
- Consultation and counseling when there is no molecular-genetic diagnosis or for inconclusive findings: Variants of uncertain significance, reproductive decisions, prognosis, etc.

Modes of genetic inheritance - I

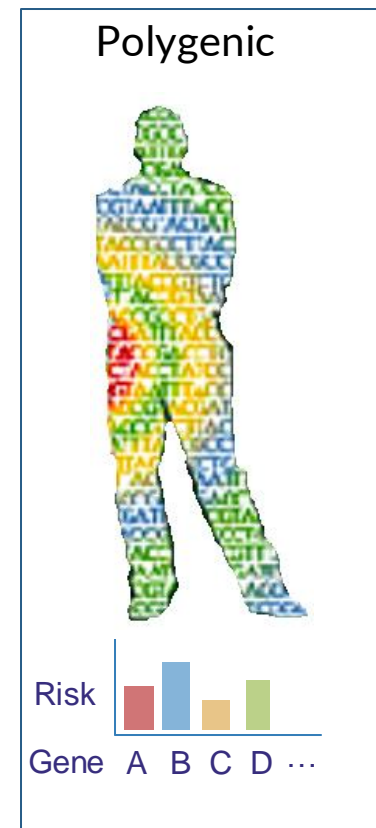
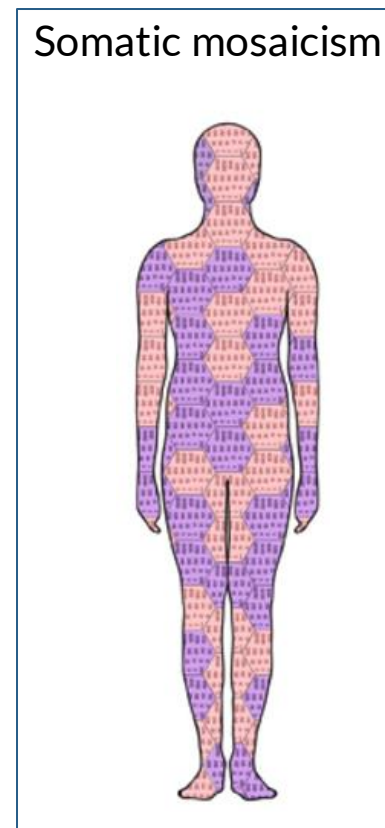
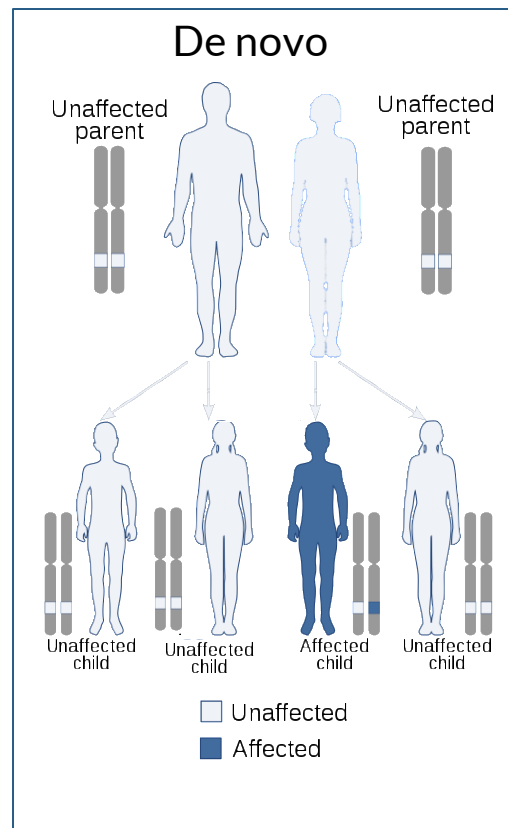
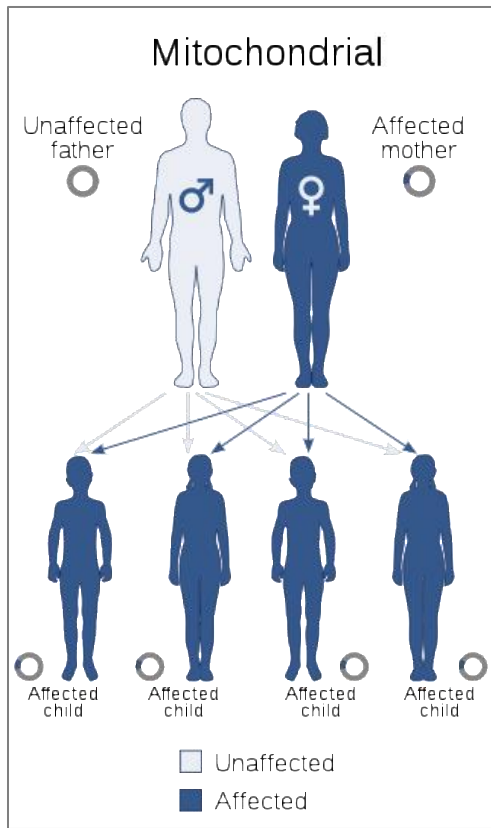


NIH/NLM



Some female carriers may be affected due to skewed X-inactivation.

Modes of genetic inheritance - II



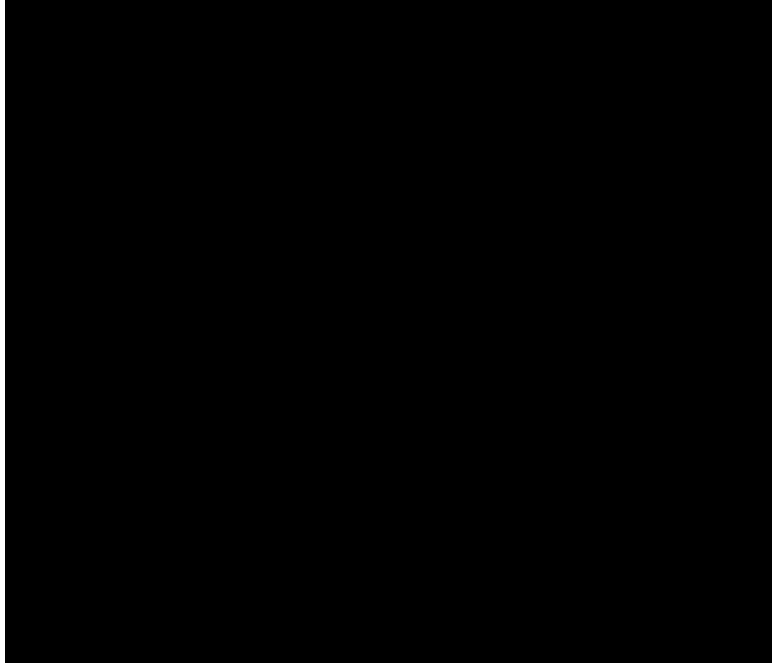
Mothers may not be affected and phenotypes may be variable, due to uneven transmission of mitochondria (heteroplasmy).

Genetic variant is not detected in either parent. May (infrequently) recur in future children.

Might not be detected by genetic testing unless affected tissue is tested.

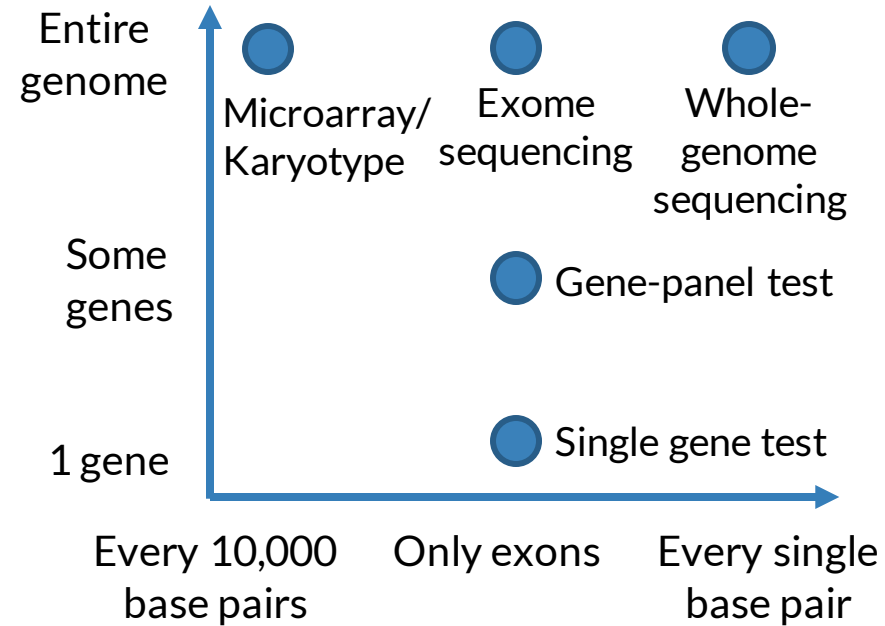
Common diseases. Can be highly heritable, but may not have simple segregation patterns.

The basic genetic tests- I



Youtube | The Element Guru

Comprehensiveness



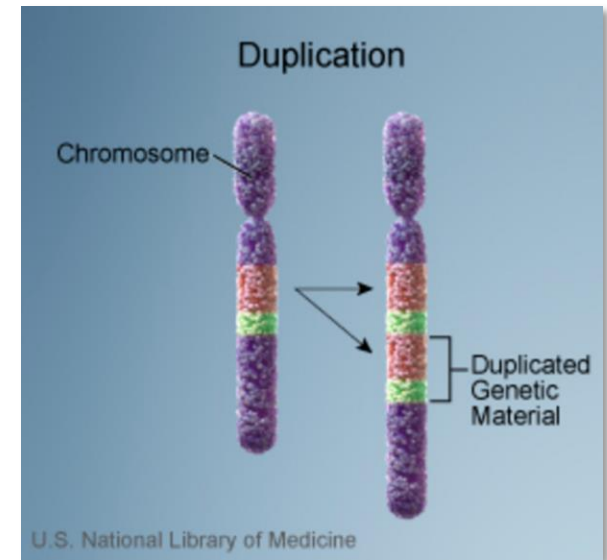
Resolution

The basic genetic tests- II

Microarray/Karyotype

- Interrogates the entire genome at low resolution.
- Useful for large copy-number changes and chromosomal aneuploidies.

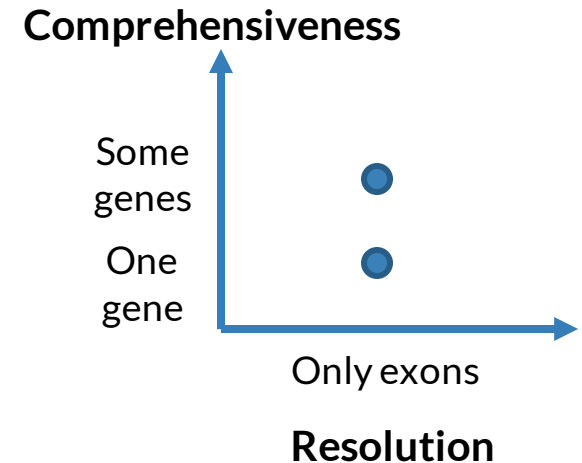
→ When to order? Always.



Single gene or gene panel tests

- Only exons of 1 to 10's of genes (gene panel) associated w/ a specific syndrome or similar syndromes.
- Usually does not detect copy number changes unless "del/dup" analysis added on.

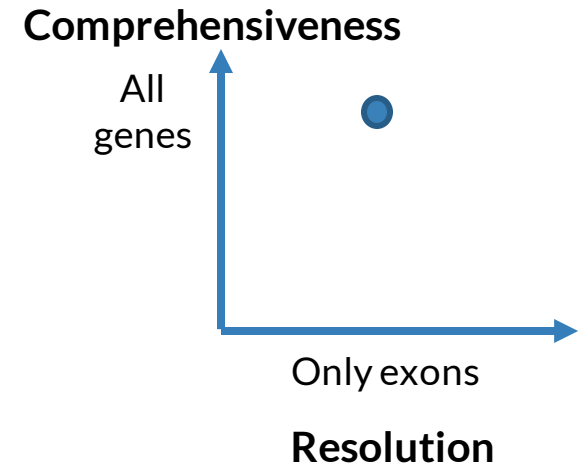
→ When to order? Specific syndrome or phenotype (e.g. Gaucher disease, or short stature).



The basic genetic tests- III

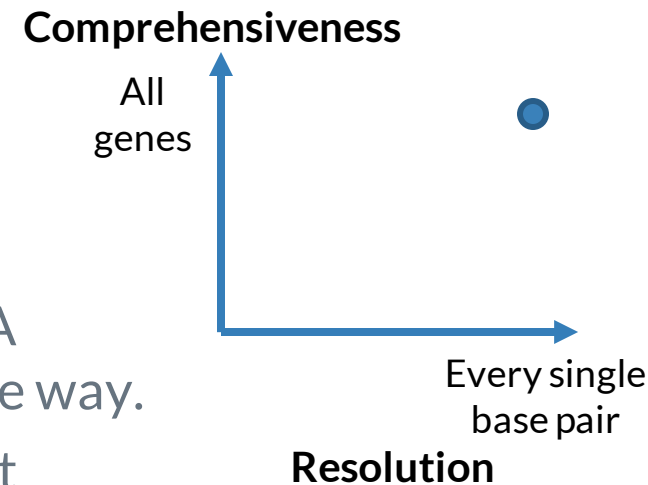
Exome sequencing

- Only exons of all genes in the genome.
 - Not good at detecting copy number changes.
- **When to order?** Rare syndrome that does not fit clear diagnosis, prior negative genetic testing, need an expedited diagnosis.



Whole-genome sequencing

- “All” base pairs of the the genome.
 - Also detects copy number and other structural changes.
 - **Still has blind spots** due to reliance on short DNA fragments: Newer ‘long-read’ technologies on the way.
- **When to order?** Rarely covered by insurance. First clinical use is rapid NICU/PICU sequencing.



Where to find clinical genetic tests you can order

NIH/NLM Genetic Testing Registry

- Search by gene or phenotype for tests registered by clinical laboratories. [Link](#)

Large and trusted commercial labs

- **GeneDx** test catalog. [Link](#)
- **Invitae** test catalog. [Link](#)
- **Baylor Genetics** test catalog. [Link](#)

There are many commercial and academic clinical labs of variable quality. Some are best only for specific syndromes.

→ If you are unsure about the quality of a company or test, please ask clinical genetics/neurogenetics.

How to order basic genetic tests - I

The current process can be complicated, due to insurance coverage and multiple test providers with different processes.

We know this need to be simplified, and we are working to improve the process.

The following are general guidelines to help you get started.

Clinical Genetics is here to help.

How to order basic genetic tests - II

Microarray

1. The process is a bit different for each hospital (NYULMC, NYU Brooklyn, NYU Winthrop, Bellevue).
2. Consent should include possibility of incidental findings and possible parental testing needed for interpretation.
3. New York State requires karyotype in addition to microarray.

How to order basic genetic tests - III

Single gene or gene panel tests

1. Inpatient testing possible only for patients with poor prognosis, prolonged hospitalization, or expedited testing before a procedure (e.g. surgical decision).
2. The large commercial labs are able to help with insurance questions and issues.
3. Always counsel patients on possibility of secondary findings, possible need for parental testing, and implications for other family members.
4. Paper requisitions and consents must be scanned into Epic.

Exome sequencing

1. Outpatient testing covered by most insurance plans.
2. Inpatient testing is rarely possible.
3. Requires consent and counseling.

NYU Undiagnosed Diseases Program

A research program for undiagnosed pediatric patients.

Whole-exome reanalysis, Whole-genome sequencing, RNA-sequencing, Patient-customized tests and assays.

Indications for referral

1. Patients with non-diagnostic clinical genetic testing, with priority for rare clinical presentations.
2. Usually after negative clinical exome sequencing, or if exome sequencing is not covered by insurance.
3. Rapid sequencing (~1 week to results) for PICU/NICU.



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Referrals or questions: PedsUDP@nyulangone.org

Who to go to for help

Clinical Genetics

- For clinical and genetic testing questions, consultation, and referrals → Dr. John Pappas

Neurogenetics

- For clinical neurogenetics questions, consultation, and referrals → Dr. Heather Lau

Center for Human Genetics & Genomics

- For research questions and challenges implementing genetic testing in your clinic → CHGGcontact@nyulangone.org

Undiagnosed Diseases Program

- For research questions and research genomic analyses for undiagnosed patients → PedsUDP@nyulangone.org

Resources

- [Genetics Home Reference](#): Brief summaries of genetics topics and genetic disorders.
- [GeneReviews](#): Detailed summaries of genetic disorders.
- [OMIM](#): Encyclopedia of all known genetic disorders.

Thanks!

Any questions?

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Part 1: The Basics

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Part 3: Genomics and exome sequencing



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Learning objectives

Part 2: Understanding genetic test reports and basic counseling

1. Interpret basic genetic variant annotations and reports.
2. Learn to respond to the most important genetic counseling questions posed by patients.
3. Recognize ethical and social issues surrounding genetic testing, and when to consult clinical genetics.

The ACMG Criteria – Is a variant pathogenic?

Standards for genetic variant reporting by the American College of Medical Genetics

Pathogenic

The variant contributes to the disease.

Likely Pathogenic

High likelihood (> 90% certainty) that the variant contributes to the disease.

Variant of Uncertain Significance (VUS)

There is not enough information to support a more definitive classification.

Likely Benign

High likelihood (> 90% certainty) that the variant does not contribute to the disease.

Benign

The variant does not contribute to the disease.

Variant classification is reported with respect to a specific condition and inheritance pattern, e.g. p.Phe508del, pathogenic, Cystic Fibrosis, Aut. Rec. GE

The ACMG Criteria – Is a variant pathogenic?

Step 1: A variant is scored against standardized criteria.

Very Strong Criteria

- Null variant (frameshift, nonsense, splice site).

Strong Criteria

- Known missense variant
- De novo variant
- Experimental support
- Increased prevalence in affected individuals

Moderate Criteria

- Absent or very rare in a control population.
- In a mutational hotspot.
- Trans inheritance for recessive disorders.

Supporting Criteria

- Variant segregates with disease in family. • Computational prediction of effect on gene.

This is a partial list of criteria, and there are similar criteria for benign classification. There is no need to memorize these.

Step 2: The combination of positive criteria determines the classification according to standardized rules.

e.g. 1 Very Strong + 2 Moderate criteria = **Pathogenic**

Only 3 Moderate criteria = **Likely Pathogenic**

Standardized nomenclature for genetic variants - I

The goal: Communicate to another individual a genetic variant's precise location and change.



1699



1750

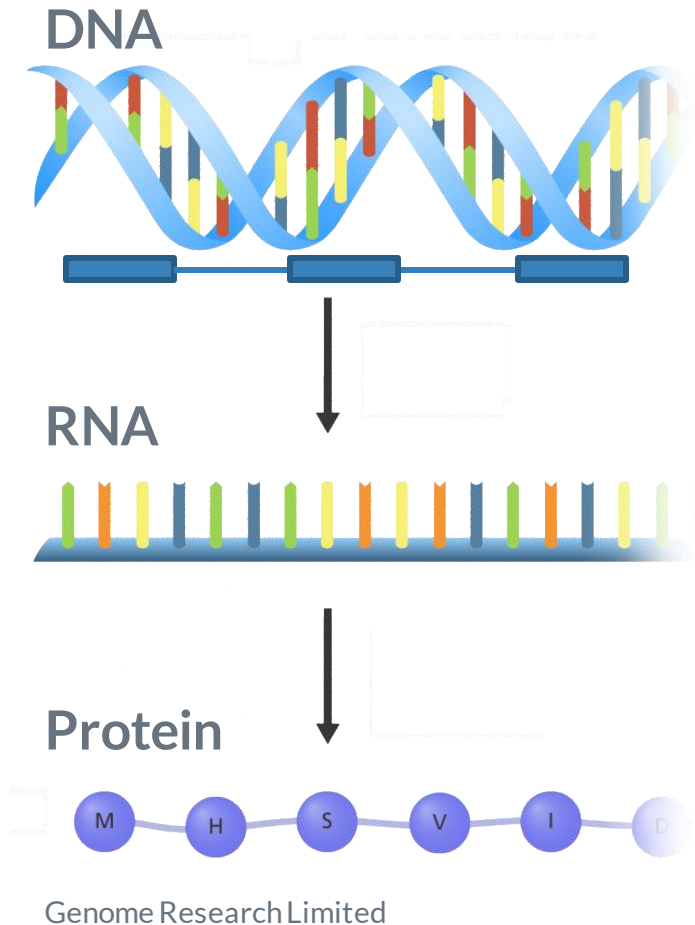


1871

Every genetic variant is always specified relative to a reference. This is the "map" both sides agree on.

Standardized nomenclature for genetic variants - II

Genetic reference “maps” can be at 3 different levels. Every variant is usually specified relative to all 3 levels.



Level 1: DNA (genome) references

e.g. **Human Genome Reference**
(hg19, hg38)

Chr11:534289 C>T (hg19)

Level 2: RNA (transcript) references

e.g. **NCBI RefSeq transcripts**

c.34G>A (NM_005343.3; *HRAS* gene)

“c.” = coding DNA

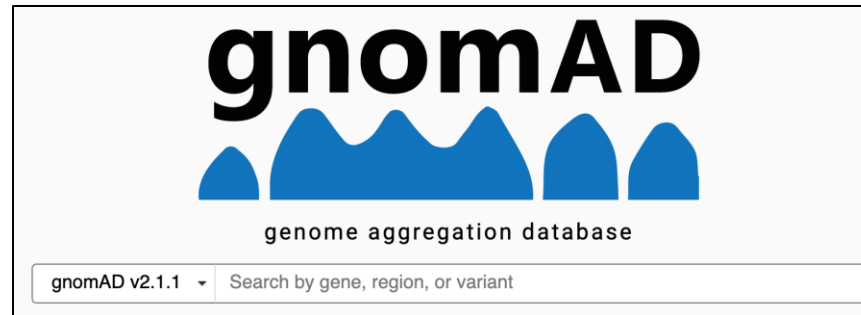
Level 3: Protein references

e.g. **NCBI RefSeq proteins**

p.Gly12Ser (NP_005334.1; Hras protein)

Population Databases - I

- In order to ascertain pathogenicity, we need to know how common the variant is in a normal (control) population.
- Population Databases: Aggregated genetic sequences of **hundreds of thousands** of “normal” individuals.



Single nucleotide variant: 11-534287-G-A

<u>Variant ID</u>	<u>Gene</u>	<u>Consequence</u>	<u>Annotation</u>	<u>Allele Frequency</u>	<u>Number of Homozygotes</u>
11-534269-C-T	HRAS	p.Ala18Ala	● synonymous	4.39e-5	0
11-534278-G-A	HRAS	p.Gly15Gly	● synonymous	1.2e-5	0
11-534281-C-T	HRAS	p.Val14Val	● synonymous	2.13e-5	0
11-534287-G-A	HRAS	p.Gly12Gly	● synonymous	9.94e-5	0
11-534290-G-A	HRAS	p.Ala11Ala	● synonymous	7.99e-6	0
11-534292-C-T	HRAS	p.Ala11Thr	● missense	1.07e-5	0
11-534293-G-A	HRAS	p.Gly10Gly	● synonymous	1.78e-5	0

<u>Population</u>	<u>Allele Frequency</u>
▶ East Asian	0.001404
▶ African	0.000
▶ Latino	0.000
▶ Ashkenazi Jewish	0.000
Female	0.0001011
Male	0.00009798
Total	0.00009941

Population Databases - II

- In order to ascertain pathogenicity, it is also helpful to know if the variant has been seen before in the same or other diseases.



ClinVar: A curated database of genetic variants reported by clinical labs from around the world, and the classification decision they made.

NM_005343.4(HRAS):c.34G>A (p.Gly12Ser)
Allele ID: 27641
Variant type: single nucleotide variant

Interpretation (Last evaluated)	Review status (Assertion criteria)	Condition (Inheritance)	Submitter	Supporting information (See all)
Pathogenic (Apr 03, 2017)	reviewed by expert panel (ClinGen RASopathy ACMG Specifications v1) Method: curation	Costello syndrome (Autosomal dominant inheritance) Allele origin: germline	ClinGen RASopathy Variant Curation Expert Panel FDA RECOGNIZED DATABASE Accession: SCV000616364.3 Submitted: (Feb 25, 2019)	Evidence details Publications PubMed (15) Other databases https://erepo.clinicalgenome.o... Comment: The c.34G>A (p.Gly12Ser) variant in HRAS has been reported as a confirmed de novo occurrence in at least 2 patients with clinical features of a ... (more)
Pathogenic (Jun 04, 2014)	criteria provided, single submitter (ACMG Guidelines, 2015) Method: clinical testing	Costello syndrome Allele origin: unknown	Molecular Diagnostics Lab, Nemours Alfred I. duPont Hospital for Children Accession: SCV000263052.1 Submitted: (Dec 22, 2015)	Evidence details Publications PubMed (9)

A walk through a genetic test report - II

4. Relevant findings - details

***CACNA2D2* c.1570G>A (p.Val524Met) - Uncertain Significance.** The c.1570G>A(p.Val524Met) missense variant results in the substitution of the valine codon at amino acid position 524 with a methionine codon. This variant has been reported 3 times in the general population (1/19/19 PMID: 27535533). *In silico* analyses imply a potentially deleterious effect on the protein function (PolyPhen-2, SIFT, MutationTaster). There is currently insufficient evidence to determine the pathogenicity of this variant, therefore the c.1570G>A(p.Val524Met) *CACNA2D2* variant is classified as a variant of uncertain significance. Clinical and biochemical correlation is required.

5. Incidental findings

Findings Unrelated To Phenotype

Diagnostic findings in genes defined as highly penetrant and medically actionable by ACMG (PMID: 27854360):

No pathogenic variants detected.

6. Detailed methods of test and test limitations

Methods and Limitations

Whole exome sequencing is performed on genomic DNA using the Agilent v6CREv2 targeted sequence capture method to enrich for the exome. Direct sequencing of the amplified captured regions was performed using 2X100bp reads on Illumina next generation sequencing (NGS) systems. A base is considered to have sufficient coverage at 20X and an exon is considered fully covered if all coding bases plus three nucleotides of flanking sequence on either side are covered at 20X or more. Low coverage regions, if any, are limited to ~1% or less of the nucleotides included in this panel unless a pathogenic variant explaining the phenotype is discovered. A list of these regions is available upon request. Alignment to the

...

Basics of counseling for genetic tests - I

I worry that genetic counseling will soon deteriorate to a short brochure or a Web page visit or a quick telephone call.

—Arthur Caplan, PhD

MAY 1997 VOLUME 22, NUMBER 5 LABORATORY MEDICINE

The genetic testing process can have implications that reach beyond the patient in front of you into the extended family. Identifying a genetic variant in a patient may also diagnose his or her close relatives without them ever consenting to testing.

Basics of counseling for genetic tests - I

Pre-test counseling

- Helps prepare an individual for the potential outcomes and limitations of genetic testing.
- Opportunity to facilitate your patient's informed consent decision about undergoing testing and ease adoption of results.
- Informed consent is an important step in the genetic testing process and is required by New York State law.
- **Comprised of 4 parts:**
 - Test information
 - Benefits
 - Limitations
 - Insurance and follow-up

Basics of counseling for genetic tests - II

Pre-test counseling - continued

1. Test information

- ▷ Purpose of testing.
- ▷ Description of the disorder being tested.
- ▷ Ability of the test to diagnose the disorder.

2. Potential test benefits

- ▷ Diagnose or identify the cause of an individual's symptoms.
- ▷ End search for a diagnosis (diagnostic odyssey).
- ▷ If predictive testing: more precise estimates of lifetime risk for disease.
- ▷ Inform personalized management and treatment.
- ▷ Enable identification of at-risk relatives.
- ▷ Identify recurrence risk and inform reproductive decision-making.

Basics of counseling for genetic tests - III

Pre-test counseling - continued

3. Potential test limitations

- ▷ Possibility of a false negative or no diagnosis; may not identify all pathogenic variants.
- ▷ Many times, a diagnosis will not alter management.
- ▷ Variant of uncertain significance – may need parental/family testing.
- ▷ Unanticipated results: non-paternity, consanguinity, diagnosis unrelated to patient's presentation, secondary findings.
- ▷ Predictive testing: not all patients w/ pathogenic variant develop disease.
- ▷ May cause anxiety, blame, guilt, or secrecy in the family.
- ▷ Labeling patient with diagnosis increases concern for discrimination.
- ▷ Confidentiality protections.
- ▷ Genetic discrimination risks and protections (GINA).

Basics of counseling for genetic tests - IV

Pre-test counseling - continued

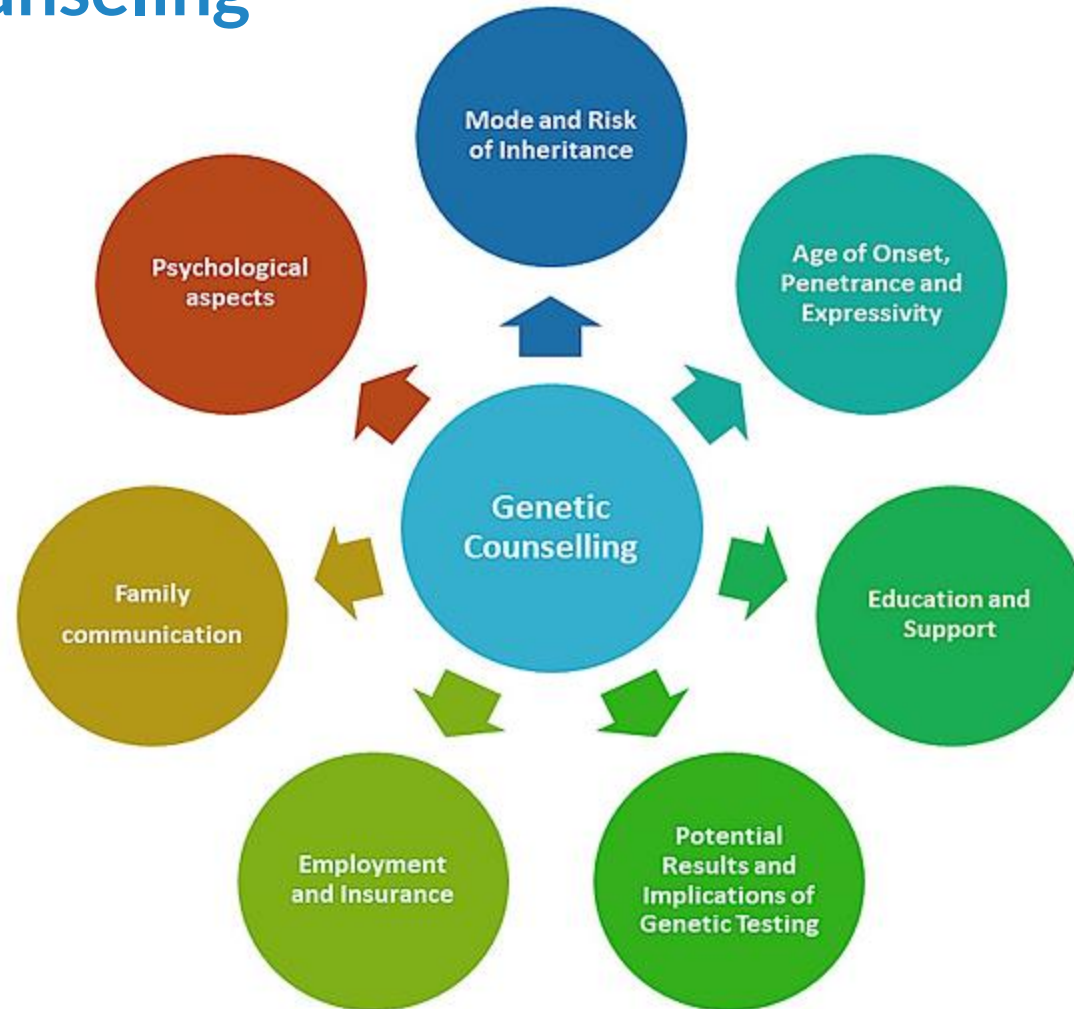
4. Insurance and follow-up

- ▷ Cost of genetic testing and possible need for insurance pre-authorization.
- ▷ Potential retention of samples by the clinical lab for internal research.
- ▷ Access to sample and genetic data.
- ▷ Disclosure of results: phone vs in-person; anticipated time to result.
- ▷ Exome sequencing:
 - Opt in/out of ACMG 59 (reviewed in seminar part 3).
 - NYS consent to hold DNA sample > 60 days.
 - Consent to be contacted for research.

Basics of counseling for genetic tests - V

Post-test counseling

Components of genetic counseling



Sarah Moharem-Elgamal et al. J Am Coll Cardiol Case Rep
2020;2:392-395

DC T 07/29/2020

Ethical and social issues

The basic tenets of beneficence, non-maleficence, autonomy, and justice are part of a framework for balancing the complex and potentially conflicting factors surrounding privacy, confidentiality, and use of genetic testing information.

- **Genetic testing is voluntary:** Patient autonomy, including the right to know or not to know, must be respected.
- **Informed consent**
- **Confidentiality**
- **Communication/non-communication of test results**
- **Predictive testing of minors for adult-onset disorders**
- **Prenatal testing for adult-onset disorders**
- **Insurance implications and potential discrimination: GINA.**

Resources

- [ACMG variant interpretation guidelines](#): Criteria for judging variant pathogenicity.
- [Human Genome Variation Society \(HGVS\)](#): Sequence variant nomenclature.
- [Genome Aggregation Database \(gnomAD\)](#): Data on population frequency for any variant.
- [ClinVar](#): Database of genetic variant classifications reported by clinical labs.
- [Informed consent & Pre-test counseling checklist](#)

Thanks!

Any questions?

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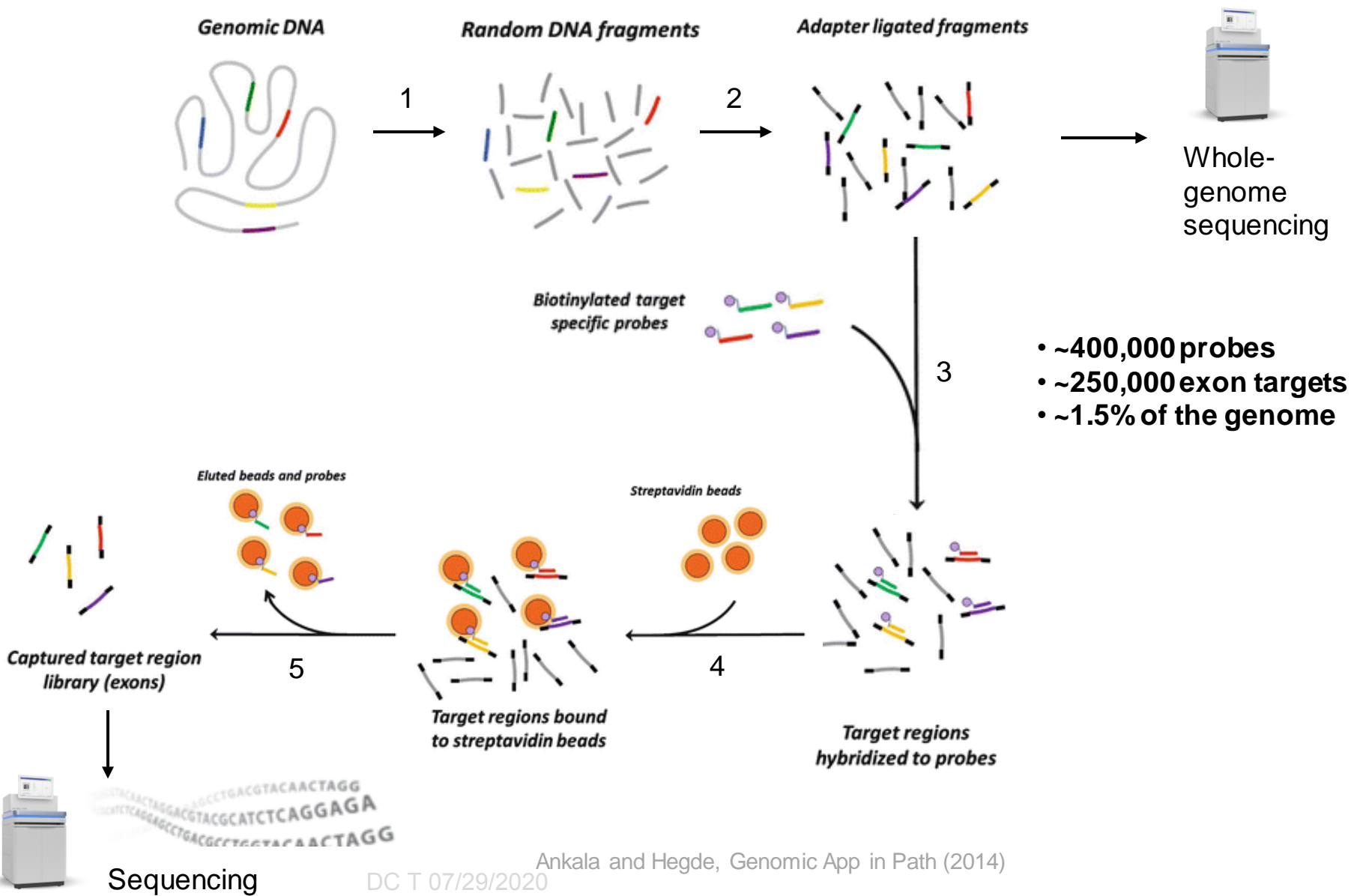
Part 3: Genomics and exome sequencing

Learning objectives

Part 3: Genomics and exome sequencing

1. Understand the basic principles of “next-generation sequencing” and exome sequencing.
2. Recognize clinical situations warranting genome-wide sequencing versus referral to genetics.
3. Become familiar with genomics service providers, how to obtain exome sequencing, and insurance considerations.

Basic principles of exome sequencing - I

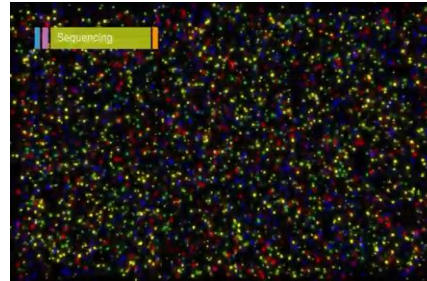


Basic principles of exome sequencing - II



ATGTCACATAGGACCTGACGCTTGCCTACAACCTAGG
GACCTGACGCTACAACCTAGG
ATGTCACATAGGACCTGACGCTTGCCTACAACCTAGG

Sequencing



Illumina, Inc.



~100 million sequencing reads

The Galaxy Project



Mapping to genome

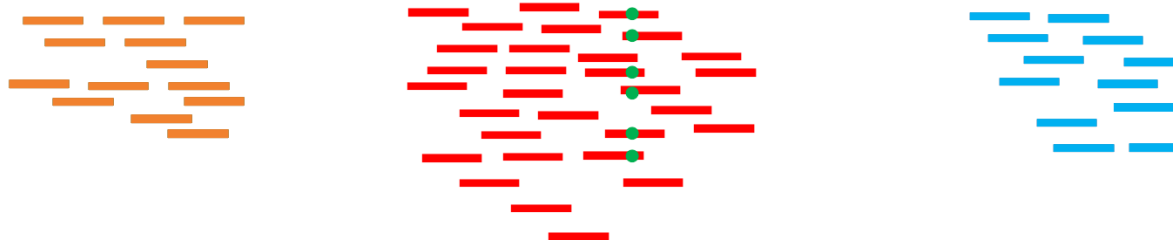
Match found on chromosome 2!

<i>GGATGAGATATTTAC</i>	<i>Read</i>
ACGGCTA <i>GGATGAGATATTTAC</i> CGAGTA	<i>Reference</i>

Reference genome



Coverage



About 50% diagnostic yield in monogenic disorders.

DC T 07/29/2020

Indications for exome sequencing

1. Suspected genetic disease, but phenotype does not correspond to a specific disorder for which a single-gene or gene-panel test is available.
2. Genetic disease with broad differential and manifestations for which exome sequencing would be more efficient than many single tests.
3. Prior single-gene or gene-panel tests are negative.
4. Rapid diagnosis when serial single-gene or gene-panel testing would take too long.

JAMA Pediatrics | [Original Investigation](#)

JAMA Pediatr. 2017;171(9):855-862.

Diagnostic Impact and Cost-effectiveness of Whole-Exome Sequencing for Ambulant Children With Suspected Monogenic Conditions

Tiong Yang Tan, MBBS, PhD; Oliver James Dillon, MD; Zornitza Stark, MD; Deborah Schofield, PhD; Khurshid Alam, PhD; Rupendra Shrestha, PhD;

In 1-2 years, it is likely exome/genome sequencing will be the first step.
→ More cost-effective and rapid than serial testing.

DC T 07/29/2020

GE

Secondary findings

Secondary findings (SF) unrelated to main condition are present in 2-3% of individuals.

Current policy: Offer 'opt-out' of SF reporting in pre-test counseling. Adult-onset findings for children are recommended by ACMG, but hotly debated.

ACMG 59 genes: Official list of genes recommended for SF reporting.

Phenotype	Gene	Phenotype	Gene	Phenotype	Gene
Hereditary breast and ovarian cancer	<i>BRCA1</i> <i>BRCA2</i>	Tuberous sclerosis complex	<i>TSC1</i> <i>TSC2</i>	Arrhythmogenic right ventricular cardiomyopathy	<i>PKP2</i> <i>DSP</i> <i>DSC2</i> <i>TMEM43</i> <i>DSG2</i>
Li-Fraumeni syndrome	<i>TP53</i>	WT1-related Wilms tumor	<i>WT1</i>	Romano-Ward long-QT syndrome types 1, 2, and 3, Brugada syndrome	<i>KCNQ1</i> <i>KCNH2</i> <i>SCN5A</i>
Peutz-Jeghers syndrome	<i>STK11</i>	Neurofibromatosis type 2	<i>NF2</i>	Familial hypercholesterolemia	<i>LDLR</i> <i>APOB</i> <i>PCSK9</i>
Lynch syndrome	<i>MLH1</i> <i>MSH2</i> <i>MSH6</i> <i>PMS2</i>	Ehlers-Danlos syndrome, vascular type	<i>COL3A1</i>	Wilson disease	<i>ATP7B</i>
Familial adenomatous polyposis	<i>APC</i>	Marfan syndrome, Loeys-Dietz syndromes, and familial thoracic aortic aneurysms and dissections	<i>FBN1</i> <i>TGFBR1</i> <i>TGFBR2</i> <i>SMAD3</i> <i>ACTA2</i> <i>MYH11</i>	Ornithine transcarbamylase deficiency	<i>OTC</i>
<i>MYH</i> -associated polyposis; adenomas, multiple colorectal, <i>FAP</i> type 2; colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas	<i>MUTYH</i>	Hypertrophic cardiomyopathy, dilated cardiomyopathy	<i>MYBPC3</i> <i>MYH7</i> <i>TNNT2</i> <i>TNNI3</i> <i>TPM1</i> <i>MYL3</i> <i>ACTC1</i> <i>PRKAG2</i> <i>GLA</i> <i>MYL2</i> <i>LMNA</i>	Malignant hyperthermia susceptibility	<i>RYR1</i> <i>CACNA1S</i>
Juvenile polyposis	<i>BMPR1A</i> <i>SMAD4</i>	Catecholaminergic polymorphic ventricular tachycardia	<i>RYR2</i>		
Von Hippel-Lindau syndrome	<i>VHL</i>				
Multiple endocrine neoplasia type 1	<i>MEN1</i>				
Multiple endocrine neoplasia type 2	<i>RET</i>				
Familial medullary thyroid cancer ^d	<i>RET</i>				
<i>PTEN</i> hamartoma tumor syndrome	<i>PTEN</i>				
Retinoblastoma	<i>RB1</i>				
Hereditary paraganglioma-pheochromocytoma syndrome	<i>SDHD</i> <i>SDHAF2</i> <i>SDHC</i> <i>SDHB</i>				

- Severe diseases with high penetrance.
- Effective interventions are available.

When to order exomes in your clinic vs. refer to genetics?

Order yourself if:

1. Your clinic is able to provide informed consent for exome sequencing. If your clinic employs a genetic counselor, they would be best qualified, but this is not required by most insurance plans.
2. The main aspects of the clinical diagnosis and phenotype of the patient are pertinent to your specialty.

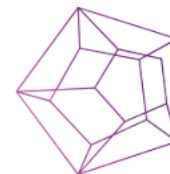
Refer to clinical genetics if:

1. There are no providers in your clinic able to conduct informed consent for exome sequencing. Contact clinical genetics and/or CHGG if you would like resources for training providers in your clinic.
2. Your patient has a multi-system disorder that would benefit from clinical genetics phenotyping and evaluation.

Main exome service providers



INVITAE



PerkinElmer
GENOMICS

- Similar quality and service: 6-8 weeks for results.
- Varying and changing support for insurance/prior-authorization help, discounts for out-of-pocket costs
- Clinical genetics and CHGG can help facilitate relationship between your clinic and these or other service providers.

Insurance issues

Pre-certification / Pre-authorization / Insurance coverage:

- Pre-certification is an initial evaluation if the patient is eligible for coverage for the genetic test.
- Pre-authorization is a second evaluation required for some genetic tests to evaluate for coverage for the genetic test based on appropriateness and cost per the insurer's criteria.
- Coverage for adults and children can differ. For example, testing for neurodevelopmental disorders in children is covered by most plans, but adult testing may need pre-authorization.
- Some insurance plans require proof of genetic counseling for coverage.

Some commercial labs offer free testing or reduced co-pay based on financial need:

- This includes parental testing and family studies.
- You can contact the commercial labs to ask about this and for help.

Ordering and sample collection

Prior to ordering:

1. Obtain a standard three-generation pedigree.
2. Obtain as detailed a phenotype as possible, which will help the lab produce a useful report.

Ordering:

1. Some tests can be ordered via Epic. Other tests are ordered directly from the commercial genetics lab.
2. Call the NYULH clinical lab to inquire about how to order the desired test.

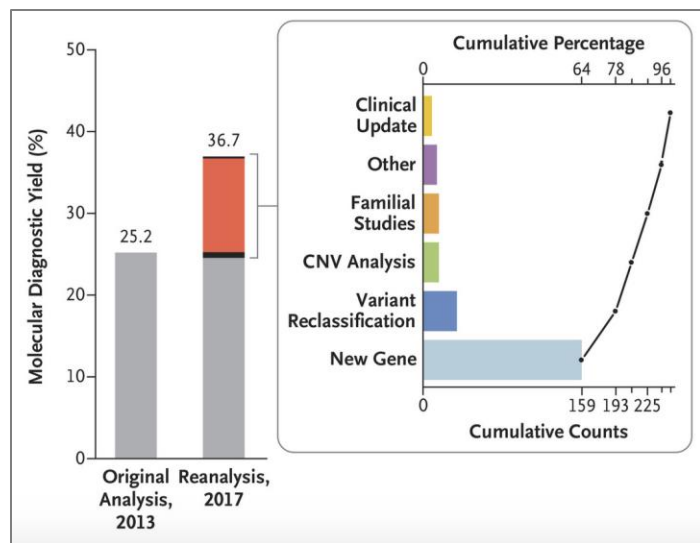
Sample collection: It is very important to collect the proper sample.

1. DNA tests: Any tissue containing nucleated cells. Purple-top (EDTA) tube for blood, special collection kits for saliva and cheek swabs (provided free by commercial labs), cultured fibroblasts from skin biopsy, tumor tissue (not fixed).
2. Chromosome analysis: Any tissue containing dividing cells. Blood (green-top tube), amniocytes, chorionic villi, skin biopsy (not fixed).

Reanalysis

Reanalysis of prior negative exome sequencing can produce a diagnosis.

- Rapid pace of discovery of new disease genes and improving interpretation of genomic data.
- Patient phenotype continues to evolve, sometimes revealing new diagnostic information.



Reanalysis of Clinical Exome Sequencing Data

The NEW ENGLAND JOURNAL of MEDICINE JUNE 20, 2019

Pengfei Liu, Ph.D.
James R. Lupski, M.D., Ph.D.
Yaping Yang, Ph.D.

- ▷ The value of existing genomic data continues to increase.
- ▷ Usually 1 free reanalysis provided per test.
- ▷ Only happens if ordered by provider. Consider reanalysis per clinical need and clinical picture, usually at least 1 year after initial test.

Thanks!

Any questions?

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