Genetics Concepts for Pediatric Hospitalists, Intensivists and Cardiologists

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Disclosure Statement

I do NOT have any relevant financial relationships to disclose or conflict of interests to resolve.
Learning Objectives/Goals

■ Understand the role of a geneticist
■ Understand when to refer a patient to genetics
■ Learn about the most common types of genetic testing and when to order them
■ Learn how to “read and explain” a genetic test result to a patient (without necessarily interpreting the result)
■ Discuss inborn errors of metabolism and newborn screening
■ Learn about new trends in genetics including direct to consumer testing and CRISPR/CAS9 (gene editing)
What is Clinical/Medical Genetics?

Applying genomic discoveries and knowledge to clinical practice.

Often involved in “translational medicine” or applying the knowledge obtained by bench research to the clinical realm.
The Role of the Clinical Geneticist

Consult on rare and common presentations involving virtually any organ system.

Evaluate all types of patients: adults, individuals with a personal/family history of cancer, couples seeking advice pre-conceptually (due to infertility or family history), expecting couples as well as pediatric patients.
Indications for a Genetics Consultation/Referral

**Growth**
- Failure to thrive
- Microcephaly
- Tall stature/connective tissue disorders- e.g. dilation/aneurysm of multiple or major arteries, poor wound healing, hyperextensibility, etc.
- Suspected skeletal dysplasia
- Hemihypertrophy
- Tall stature/increased weight/macrocephaly
Indications for a Genetics Consultation/Referral (cont.)

**Neurology**

Unexplainable and/or recurring seizures sometimes unresponsive to conventional management.

Mental illness such as schizophrenia, depression, bipolar disorder, etc.

Tremors, dystonia, chorea, unusual tics, muscular dystrophy, myotonic dystrophy

Hypo/hypertonia

Cerebral palsy

Developmental delay/learning disabilities/autism (anywhere on the spectrum), intellectual disability.
Indications for a Genetics Consultation/Referral (cont.)

**Hematology**
Excessive bleeding or clotting (as evidenced by recurrent deep vein thrombosis or pulmonary emboli).
Suspicion of inherited forms of anemia e.g. sickle cell, thalassemia etc.

**Cardiology**
Cardiomyopathy, conduction disorders, early onset hyperlipidemia or family history of these.
Any cardiac defect. Some examples include: atrial septal defect, ventricular septal defect, coarctation of the aorta, vascular ring, tetralogy of Fallot etc.
Dilatation of the aorta or other vessels, aneurysm of any vessel.
Indications for a Genetics Consultation/Referral (cont.)

**Birth Defects**
Any patient with a birth defect.

**Inborn Errors of Metabolism**
Acidosis
Hypoglycemia
Elevated anion gap (greater than 14)
Unexplained elevated CPK
Abnormal newborn screen
Interpretation of newborn screen
Indications for a Genetics Consultation/Referral (cont.)

**Ophthalmology**
- Retinitis pigmentosa
- Early onset macular degeneration
- Early onset cataracts
- Coloboma
- Duane anomaly
- Albinism
- Early onset glaucoma
- Lens dislocation
- Strabismus and nystagmus

**ENT**
- Hearing loss (syndromic vs. non syndromic)
- Cleft lip
- Cleft palate
- Velopharyngeal insufficiency
- Choanal atresia
Indications for a Genetics Consultation/Referral (cont.)

**Dermatology**
6 or more café au lait macules >1.5 mm diameter
Ash leaf spots
Increased number of nevi.
Unusual patterns of hypo or hyper pigmentation
Albinism

**Orthopedics**
Skeletal dysplasia
Multiple fractures
Indications for a Genetics Consultation/Referral (cont.)

Conditions involving any organ system/multiple organ systems where presentation is unexplainable or unusual. “Symptoms don’t seem to fit together”. If the presentation is strange/weird-the diagnosis is likely genetic.

Recognized genetic disorder including chromosomal, single gene or metabolic (inborn error of metabolism).

Family history of a recognized genetic disorder.

Relative with a sudden, unexplained death particularly at a young age e.g. SIDS, sudden death due to myocardial infarct before the age of 50.
Indications for a Genetics Consultation/Referral (cont.)

Any condition that appears genetic on family history or physical exam e.g. similar symptoms in multiple family members, consanguinity, dysmorphic features + birth defect/developmental concern.

Extensive family history of a single cancer or different cancer cluster types occurring in 2 or more first degree relatives. Unusual cancer for patient’s age. A presentation that may be indicative of an underlying cancer syndrome.

Infertility in both men and women.

Couples who wish to conceive and have significant results through carrier testing, a family history of a genetic disorder, a previous fetus with birth defects, advanced maternal/paternal age (35 years and 40 years respectively).
Indications for a Genetics Consultation/Referral (cont.)

Couples undergoing in vitro fertilization whose fertilized eggs have undergone genetic testing (pre implantation genetic testing) where results are significant.

Expecting couples with positive genetic test results whether through carrier testing, cell free DNA or through chorionic villus sampling/amniocentesis.

Expecting couples with a family history of a genetic disorder.

Expecting couples with a fetus that is highly suspicious for a genetic disorder e.g. birth defects, abnormal findings on sonogram.
Genetic Testing

- Sequencing
- Chromosomes
- Chromosome Array
- Inborn Errors of Metabolism
Chromosomes and Microarray

Sequencing

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Sequencing

“Genome sequencing is figuring out the order of DNA nucleotides, or bases, in a genome—the order of As, Cs, Gs, and Ts that make up an organism's DNA. The human genome is made up of over 3 billion of these genetic letters.”
Sequencing

Primer for replication

Strand to be sequenced

Prepare four reaction mixtures; include in each a different replication-stopping nucleotide

C G A T

Primed DNA

Replication products of “C” reaction

Separate products by gel electrophoresis

Read sequence as complement of bands containing labeled strands

T T G T T A T C C G C T C A C A A T T C C A C A C A A C

120 130 140
Sequencing

**Panels** - tests are ordered based on a specific symptom e.g. cardiomyopathy or seizures. Genes tested in the panel are associated with that symptom.

**Whole Exome Sequencing**: sequences all the exons or “coding region” of the genome. Used mostly if diagnosis is not clear. More accurate if both parents’ DNA is available.

**Whole Genome Sequencing**: sequences the exons and introns. Introns used to be considered “junk”. We now know that they can contribute to human disease through epigenetics. These are tests used by some “direct to consumer” genetic testing companies. We do not routinely order this test in clinical practice (at this time, it will change soon) as research is ongoing. Used for “personalized medicine”.

Chromosome analysis

“A laboratory procedure that isolates the chromosome pairs so that they may be visualized”.

Chromosome analysis
Chromosome array

“Detection of chromosome imbalances that are too small to be detected by looking down the microscope”.
Chromosome Microarray

- Works by exploiting the ability of a DNA molecule to hybridize to another DNA molecule.
- Comprises tens of thousands of short sequences of DNA ("probes"), arranged in a precise grid on a glass slide called a chip. DNA from the patient is digested and the fragments are labelled with a fluorescent dye.
- Reference DNA (from pool of people with no genetic abnormalities) is labelled with a different colored fluorescent dye.
- Reference and patient samples are mixed together and applied to the chip and the fragments of DNA hybridize with their matching probes on the array. The chip is then scanned in a machine called a microarray.
Patient sample Labelled with red fluorescent dye

Reference sample Labelled with green fluorescent dye

Mix together and apply to slide

Hybridisation

Microarray scanner

Computer analysis

Red/Green fluorescence ratio

Gains (duplications)

Losses (deletions)
Chromosomal Microarray Report

Clinical Indications: Dysmorphic features in setting of significant murmur and FTT; Failure to thrive; Other: Murmur; pending work-up; Dysmorphic facial features; 37.5;

Specimen(s) Source/Type: Peripheral Blood, EDTA

CMA Summary: Microarray: CytoScan HD, Lot#: 4255763

Coverage: 2.6 million markers for CNA, 750,000 SNPs

Genome build: GRCh37/hg19, Resolution: 1.15 kb

ABNORMAL Male with a Pathogenic Deletion and Long Contiguous Stretches of Homozygosity

CMA Nomenclature: arr[hg19] 22q11.21(18,916,842-21,465,659)x1

5q31.1q31.3(133,238,687-142,872,746)x2 hmtz 11p11.2p11.12(45,768,400-51,563,636)x2 hmtz
6p21.1p12.1(43,811,761-55,134,483)x2 hmtz 12q14.1q14.3(58,817,621-67,178,262)x2 hmtz
6q12.1q16.1(69,922,461-97,131,684)x2 hmtz 13q31.3q32.2(93,124,104-98,388,811)x2 hmtz
6q15.3q21(100,810,621-107,700,192)x2 hmtz 14q24.2q24.3(73,044,048-76,301,078)x2 hmtz
6q23.2q23.3(132,571,888-137,231,923)x2 hmtz 16p11.2p11.1(29,638,640-35,220,544)x2 hmtz
7p21.1p14.3(16,625,164-31,082,151)x2 hmtz 17p13.1p11.1(7,766,266-22,217,983)x2 hmtz
7q11.22q21.11(71,429,947-77,996,863)x2 hmtz 18q12.1q12.2(30,169,255-33,689,863)x2 hmtz
7q32.3q33(131,739,664-136,236,402)x2 hmtz
8p23.1(8,107,313-12,626,394)x2 hmtz

A 2,548.8 kb genomic deletion of chromosome 22 within the typical critical region associated with CHROMOSOME 22q11.2 DELETION SYNDROME\(^1,2\) was detected by Chromosome Microarray Analysis (CMA). Parental testing may be considered (at no charge, excluding blood draw fees) to discriminate between a familial variant and a de novo genomic alteration. If follow-up testing is desired, please enter 2 orders as "CHLA Microarray Family follow-up"; one for each parent. Parental results will be provided in separate reports. Genetic counseling, clinical correlation and continued surveillance of the literature regarding the clinical relevance of the genes at these loci are recommended.

In addition, SNP analysis showed 16 segments of allelic homozygosity totaling 136Mb, encompassing at least 73 OMIM recessive genes, and greater than 4.72% of the individual's genome. The implications of such copy neutral segments showing homozygosity are unclear at present. In theory, an increased risk for recessive Mendelian disorders within these regions may be considered. Additionally, this result could indicate a familial relationship between this individual's parents. A genetics consultation is recommended. Interpret with caution. (Further testing and evaluations may be indicated). Candidate genes* may be checked for inclusion in the homozygotic regions listed above. See methodology section for reporting criteria and limitations of this assay.
Guide to Ordering Genetic Testing in the Pediatric Population

American College of Medical Genetics and Genomics
ACT Sheets

Discuss protocols for genetic testing and evaluation for many different conditions/carrier status as well as guidelines for following up on positive newborn screen results.
Guide to Ordering Genetic Testing in the Pediatric Population (cont.)

If the child has one or more organ system involvement and +/-dysmorphic features it is reasonable to consider a micro array +/- chromosomes.

Developmental Delay/Intellectual Disabilities

ACMGG guidelines include the following:

- Micro array
- Fragile X testing
- Inborn Error of Metabolism work up (if needed)
Testing for Inborn Errors of Metabolism

First Line Testing is metabolite testing:

- Plasma Amino Acids
- Urine Organic Acids
- Acyl carnitine Profile
- Total and Free Carnitine
- Ammonia
- Chemistry 14
- Lactate/Pyruvate
- Uric Acid

These test for certain metabolites. They are a screening tool and not necessarily diagnostic. They can be affected by diet, fasting status, TPN, and an immature liver.
Testing for Inborn Errors of Metabolism/Newborn Screen

If the metabolite screen is positive, enzyme testing and/or genetic testing/sequencing is warranted. These are diagnostic.

For some metabolic disorders, the gene involved is not known or well understood. In these cases, a diagnosis can be made by studying the enzyme function.
Newborn Screening Nevada

“31 core and 29 secondary conditions recommended by the College of Medical Genetics and the March of Dimes.”

New testing:

- X-linked adrenoleukodystrophy
- Pompe Disease
- Mucopolysaccharidosis I (MPS I)/Hurler syndrome (MPS I-H), Hurler-Scheie syndrome (MPS I-H/S), and Scheie syndrome (MPS I-S)
- Spinal muscular atrophy
Mitochondrial DNA Sequencing vs. Nuclear DNA Sequencing for Mitochondrial disorders

Mitochondrial disorders can be due to mutations in the nuclear DNA (derived from the nucleus of the cell) or mitochondrial DNA (derived from the DNA of the mitochondria).

Mitochondrial disorders derived from nuclear DNA abnormalities are usually autosomal recessive in nature and manifest in infancy.

Mitochondrial disorders derived from mitochondrial DNA abnormalities are maternally inherited and have later onset.
New Trends in Genetics

Direct to Consumer Genetic Testing

Genetic testing marketed directly to the patient/consumer without a geneticist or physician order. **Whole genome sequencing** that looks at all genes not just those for rare disorders. Gives “susceptibilities or likelihoods” for more common disorders or cancers. Can be inaccurate or incomplete. This is the concept of “personalized medicine”.

When patients receive the results, they can post them to a public database in order to gain more information about their results.

This is how the “Golden State Killer” was obtained by authorities.
Direct to Consumer Genetic Testing

*What do you do when you receive these genetic testing results that you did not order?*

A. Call Dr. Sorrentino! I have her on speed dial....

B. Place a referral to genetics then see above....

C. Explain to the patient that you do not know how to interpret the results and hope they don’t ask for a referral to genetics.

D. Explain to the patient that these tests are not yet validated for clinical use. Although the information is reported, a physician or other health care provider cannot clinically act on them. This may change in the future. If the patient has a concern about a genetic disorder in their family/child (whether based on the test results or not) further genetic testing may need to be conducted by a CLIA certified lab.

Then see A or B or just place the referral!
Whole Exome/Genome Sequencing and Rapid Genomic Testing in the ICU/Inpatient Unit for the Pediatric Population-Most Recent Data

Health care costs for children with genetic disorders are higher. One study estimated that direct health care costs of children diagnosed with genetic diseases were anywhere from 4.54 to 19.76 times greater than that of the general population and 1.77-8.27 times greater than children with other chronic diseases such as asthma and diabetes. This study matched cohorts based on sex, date of birth, family income, rural vs. urban household at birth and date of diagnosis.

They were also more likely to visit specialists and less likely to see their primary care physician.

Studies have demonstrated that rapid genome sequencing can result in reductions in cost to delivering care to this population.
Whole Exome/Genome Sequencing and Rapid Genomic Testing in the ICU/Inpatient Unit for the Pediatric Population-Most Recent Data (cont.)

Reduction in healthcare costs is attributable to the following:
Less testing, diagnostic studies and procedures due to minimizing or eliminating the diagnostic odyssey.

May guide treatment and management:
This includes specialist consultations, change/add medication, diagnostic studies to order, determination if surgery should proceed, type of surgery done and making the decision to initiate palliative care.

This may also mean that a rare disease that has a treatment is discovered and that treatment is started in a timely fashion instead of trying other treatments first.
Guide RNA brings complex to area of mutation. Cas9 is an enzyme that cleaves the area of the mutation. The “normal or typical” gene sequence then can be introduced.

In a recent article, there was successful in vivo use of CRISPR/Cas9 for genome editing in order to correct an inherited cardiac arrhythmia in mice (R176Q pathogenic variant in the RYR2 gene).
CRISPR/Cas9
“Eat less, exercise more and invent a time machine so you can go back and choose parents with better genetics.”
Resources

The Busy Physician's Guide to Genetics, Genomics and Personalized Medicine

The Human Genome

The Book of Essential Knowledge

John Quackenbush, Ph.D.

Foreword by John Sulston, Ph.D., Nobel Laureate
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