Genetic Testing From Basic To COMPLEX

SHOBANA KUBENDRAN,MBBS,MS,CGC
GENETIC COUNSELOR
ASSOCIATE PROFESSOR
DIRECTOR, DIVISION OF GENETICS
DEPT OF PEDIATRICS
KUSM- WICHITA
OCTOBER 6,2017

KU WICHITA PEDIATRICS
The University of Kansas

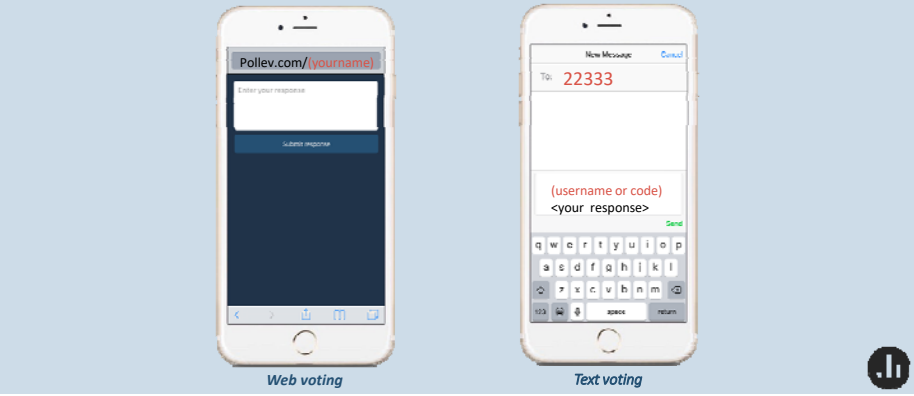
Disclosure

- I HAVE NO RELEVANT FINANCIAL RELATIONSHIPS WITH THE MANUFACTURERS(S) OF ANY COMMERCIAL PRODUCTS(S) AND/OR PROVIDER OF COMMERCIAL SERVICES DISCUSSED IN THIS CME ACTIVITY
- I DO NOT INTEND TO DISCUSS AN UNAPPROVED/INVESTIGATIVE USE OF A COMMERCIAL PRODUCT/DEVICE IN MY PRESENTATION.

Objectives

- Knowledge
  - List four methods of genetic testing
- Application
  - Practice how to choose right genetic test
- Analysis
  - Distinguish benefits and limitations between two genetic testing methods
- Synthesis
  - Propose models of integrating genetic testing into primary care practice

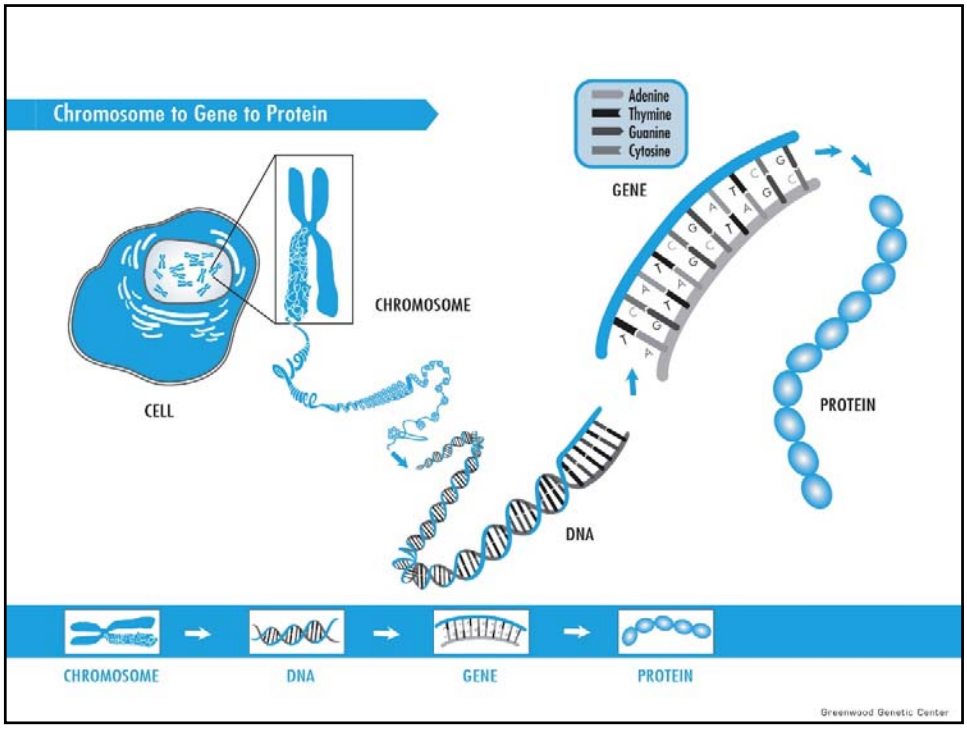
Responding with Poll Everywhere



Knowledge

Genetic Testing Methods

- Sequencing
- Microarray
- Targeted mutation analysis
- Methylation analysis

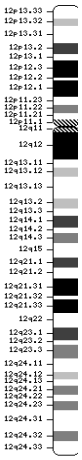


Genetic Testing Analogies

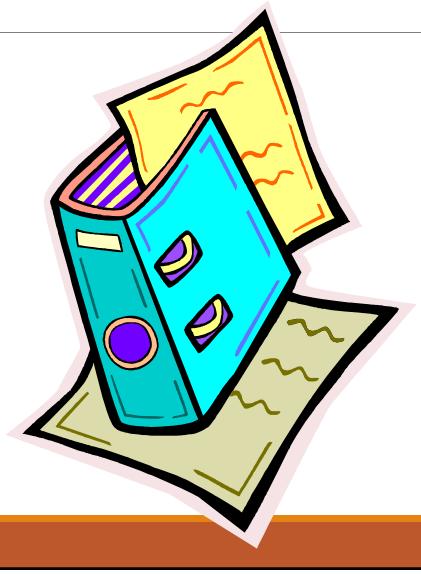
46 chromosomes

6 billion letters

Chromosome testing is like looking for chapters in a book

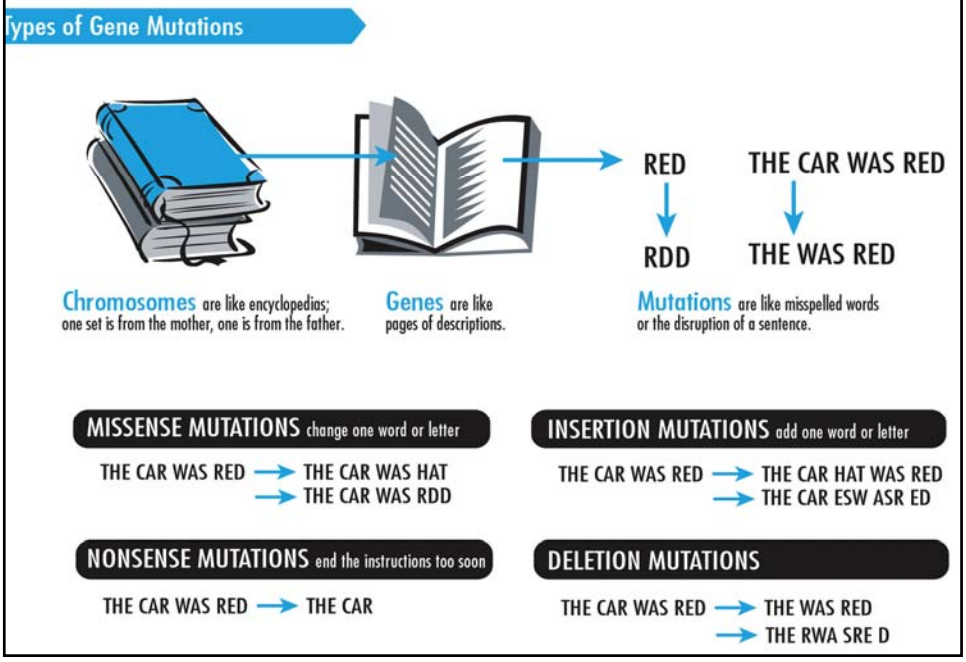


Microarray testing looks closer to see if <u>pages</u> with the recipe(s) are missing/extra



Sequencing is like reading

- Single gene sequencing – reading one gene (one recipe)
- Multi gene panel sequencing – reading a group of genes (group of recipes) at the same time
- Exome sequencing – reading the protein coding regions of all 20,000 exons (20,000 recipes) at the same time
- Genome sequencing – reading all the protein coding and non coding regions of genome such as exons and introns (recipes and the "blank" pages in between) at the same time



Genes With Multiple Mutations

Some conditions, like Sickle Cell Anemia, are caused by one specific mutation.

Some conditions, like Cystic Fibrosis, can be caused by multiple mutations in the same gene.

Some mutations are more common in a specific population.
One example is the BRCA1 gene, which has 2 mutations that occur with a high frequency in the Ashkenazi Jewish population.

Some genes do not have any common mutations. To find mutations, the laboratory has to sequence the gene. This is like taking a 100,000 piece puzzle and looking at each piece to find a problem.

Greenwood Genetic Center

Mutation vs Variation

The cat in the hat

The cat in the zat

The cat in the mat

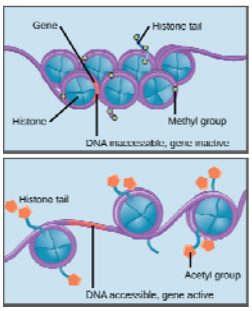
ACMG Variant Classification

Classification	Evidence	Report status
Pathogenic	Mutation known to be associated with disease	Yes
Likely pathogenic	Mutation known to be associated with disease, but not sufficient evidence	Yes
Uncertain significance	Does not meet criteria to be benign or pathogenic	Usually
Likely benign	Does not meet criteria to be benign or pathogenic and functional studies argue against damaging effect	No
Benign	Variation present at greater than 5% frequency in the general population	No

Whole Exome vs Whole Genome

WES	WGS
Protein coding regions of genome - 20,000 genes or 1% genome	Coding and non coding regions
96%-99% coverage of exons	Uniform coverage
85% of mutations are in the exome	15% mutations in intron/exon boundary or introns
~200,000 variations	2-4 million variations
Results in 2-4 months*	Results in 4-6 months*
Incidental findings 3%	Incidental findings 3%

Methylation analysis: is the gene "turned on" or "off"?

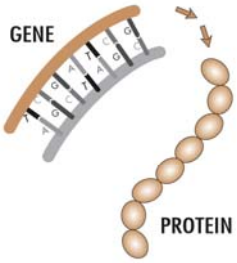


Methylation of DNA and histones causes nucleosomes to pack tightly together. Transcription factors cannot bind the DNA, and genes are not expressed.

Histone acetylation results in loose packing of nucleosomes. Transcription factors can bind the DNA and genes are expressed.

Targeted mutation analysis

Trinucleotide Repeat Disorders



A **trinucleotide** is a group of 3 chemical bases within a gene. Trinucleotides code for **amino acids**. Amino acids are the building blocks of **proteins.**

TRINUCLEOTIDE C G G

Normal number of repeated trinucleotide segments within a gene = **Normal Protein Function.**

CGG CGG CGG CGG

Mildly expanded number of repeated trinucleotide segments = **Normal Protein Function.**

CGG CGG CGG CGG CGG CGG CGG

Fully expanded number of repeated trinucleotide segments = **Abnormal or Absent Protein.**

CGG CGG CGG CGG CGG CGG CGG CGG CGG CGG CGG

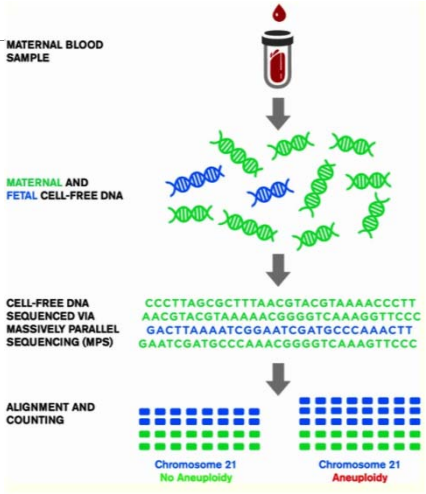
Greenwood Genetic Center

Non Invasive Prenatal Screening

Analyze cell free fetal DNA
- Massively parallel shotgun sequencing (MPSS)

10%-15% of cell free DNA circulating in maternal blood is from fetus (placenta)

Quantitative differences in chromosome fragments in maternal blood can be used to distinguish fetuses affected with Trisomy 13, 18, 21



Non-invasive Prenatal Testing: Technologies, Clinical Assays and Implementation Strategies for Women's Healthcare Practitioners
Curr Genet Med Rep. 2013 June; 1(2): 113–121.

Non Invasive Prenatal Testing Factsheet – NCHPEG & NSGC

Non Invasive Prenatal Screening

Testing can be done after 10 weeks

Results 7 -10 days

Indications
- AMA
- Abnormal serum screen
- Personal or family history of aneuploidy
- Abnormal ultrasound

Non Invasive Prenatal Testing

Detection rate for trisomy 21 and 18
- 99% detection rate
- <1% false negative rate
- 0.2% false positive rate

Detection rate for trisomy 13
- 79%-92% detection rate
- <1% false positive rate

Comparing Down syndrome Screening Methods

Method	Detection Rate	FPR	PPV high risk population (1/100)	PPV low risk population (1/500)
Quad screening	80%	5%	17%	4%
Non invasive prenatal screening	98.6%	0.2%	91%	67%

Positive Predictive Value – dependent on the prevalence of the condition

PPV for microdeletion conditions 5%-10%

Interpretation of NIPS results

Positive results are “near diagnostic”
- should be confirmed with CVS, amnio or postnatal testing

Negative results – highly sensitive and specific but not 100%

Terminology

1 base pair

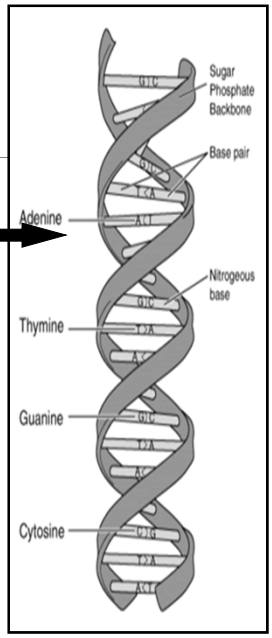
1000 bp = 1 kilobase pair

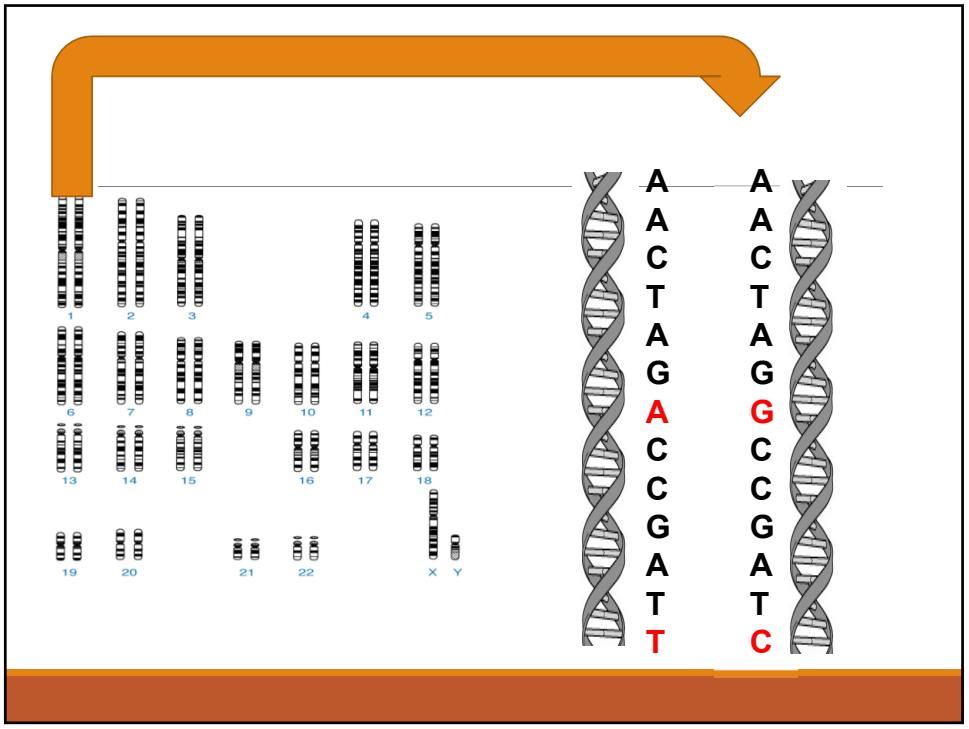
1000 kb = 1 megabase pair

1 Mb = 1 million bp

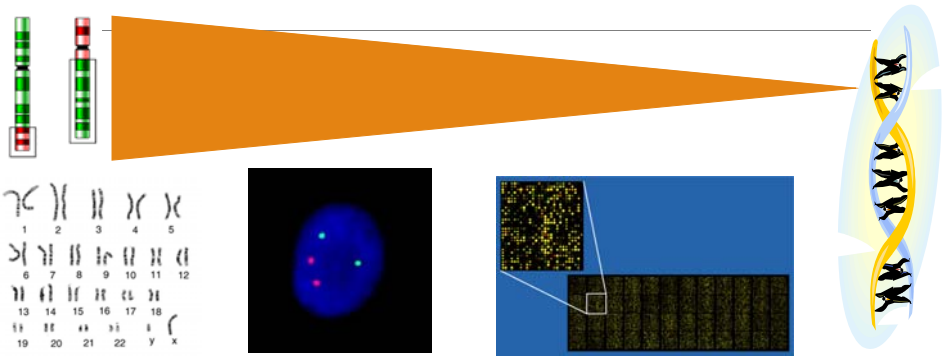
Size of Human genome

3 billion base pairs





Genetic Test Resolution



Karyotype

~ 4 Mb

FISH

40 bp – 250 kb

Array Comparative Genomic Hybridization

20 bp - 100 kb

Sequencing

A T G C

1 base pair

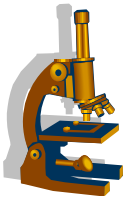
Karyotype

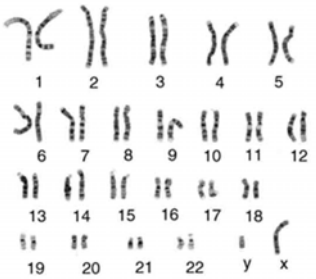
BENEFITS

- Detect
  - Deletion
  - Duplications
  - Trisomies
  - Unbalanced translocations
  - Balanced translocations
  - Inversions

LIMITATIONS

Detect copy number variations ~ bigger than 4 Mb





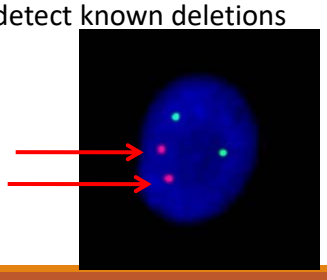
"FISH" using Probes

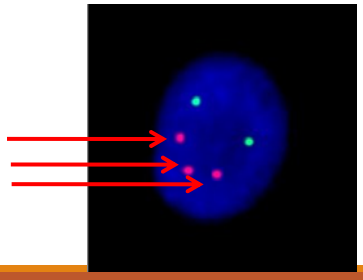
BENEFITS

- cell growth not required
- rapid screening aneuploidies (24h)
- mosaicism
- detect known deletions

LIMITATIONS

- Sensitivity limited by size of probe
- Requires clinical suspicion of a condition



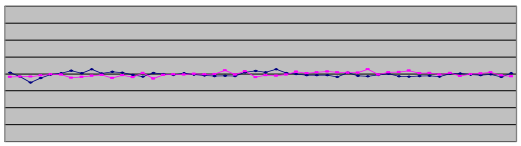


FISH or CHIPS?

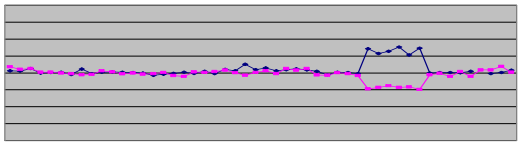
"FISH"ING
ONE FISH AT A TIME

MICROARRAY "CHIP"
CASTING A WIDE NET

Array CGH Diagnostic Applications: Characterization of Microdeletions

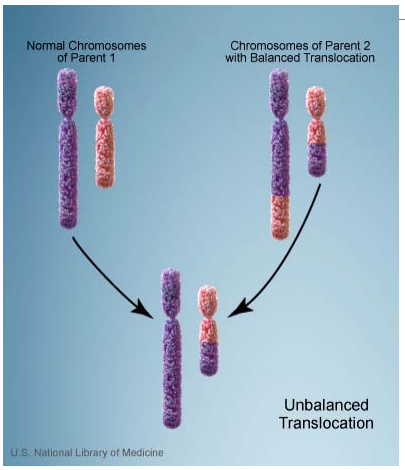


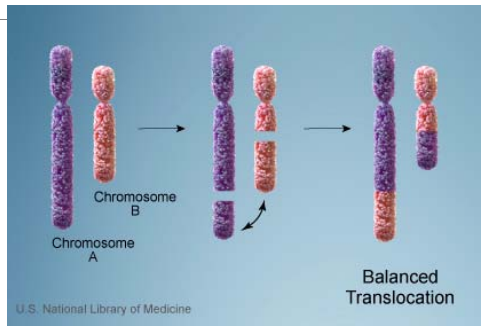
Normal Chromosome 7

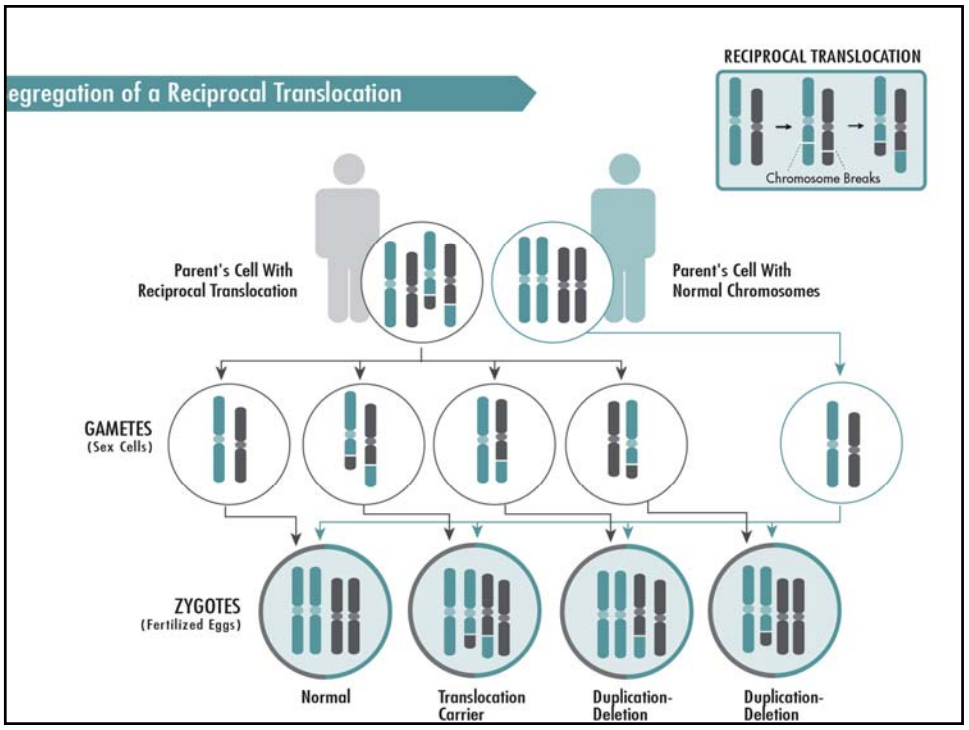


Large Deletion

Array CGH will not detect balanced alterations





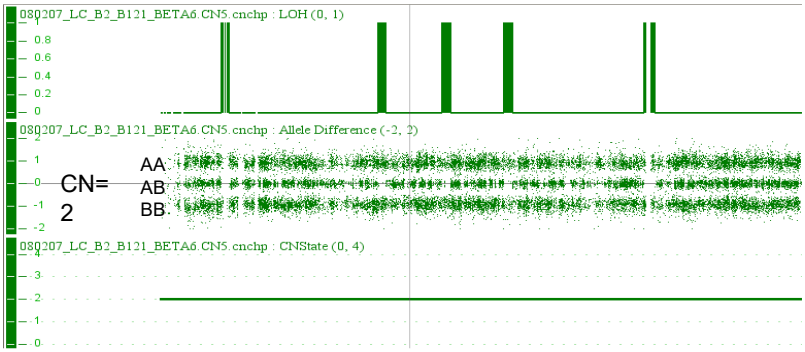


COPY NEUTRAL HOMOZYGOSITY;

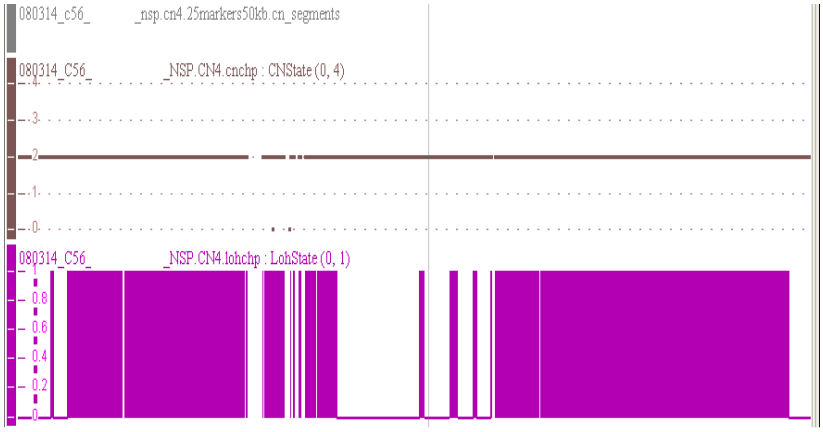
Identical alleles at polymorphic sites

AATGCGGTAGCGGTTTAGCGCAGGATGT

AATGCGGTAGCGGTTTAGCGCAGGATGT

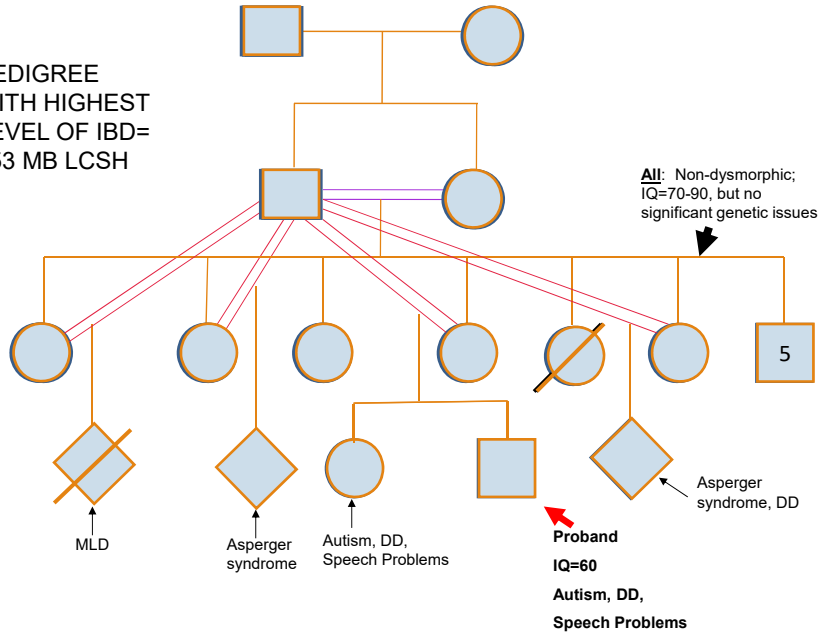


IDENTITY BY DESCENT



Chromosome 10: 97Mb Interval Total

PEDIGREE
WITH HIGHEST
LEVEL OF IBD=
953 MB LCSH



Application

- Practice how to choose right genetic test
- Genereviews.org
- AAP Guidelines on Healthcare Supervision

http://pediatrics.aappublications.org/cgi/collection/committee_on_genetics?page=1

How to interpret results?

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Recurrent Rearrangements of Chromosome 1q21.1 and Variable Pediatric Phenotypes

H. Mefford, A. Sharp, C. Baker, A. Itsara, Z. Jiang, K. Buysse, S. Huang, V. Maloney, J. Crolla, D. Baralle, A. Collins, C. Mercer, K. Norga, T. de Ravel, K. Devriendt, E. Bongers, N. de Leeuw, W. Reardon, S. Gimelli, F. Bena, R. Hennekam, A. Male, L. Gaunt, J. Clayton-Smith, I. Simonic, S. Park, S. Mehta,

NIH Public Access
Author Manuscript
Nat Genet. Author manuscript; available in PMC 2009 December 1.

NIH-PA Author Manuscript

Published in final edited form as:
Nat Genet. 2008 December ; 40(12): 1466–1471. doi:10.1038/ng.279.

Recurrent reciprocal 1q21.1 deletions and duplications associated with microcephaly or macrocephaly and developmental and behavioral abnormalities

Nicola Brunetti-Pierri[1,23], Jonathan S Berg[1,23], Fernando Scaglia[1,2], John Belmont[1], Carlos A Bacino[1,2], Trilochan Sahoo[1], Seema R Lalani[1], Brett Graham[1], Brendan Lee[1,3], Marwan Shinawi[1], Joseph Shen[1], Sung-Hae L Kang[1], Amber Pursley[1], Timothy Lotze[4], Gail Kennedy[5,6], Susan Lanpher-Shafer[5,6], Christine Weaver[5,6], Elizabeth P Roeder[7], Theresa A

Analysis

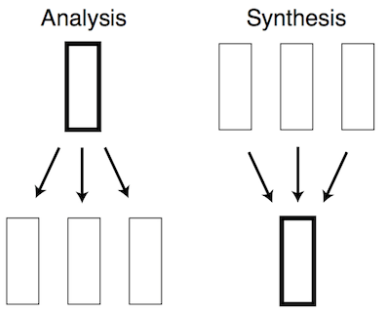
Components of Informed Consent

- Variable expression
- Incomplete penetrance
- Does not detect all variations
- Interpretation of negative result
- Variants of uncertain significance
- Familial implications
- Reproductive implications
- Option to participate in research
- Incidental findings
- Insurance discrimination

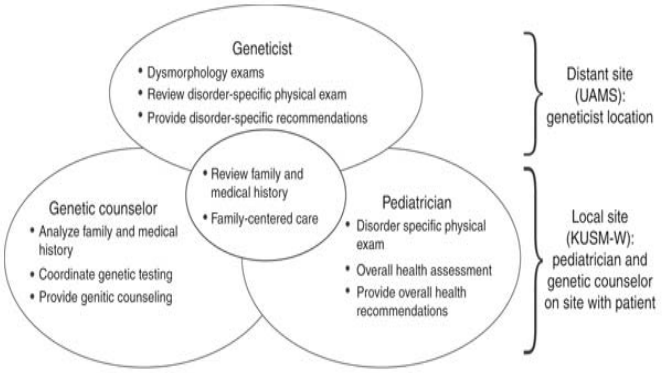
Points to consider

- Medical status of infant/child
  - Turn around time for results
  - Tiered genetic testing strategy vs "comprehensive"
- Specific features vs broad differential
  - Targeted mutation analysis vs whole exome
- Cost and insurance coverage

Synthesis



KU Wichita Pediatrics Primary Genetic Evaluation Clinic



GENETICS IN MEDICINE | ORIGINAL RESEARCH ARTICLE OPEN

A novel approach in pediatric telegenetic services: geneticist, pediatrician and genetic counselor team

Shobana Kubendran, Siddharthan Sivamurthy & Gerald Bradley Schaefer

Affiliations | Corresponding author

GENETICS in MEDICINE (2017) | doi:10.1038/gim.2017.45

Received 11 October 2017 | Accepted 10 March 2017 | Published online 04 May 2017

http://bd4uz2kj6y.search.serialssolutions.com/OpenURL_local?sid=Entrez:PubMed&id=pmid:28471436

Challenges

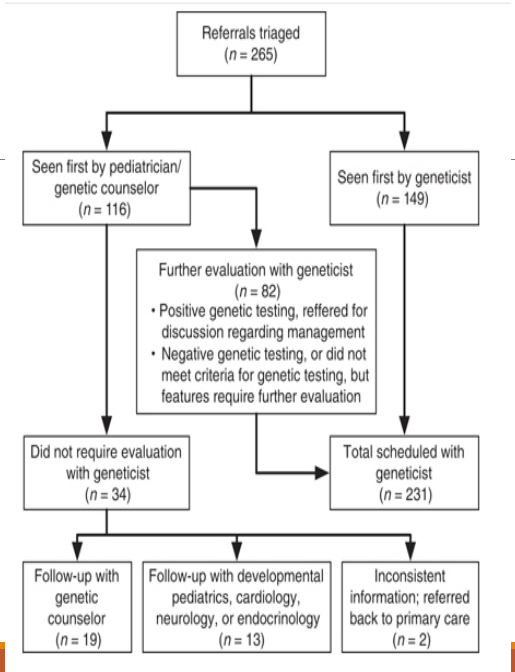
National average 3.5 geneticists/million population

Kansas <1 geneticists/million population

Genetics clinics located

- Wichita
- Kansas City

KU School of Medicine-Wichita Wesley Pediatric Subspecialists



Outpatient Telegenetics Clinic

KU SCHOOL OF MEDICINE WICHITA The University of Kansas

UAMS UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

Results – Patient Satisfaction with Telegenetics

Before today's visit, what made it hard to get the specialty services your child needs?

The information I received before the visit helped me understand what was going to happen.

The equipment worked well.

The use of technology did not get in the way with being able to have a good conversation with the specialist.

Despite the obstacles to receiving care in person, I would STILL prefer to travel to see the specialist.

If you had not been able to use telemedicine to get specialty care today, what would you have done?

71 patients

All patients
- Satisfied - highly satisfied

More convenient/less gas

I really like the telemedicine, it is really nice not having to travel

I hope the other specialist begin to appreciate this technology. We live in great times!

Awesome love the people here

Thank you for providing us with wonderful care that is so easily accessible

Results – Patient Satisfaction with Pediatrician/GC

The information I received before the visit helped me understand what was going to happen

The pediatrician and genetic counselor responded to all of my questions

I feel confident in the quality of care provided today

I feel confident in the recommendations from today's visit

I would recommend the genetics clinic to other families

Overall I was satisfied with the visit today

30 responses

All patients –
- Satisfied or highly satisfied

Yes, even though we didn't have genetic doctor here I got the information we needed

I feel very confident in the pediatrician and counselor to handle my child's case

Heartland Genetics Network

- KU Wichita Pediatrics Subaward to work with PCP's and communities to
  - Increase access to genetic services in remote sites
  - Genetics education for primary care providers
  - Support families of children with special health care needs

Review Objectives

- Knowledge
  - List four methods of genetic testing
- Application
  - Practice how to choose right genetic test
- Analysis
  - Distinguish benefits and limitations between two genetic testing methods
- Synthesis
  - Propose models of integrating genetic testing into primary care practice

Resources

GeneReviews

AAP Guidelines on Healthcare Supervision

http://pediatrics.aappublications.org/collection/committee-genetics

Genetics in Primary Care

- http://www.geneticsinprimarycare.org/Pages/default.aspx

THANK YOU !

Direct clinic number for appointments– 316-962-2153

Fax – 316-962-2147

Pager – 316-962-3030

Shobana.kubendran@wesleymc.com

Resources

GeneReviews

AAP Guidelines on Healthcare Supervision

- http://pediatrics.aappublications.org/cgi/collection/committee_on_genetics?page=1

References

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., ... & Voelkerding, K. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in medicine*, *17*(5), 405-423.

Grody, W. W., Thompson, B. H., & Hudgins, L. (2013). Whole-exome/genome sequencing and genomics. *Pediatrics*, *132*(Supplement 3), S211-S215.

ACMG Board of Directors. (2012). Points to consider in the clinical application of genomic sequencing. *Genetics in Medicine*, *14*(8), 759-761.

Green, R. C., Berg, J. S., Grody, W. W., Kalia, S. S., Korf, B. R., Martin, C. L., ... & Rehm, H. L. (2013). ACMG recommendations for reporting of incidental findings in clinical exome and genome sequencing. *Genetics in medicine: official journal of the American College of Medical Genetics*, *15*(7), 565.