Tuesday, 10:00 – 11:30, A1

What's New in Genetics

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Objective:

Identify advances in clinical assessment and management of selected healthcare issues related to persons with developmental disabilities

Notes:

What's New in Genetics

Helga V. Toriello, PhD

PRESENTATION TOPICS

- THE ISSUE OF THE UNDIAGNOSED PATIENT
- TESTING FOR GENETIC CONDITIONS
- TREATMENT OPTIONS FOR THE FUTURE

UNDIAGNOSED PATIENT

- Genetic disorders are individually rare, but cumulatively affect 25 million in the US alone
- Harder for physicians to recognize most of these rare disorders; initial findings non-specific, e.g., weight loss, growth failure, fatigue, fever
- Tendency is to make diagnosis of common disorder rather than rare disorder

UNDIAGNOSED PATIENT

- Undiagnosed diseases remain undiagnosed if incorrectly diagnosed as something else. example.
- Problem if wrong diagnosis persists – wrong treatment, wrong natural history, evaluations which are unnecessary are done and vice versa
- Clinic experience with the "why isn't this child deceased yet?" referrals

EXAMPLES

Initial diagnosis	Final diagnosis
Late-onset autistic regression	Kleefstra syndrome
Hypotonia	Nemaline myopathy
Seizures	SCN2B mutation
IBD	XIAP
Arthritis	Hyper-IgD syndrome
Hepatic failure	Citrin deficiency

DIAGNOSTIC PITFALLS

- Inherent to disease process
- Patient-specific
- Physician-specific
- Limitations in diagnostic modalities

DISEASE PROCESS

- "diseases do not read the textbooks"
- Individuals with periodic fever syndromes do not have fever
- Individuals with hyper-IgD have normal levels of IgD

PATIENT SPECIFIC

- Parents often anxious, and viewed with negative bias
- Tend to over-report tiniest details in hopes of finding clue that may provide the diagnosis
- Overwhelming amount of information for the physician – all of it important to the family
- physicians might even suspect some information exaggerated or even fabricated
- Leads to doctor-shopping, may even lead to suspicion of Munchausen by proxy

PHYSICIAN SPECIFIC

- Two rules – expect the unexpected, AND never say never
- Wrong things to say –
- "I have never seen this symptom in this disease"
- "It can't be this"
- "It must be this"
- Once diagnosis in record, it becomes fixed –

PHYSICIAN SPECIFIC

- Might seek symptoms which support the diagnosis, ignores those which don't
- Called confirmation bias – especially problematic if disease is one senior physician is an expert
- E.g., the case of "C syndrome"
- Especially if rare, only have a few patients on whom to base the phenotype

C SYNDROME

- Patients reported to have C syndrome – only commonality is trigonocephaly and ID
- Prenatal exposure to Depakote can also yield this phenotype

PHYSICIAN SPECIFIC

- Need to revisit diagnosis from time to time –
- Child with autism diagnosis shows regression and behavioral changes, think metabolic
- Repeated episodes of swelling not allergic, but may be hereditary angioedema
- Abdominal pain not constipation, but porphyria, Fabry disease, or familial Mediterranean fever

PHYSICIAN SPECIFIC

- Reliance on diagnostic criteria: helpful, but variation in phenotype can make these less than useful
- Marfan – over 300 unique mutations, with some having lens dislocation, others aortic aneurysm, and others primarily skeletal manifestations
- 12% of patients not meeting criteria have mutation; 66% who do meet criteria do not have mutation

UNDIAGNOSED PATIENT AND GENETIC TESTING

- Tendency is towards "genotype first" approach
- Compared to "phenotype first"

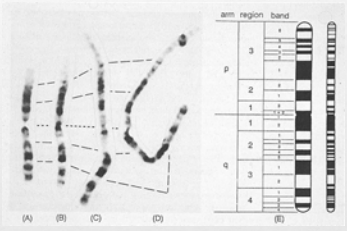
TESTING OPTIONS – PAST AND PRESENT

- Brief Review
  - What are chromosomes?
  - What are genes?
  - How are they involved in causing disorders?
- How do we test for changes in genes or chromosomes?
  - Old and new technologies for detecting chromosome anomalies
  - Old and new technologies for detecting single gene disorders

CHROMOSOMES

- Composed of DNA and protein
- Contain all of the genes in the nucleus
- Humans have 23 pairs
- Extra or missing chromosome material usually causes ID and anomalies ("birth defects")

CHROMOSOME - DETAIL



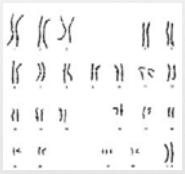
TYPES OF ANOMALIES

- Aneuploidy (extra or missing chromosomes) – example is Down syndrome (trisomy 21)
- Deletions (missing part of a chromosome)
- Duplications (extra parts of a chromosome)
- Other

TYPES OF ANOMALIES

Down syndrome

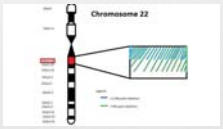
Trisomy 21



TYPES OF ANOMALIES

DEL 22q

Del 22q



OTHER CHROMOSOME ANOMALIES

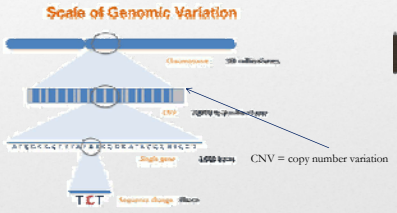
DUP 16p11.2

Ring 20

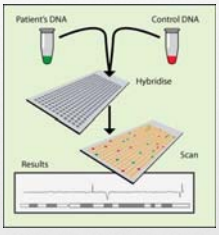
HOW ARE CHROMOSOME ANOMALIES IDENTIFIED?

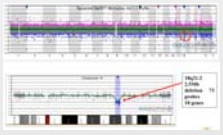
- Karyotype
- FISH
- Oligo array
- SNP array
- Note: Both oligo array and SNP array are types of chromosomal microarray

MORE ON MICROARRAY



MORE ON MICROARRAY





BENEFITS OF MICROARRAY

- With greater resolution, identification of new syndromes
  - Example: del 1q43q44 (microcephaly, lack of speech, minor facial and limb anomalies)
- Consolidation of previously distinct syndromes
  - E.g.,Shprintzen syndrome, velocardiofacial syndrome, diGeorge syndrome, and some cases of Opitz BBB syndrome
- Recognition of deletion and duplication syndromes at same site e.g., 22q deletion and 22q duplication

ISSUES WITH MICROARRAY

- Reduced penetrance and variable expressivity
- 16p11.2 (60% penetrance)
- 15q11.2 (10% penetrance)
- 1q21.1 (TAR syndrome only if additional variant present)
- For some of these, there may still be some phenotypic effects, e.g., obesity, reduced fertility, other

MICROARRAYS

- Genotype-phenotype correlation:
- Single gene within CNV compared to true microdeletion syndrome
- Location within gene can influence phenotype
  - E.g., in-frame versus out-of-frame in dystrophin so Becker versus Duchenne

CLINICAL UTILITY

- Now first tier test for ID, autism and MCA
- Cannot find balanced translocations; but we now know many are in fact unbalanced
- Still do karyotype to detect parental balanced however

DETECTION RATE

- Depends on phenotype and array:
- Higher in cardiovascular or craniofacial than in those with epilepsy or autism
- Combinations higher anomaly rate e.g. epilepsy and ID higher than epilepsy alone
- Those with ASD, associated ID, congenital anomaly, or dysmorphic features
- Array type
- SNP also detect areas of homozygosity – consanguinity or UPD
- Number of probes and placement can increase detection
- However, also more likely to find benign CNV

INCREASING LAB EXPERIENCE

- Databases – know what is benign and what isn't
- Better interpret meaning of those inherited from an unaffected parent – reduced penetrance versus benign CNV

MANAGEMENT

- Might find coincidental variant which affects management
- CNV contains gene which has relevant management issues
- Other issues – parental mosaicism more common than thought – increased recurrence risk, but technology not quite there to identify it on a parental blood sample

WHAT CAN EACH TEST TELL YOU?

Condition	Karyotype	FISH	Oligo Array	SNP Array
Aneuploidy	Yes	Yes*	Yes	Yes
Deletion or Duplication syndrome	Usually no	Yes*	Yes	Yes
Mosaicism	Yes, to a certain level	Yes*	Yes	Yes
Homozygosity	No	No	No	Yes
Balanced translocation	Yes	Yes	No	No
Cause of ID/DD	5%	Subtelomeres 8%	14-15%	18% +

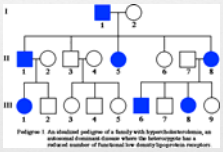
*if use the correct probe

SWITCHING TO MOLECULAR TESTS AND GENES

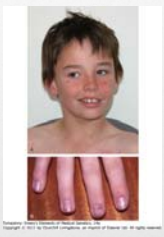
- Unit of heredity
- Code for proteins which can have several different functions
- Change in genetic code is called mutation
- Mutations may be harmful or benign

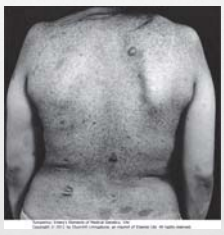
MUTATIONS CAN CAUSE GENETIC DISORDERS

- When only one copy of a mutant gene is necessary to cause the condition, the resultant condition is inherited as an autosomal dominant trait



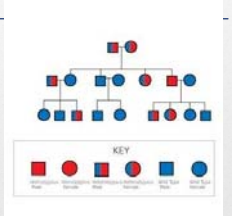
EXAMPLES OF DOMINANT DISORDERS





MUTATIONS

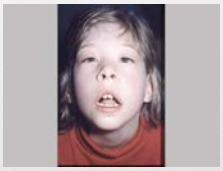
- When both copies of the gene need to be mutated to cause the condition, the inheritance pattern is autosomal recessive



TYPES OF MUTATIONS

- Missense mutations – switching of one base pair for another; leads to coding for a different amino acid, which may affect protein structure. Example: "one cup of nuts" becomes "one cup of nuns"
- Nonsense mutations – base pair change leads to the coding of a stop codon. Protein is shortened. Example: "add one cup of nuts to the batter" becomes "add one cu"
- Other types

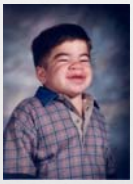
EXAMPLES OF AUTOSOMAL RECESSIVE DISORDERS



X-LINKED DISORDERS

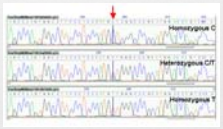
XL Dominant

XL Recessive



HOW DO WE LOOK FOR MUTATIONS IN GENES?

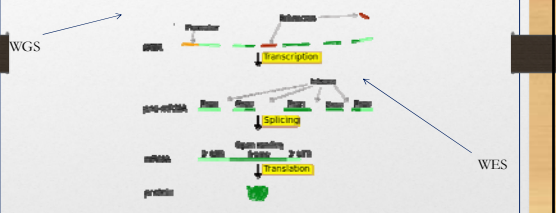
- Sequencing of single gene – Sanger sequencing
- Able to find base pair changes (mutation) and very small deletions (of a couple of base pairs)
- Not able to find larger deletions (CNV)



NEXT GENERATION SEQUENCING

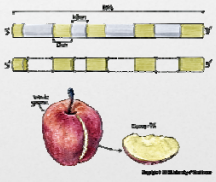
- TEST PANELS
  - Simultaneously test for most if not all genes that cause a particular phenotype
- WHOLE EXOME SEQUENCING
  - Sequence all coding regions (exons) of genes - ~1% of genome that includes sequences that code for proteins
- WHOLE GENOME SEQUENCING
  - Sequence "everything"

WES versus WGS



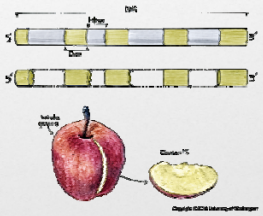
EXOME SEQUENCING

- Sequencing all of the coding regions of the genes
- Account for ~80-90% of disease-causing mutations
- Still only small part of the genome
- Numerous variants identified, many of which are likely benign



GENOME SEQUENCING

- Sequences everything, including non-coding regions



CHOOSING THE RIGHT TEST

- Single-gene tests
- Ideal for conditions known to be caused by a single gene, e.g., cystic fibrosis
- Efficient, high sensitivity and specificity
- Uses Sanger sequencing – highly accurate, but time consuming

CHOOSING THE RIGHT TEST

- Gene panels
- Best for conditions with locus heterogeneity
- Not quite as good at finding all mutations; might also need to do Sanger to verify or cover regions not covered by panel
- Greater chance of finding VUS
- Inclusion of genes on panels varies from lab to lab

COMPARISON OF PANES (SEARCHED FOR EPILEPSY PANELS)

Indication	Number of genes	comment
Pyridoxine-dependent	85	
Epilepsy panel	343	Includes achondroplasia, Apert syndrome, etc.
Epilepsy with LD and/or behavioral concerns	95	

Cost not provided by any of these labs

CHOOSING THE RIGHT TEST

- Exome sequencing
- Becoming more acceptable as test
- Used to be test of last resort, now becoming first tier test
- Interpretation may be problematic
- Little information on cost-effectiveness, however
- Mendeliome another option – sequencing of all currently known disease genes

VALUE OF MOLECULAR DIAGNOSIS

- Provides information regarding disease mechanism
- Adds information to database of variants
- Ends diagnostic odyssey
- Some cases, management affected

PATIENT'S PERSPECTIVE

- Know what they have
- How it was caused
- What the disease progression might be
- Whether treatment is available
- How condition might affect family members

WHAT IS THE DIAGNOSTIC YIELD?

- Basically around 20-30%
- Higher yield in trios than in proband testing
- Age of patient – lower yield in adults
- Classification of results (e.g., include possible pathogenic variants or not)

WHAT ARE THE COSTS?

- In general, Sanger sequencing is more accurate, but also more costly if done on each gene individually
- Panels are becoming a good alternative to Sanger sequencing, although there is still a little bit less accuracy involved
- Currently, WES is around $4600-$5000, and rapidly coming down in cost.

COSTS, Con't.

- **Bardet-Biedl Syndrome each gene**
- 252 BBS1 $890
- 262 BBS10 $580
- 263 BBS11/TRIM32 $580
- 264 BBS12 $580
- 253 BBS2 $910
- 254 BBS3/ARL6 $610
- 255 BBS4 $910
- 256 BBS5 $780
- 257 BBS6/MKKS $650
- 258 BBS7 $990
- 259 BBS8/TTC8 $870
- 261 BBS9 $1120
- 659 SDCCAG8 $1220

- 251 Sequential Panel $580-**$9090**
- (BBS1, BBS2, ARL6, BBS4, BBS5, MKKS,
- BBS7, TTC8, BBS9, BBS10….
- 1053 NextGen Panel **$1990**
- (BBS1, BBS2, ARL6, BBS4, BBS5, MKKS, BBS7, TTC8, BBS9, BBS10, TRIM32, BBS12, CEP290, SDCCAG8, TMEM67, WDPCP)

LIMITATIONS ON TESTING

- VUS – plus change in categorization over time
- CNV – currently not readily identifiable
- Complex mechanisms – trinucleotide repeats, UPD
- Intronic or regulatory changes
- Mitochondrial mutations – not detected
- Gaps – not all regions of genes are covered
- Presence of pseudogene – variant calling unreliable

INCIDENTAL FINDINGS

- Biggest problem associated with WES
- Three types of results available:
  - Pathogenic mutation in gene which explains phenotype
  - Mutation in other gene, but which is actionable
  - Mutation in other gene, but which is not-actionable
- Example: baby with dysmorphic facial appearance, cardiac defect, and hypotonia has WES. Found to have mutations in:
  - MLL2 (cause of condition)
  - APC
  - C9ORF72

OUTLINE – FORMS OF TREATMENT

- Established forms of treatment – brief review
- Newer forms of treatment
  - Silencing extra chromosomes
  - Exon skipping
  - Nonsense mutation read-through
  - Genome editing

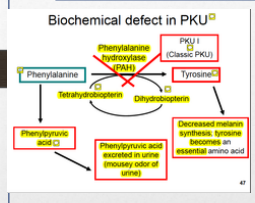
TREATMENT

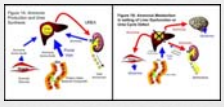
- Standard forms –
  - Modification of diet
  - Enzyme replacement
  - Gene therapy
- Newer forms –
  - Dosage compensation
  - Exon skipping
  - Nonsense mutation read-through
  - Genome editing

STANDARD FORMS

- Diet modification
  - PKU
  - OTC
- Enzyme replacement
  - MPS
- Gene therapy

DIET MODIFICATION – PKU and OTC

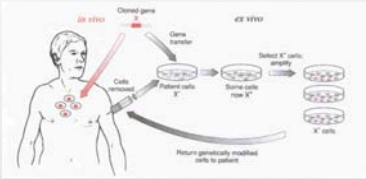




ENZYME REPLACEMENT THERAPY

- Hunter syndrome
- ERT improves physical manifestations (e.g., reduced respiratory problems, improved joint motion)
- Little, if any effect on cognition
- Expensive – 300K – 500K per year per patient

GENE THERAPY



GENE THERAPY – POTENTIAL APPLICATIONS

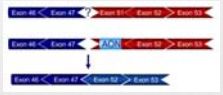
- Leber congenital amaurosis (form of vision loss)
- Lysosomal storage disorders
- Rett syndrome

NEWER FORMS OF THERAPY

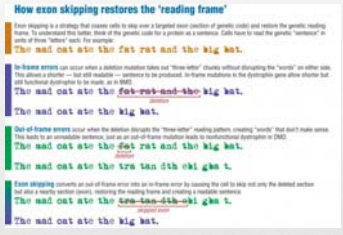
- Exon skipping
- Nonsense mutation read through
- Chromosome silencing
- Genome editing

NEWER FORMS

- Exon skipping
- A molecular patch leads to "skipping over" the missing exons
- Transcription proceeds normally beyond the patch
- Primary use is in Duchenne MD so far

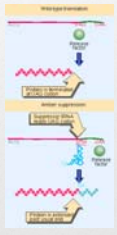


EXON SKIPPING



NEWER FORMS

- Nonsense mutation read-through
- Drug binds to tRNA and prevents recognition of stop codon
- An amino acid is inserted into the site, and the rest of the protein is made
- Not quite as good as normal protein, but better than shortened form



POTENTIAL USES

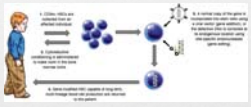
- Duchenne MD
- Some metabolic disorders
- Usher syndrome
- Spinal muscular atrophy
- Retinal disorders

NEWER FORMS

- Silencing of extra chromosome
- Gene on X chromosome – XIST
- This gene inserted into extra chromosome 21 (in Down syndrome)
- One copy of chromosome 21 no longer expressed
- Not much research done on this technique recently however

GENOME EDITING

- Basically replace dysfunctional gene with functional copy
- Ultimate goal is to correct disease-causing mutation via single treatment at birth
- Especially good for metabolic disorders



ETHICAL CONSIDERATIONS

- Goal of treatment might be elimination of the disorder – sends message that a "cure" is good, whereas the disorder is bad
- Health care workers need to be aware that they and the individual might disagree on what is best for that individual
- Medical model versus social model
- Consider what is being treated

CONCLUSION

- "I would much rather have society adapt to accommodate my children rather than have my children change to meet society's expectations."
  -Sarah F., mother of 2 children with Down syndrome
- "So, would I want my daughter "cured"? Since I don't perceive her as "afflicted," the answer is no. Do I do what I can to put her in situations and therapies that can support and build on her needs and strengths? Absolutely. But so does every parent who puts their child in sports, music lessons, etc. The potential neurodegenerative brain changes are what give me the most pause, at this point, and there I find future medical research can be the most beneficial."
- Andrea G., mother a child with Down syndrome