The Diagnostic Odyssey: Changing Genetic Technologies
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Objectives
- Present four clinical cases
- Case 1: 19-year-old with microcephaly, seizures and severe intellectual disability who had been followed in Genetics Clinic since age 2 years
- Case 2: 3-year-old with multiple minor malformations, deafness and developmental delays
- Case 3: newborn with severe hypotonia
- Case 4: 13-month-old with new onset seizures

Case 1
- Initial genetics evaluation at age 2 years
- Chief complaint: deceleration in head growth beginning at age 6 months and global developmental delays
- Family history: negative for birth defects, developmental delays and/or autism

Objective
- Demonstrate how changing genetic technologies allowed for the provision of a specific etiologic diagnosis in all four cases
- Discuss some of the newer genetic technologies
- Discuss the practical implications of identifying a specific diagnosis in each case

Case 1
- Pregnancy/delivery
  - 32-year-old primigravid woman and her 34-year-old healthy unrelated husband
  - Pregnancy complicated by mild pre-eclampsia; delivery induced at term for oligohydramnios
  - Birthweight: 6 pounds 11 ounces; no complications; infant discharged to home with mother

Case 1
- Developmental History
  - Cruising but not yet walking at age 2 years
  - Speaking a few single words
- Diagnostic studies
  - EEG study: normal
  - MRI study of the brain: normal
Case 1

- Physical Examination
  - Growth
    - Height: 75th centile
    - Weight: 25th centile
    - OFC: <5th centile
  - Craniofacial: wide mouth; prominent mandible
  - Remainder of structural examination: normal
  - Neurologic: hypotonic with unsteady gait
  - Other: frequent smiling

Case 1

- Diagnostic considerations at time of initial presentation:
  - Chromosomal abnormality
    - Routine karyotype: normal 46,XX
    - Angelman syndrome
      - FISH for 15q deletion: negative

Case 1

- Interval history
  - Began walking at age 27 months
  - Seizures recognized at age 8 years which became refractory to treatment
  - Chronic constipation
  - Scoliosis diagnosed at age 12 years
  - Severe intellectual disability

Case 1

- Negative diagnostic tests:
  - Routine karyotype
  - Plasma amino acids and urine organic acids
  - Lysosomal enzyme panel
  - Angelman syndrome testing including FISH study, methylation testing and UBE3A sequencing
  - Multiprobe subtelomeric FISH study

Case 1

- Negative diagnostic tests (continued)
  - Rett syndrome testing: MECP2 sequencing and deletion/duplication testing
  - Atypical Rett syndrome testing: CDKL5 sequencing and deletion/duplication testing
  - Testing for congenital disorders of glycosylation
  - Creatine
  - Guanadinoacetate
  - Chromosomal microarray (BAC and SNP)
    - Paternally inherited variant - dup 10p11.21
Chromosomal microarray

- Higher sensitivity than a karyotype
- Looks for extra or missing chromosomal segments (duplications or deletions)
- SNP technology also detects regions of homozygosity (autosomal recessive and imprinted disorders)
- Uses a microchip-based testing platform which allows for high volume automated analysis of many pieces of DNA simultaneously
- Computer analysis compares patient’s genetic material to reference sample

Chromosomal microarray

- Does NOT detect:
  - Small changes in gene sequence (point mutations)
  - Trinucleotide repeat disorders
  - Balanced chromosomal rearrangements

Case 1

Whole Exome Sequencing (WES)

- Sequencing of the coding regions (exons) of the genome
  - 1-2% of the genome
  - 180,000 exons
  - 30,000,000 base pairs
  - Includes mitochondrial genome screening
  - Accounts for ~85% of known disease causing variants

Whole Exome Sequencing

- Indicated when a genetic etiology is suspected and the diagnosis remains unknown
- Requires trios (proband and both parents): targeted sequencing rather than WES performed on parental samples based upon proband’s results
- Disorders not detected by WES
  - Trinucleotide repeat disorders
  - Disorders caused by intronic mutations
  - Disorders caused by uniparental disomy

Whole Exome Sequencing

- Detailed informed consent; turn-around time of ~4 months
- Report:
  - Mutations and variants in genes related to clinical phenotype
  - Mutations in genes causing medically actionable disorders (cancer and arrhythmia syndromes)
  - Optional categories
  - Carrier status for autosomal recessive disorders (cystic fibrosis, sickle cell anemia)
  - Pharmacogenetic information (warfarin, plavix metabolism)
  - Exclusions: genes causing adult onset dementia for which there is no treatment
Case 1

WES Results:
- De novo likely pathogenic variant in KIF5C gene (kinesin family member 5C which produces a microtubule associated protein)
- De novo missense mutations in KIF5C have been detected in other patients with intellectual disability, microcephaly, seizures and cortical dysplasia
- An identical variant was reported in an unrelated patient with microcephaly, seizures and severe developmental delay

Case 1: Practical Implications
- Genetic etiology for longstanding developmental disability disorder identified after extensive diagnostic testing
- Identification of a novel gene that causes severe intellectual disability with seizures
- Confirmation of de novo mutation provides important information and reassurance regarding recurrence risks to siblings’ future offspring

Case 2

Genetics consultation at age 3 years shortly after adoption from China
- Chief complaint: small for age, dysmorphic features, profound bilateral sensorineural hearing loss and developmental delays
- Family history: unknown
- Pregnancy/delivery: unknown
- Developmental history: walked prior to age 3 years; no true words; uses a few signs

Case 2

Physical Examination
- Growth: Height, weight and OFC < 5th centile; weight and OFC < 5th centile relative to height
- Craniofacial: synophrys, normal lashes, upturned nasal tip, long philtrum, downturned corners of the mouth
- Thorax: hypoplastic nipples
- Back: mild hirsutism
- Limbs: short fifth fingers with clinodactyly

Case 2

Suspected a diagnosis of Cornelia de Lange syndrome

Cornelia De Lange Syndrome
Case 2
- Interval history
  - Nonverbal at age 8 years
  - S/P bilateral cochlear implants
  - Developed pica

Case 2
- NIPBL sequencing results:
  - Original interpretation (1/26/10) - sequence change in NIPBL classified as a variant of unknown significance (adopted, therefore parental studies were not possible)
  - Updated interpretation (5/19/14) - subsequent testing identified the same de novo sequence change in another patient suspected to have CdLS
  - Sequence change now classified as likely pathogenic

Case 2: Practical Implications
- Confirmation of clinical impression
- Medical evaluations to consider
  - Cardiology: cardiac septal defects in 25%
  - Ophthalmology: ocular problems in 50%
  - Audiology: SNHL in 80%; 40% profound
  - Neurology: increased incidence of seizures
  - Gastroenterology: severe GERD and intestinal malrotation
- Recurrence risks: 50%

Case 3
- Initially evaluated shortly after birth
- Chief complaint: severe hypotonia
- Family history: negative for neuromuscular disease, birth defects and/or intellectual disability
- Birth history:
  - Born to 23-year-old primigravid woman and her unrelated 28-year-old husband
  - Delivered via Caesarean section for premature rupture of membranes at 31+ weeks
  - Birth weight: 1.3 kg; Apgars 2 and 7

Case 3
- Review of Systems
  - Otolaryngology
    - Vocal fold paresis
  - Respiratory
    - Inability to clear secretions
    - Respiratory insufficiency with recurrent atelectasis
    - Multiple failed attempts at extubation necessitating tracheostomy placement
  - Gastroenterology
    - Feeding difficulties necessitating ND feeds

Case 3
- Physical Examination
  - Growth
    - Length: 10th-25th centile
    - Weight: 10th centile
  - OFC: 20th-50th centile
  - Craniofacial: myopathic facies; nondysmorphic
  - Limbs: long, slender fingers
  - Neurologic
    - Severe hypotonia
    - Diminished to absent DTRs
Case 3

Initial diagnostic considerations
- Neonatal Marfan syndrome
- Spinal muscular atrophy
- Prader-Willi syndrome

Negative diagnostic tests
- Plasma amino acids
- Methylation testing for Prader-Willi syndrome
- Spinal muscular atrophy testing
- Myotonic dystrophy testing
- Chromosomal microarray study

Additional diagnostic studies
- MRI study of the brain: maturational delay; grade 1 left caudothalamic groove hemorrhage
- Muscle biopsy: EM showed myofibers with central nuclei and myofibrillatory disorganization; abnormal mitochondria also present; findings suggested centronuclear/myotubular myopathy
- Sequencing of the MTM1 gene (myotubularin gene): pathogenic mutation in exon 8

Case 3: Practical Implications
- Confirmation of a diagnosis of myotubular myopathy
- Prognosis: variable, although patient’s specific mutation is associated with a more severe phenotype
- Respiratory failure with chronic ventilator dependence
- Delayed motor skills; most non-ambulatory
- High incidence of infant death or death in early childhood

Pattern of inheritance: X-linked with 10-20% of cases due to de novo mutation
- Results of mother’s DNA testing are pending; if mother is a carrier, recurrence risks will be 25% (50% to a male fetus)
- If mother is a carrier, prenatal diagnosis will be possible
Case 4
- Genetics consultation at age 13 months
- Chief complaint: new onset febrile seizures in otherwise healthy child; 7 seizures within 5 months
- Family history
  - Maternal cousin with single seizure, age 18 years
  - Two paternal cousins with seizures
  - One paternal cousin with childhood epilepsy
- Pregnancy/delivery and developmental history: normal
- Physical examination: normal growth parameters and normal morphology

Seizure panel testing
- Testing of saliva
- Sequencing of 71 genes known to be associated with infant and early childhood seizure disorders
- Pathogenic variant detected in the SCN1A gene
- Results of SCN1A testing in parents were normal

Case 4: Practical Implications
- Diagnosis of SCN1A related seizures affects medical management
- SCN1A channels disproportionately affect GABA neurons
- Based upon above, seizures respond best to anti-epileptic drugs (AEDs) that bind to GABA receptors, including clobazam (onfi) and stiripentol
- Certain AEDs worsen symptoms - tegretol and phenytoin

SCN1A Related Seizure Disorders
- Clinical spectrum ranging from simple febrile seizures to intractable childhood epilepsy, inclusive of Dravet syndrome (severe myoclonic epilepsy of infancy) and some cases of Lennox-Gastaut syndrome
- Incomplete penetrance and variable expressivity, even within families
- Most severe phenotypes are the result of de novo mutations

Genetic test panels
- Epilepsy/seizures
- Hereditary motor neuropathies
- Hearing loss
- Visual loss
- Cancer syndromes