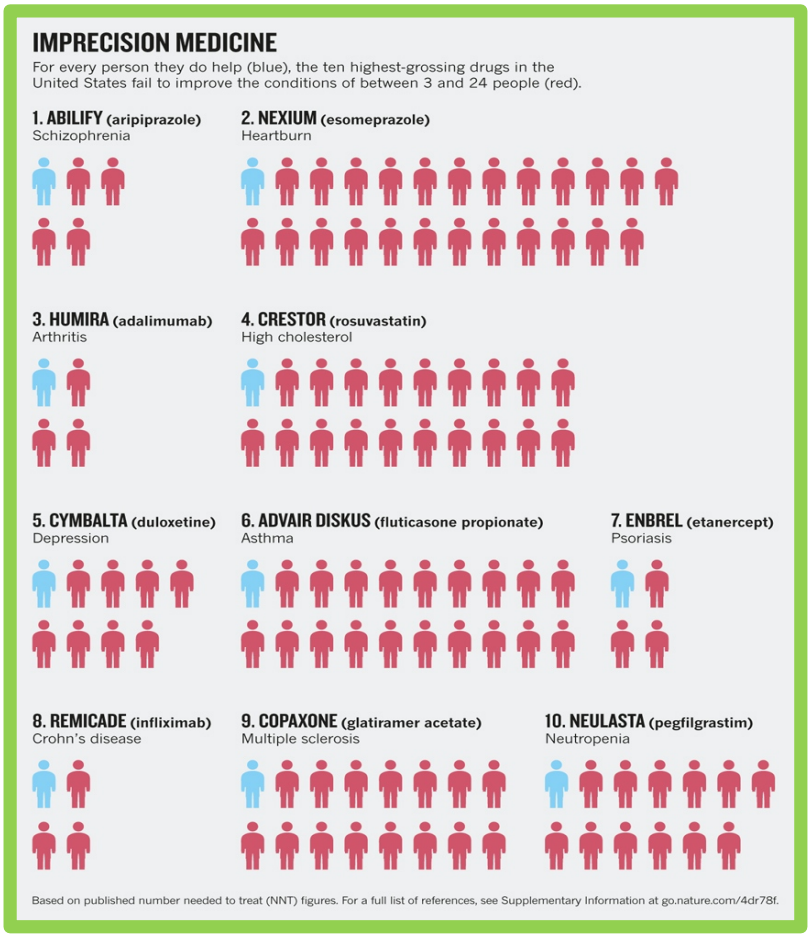
Panel 2

Individual Genetic Variability Matters, Including ADME Genes

Len van Zyl, Ph.D.

26 May 2021

IMPRECISION MEDICINE



- Only 4-33% of patients experience improved health outcomes from the 10 highest grossing commercial pharmaceutical drugs.
- The remaining **67-96% DO NOT BENEFIT!**

Schork, 2015

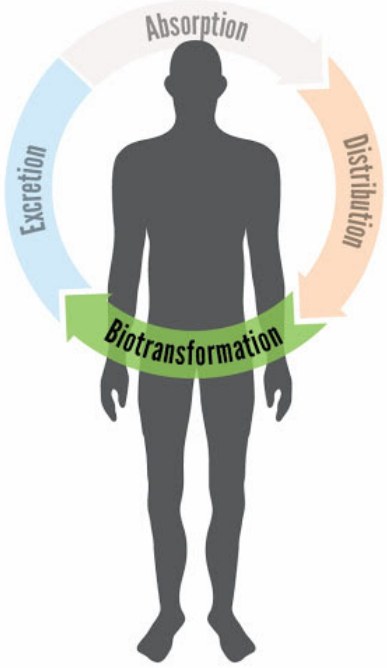
Why?

Every Person is Genetically Unique!

Individual Genetic Variability Matters

ADME Genes

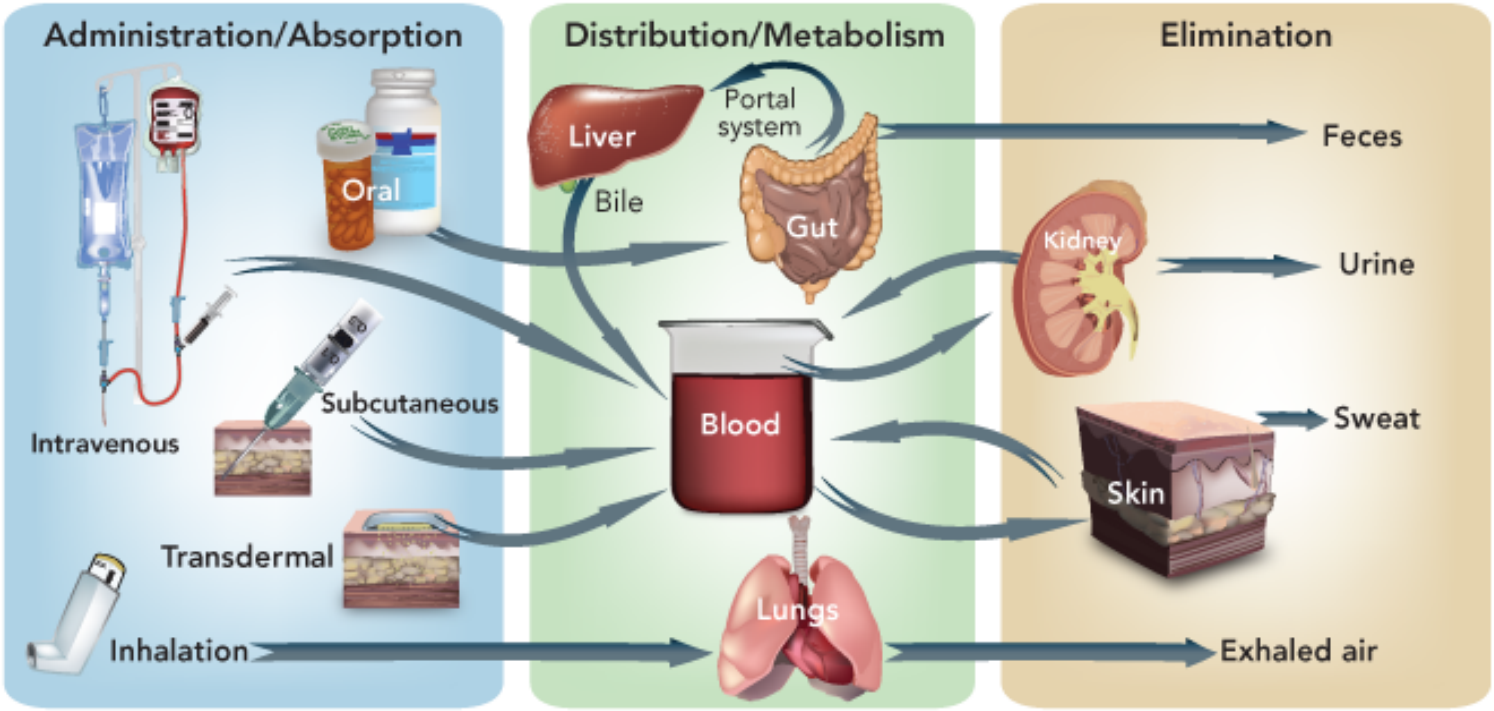
- Genetic variation in genes that function in the Absorption, Distribution, Metabolism, and Elimination (ADME) of drugs contributes significantly to the interindividual variability in efficacy and toxicity of numerous drugs from practically all therapeutic categories.



http://pharmaadme.org/joomla/index.php?option=com_content&task=view&id=12&Itemid=27

Arbitrio *et al.*, 2018; Klein *et al.*, 2019; Zaid et al., 2019; Hu *et al.*, 2020

ADME

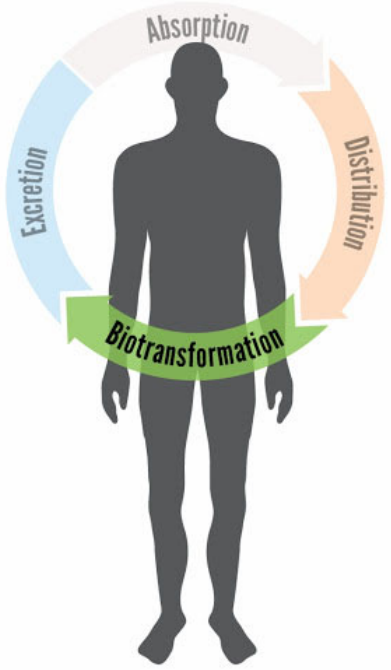

Administration/Absorption
Distribution/Metabolism
Elimination
Oral
Intravenous
Subcutaneous
Transdermal
Inhalation
Liver
Portal system
Bile
Gut
Blood
Lungs
Kidney
Feces
Urine
Sweat
Skin
Exhaled air

[Cite]

Individual Genetic Variability Matters

ADME Genes

- In the past half century, pharmacogenetic research has unraveled many clinically meaningful association between germline genetic variants and pharmacokinetic or drug response phenotypes.
- However, a considerable proportion of genetic variability remains unexplained, even for well-studied genes like *CYP2D6*.



Arbitrio et al., 2018; Klein et al., 2019; Hu et al., 2020

Individual Genetic Variability Matters

ADME Genes

- It is now known that very rare deleterious variants fill this gap, and contribute significantly to functional variability, a finding further supported by the fact that rare variants are enriched for deleterious alleles due to purifying selection (1000 Genomes Project Consortium, 2012)
- As an example, the Pharma ADME consortium uses 2,794 known genetic variations within 369 ADME genes to clinically determine the effective concentration of a drug at its effective site, which in turn is a key determinant regarding the safety and efficacy of the drug

Arbitrio *et al.*, 2018; Klein *et al.*, 2019; Zaid *et al.*, 2019; Hu *et al.*, 2020

Metabolism of Benzene

The Same Metabolizing Genes are Involved

- GSTs (glutathione-S-Transferase)
- Cytochrome P450 2E1
- MPO (Myeloperoxidase)
- NQO1 (NAD(P)H:quinone acceptor oxidoreductase)
- Inter-individual variability in benzene metabolism has been shown, and genomic analyses are being used in tort litigation to assess the situation in a person

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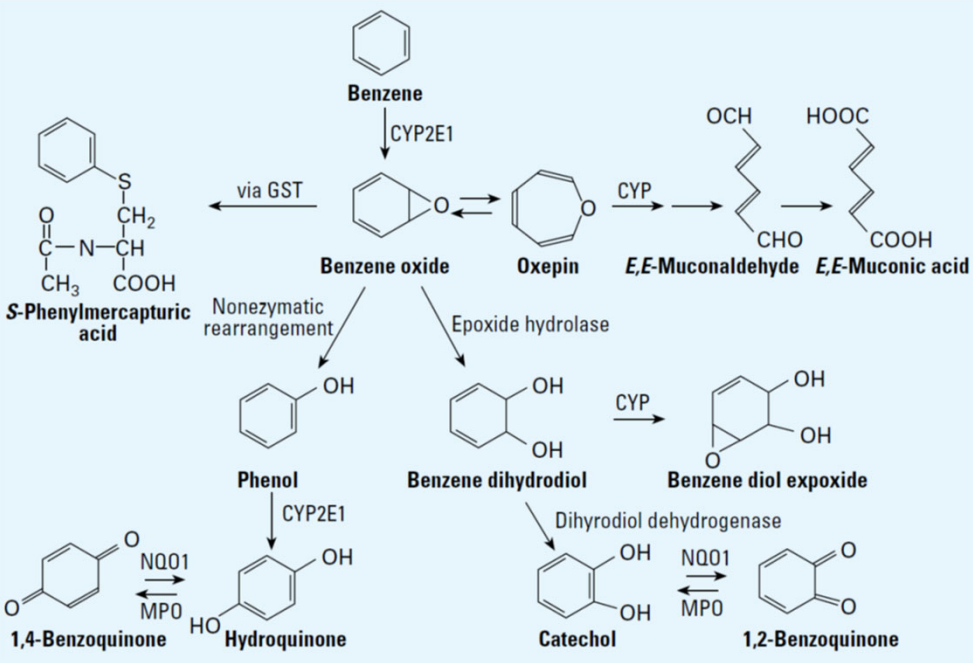
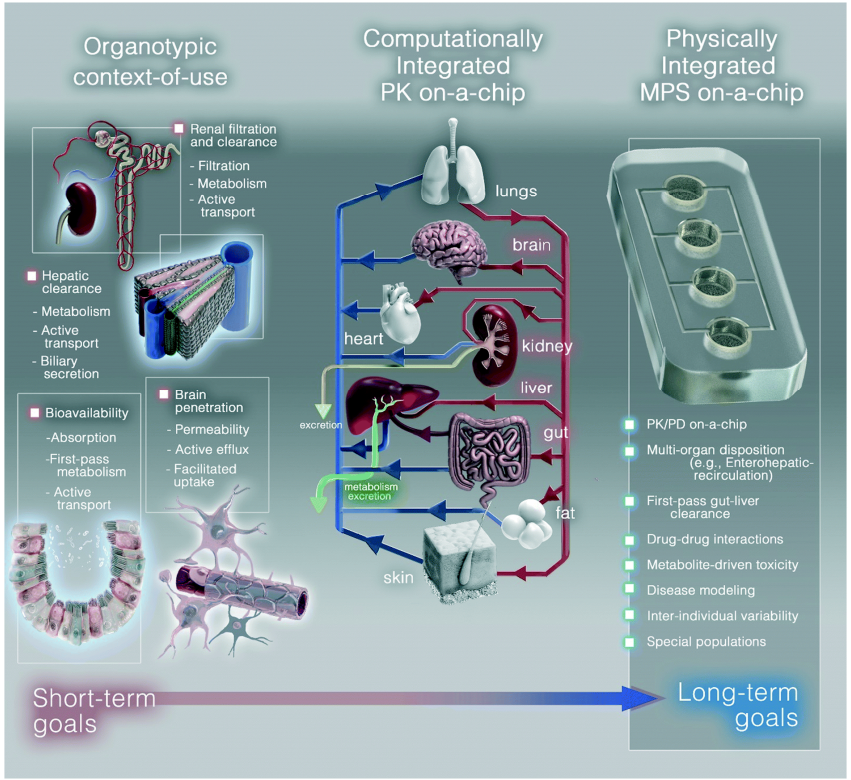


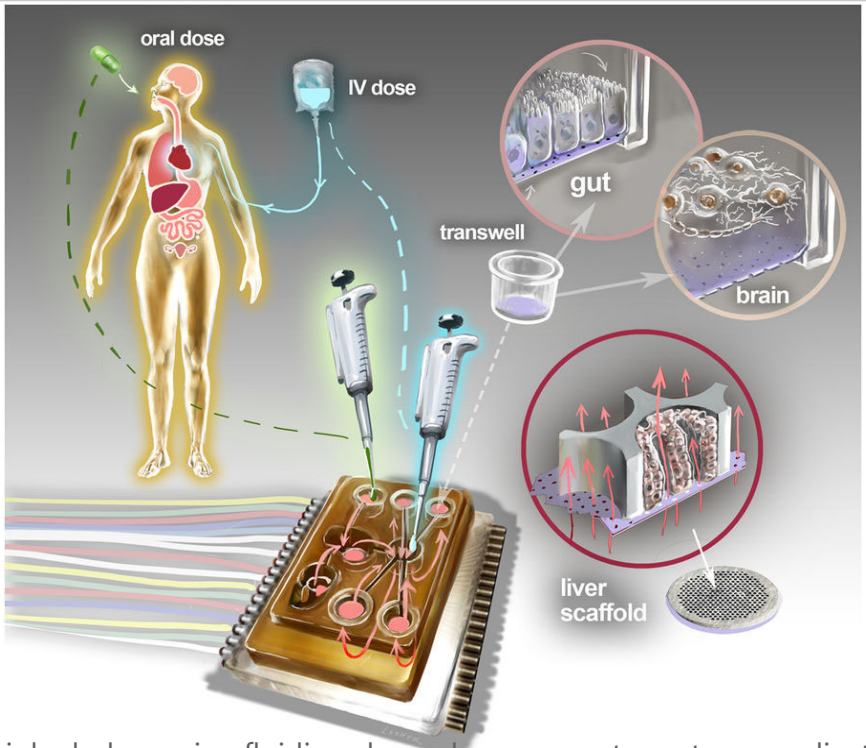
Kim et al., 2006; Gobba et al., 1997; Rappaport et al., 2009

Microphysiological Systems for ADME-related Applications on a Chip

"Body on a Chip" Systems Biology Approaches



Fowler *et al.*, 2020



Linked by microfluidic channels, compact system replicates interactions of 2 million human-tissue cells in 10 "organs on chips," replacing animal testing

Genomics And Federal Courts

Andrew Gendron, Esq.
Lewis Brisbois Bisgaard & Smith LLP
May 26, 2021

Federal Rule of Civil Procedure 35(a)

Where the mental or physical condition of a party or someone in the party's custody or subject to the party's legal control is **in controversy**, upon motion and for **good cause** the court presiding over the action may order a mental or physical examination.

Fed. R. Civ. P. 35(a); *Schlagenhauf v. Holder,* 379 U.S. 104, 114 (1964) (quoting *Hickman v. Taylor*, 329 U.S. 495, 507 (1947))

Potential Limitation of Rule 35 Orders

- *Schlagenhauf*, 57 years ago: "[T]he person to be examined must be a party to the case."
- 1970 amendment expanded rule's reach to someone in the party's custody or subject to the party's legal control.
- Cases are at best split on power to order parental blood draw.

Schlagenhauf, 379 U.S. at 115 & n.12
Young v. U.S., 311 F.R.D. 117, 122-23 (D.N.J. 2015)
Cruz v. Super. Ct., 121 Cal. App. 4th 646, 652 (2004)

Practical Implications If Trio WES Foreclosed

- Exome represent 1.5% of genome.
- Exome harbors ~ 85% of all known pathogenic variants.
- Trio WES diagnoses 34-41% of hitherto-unexplained cases.
- This number drops to 27% when child's DNA alone is tested.

N. Dragojlovic et al., *The Cost and Diagnostic Yield of Exome Sequencing for Children with Suspected Genetic Disorders: A Benchmarking Study*, Genet. Med., Jan. 4, 2018.

S. Srivastava et al., *Clinical Whole Exome Sequencing in Child Neurology Practice*, 76 Ann. Neurol. 473–83 (2014).

Satisfying "In Controversy" & "Good Cause" under Rule 35?

- Some courts, even those dealing with PI claims under tort theories, undertake extended meditations on these issues. *E.g., Young v. U.S.; Fisher v. Winding Waters Clinic*.
- While "in controversy" and "good cause" may not rest on "mere conclusory allegations of the pleadings" or "mere relevance," sometimes "the pleadings alone" suffice.
- "**A plaintiff in a negligence action who asserts mental or physical injury places that mental or physical injury clearly in controversy and provides the defendant with good cause for an examination.**"

Schlagenhauf, 379 U.S. at 118-19 (citations omitted)

Two Divergent Approaches: One Right; One … Not

- Only 2 published federal decisions on propriety of WES under R. 35.
- Both raised causation question re med negligence and birth injuries.
- *Fisher*: child born with neurodevelopmental delay, seizures, hypospadias, urethral stenosis, anal atresia, IGR, and HIE.
- *Burt*: HIE, seizures, visual damage, motor delays, profound devel. disability.
- In both, only WES of child sought.
- Battle of experts over appropriateness of WES.
- In *Fisher*, this should have satisfied Rule 35; but …

Fisher – Neither 'In Controversy' Nor 'Good Cause'

WES is "overbroad" because …

- "defendants have not shown that the near entirety of X.S.F.'s genome … is in controversy."
- "it could uncover genetic predispositions to … conditions unrelated to X.S.F.'s known injuries."
- defense expert hasn't specified what caused condition.
- plaintiff's expert said that brain damage unlikely to have genetic cause.
- CMA turned up nothing.

Fisher v. Winding Waters Clinic, 2017 U.S. Dist. LEXIS 19691, at *11, 2017 WL 574383, at * 4 (Feb. 13, 2017), *aff'd*, 2017 U.S. Dist. LEXIS 179580, 2017 WL 4870616 (D. Or. Oct. 22, 2017)

Is WES "Overbroad"?

- Genome-wide? So is CMA, which can also uncover genetic predispositions to conditions unrelated to plaintiff's known injuries;
- All medical diagnostic procedures run the risk of incidental findings unrelated to disease of concern;
- This can be addressed in instructions to lab not to report incidental or even secondary findings;
- Protective orders can control use and dissemination of information;
- Like CMA, WES is hypothesis-neutral;
- Brain damage can't cause hypospadias, anal atresia, urethral stenosis.

A. Gendron & T. Morgan, *Incomplete Penetrance: Whole-Exome Sequencing and Federal Courts*, For the Defense Ja. 2019, pp. 22-29

Is WES "Unnecessary" If CMA Turns Up Nothing?

- CMA and WES complementary.
- CMA looks for chromosomal structural anomalies that WES cannot detect.

Fisher's Non Sequitur

- WES looks for DNA variants (single-gene mutations) underlying Mendelian disorders that CMA cannot detect.

- The bureau and the socks.

Fisher's Greatest Error

- The court decided the merits of the causation dispute.
- Determining whether "in controversy" and "good cause" have been met "does not, of course, mean that the movant must prove his case on the merits in order to meet the requirements for a mental or physical examination."

Burt v. Winona Health

- Birth injury – medical negligence or genetic cause.
- Magistrate Judge followed *Fisher*; denied request for WES.
- Defts: "Magistrate Judge had inappropriately burdened [them] with proving their case on the merits" during discovery.
- District Judge: "definite and firm conviction that a mistake ha[d] been committed."
- Genetic testing "truly and genuinely in controversy."

Burt v. Winona Health, 2018 U.S. Dist. LEXIS 128944, at *9, 2018 WL 3647230, at *4 (D. Minn. Aug. 1, 2018) (quoting *Schlagenhauf*, 379 U.S. at 109)

Genetic Testing "May Best Serve" Both Sides

- If Court had denied request, "the parties and Court surely would have faced significant pretrial evidentiary issues, including Daubert motions, concerning the experts' competing causation opinions and the effect of Plaintiffs not completing WES testing."

- WES "may bolster" plaintiffs' claim that defendants' conduct or product caused injury.

- Leaving no stone unturned.

Burt, 2018 U.S. Dist. LEXIS 128944, at *9, 2018 WL 3647230, at *4 (D. Minn. Aug. 1, 2018)

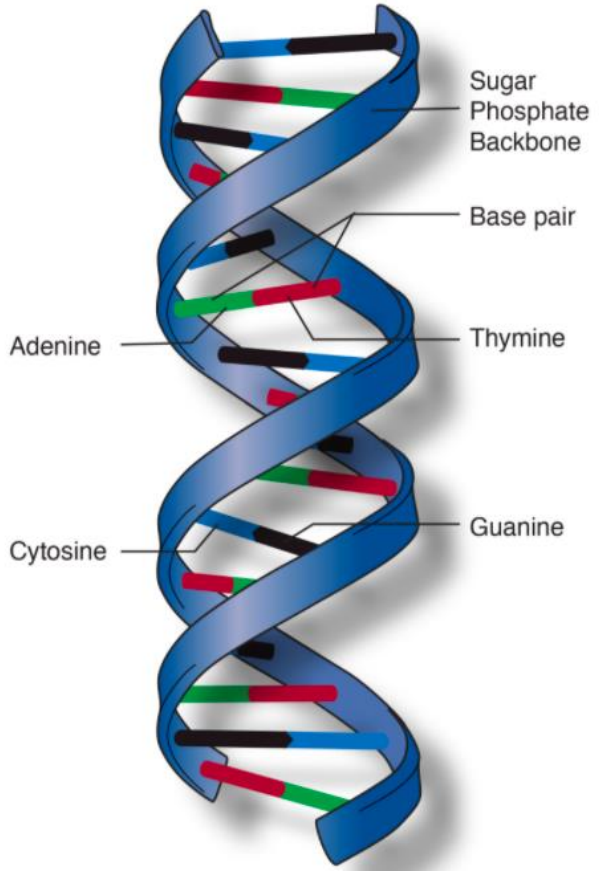
ATGC-you in court: genomic analysis of tort claims

Thomas Morgan, MD FACMG
Vanderbilt University School of Medicine
May 26, 2021

Clinical Human Genomics

Genomewide testing is standard medical care

- Full ATGC sequence, 2001
- > 7,500 genetic syndromes
- Unbiased diagnostic investigation:medical anomaly
- Why, how to treat, what else to worry about, next baby?
- Exome/genome sequencing
- $1250 (cash price)



https://www.genome.gov/genetics-glossary/Double-Helix

What caused an alleged medical harm?

- Probative value of genetics
- Did in utero drug exposure cause fetal malformations?
- Or was it a gene mutation?
- Analytical validity of test
- Clinical validity of physician's genetic test interpretation
- Medical/legal actionability of the genetic diagnosis

DRUG AND MEDICAL DEVICE

Incomplete Penetrance

Whole-Exome Sequencing and Federal Courts

By Andrew Gendron and Thomas M. Morgan

Although anecdotal evidence indicates that knowledgeable defense counsel appreciate whole-exome sequencing's benefits, to date only two federal courts have issued publicly available decisions addressing requests for it under Federal Rule of Civil Procedure 35.

A plaintiff's child was born with neurodevelopmental delays, seizures, and major congenital malformations of the genitourinary and digestive tracts. The plaintiff claims that in utero exposure to your client's drug caused these birth defects. Your expert suspects a genetic cause. Anticipating this, the plaintiff's expert ordered a chromosomal microarray (CMA) test, which examined over 2.6 million DNA probes covering the entire human genome. He claims that the normal test results rule out a genetic cause. Your expert recommends another test, whole-exome sequencing (WES), which also covers the human genome and looks

■ Andrew Gendron, a partner in Venable's Baltimore office, has represented pharmaceutical, medical device, tobacco, and paint/pigment manufacturers in product liability litigation, energy companies in toxic tort cases, and corporate defendants in stockholder suits and complex commercial litigation. He has led expert and subject-matter teams, and he belongs to DRI and the IADC. Thomas M. Morgan, MD, Associate Professor of Pediatrics at Vanderbilt, is board certified in Medical Biochemical Genetics, was head of Human Disease Genetics at Novartis (2007–2014), and has served as an expert witness in litigation involving a major pharmaceutical manufacturer. His work was supported by LawSeqTM (NHGRI/NCI 1R01HG00860501A1, principal investigators Wolf, Clayton).

Fisher case medical claims

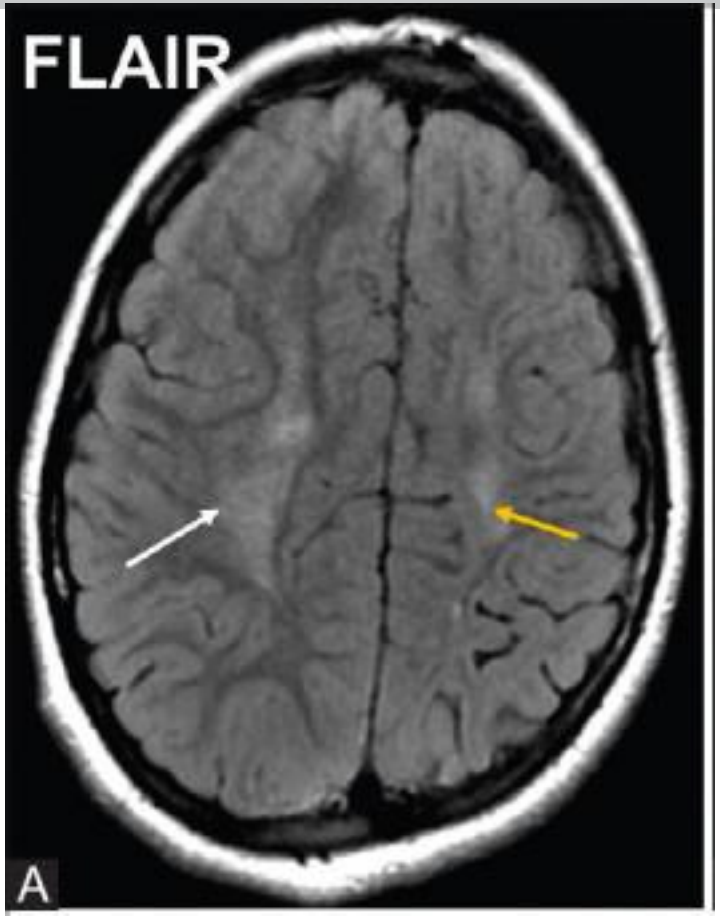
- Alleged hypoxic-ischemic encephalopathy/birth injury case
- Neurodevelopmental delay, seizure
- However, X.S.F. was also born with several birth defects
- Genitourinary malformations (hypospadias and urethral stenosis) as well anal atresia (incomplete anal opening)
- He also had intrauterine growth retardation
- Defense: undiagnosed genetic syndrome
- Clinical exome sequencing order declined by courts

Fisher for X.S.F. v. Winding Waters Clinic, PC, 2017 WL 574383 (Feb. 13, 2017), *aff'd*, 2017 WL 4780616 (D. Or. Oct. 22, 2017).

Fisher - "really and genuinely in controversy"

Could it have been COL4A1 de novo mutation?

- COL4A1 mutation —> “cerebral palsy”
- EFNB2/COL4A1 at 13q33.1-q34, known hypospadias/anal atresia
- COL4A1 mutation - broad range of congenital anomalies of kidney and urinary tract (CAKUT)
- Dr. Ward lacked recent COL4A1 data
- “Highly remote” “Court is not satisfied”



https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7954152/

Fisher case - clinical logic

- *Schlagenhauf* specificity trap (guess diagnosis or no exome?)
- Suplexes the hypothesis-neutral logic of exome on its head
- Exome diagnosis in 1/3-1/2 of cases
- Occam's Razor - most parsimonious, most likely explanation
- Hickam's dictum: the patient "may have as many diagnoses as he damn well pleases."
- In clinical genetics, ~4-5% of patients have 2+ rare diseases
- Strange cases concentrate in courtrooms and clinics

Genetics syndromes often complicate tort cases

- Aubrey Milunsky, MD, DSc, FRCP, FACMG, DCH

Key message

Unexpected adverse pregnancy outcome always spawns a search for the cause(s). Genetic disorders, syndromes, or congenital malformations may cause or compound obstetrical "complications". Causal recognition may take years, well after the universal experience of mostly unnecessary claims of medical negligence.

TABLE 1 Diagnoses of genetic and other disorders following claims of hypoxic ischemic encephalopathy in association with a range of complications in pregnancy and labor (131 cases)

Chromosomal abnormalities					n
Unbalanced t (4/8)		47XXY (Klinefelter syndrome)			
Trisomy 21		47XXY			
Unbalanced t (4/9)		Inv. 7p21q34			
Unbalanced t (21/21)					7
Chromosomal microdeletions/microduplications					
1q21.1 dupl (2)		5q12.1 del	13q31 del	16p dupl	
1q43 dupl		5q12.2 del	14q22 del	17p del (2)	
2q21 dupl		6q24.3 del	14q21.1-21.3 del mosaic	17q12 dupl (1.43mb)	
4p del		7p14 del	15q13.3 del	18p del	
4q del		9p21.31 del	15q del	20p12.1 del	
4q dupl		11p11.2 del	15q dupl	22q11.2 del (4)	29
Monogenic disorders					
Neurological		*Hematological*	*Myopathy*	*Metabolic*	
Epileptic encephalopathy (4)	Spastic paraparesis	Prothrombin gene mutation	Congenital muscular dystrophy	Maple syrup urine disease	
Pontocerebellar hypoplasia	Neurocutaneous disorder	Factor IX deficiency	Myotonic muscular dystrophy	Glutaric aciduria	
Neurofibromatosis	Autism, macrocephaly, PTEN gene mutation	Factor V Leiden deficiency	Congenital myopathy	Mabry syndrome	
Incontinentia pigmenti	Oligodontia and deafness	Factor VII deficiency			
		Protein C deficiency and PA inhibitor			22

Milunsky A. Obstetrics, genetics, and litigation. Acta Obstet Gynecol Scand. 2021 Jan 22. doi: 10.1111/aogs.14095. Epub ahead of print. PMID: 33483959.

Genomic alchemy: diagnosis from blood

- Biospecimen collection & transport to laboratory
- DNA extraction
- Chemical reagents fragment DNA into short fragments that can be analyzed simultaneously in parallel
- Analytical phases follow, “bioinformatics pipeline”

- Instrumentation (sequencing analyzers/physical devices)
- Consumables and supplies (e.g., chemical reagents)
- Analytical software algorithms run by skilled personnel

Evans BJ, Javitt G, Hall R, Robertson M, Ossorio P, Wolf SM, Morgan T, Clayton EW; LawSeq Quality Task Force. How Can Law and Policy Advance Quality in Genomic Analysis and Interpretation for Clinical Care? J Law Med Ethics. 2020 Mar;48(1):44-68. doi: 10.1177/1073110520916995. PMID: 32342785; PMCID: PMC7447152.

Analytical validity

MOLECULAR diagnosis is assured by quality standards

ACMG PRACTICE GUIDELINES | Genetics inMedicine

ACMG clinical laboratory standards for next-generation sequencing

Heidi L. Rehm, PhD[1,2], Sherri J. Bale, PhD[3], Pinar Bayrak-Toydemir, MD, PhD[4], Jonathan S. Berg, MD[5], Kerry K. Brown, PhD[6], Joshua L. Deignan, PhD[7], Michael J. Friez, PhD[8], Birgit H. Funke, PhD[1,2], Madhuri R. Hegde, PhD[9] and Elaine Lyon, PhD[4]; for the Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee

Rehder, C., Bean, L.J.H., Bick, D. *et al.* Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* (2021). https://doi.org/10.1038/s41436-021-01139-4

Clinical Validity

CLINICAL-MOLECULAR diagnosis: technical/professional judgment

Genetics inMedicine

www.nature.com/gim

Check for updates

ACMG TECHNICAL STANDARD

Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG)

Catherine Rehder[1], Lora J. H. Bean[2], David Bick[3], Elizabeth Chao[4], Wendy Chung[5], Soma Das[6], Julianne O'Daniel[7], Heidi Rehm[8,9], Vandana Shashi[10], Lisa M. Vincent[11,12] and ACMG Laboratory Quality Assurance Committee[13]*

ACMG STANDARDS AND GUIDELINES | Genetics inMedicine

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

Sue Richards, PhD[1], Nazneen Aziz, PhD[2,16], Sherri Bale, PhD[3], David Bick, MD[4], Soma Das, PhD[5], Julie Gastier-Foster, PhD[6,7,8], Wayne W. Grody, MD, PhD[9,10,11], Madhuri Hegde, PhD[12], Elaine Lyon, PhD[13], Elaine Spector, PhD[14], Karl Voelkerding, MD[13] and Heidi L. Rehm, PhD[15]; on behalf of the ACMG Laboratory Quality Assurance Committee

Rehder, C., Bean, L.J.H., Bick, D. *et al.* Next-generation sequencing for constitutional variants in the clinical laboratory, 2021 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). *Genet Med* (2021). https://doi.org/10.1038/s41436-021-01139-4

Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. 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. Genet Med. 2015 May;17(5):405-24. doi: 10.1038/gim.2015.30. Epub 2015 Mar 5. PMID: 25741868; PMCID: PMC4544753.

Assessing pathogenicity of variants

- Population frequency data (ethnicity-specific)
- Bioinformatic model prediction of damage to protein
- Functional mutation effect in cells or organisms
- Family tree segregation of mutation with disease status
- New mutation in child (not found in parents - rare event)
- Allelic phase data (did each parent transmit one mutation?)
- Correlation of mutation with clinical syndrome (ClinVar, etc)

[Cite]

Doll-Hill criteria for (genetic) causation

- Strength of association
- Consistency of studies
- Specificity/nonconfounding
- Temporality
- Bio-gradient (mutation category/number of alleles)
- Bio-plausibility
- Coherence of evidence
- Experimental confirmation
- Analogy (similar mutation in same protein motif, etc)
- Exclusion of chance

Geneletti S, Gallo V, Porta M, Khoury MJ, Vineis P. Assessing causal relationships in genomics: From Bradford-Hill criteria to complex gene-environment interactions and directed acyclic graphs. *Emerg Themes Epidemiol*. 2011;8(1):5. Published 2011 Jun 9. doi:10.1186/1742-7622-8-5