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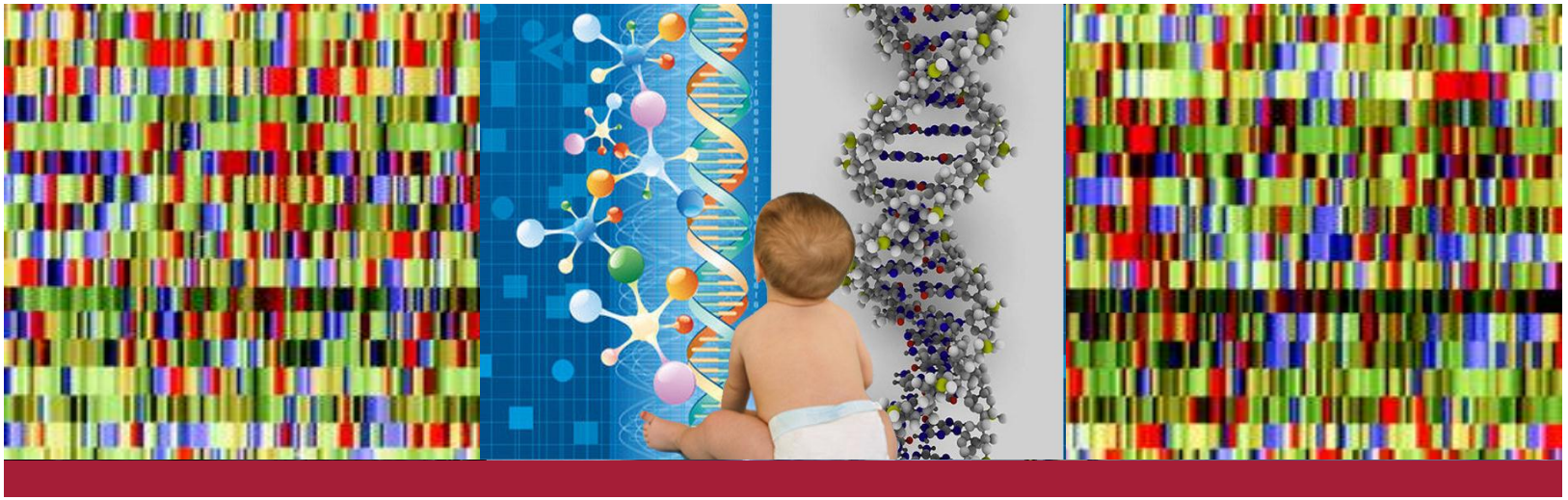
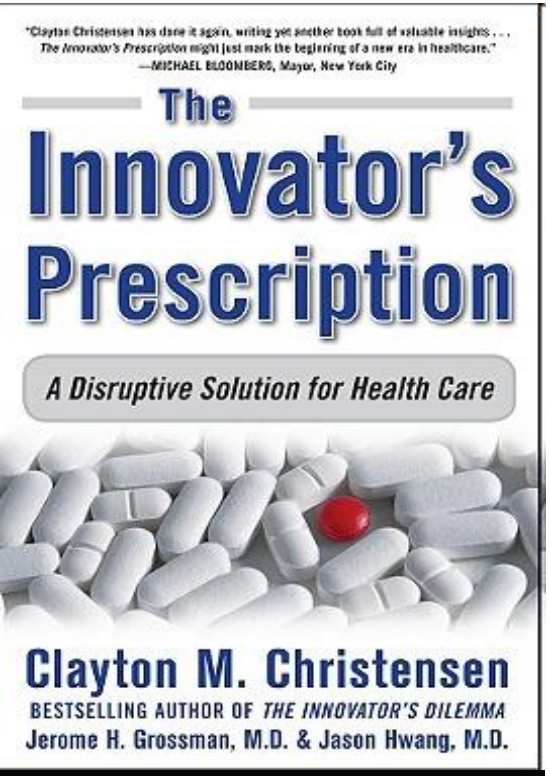


Complexity of NGS approaches in neurological disorders:

A new role of Medical Genetics in clinical guiding

Olaf Riess
Institute of Medical Genetics and Applied Genomics, Tübingen, Germany

„Disruptive technological enablers in Health Care“



1. Molecular genetic diagnostics
2. Imaging technologies
3. Telecommunication

USA: appr. 17-29$ Billion per year spend due to wrong clinical diagnosis

Efficacy of NGS

1st generation

Applied Biosystems
3730xl
0,08 Mb / run
1 Mb / 24 h

2nd generation

Applied Biosystems
SOLiD
3000 Mb / run
(120h)

Roche / 454
Genome Sequencer FLX
400 Mb / run (8h)

Illumina / Solexa
Genetic Analyzer
2000 Mb / run (96h)

300 : 1

Costs in health care system

- **Routine in neurodevelopmental disorders**
  - » Imaging, electrophysiological studies such as EEG, biochemical analysis, biopsy add to more than 10.000$ per patient (Kingsmore and Saunders 2011, SciTransMed 3, 87ps23)

- Neurodevelopmental disorders effect 4 to 6% of the general population, most notably children (incl intellectual disability, epilepsy, autism, structural brain diseases, neuromuscular diseases)

- Neurodevelopmental disorders account for 5-10% of total health care expenditure in the US (Center for Disease Control and Prevention (CDC): Economic costs associated with mental retardation, cerebral palsy, hearing loss and vision impairment-United States, 2003, MMRW Morb Mortal Wkly Rep 53, 57-59 (2004)

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Genetic testing:

Minimize, Maximize or Personalize?

Minimize?

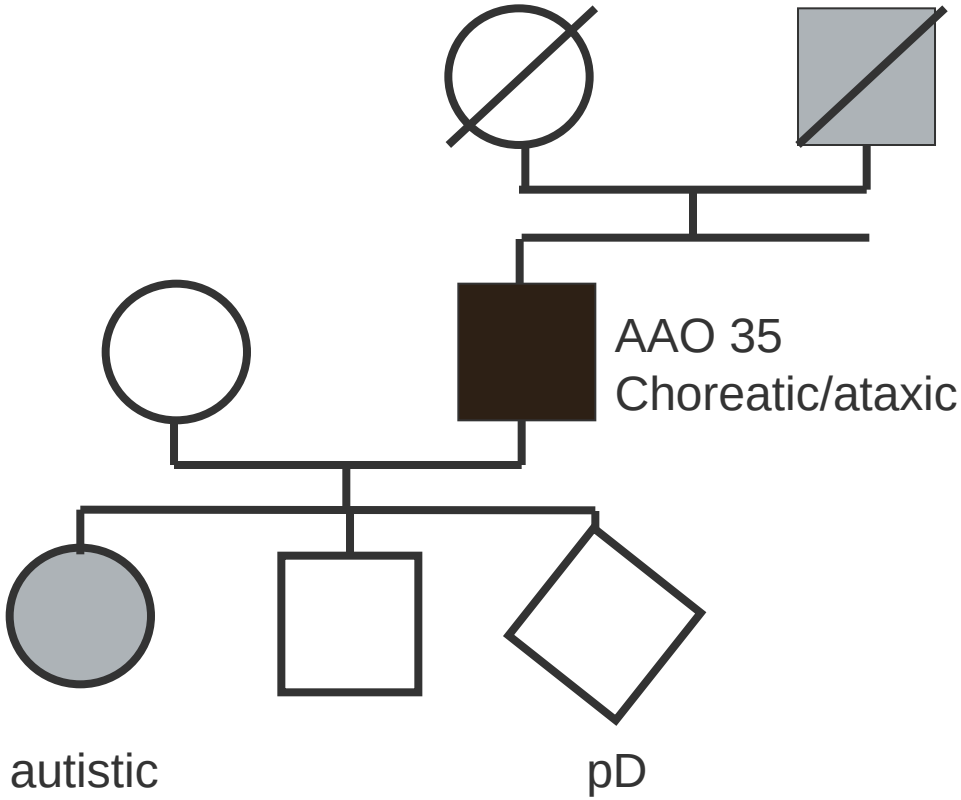
Arguments in favour of testing single gene loci:

- **Ethical dimension** of WES/WGS even if defined for the society, is never prepared for the single individual and family

- **Costs** (not of consumables, but rather of follow up analysis)

Health insurance companies:
We do not need genetic analysis at all, as they have limited diagnostic value for treatment, and family testing is not a declared goal of the insurance.

Minimize?



Genetic analysis: Huntington‘s disease
SCA17 √

Minimize?

- Clear diagnosis to be genetically confirmed

- Repeat expansion diseases (still technical limits of NGS)

Patients with rare causes of a disease and
Patients with unclear diagnosis deserve the same right
of a correct diagnosis as patients with frequent diseases!

Examples

- Ataxias:	>	140 known genes
- Paraplegias:	>	70 known genes
- Intellectual disability:	>	1000 known genes

Only ~ 50% of the genes of these disease groups have been discovered yet!

- Polyneuropathies:	>	330 known genes (5600 exons)
- Deafness:	>	70 known genes (1300 exons)
- Ciliopathies:	>	258 known genes (4700 exons)
- Retinitis pigmentosa:	>	60 known genes (800 exons)

Maximize?

= WES/WGS

Challenges of human genetics in the area of genome sequencing

DIAGNOSTICS: Whole Exome Sequencing

Problem „Sequencing depth“ for diagn. sensitivity solved in 2 years (currently 25% „strike outs“)

Advantages: - Identification of novel disease genes even in single patients possible without major ressources
- Diminish categories of clearly defined phenotypes
- Discovery of at least 5% wrong diagnosis of current genetic reports

Disadvantage: - How to deal with „unwanted“ results

Challenges of human genetics in the area of genome sequencing

DIAGNOSTICS: Whole Genome Sequencing

Still limited diagnostic value for the next 5 years

- Advantages: - Potential for better prediction of disease modification such as penetrance, age at onset and progression

- Huge time advantage (Kingsmore within 50h CAVE of 8 patients/families 2 = 25% had „strike outs“)

- Disadvantages: - Bioinformatic

- Long process of defining significance of base pair changes for disease process

- Cost

-How to deal with „unwanted“ results

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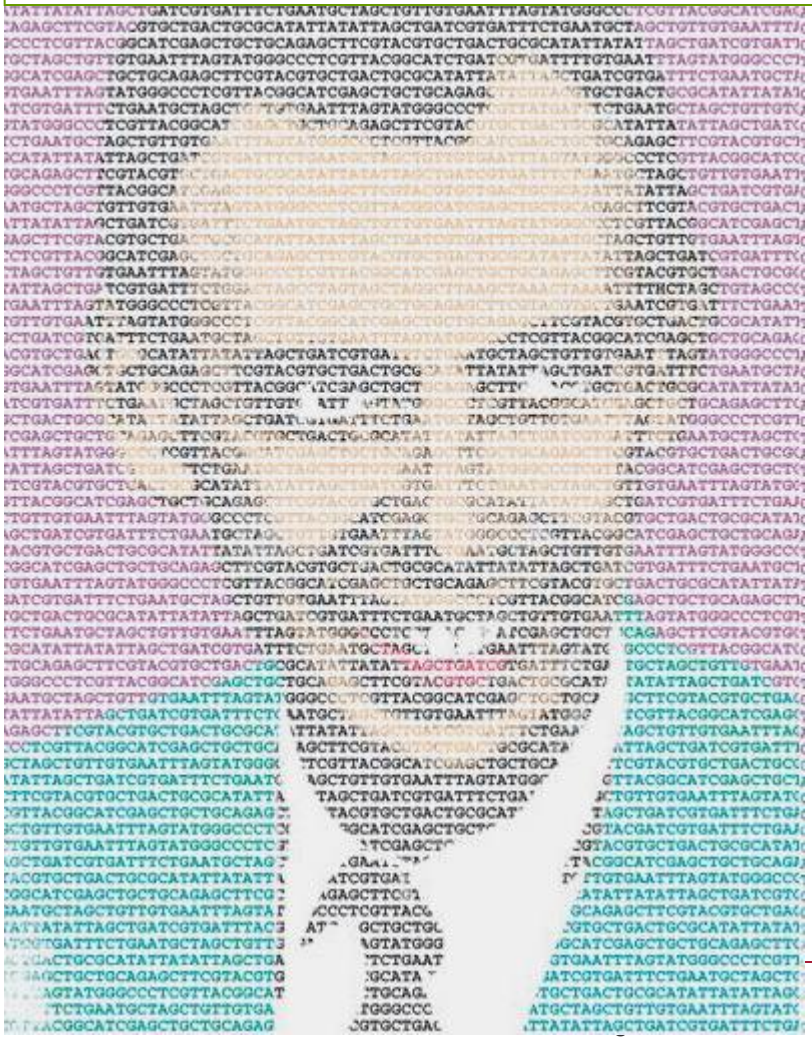
James Watson‘s Genome

☒ I want to be informed on „**clinical relevant**“ data not directly related to my condition



- Retinitis pigmentosa (PDE6B)
- Rod-cone-dystrophy (RPGRIP1)
- Usher Syndrom 1b (MYO7A)
- Metachromatic Dystrophy
- Cong. Nephrotic Syndrom
- Cockayne Syndrom (ERCC6)
- Myopathy (GNE)
- Glycogene storage disease
- Many other disease relevant

Disclosure of diagnostic data: Counseling

Roughly 100 genetic risks discovered in each individual genome.

Even if per disease / risk information will only be provided for 15 min,

direct patient contact would last 25 hours !

Taken that each patient / individual can only stay concentrated for 5 h / day,

one would need 5 days of counseling ONE individual!

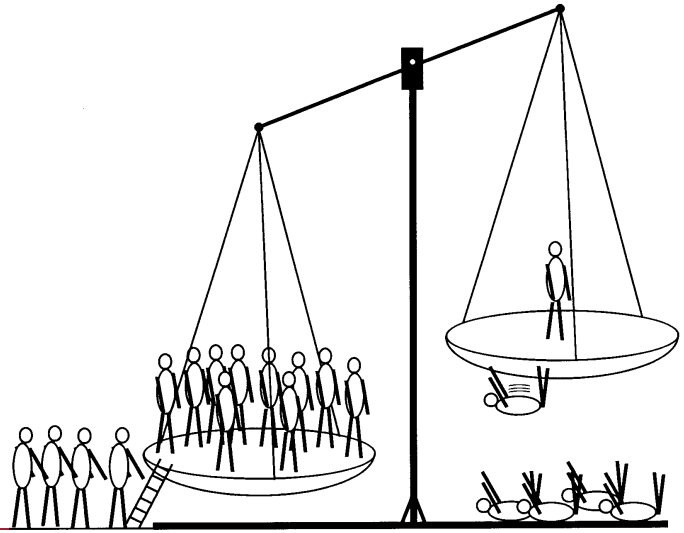
Background research into the importance of genomic data takes manyfolds longer.

Arguments against Maximize

Complexity of information:

Risk, that clinical, for the patient relevant information, will get missed due to the whole mass of information!

Meaningful



Information not wanted
No relevant information

Disadvantages of Maximize

Different interpretations of data and different judgement of „clinical relevance“ in different centers

Different judgement / interpretation of the same data ?

„Only“ 213 conditions of the complex diseases in 5 genetic DTC companies predicted

Pre-test: Average life time risk for	Obesity ranges between	34%	and 64%
	Thromboembolism	3%	and 12%
	Heart attack	21%	and 42%
	Glaucoma	1%	and 15%
Risk allele quantification	Celiac disease HLA-DQA1	0.30	and 7.00
	Glaucoma	0.03	and 1.00

Swan M. Genetics in Medicine 2010

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WES analysis

However, it may solve difficult disease causes!

5 month old boy suffering from dehydration and chronic diarrhea indicating Bartter syndrome

- Exome sequencing = Congenital Chloride Diarrhoe (SLC26A3)

2 Patients with likely cystic fibrosis for which CFTR mutations have been excluded

- Exome sequencing = Primary ciliary dyskinesia (Kartagener S)

Choi et al. PNAS USA 2009; Ng et al. Nat Genet 2010; Roach et al. Science 2010

Personalize?

NGS –

Disease related gene panel diagnostics

Panel: 1 - 1222 genes

Advantages:
- Disease „specific“ reduces unwanted results
- All data are releated to the disease
- High coverage, high diagnostic value

Disadvantages:
- Design is not very flexible
- Different groups use different designs

107 genes implicated in XLMR:

ACSL4, AFF2, AGTR2, AIFM1, AP1S2, ARHGEF6, ARHGEF9, ARX*, ATP6AP2, ATP7A, ATRX, BCOR, BRWD3, CASK, CDKL5, CLCN4, CUL4B, DCX, DKC1, DLG3, DMD*, EIF2S3, FANCB, FGD1, FLNA, FMR1, FTSJ1, GDI1, GK, GPC3, GRIA3, HCCS, HCFC1, HDAC8, HPRT, HSD17B10, HUWE1, IDS, IGBP1, IKBKG*, IL1RAPL1, IQSEC2*, KDM5C, KIAA2022, KLF8, L1CAM, LAMP2, LAS1L, MAGT1, MAOA, MBTPS2, MECP2, MED12, MID1, MTM1, NAA10, NDP, NDUFA1, NHS*, NLGN3, NLGN4X, NSDHL, NXF5, OCRL, OFD1, OPHN1, OTC, PAK3, PCDH19, PDHA1, PGK1, PHF6, PHF8, PLP1, PORCN, PQBP1, PRPS1, PTCHD1, RAB39B, RAB40AL, RBM10, RPL10, RPS6KA3, SHROOM4, SLC6A8, SLC9A6, SLC16A2, SMC1A, SMS, SOX3, SRPX2, SYP, SYN1, THOC2, TIMM8A, TSPAN7, UBE2A, UPF3B, ZDHHC15, ZDHHC9, ZMYM3, ZNF41, ZNF674, ZNF711, ZNF81

Gene panel diagnostics

Always simple?

Case report: 25 years old male patient
complex hereditary paraplegia with axonal polyneuropathy
MRI normal
Most frequent isoforms SPG4, SPG5, SPG3 excluded…
Gene panel of 62 genes causing paraplegic phenotype

KIF5A = SPG10 heterozygote p.R204W (VUS5) mutation

This mutation has been described in autosomal dominant and autosomal recessive SPG10.

What is the risk of his future children?
Should we sequence KIF5A in his wife?

Gene panel diagnostics

Always simple? – continued -

Case report: 25 years old male patient
complex hereditary paraplegia with axonal polyneuropathy
MRI normal
Genetically SPG4, SPG5, SPG3 excluded…
Gene panel of 62 genes causing paraplegic penotype

KIF5A = SPG10 heterozygote p.R204W (VUS5) mutation

Additionally, **compound heterozygote for GCH1 p.P23L/p.P69L**
Indicating **dopa-responsive Dystonia**
Both mutations have been described in cis and trans
If in trans, the patient has also dopa-responsive dystonia
And may require treatment
Or he will develop symptoms later
Should we test his wife?

Why genome analysis are no luxury but necessary diagnostic steps in health care of the 20th century

How much is a genome analysis worth for the health care system?

- In the US, health care costs of a person is about 9000 $ per year, and a patient bed in neonatology is 8.000 $ per day

- Consumables for WES is about 1.000 $, with personal, overhead, equipment at about 5.000 $

- A genome analysis is „static“ and must not be repeated

- Average age of an individual in developed countries is about 78 years, thus costs of WES per year would add to 65 $ per year

No luxury

A new role of Medical Genetics in clinical guiding

• Child with inflammatory bowel disease reminding to Crohn disease but with more severe and faster progression. 3 years continuous hospitalization, more than 100 surgeries and clinical consultations with doctors around the world, weekly „clinical care meetings“

• WES identified XIAP mutation, unknown for inflammatory bowel disease but rather for hemophagocytotic lymphohistocytosis (HLH). Liver biopsy, bowel biopsy and bone marrow analysis did not reveal any clinical indication for active HLH

• However, as due to the XIAP mutation a low survival chance was predicted, doctors performed hematopoetic stem cell transplantation

• After 5 month, child was basically **cured** !

Necessary!

Worthey et al. 2011 „XIAP deficiency in inflammatory bowel disease“ Genet Med

Child with Intellectual Disability

Josua, 7 years old boy

- Clinical manifestation:
  - MR, epilepsy, „non-syndromic“
  - „global metabolic failure“
  - after infect with loss of consciousness
  - length below 3rd percentile
  - head circumference at p25
  - all developmental milestones delayed
  - with 5 years spasticity
- Diagnostic work up:
  - Genetically: karyotying, subtelomere, Angelman
  - Biochemically: amino acids organic acids, oligosaccharides, lactate in Liquor, spectroscopy, ammoniak, mitochondriopathy
  - Imaging: bilateral lesions in frontal globus pallidus

- Therapeutic options: fostering, stabilizing, computer with a voice synthesizer

Joshua

Diagnostic options:

- WES
- Epilepsy/MR panels (3)
- „Kingsmore“ panel version 2 (1222 genes)
- Skewed X inactivation in mother pointed us to sequence the X chromosome

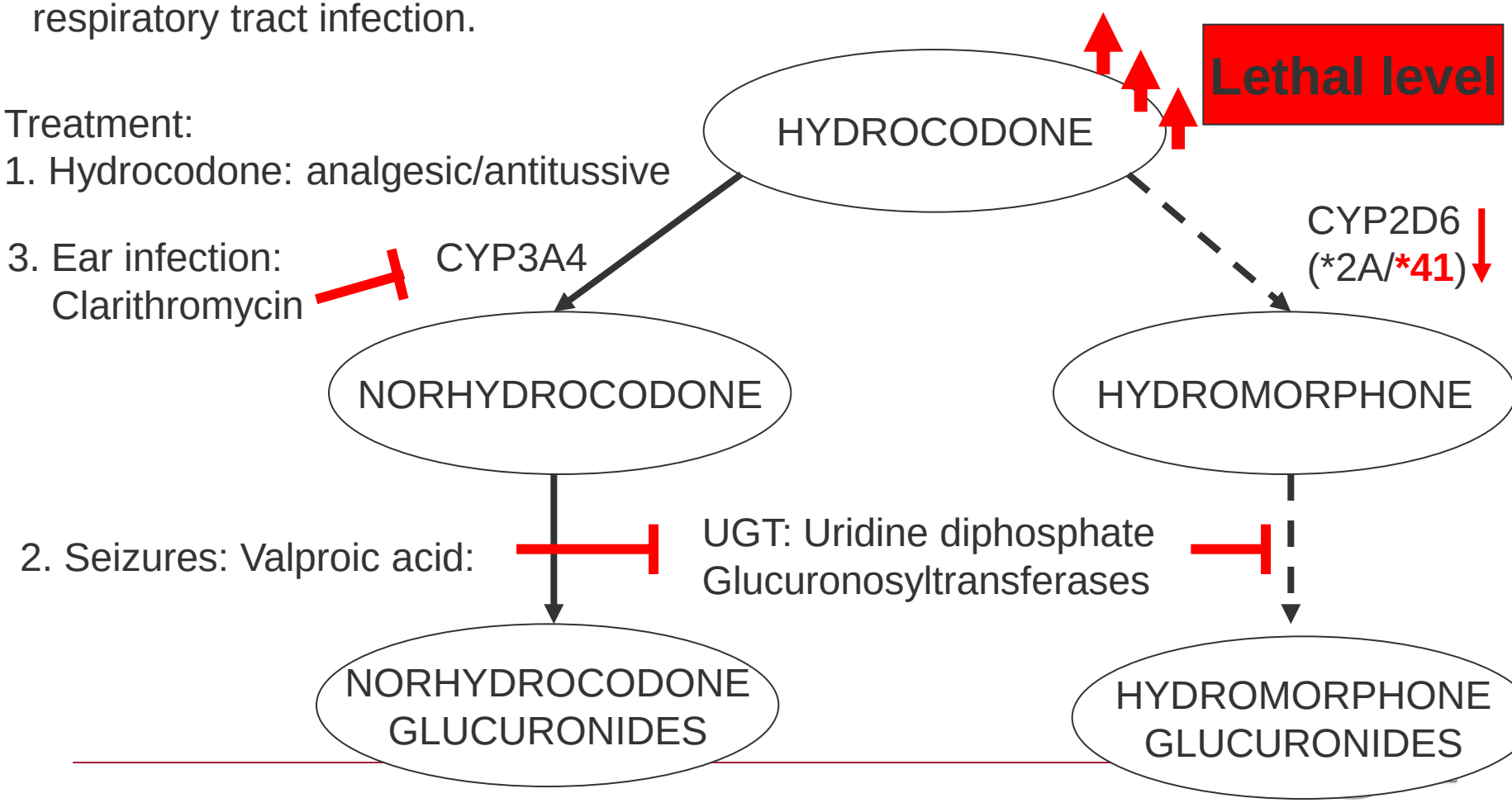
c.G1058A:p.G353D hemizygote mutation in the Glycerol kinase gene causing

Glycerol Kinase Deficiency causing Hyperglycerolemia
= life-threatening metabolic crisis with developmental delay and MR

Therapeutic options: low fat diet, in acute situations glucose infusion, consider corticosteroids in crisis

Prevention:
Pharmacogenetics and drug interactions

6 year old developmentally delayed **child died** after high dose of hydrocodone for respiratory tract infection.



Madadi et al. Pediatrics 2010

The 20th century saw great advances in treatment of infectious disease. Today in the 21st century, we should be making similar gains against genetic diseases, but we aren‘t attacking them as hard as our predecessors did infectious disease.

Stephen Braddock, MD