New gene sequencing technology is changing diagnosis and management

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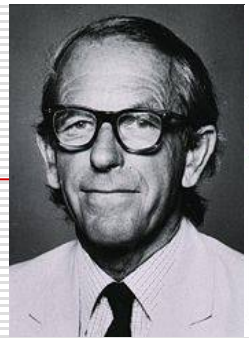
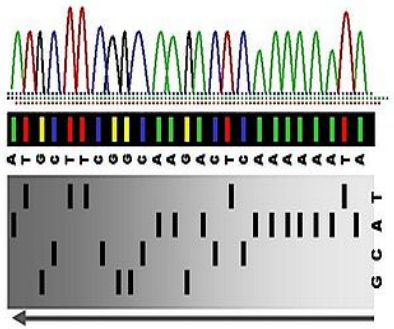
Sanger sequencing from 1977





- A low throughput technology that is expensive

  One gene at a time, one patient at a time
  $500-1500 per gene per case

- Cannot deal with genetic heterogeneity, because a number of genes have to be sequenced

  e.g. deafness >100; retinitis pigmentosa >50; familial spastic paraplegia >40

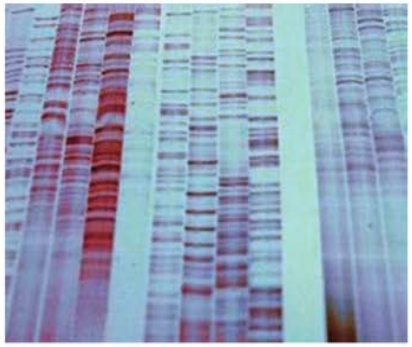
Next generation (massively parallel) sequencing (NGS/MPS)

METHOD OF THE YEAR | **SPECIAL FEATURE**

The year of sequencing

In 2007, the next-generation sequencing technologies have come into their own with an impressive array of successful applications. Kelly Rae Chi reports.

In the toxicology building of North Carolina State University in Raleigh, Nigel Deighton, head of a small genome research facility, and a few others unpack the facility's first next-generation sequencing machine, a 454 GS FLX, on loan from Roche Diagnostics for three months. They train for a few days, nebulize a colleague's bacterial DNA and PCR-amplify "the living daylights out of it," Deighton recalls. They load the bead-bound PCR products onto a plate with holes that are not visible to the naked eye, pop the plate into the

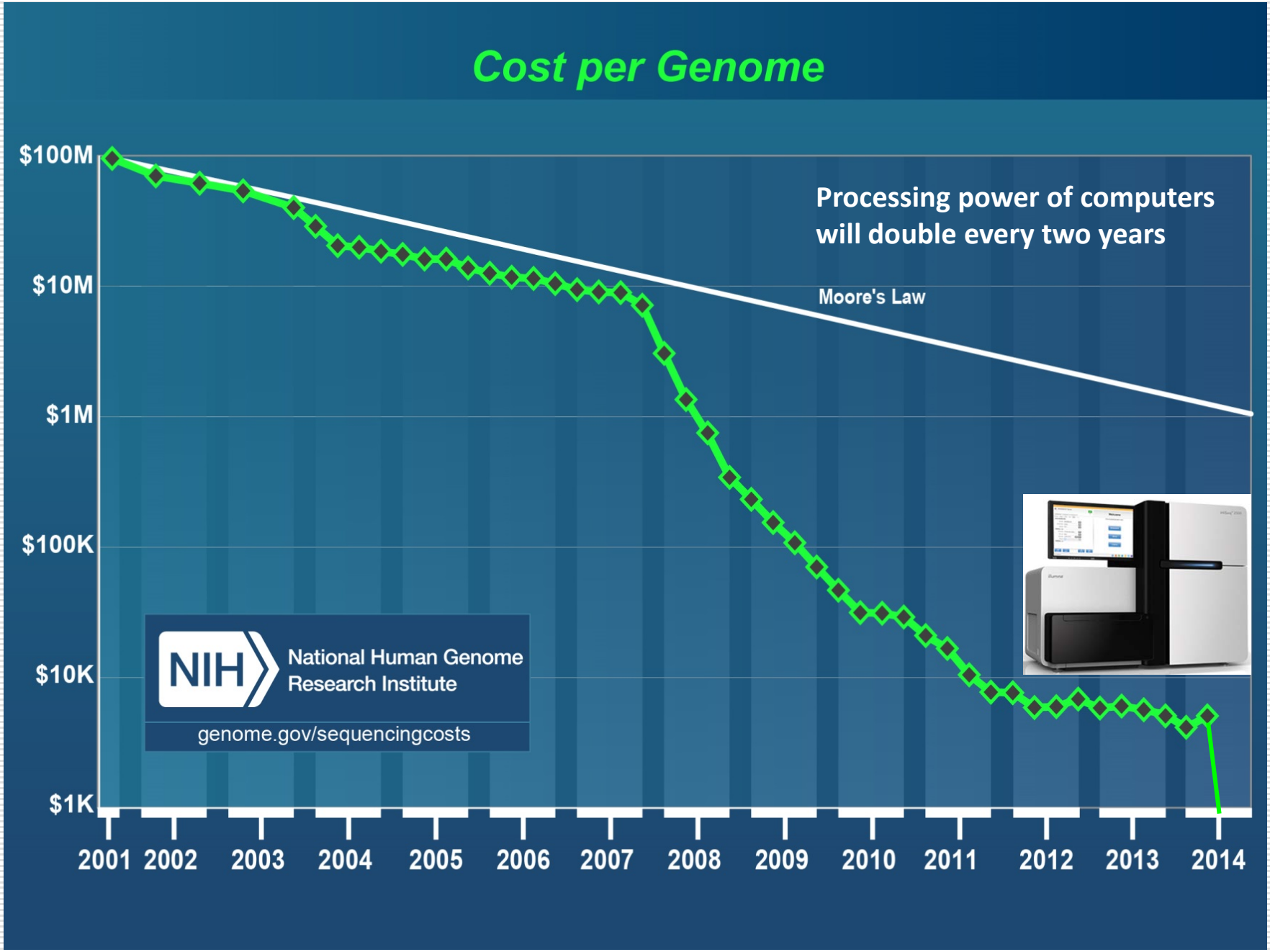


Sanger sequencing becomes the 'old' generation.

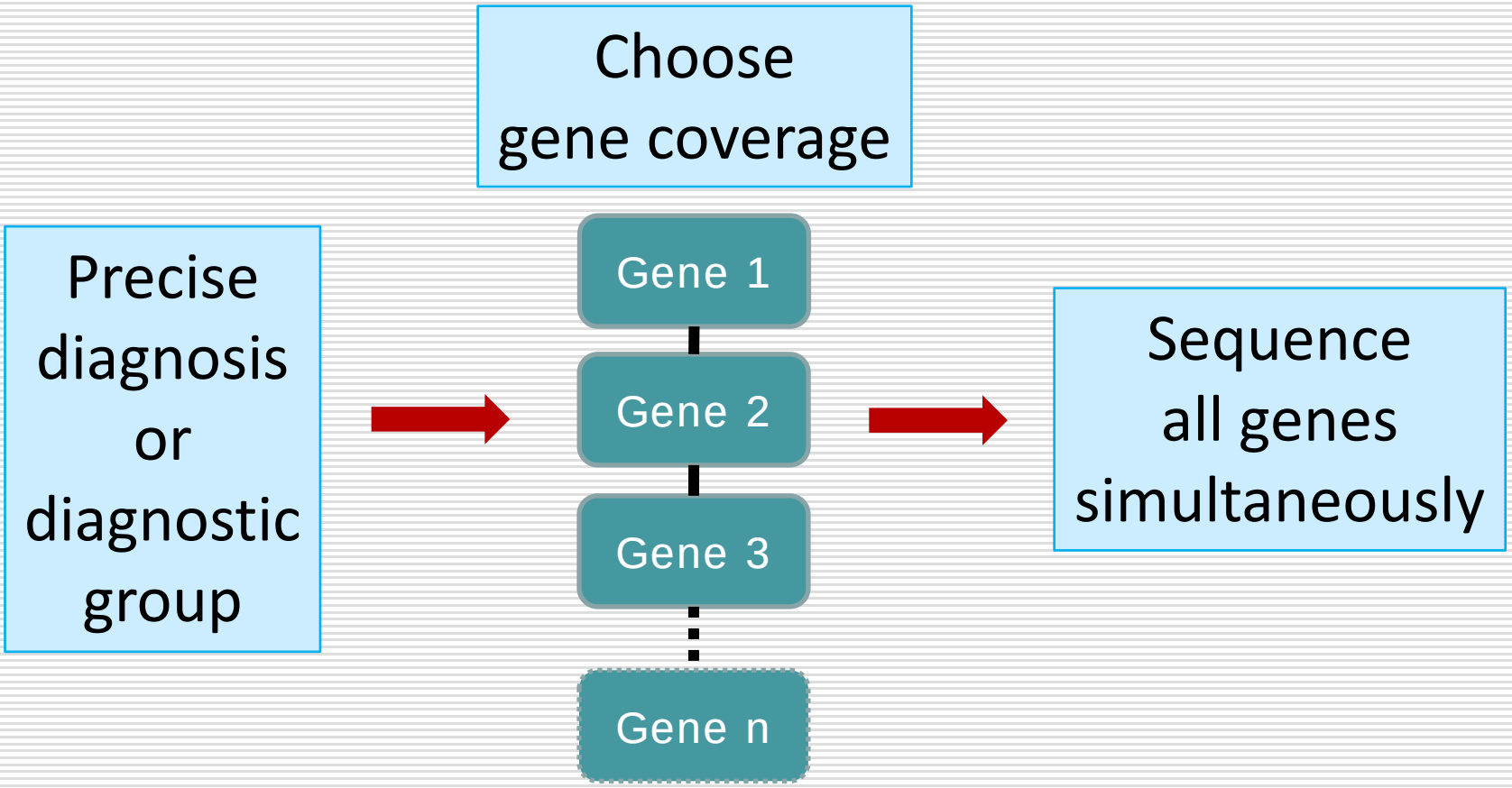
In 2007, researchers performed whole-genome human sequencing using old and new platforms. Researchers at Baylor College of Medicine and 454 Life Sciences sequenced James Watson's genome in two months, for about $1 million. Two other personal genomes were sequenced: Craig Venter's, at the Institute he founded, and that of a Chinese individual, at the Beijing Genomics Institute. The J. Craig Venter Institute used Sanger technology for sequencing Venter's DNA, which cost an estimated $70 million and took sev-

NATURE METHODS | VOL.5 NO.1 | JANUARY 2008 |

Cost per Genome



Genetic diagnosis with NGS



1- 5,000 genes with known phenotypes in one go

Neurological

Aicardi-Goutieres Syndrome	5
Amyotrophic Lateral Sclerosis	36
Ataxia with Oculomoter Apraxia	2
Autism	100
Basal Ganglia Calcification	2
Cerebellar Hypoplasia	8
Cerebral Cavernous Malformations	3
Charcot Marie Tooth Disease Extended	49
Ciliopathies	95
Cockayne Syndrome	2
Cornelia De Lange Syndrome	5
Distal Hereditary Motor Neuropathy	15
Dystonia Dyskinesia	14
Early Onset Familial Alzheimer Disease	3
Epilepsy	343
FGFR-Related Craniosynostosis	3
Hemiplegia/Stroke	10
Hereditary Neuropathies	33
Hirschsprung Disease	5
Holoprosencephaly	7
Intellectual Disability	391
Joubert and Meckel	22
Joubert Syndrome	18
Leukoencephalopathy	26
Lipodystrophy	10
Lissencephaly	14
Lysosomal Disorders	106
Microcephaly	35
Migraine	18
Neuromuscular	50
Neuronal Ceroid Lipofuscinoses	10
Noonan Syndrome	12
Nuclear-Mito	504
Parkinson-Alzheimer-Dementia	37
Polymicrogyria	7
Pontocerebellar Hypoplasia	8
Rett-Angelman	18
Rubenstein-Taybi Syndrome	2
Septo-optic Dysplasia	5
Spastic Paraplegia	25
XLID	107

Choosing the right sequencing test

Simple clinical phenotypes/confident diagnosis:

- **Single gene** *e.g. Cystic fibrosis, familial adenomatous polyposis*
- **Focussed gene panel** *e.g. familial Alzheimer, auto-inflammatory disorders*

Heterogeneous disorders and complex clinical phenotypes:

- **Broad panel** *that covers the diagnostic group*
  e.g. intellectual disability 400 genes; retinal dystrophy 127 genes
- **Clinical exome** *(~5,000 genes associated with known phenotypes)*
  e.g. patient with epilepsy and intellectual disability
- **Whole Exome Sequencing** *(~20,000 protein coding genes)*
  e.g. long differential with possibility of a novel gene cause
- **Whole Genome Sequencing** *e.g. long differential with possibility of a novel gene cause and/or a cause outside the exome*

Decoding Massimo Damiani's rare genetic disease

DARS gene:
Hypomyelination with brain stem and spinal cord involvement and leg spasticity

The First Child Saved By DNA Sequencing

Since he was a toddler, six-year-old Nicholas Volker's intestine had been dangerously inflamed, necessitating a hundred surgeries including the removal of his colon. No one knew the cause, but it seemed certain that the boy was dying. In a desperate attempt to figure out what was wrong, doctors at the Medical College of Wisconsin did something desperate and unproven: they sequenced his DNA.

XIAP gene: X-linked lymphoproliferative syndrome
Treated with cord blood cell transplant

Genome study solves twins' mystery condition

Sequencing ends years of speculation over children's rare disorder.

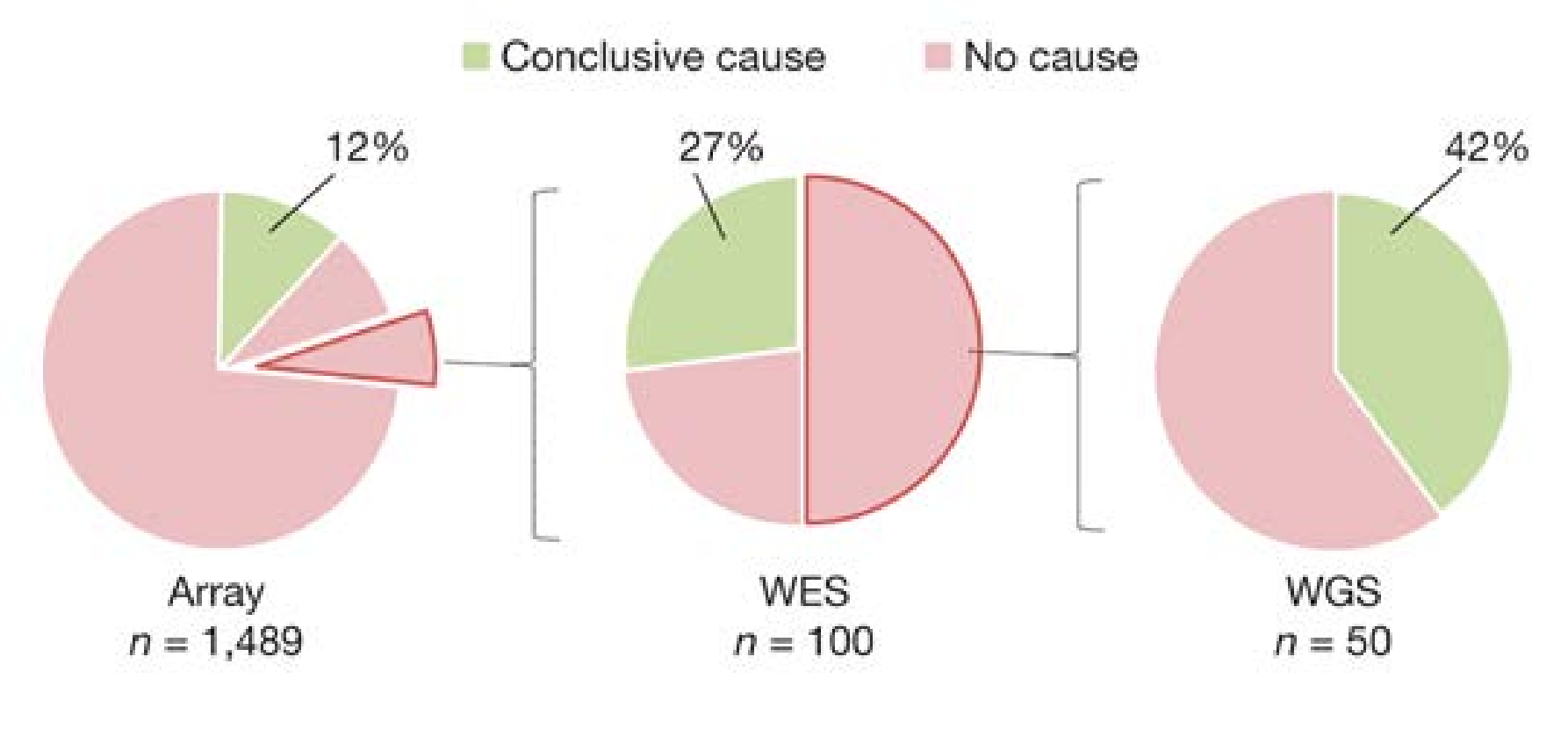
Erika Check Hayden

Two years ago, 13-year-old Alexis Beery developed a cough and a breathing problem so severe that her parents placed a baby monitor in her room just to make sure she would survive the night. Alexis would often cough so hard and so long that she would throw up, and had to take daily injections of adrenaline just to keep breathing. Yet doctors weren't sure what was wrong.

Genome sequencing suggested a new approach to treatment for twins Noah and Alexis Beery, shown here with their parents. *Life Technologies*

SPR gene: Dopa-responsive dystonia due to sepiapterin reductase deficiency
Treated with L-Dopa and 5-hydroxytryptophan

Identifying the cause of 'severe' intellectual disability



Extrapolated diagnostic yield 60%

Gilissen et al. Nature 2014; 511, 344

Rapid diagnosis of Glycogen Storage Diseases by NGS

Abbs et al. International Congress of Human Genetics 2011, Abstract 265

Patient with clinical and biochemical features of GSD type III, VI, IX

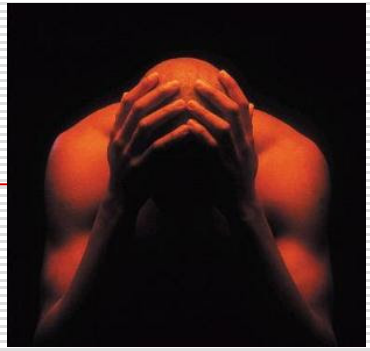
Over 12 years, patient had repeated enzyme analysis, a liver biopsy and conventional sequencing of three GSD IX genes, without a definite diagnosis

NGS identified a homozygous pathogenic variant in the *PYGL* gene, finally confirming a diagnosis of GSD type VI

NGS made a diagnosis in weeks, at 10% of the combined cost of all the inconclusive tests performed over 12 years - $1,700 vs $17,000

The 'diagnostic odyssey' can become a 'diagnostic stroll in the park', which is time- and cost-effective and minimises invasive procedures

Variants of uncertain significance
An annoyingly common outcome



TEST RESULT

No clearly pathogenic variant detected

Variant of uncertain pathogenic potential detected: *COL4A5* c.892-18T>G

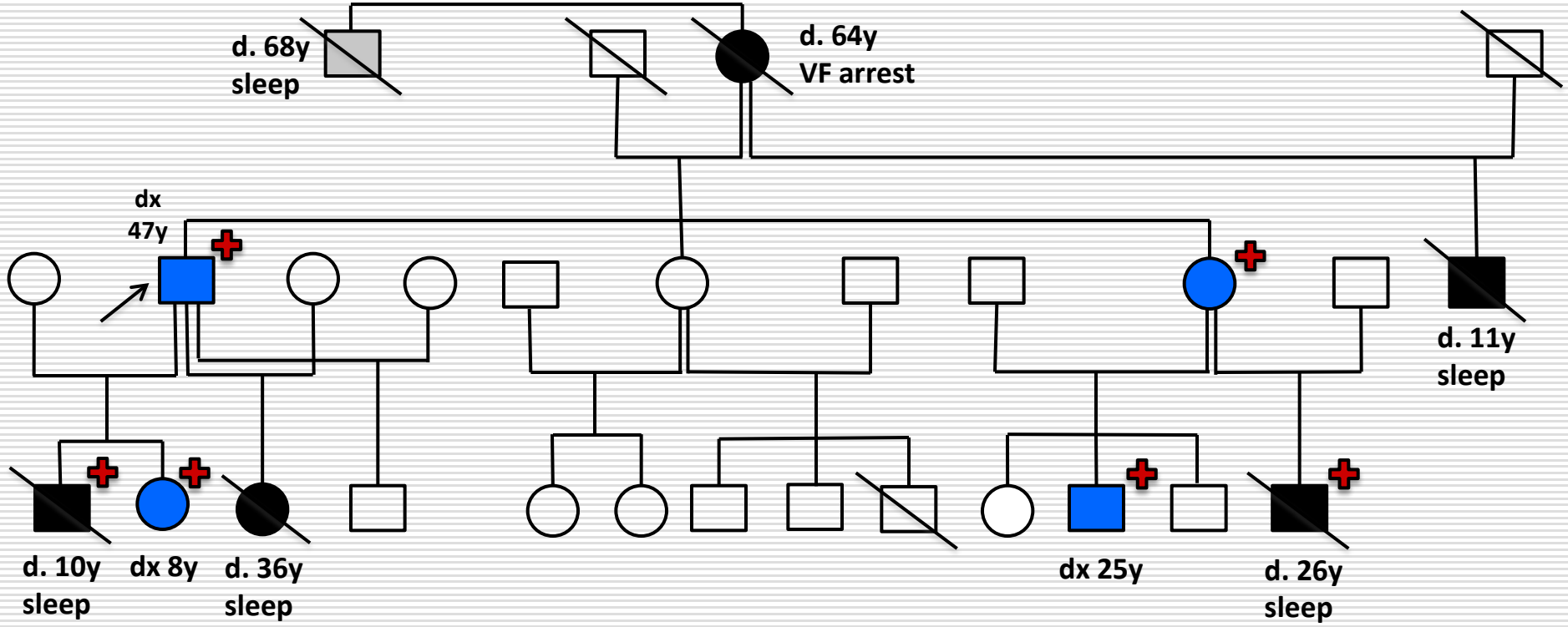
SUMMARY

The *COL4A5* c.892-18T>G sequence variant may affect the splicing of the *COL4A5* transcript (in silico analysis shows the creation of a new acceptor splice site), however the pathogenic potential of this sequence change is uncertain.

The diagnosis of Alport syndrome or TBMN has NOT been confirmed.

Significance may become clear over time or after family studies

Family studies can be helpful



= Sudden death

= Long QT syndrome

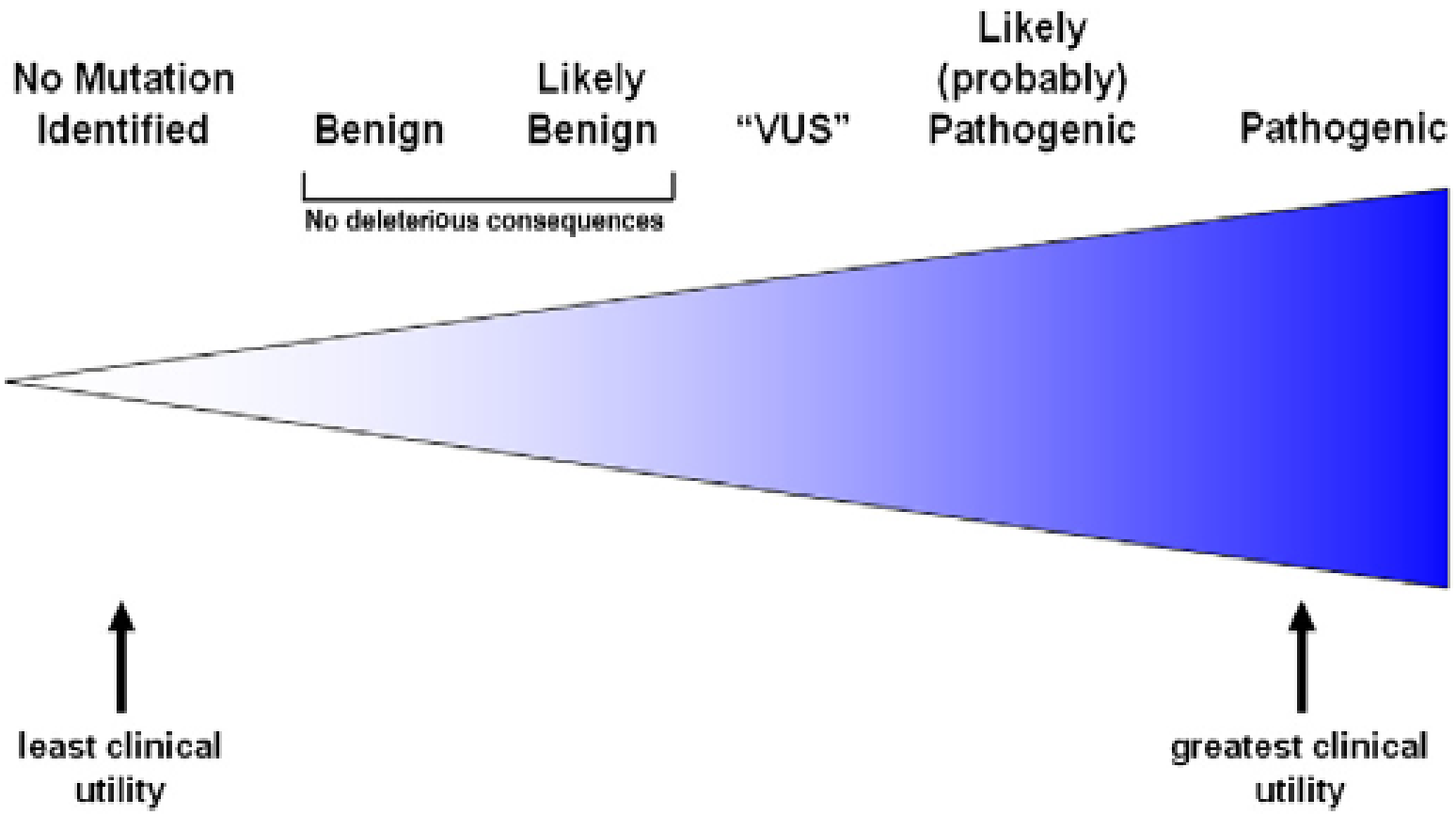
= SCN5A mutation present

Incidental (secondary) findings: 1-3% of genome studies

Incidental Findings according to the genes included in the ACMG recommendations:

Gene	c-position	p-position	Zygosity	significance	Disease (according to OMIM)
BRCA2	c.1514T>C	p.Ile505Thr	Heterozygous	Variant of uncertain clinical significance	Breast cancer
RYR1	c.7844G>A	p.Arg2615His	heterozygous	Variant of uncertain clinical significance	Malignant hyperthermia susceptibility
SCN5A ★	c.659C>T	p.Thr220Ile	Heterozygous	Variant of uncertain clinical significance	Brugada syndrome 1
APC	c.2586C>G	p.Asn862Lys	Heterozygous	Variant of uncertain clinical significance	Familial adenomatous polyposis

Genetic testing outcomes



Maron, Maron and Semsarian. J Am Coll Cardiol 2012; 60: 705-15

Consent – things to discuss

- What will be done: gene/panel/WES/WGS
- Possibility of finding:
  - ≥1 pathogenic variant
  - nothing relevant – clinical diagnosis/inheritance unchanged!
  - variants of uncertain significance, incidental findings and variants causing recessive / X-linked disorders – *and does the patient want to know about them*
  - Non-paternity/maternity
- Implications of finding a disorder-causing variant – for tested individual and family (including for some types of insurance)
- Use of results and DNA for research

Genetic diagnosis made – does it help the patient?

- Provide or support a clinical diagnosis
- Determine/confirm inheritance pattern
- Allow prognostication/risk stratification
- May lead to improved management
- Reproductive options

Genetic diagnosis made – useful for relatives

Makes possible predictive testing to assess susceptibility:

- ***if mutation present,*** possibilities may include
  - surveillance for early diagnosis
  - preventative strategies
  - early management
  - genetic counselling, including reproductive options
- ***if mutation not present,*** risk becomes low for patient and offspring, and surveillance, if in place, can stop

Diagnostic technologies converge

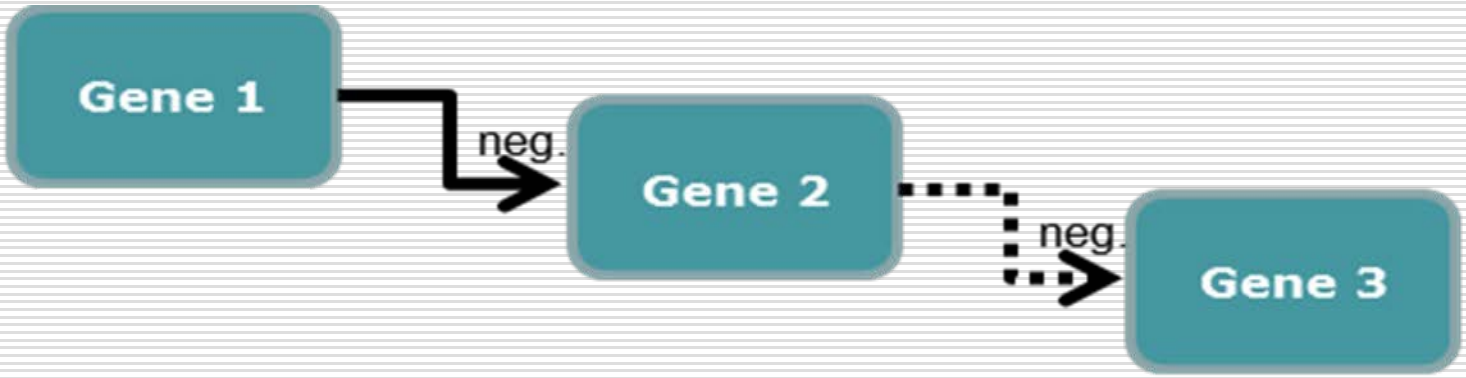
A single platform for common genetic mechanisms (?)

- Single nucleotide variants
- Copy number variants (deletions/duplications)
- Repeats
- Epigenetic modifications
- RNA sequence variation (gene expression)

PacBio RS II

Genetic diagnosis the 'old' way

Decide on most likely diagnosis in the genetic differential, or most likely gene in the diagnostic group



Spending limits reached quickly !