Next generation sequencing: Wat is vandaag (niet) mogelijk?

6 mei 2017

Antwerpen

Prof.dr. Martina Cornel, VU University Medical Center, Amsterdam

www.vumc.com/researchcommunitygenetics

Overzicht

- Historische terugblik vanaf 2000, wat is NGS?

- Personalized medicine – waar gaat het heen?
    - Behandelen op maat
    - Voorspellen
    - Testen van familieleden

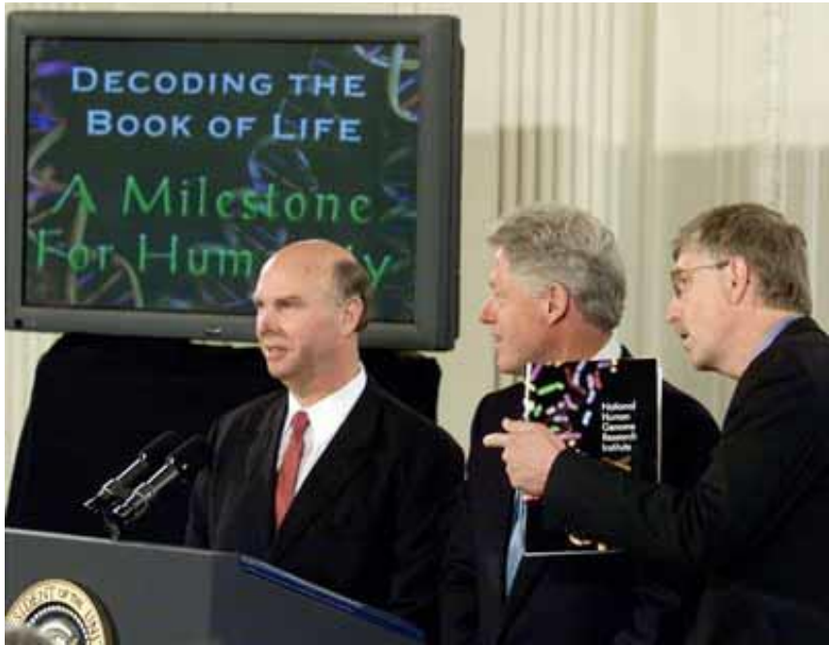
- Ethische en juridische kanten
    - Past preventie in de gezondheidszorg?
    - Wie mag de test leveren en de kwaliteit controleren?
    - Anonimiteit bestaat niet?

2000: genome sequence published

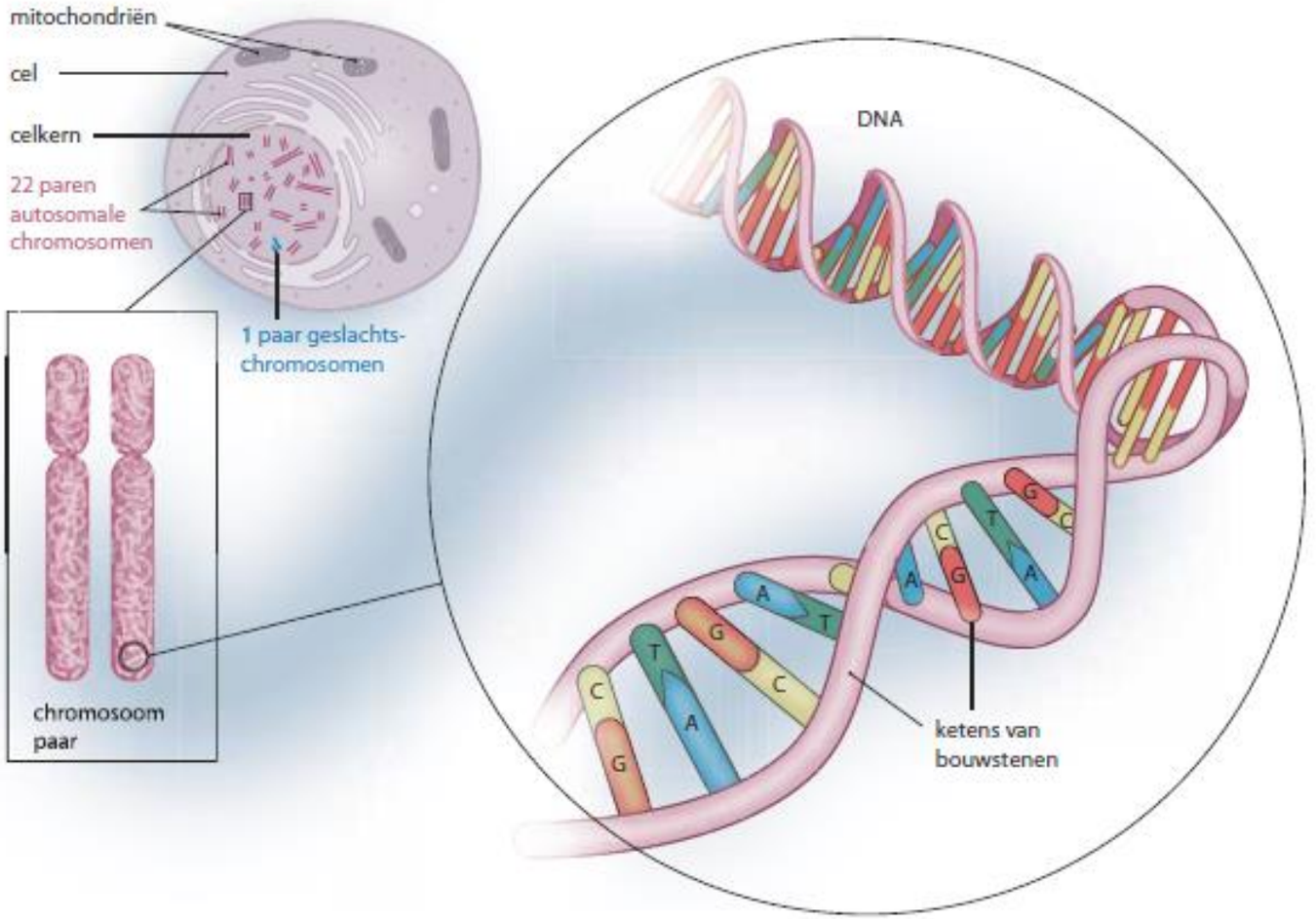
Bill Clinton: We are here to celebrate the completion of the first survey of the entire human genome …

With this profound new knowledge, humankind is on the verge of gaining immense, new power to heal. Genome science will have a real impact on all our lives -- and even more, on the lives of our children.

It will revolutionize
the diagnosis, prevention
and treatment of most,
if not all, human diseases.



Collins FS (Right at photo). Nature 2010 &



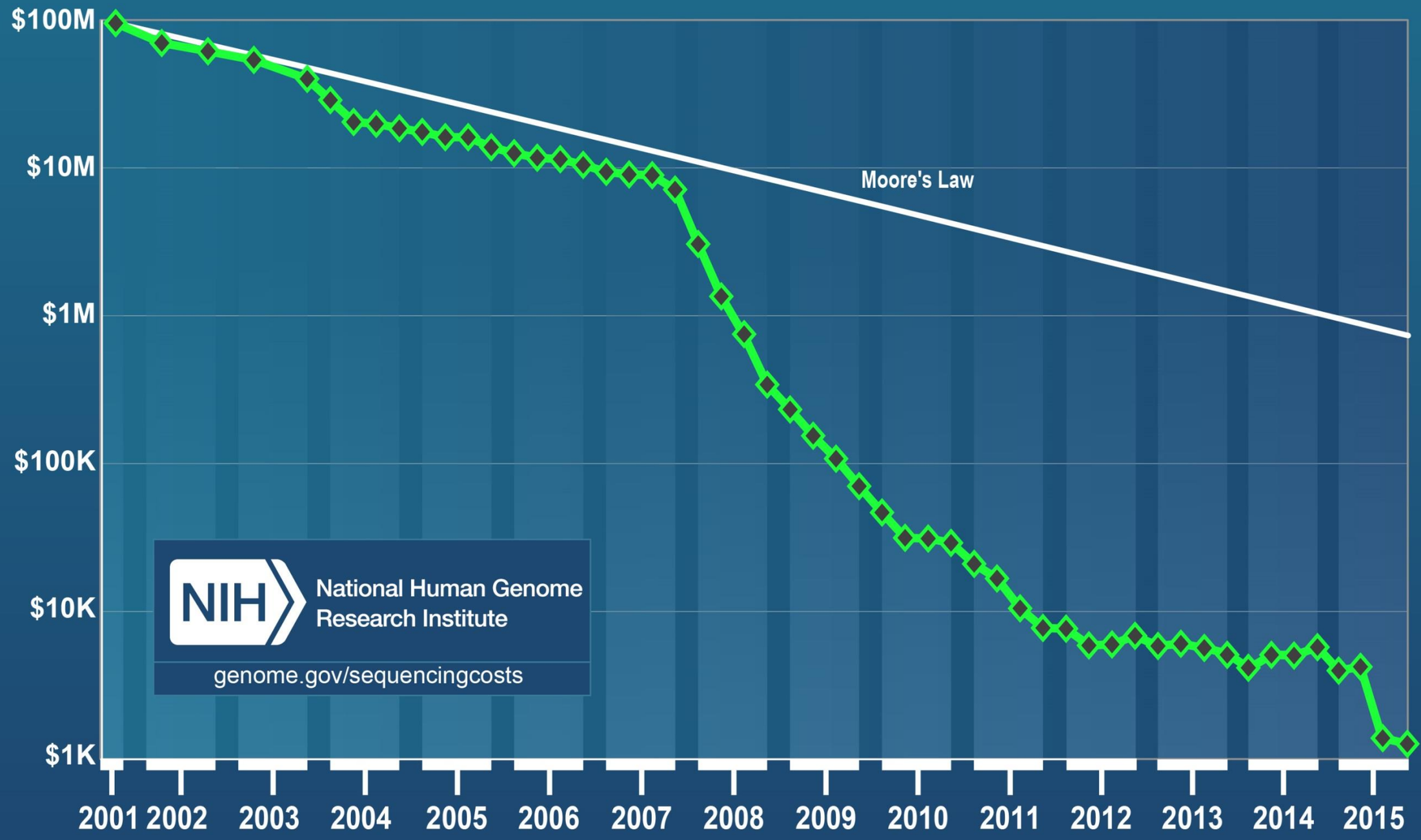
Illustratie 1: In de kern van de cel bevinden zich 22 paren autosomale chromosomen en één paar geslachts-chromosomen. In de chromosomen zit, omgeven door eiwitten, het DNA. Elk DNA-molecuul is samengesteld uit twee ketens van bouwstenen, die in een dubbele spiraal met elkaar zijn verviochten. Elke bouwsteen in de ene keten is met een bouwsteen in de andere keten verbonden. Er zijn vier soorten bouwstenen: A (Adenine), T (Thymine), C (Cytosine) en G (Guanine). Bouwsteen A is altijd verbonden met bouwsteen T (en omgekeerd); bouwsteen G is altijd verbonden met bouwsteen C (en omgekeerd). Illustratie R.S. Enterprises

Bron: NFI

Genome sequence?

Bron: Wikipedia, foodpoisonjournal

Cost per Genome



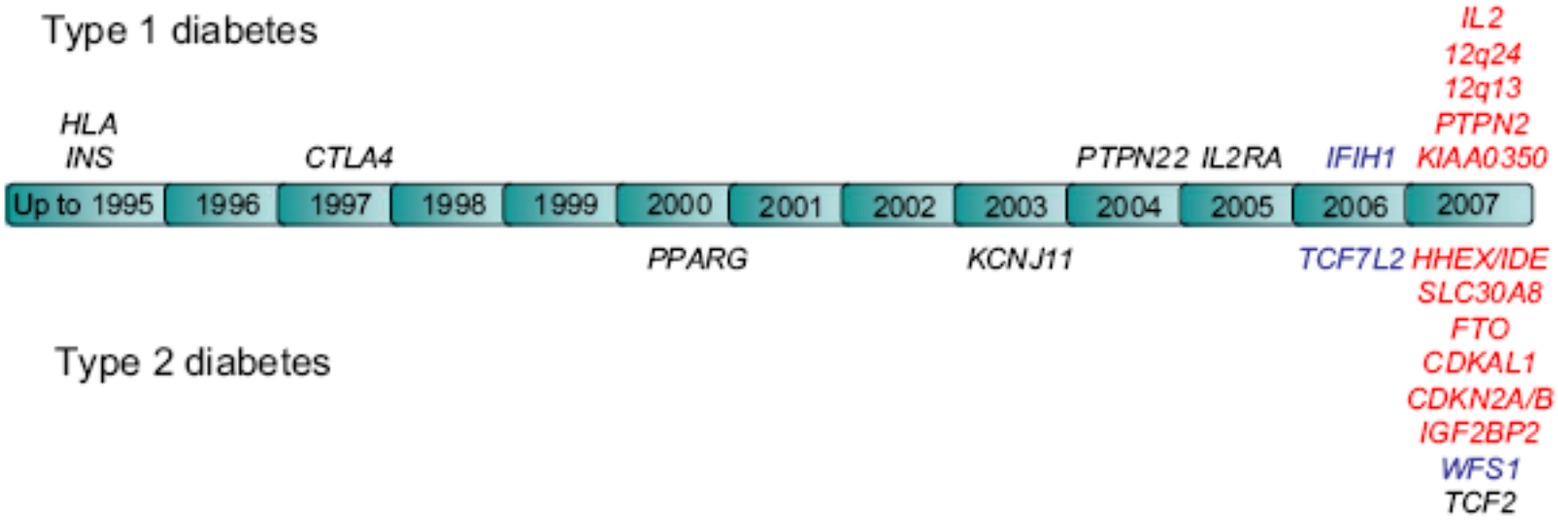
Prijs per genoom

- (bouwstenen van erfelijke code, erfelijke aanleg van een individu)

- 100.000.000$ in 2001
- 1000$ in 2017

2000-2010:
GWAS, SNPs, Low risk genes

As a result of (genome wide) association studies, increasing number of low-penetrance gene variants associated with disease identified



Frayling et al. Diabetologia 2007

De jaren 2000-2010

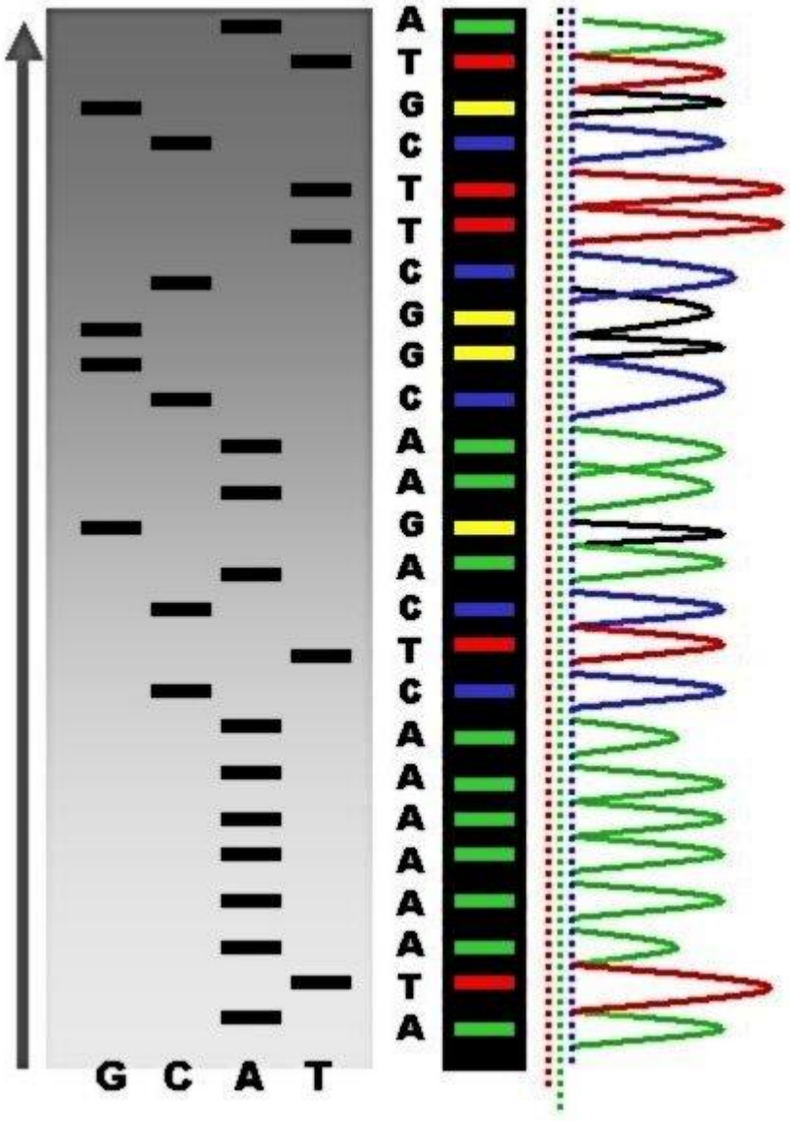
- Snelle technische ontwikkelingen
  - SNPs in GWAS – vooral laagrisicogenen
  - CNVs (deleties) als belangrijke bron van ziekte
  - Volgende technische stap = ***sequencen***
  - Gelijktijdig testen op meerdere aandoeningen, bij eerste diagnostische vraag hele genoom in kaart brengen?

- Potentiële toepassingen in gezondheidszorg rond diagnose, behandeling en preventie van talrijke aandoeningen

- Veel in vroege translationele fase (T1)
  - wel wetenschap, geen klinische toepassing

Sequencen?

- De (al dan niet complete) DNA sequentie “aflezen”
- **Data** GCATGCATG moet daarna geïnterpreteerd worden
- Aanvankelijk “**Sanger** sequencing” = letter voor letter
- Rond 2007 **next generation** sequencing = parallel



Sequencen?

© Borry & Matthijs

- **WES**= whole **exome** sequencing: alleen genen aflezen die coderen voor eiwitten = 1% van het genoom, waarin meeste monogene aandoeningen zitten die we nu kennen (CF, Hb).

- **WGS**= whole **genome** sequencing: de analyse wordt nog veelal gericht (***targeted***) op bijv. varianten die wel bij kind maar niet bij ouders voorkwamen (oorzaak van ontwikkelingsstoornis)

- **Coverage**: hoeveel kopieën van het genoom, resp. van gen

- **Unsolicited findings**: niet gezocht, wel gevonden

Collins, 10 years later (Nature 2010;464:674-5)

Has the revolution arrived?

The consequences for clinical medicine, however, have thus far been modest.

Those who somehow expected dramatic results overnight may be disappointed, but should remember that genomics obeys the First Law of Technology:
we invariably overestimate the short-term impacts of new technologies and underestimate their longer-term effects.

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Personalized medicine

- De juiste therapie voor de juiste patiënt op het juiste moment
- **Diagnostiek** bij jonge borstkankerpatiënt (of 2e BC of triple neg)
- **Presymptomatische diagnostiek** bij eerstegraadsverwanten
  - De kanker vóór zijn
- De juiste **medicatie (PARP inhibitors** bij BRCA1/2 positieve ovariumcarcinoom die niet reageert op reguliere chemotherapie)

Personalized medicine & therapy

- Companion diagnostics (Delaney 2016)

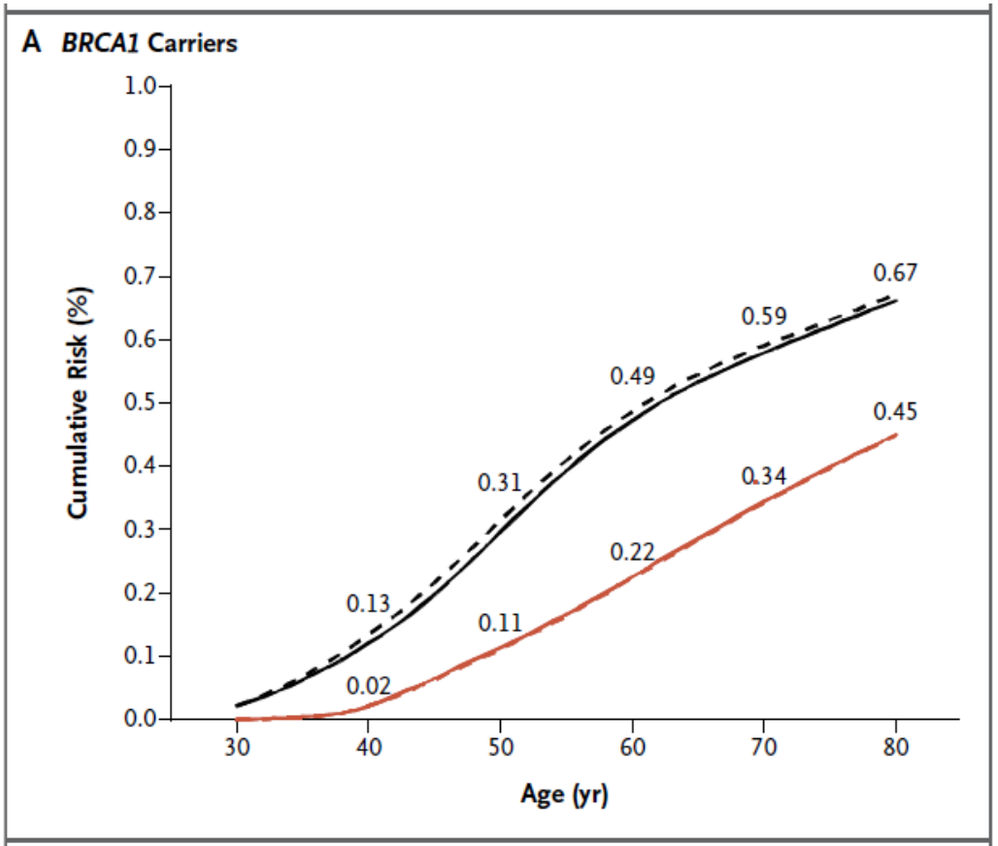
Table 2. List of FDA-cleared or approved companion diagnostic devices (*in vitro* and imaging tools).

Drug trade name (generic name)	Device trade name	Intended use (IU)/indications for use (IFU)
Tagrisso® (osimertinib)	cobas® EGFR mutation test v2	To aid in identifying patients with NSCLC whose tumors have defined EGFR mutations and for whom safety and efficacy of a drug have been established.
Keytruda® (pembrolizumab)	PD-L1 IHC 22C3 pharmDx	To aid in identifying NSCLC patients for treatment with Keytruda® (pembrolizumab).
Iressa (gefitinib)	therascreen® EGFR RGQ PCR kit	To select patients with NSCLC for whom GILOTRIF® (afatinib) or IRESSA® (gefitinib), EGFR tyrosine kinase inhibitors (TKIs), is indicated.
Xalkori (crizotinib)	VENTANA ALK (D5F3) CDx assay	To aid in identifying patients diagnosed with non-small cell lung carcinoma (NSCLC) eligible for treatment with XALKORI® (crizotinib).
Erbitux (cetuximab); Vectibix (panitumumab)	The cobas® KRAS mutation test	To aid in the identification of CRC patients for whom treatment with Erbitux® (cetuximab) or with Vectibix® (panitumumab)
Lynparza™ (olaparib)	BRACAnalysis CDx™	Results of the test are used as an aid in identifying ovarian cancer patients with deleterious or suspected deleterious germline BRCA variants eligible for treatment with Lynparza™ (olaparib).
Erbitux (cetuximab); Vectibix (panitumumab)	*therascreen* KRAS RGQ PCR kit	To aid in the identification of CRC patients for treatment with Erbitux (cetuximab) and Vectibix (panitumumab) based on a KRAS no mutation detected test result.
	DAKO EGFR PharmDx kit	To aid in identifying colorectal cancer patients eligible for treatment with Erbitux (cetuximab) or Vectibix (panitumumab).
Exjade (deferasirox)	Ferriscan	To measure liver iron concentration to aid in the identification and monitoring of non-transfusion-dependent thalassemia patients receiving therapy with deferasirox.
Gilotrif (afatinib)	therascreen EGFR RGQ PCR kit	To select patients with NSCLC for whom GILOTRIF (afatinib), an EGFR tyrosine kinase inhibitor (TKI), is indicated
Gleevec/Glivec (imatinib mesylate)	DAKO C-KIT PharmDx	To aid in the differential diagnosis of gastrointestinal stromal tumors (GIST). After diagnosis of GIST, results from c-Kit pharmDx may be used as an aid in identifying those patients eligible for treatment with Gleevec/Glivec (imatinib mesylate).

Personalized medicine ?

1. Soms is het risico anders dan bij anderen
   Borstkanker screening voor alle vrouwen vanaf 50 jaar
   Maar bij BRCA1/2 vanaf 25 jaar

2. Soms is de gevoeligheid
   van de tumor anders



Figuur: Hartman 2016 NEJM

Voorspellen?

- Kans groter
- Gevoeligheid groter, dus meer kans op succes therapie
- Maar niet: Op leeftijd XX gaat YY gebeuren

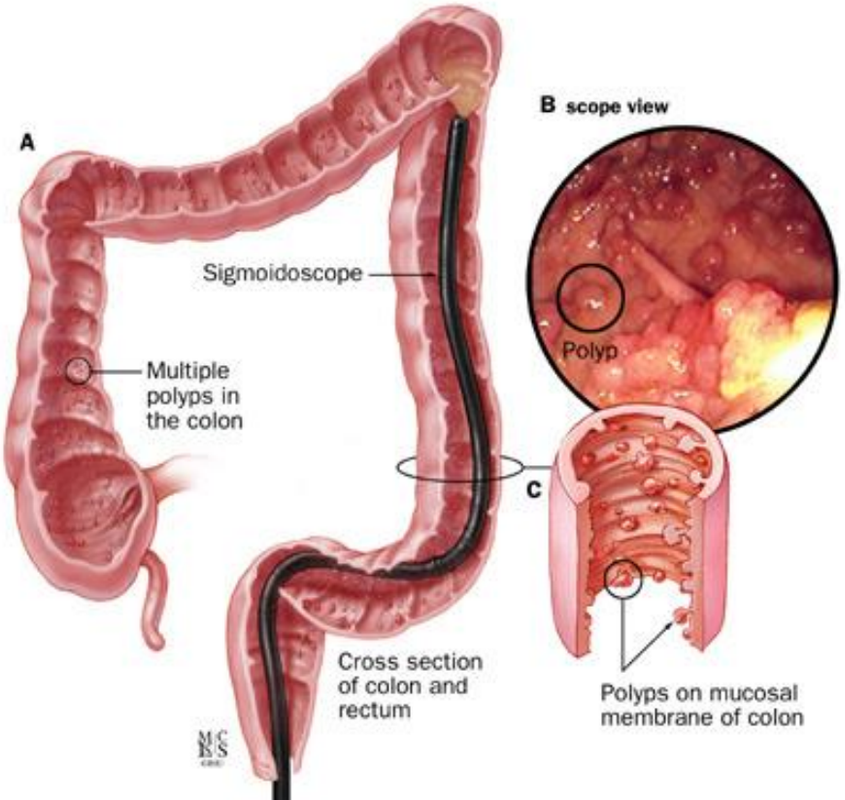
Personalized medicine?
Early recognition

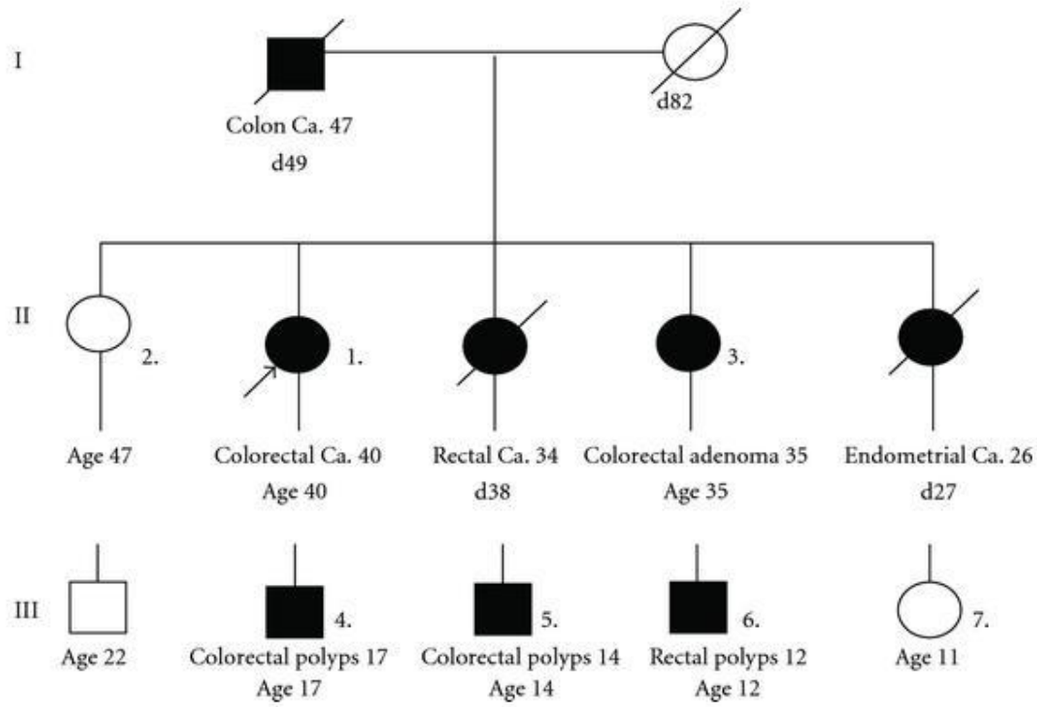
1. Breast cancer in the family at a young age
2. Potentially high risk
3. Interventions available

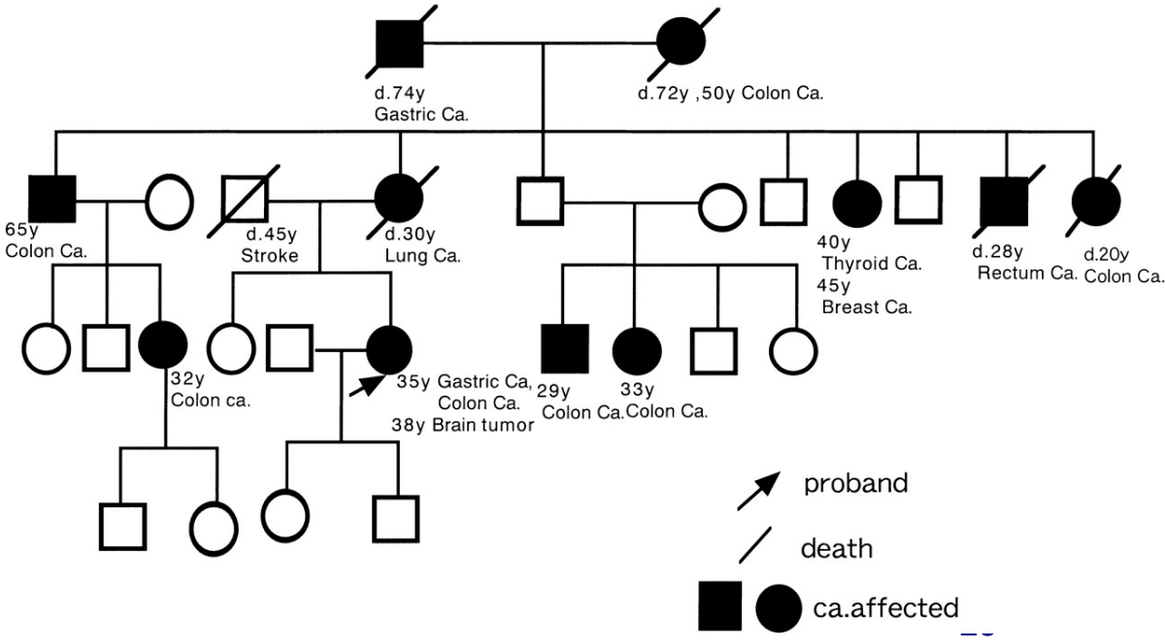
"I CARRY A “FAULTY” GENE, BRCA1, WHICH SHARPLY INCREASES MY RISK OF DEVELOPING BREAST CANCER AND OVARIAN CANCER. [...] ONCE I KNEW THAT THIS WAS MY REALITY, I DECIDED TO BE PROACTIVE AND TO MINIMIZE THE RISK AS MUCH I COULD. I MADE A DECISION TO HAVE A PREVENTIVE DOUBLE MASTECTOMY. I STARTED WITH THE BREASTS, AS MY RISK OF BREAST CANCER IS HIGHER THAN MY RISK OF OVARIAN CANCER, AND THE SURGERY IS MORE COMPLEX. [...] I CAN TELL MY CHILDREN THAT THEY DON’T NEED TO FEAR THEY WILL LOSE ME TO BREAST CANCER."

Colon cancer?

- FAP, Lynch (HNPCC)
- Colonoscopy
- Aspirine decreases cancer risk in Lynch syndrome (CAPP3 trial)







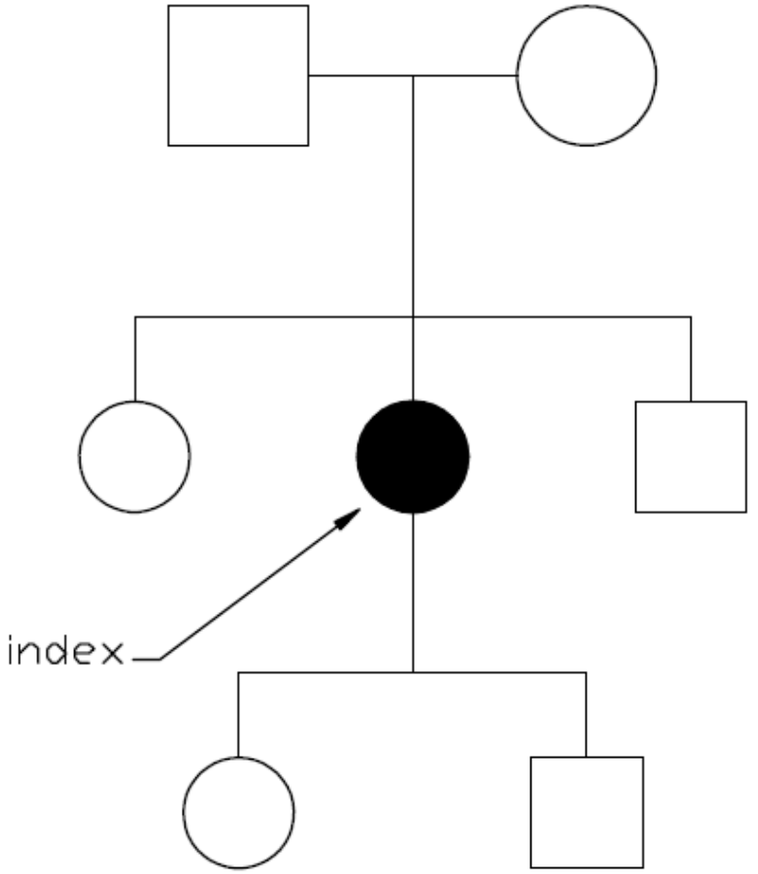
What to prioritize?

considerations of medical benefit, health need and costs

- evidence of benefit in terms of **clinical benefit**,
- benefit of information for important life decisions,
- benefit **for other people** apart from the person tested and the patient specific **likelihood** of being affected by the condition tested for

Severin et al. European Journal of Human Genetics (2015) 23, 729–735

Screening? Cascade screening!



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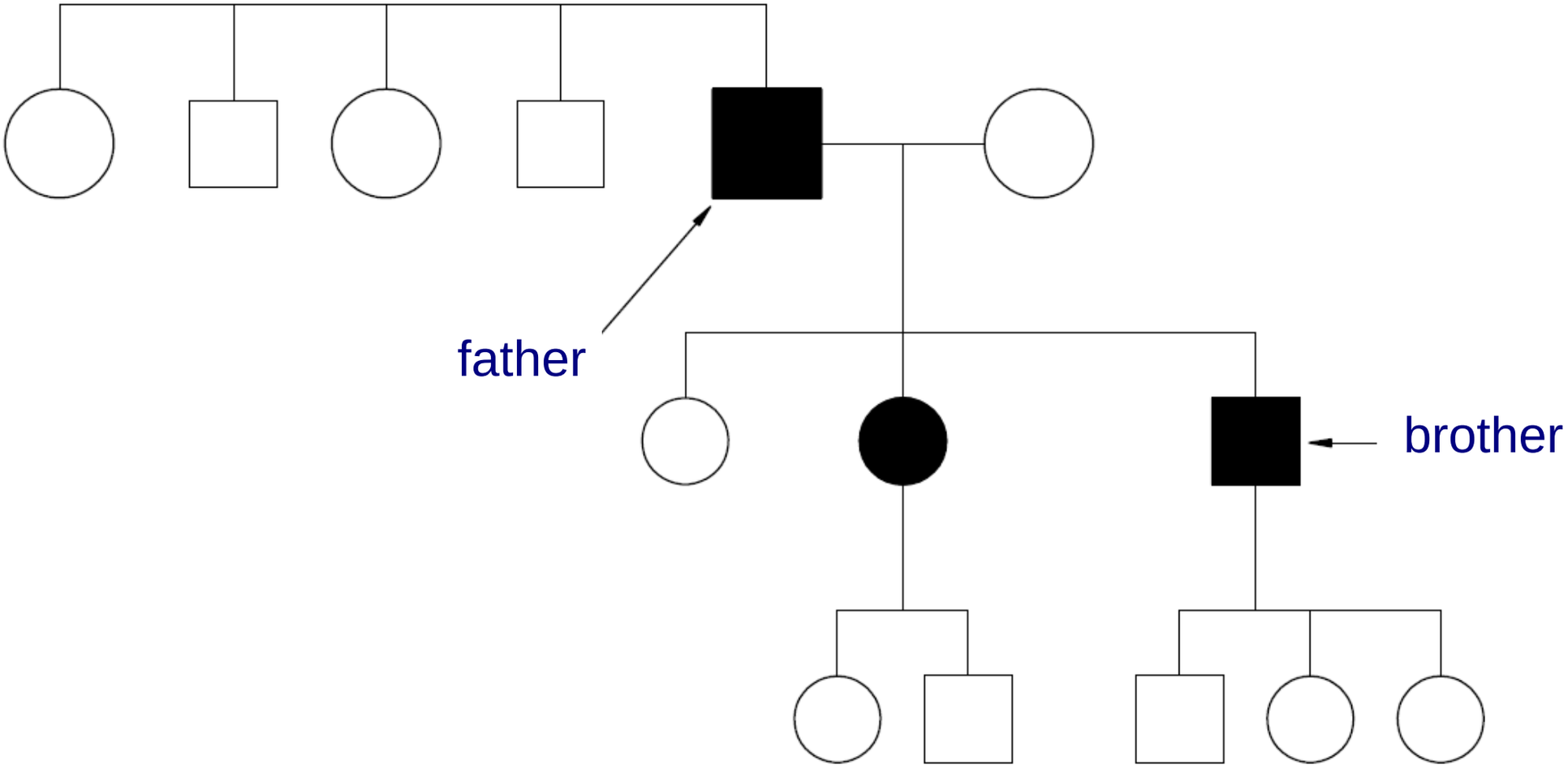


Eén gen of het hele exoom/genoom

- BRCA voor diagnostiek
- BRCA voor therapie (PARP inhibitor)

- Bij ontwikkelingsachterstand: WES (whole exome sequencing) voor diagnose
- Bij ontwikkelingsachterstand: ouders en kind whole genome sequencing, alleen nieuwe mutaties rapporteren voor diagnose (Nijmegen: 30-50% diagnoses bij tot dusver niet begrepen casuïstiek).
- Sequencing steeds goedkoper, analyse nog in ontwikkeling
- “Blurring boundaries of Reseach and Care”

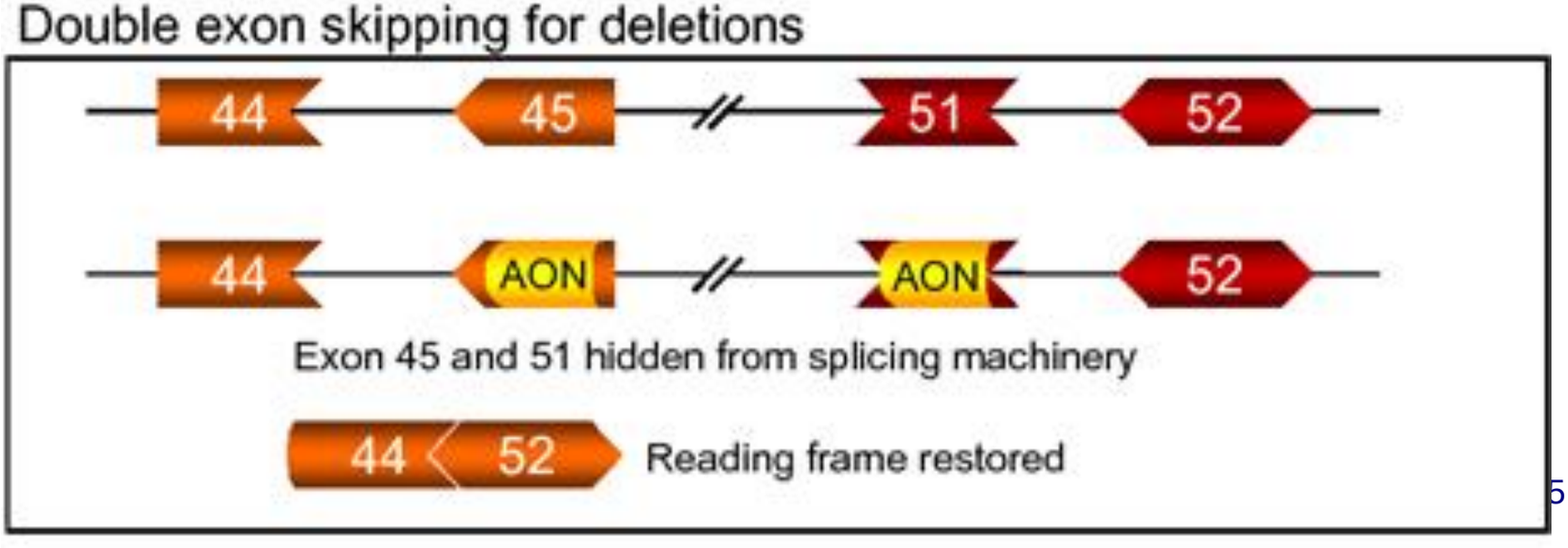
Personalized medicine & therapy

- Mammaprint ® (expressie 70 genen) en GEEN chemotherapie

- Expressie van genen in tumoren laat overeenkomsten zien waardoor middel voor één kanker wellicht ook voor andere werkt
  - *On August 26, 2011, the U.S. Food and Drug Administration approved **crizotinib** (Xalkori) to treat certain late-stage (locally advanced or metastatic) non-small cell lung cancers that express the abnormal anaplastic lymphoma kinase (ALK) gene. Approval required a companion molecular test for the EML4-ALK fusion.*
- Mogelijk speelt dat fusie-gen ook bij andere tumoren een rol.

Personalized Medicine & Therapy

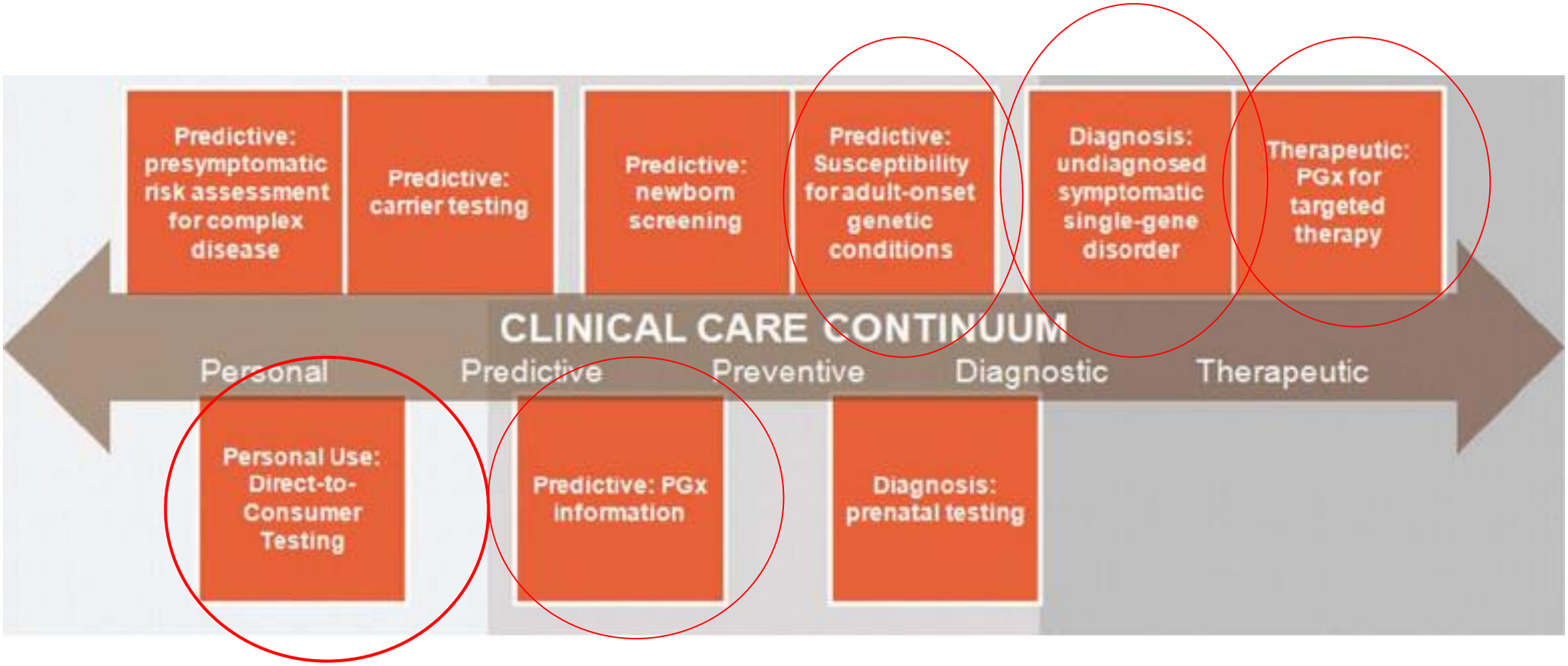
- Duchenne spierdystrofie
- Voor verschillende exonen verschillende exon-skipping therapieën in ontwikkeling
- Trials lopen, o.a. vanuit LUMC



Pharmacogenomics

- Bij statines mogelijk snel spierpijn en/of ernstige spierpijn
- Eerst SLCO1B1 bepalen? Relatief veel bijwerkingen
- Geen verband met effectiviteit

- Wanneer doe je PGX profiel?
- G-standaard vertelt wel WAT je moet doen bij bepaald PGX profiel, maar implementatie vóór voorschrijven is er nog niet.



Source: Delaney 2016

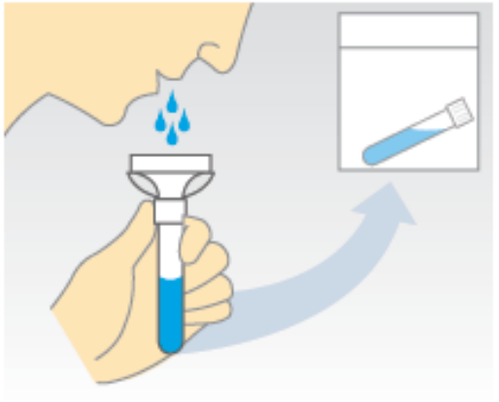
Personal Genome Service™

Get to know your DNA. All it takes is a little bit of spit.

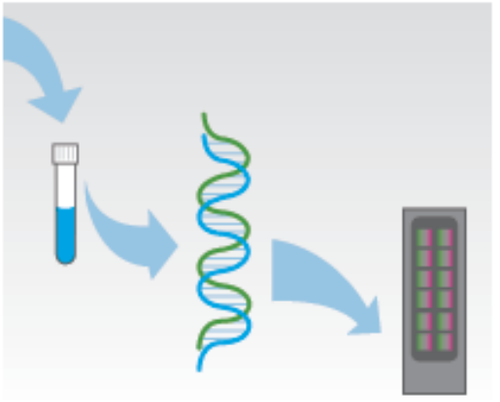
Here's what you do:

PGS™

1. Order a kit from our online store.



2. Register your kit, spit into the tube, and send it to the lab.



3. Our CLIA-certified lab analyzes your DNA in 6-8 weeks.

4. Log in and start exploring your genome.

Frequently Asked Questions

- How does 23andMe genotype my DNA?
- Why can't 23andMe diagnose me?
- What is the difference between genotyping and sequencing?
- What technology is used for the analysis?

visit the store

try a demo

Our Technology and Standards

Department of Health and Human Services

Public Health Service
Food and Drug Administration
10903 New Hampshire Avenue
Silver Spring, MD 20993

Nov 22, 2013

Ann Wojcicki
CEO
23andMe, Inc.
1390 Shoreline Way
Mountain View, CA 94043

Document Number: GEN1300666
Re: Personal Genome Service (PGS)

WARNING LETTER

Dear Ms. Wojcicki,

The Food and Drug Administration (FDA) is sending you this letter because you are marketing the 23andMe Saliva Collection Kit and Personal Genome Service (PGS) without marketing clearance or approval in violation of the Federal Food, Drug and Cosmetic Act (the FD&C Act).

This product is a device within the meaning of section 201(h) of the FD&C Act, 21 U.S.C. 321 (h), because it is intended for use in the diagnosis of disease or other conditions or in the cure, mitigation, treatment, or prevention of disease, or is intended to affect the structure or functior of the body. For example, your company's website at www.23andme.com/health (most recentl viewed on November 6, 2013) markets the PGS for providing "health reports on 254 diseases and conditions," including categories such as "carrier status," "health risks," and "drug response," and specifically as a "first step in prevention" that enables users to "take steps toward mitigating serious diseases" such as diabetes, coronary heart disease, and breast cancer. Most of the intended uses for PGS listed on your website, a list that has grown over time, are medical device uses under section 201(h) of the FD&C Act. Most of these uses have not been classified and thus require premarket approval or de novo classification, as FDA has explained to you on numerous occasions.

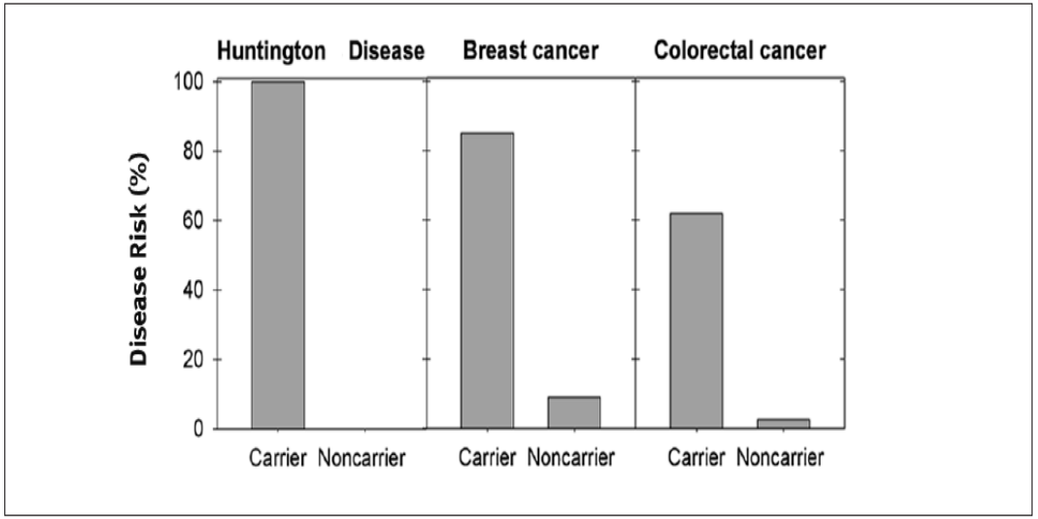
Some of the uses for which PGS is intended are particularly concerning, such as assessments for BRCA-related genetic risk and drug responses (e.g., warfarin sensitivity, clopidogrel response, and 5-fluorouracil toxicity) because of the potential health consequences that could result from false positive or false negative assessments for high-risk indications such as these. For instance, if the BRCA-related risk assessment for breast or ovarian cancer reports a false positive, it could lead a patient to undergo prophylactic surgery, chemoprevention, intensive screening, or other morbidity-inducing actions, while a false negative could result in a failure to recognize an actual risk that may exist. Assessments for drug responses carry the risks that patients relying on such tests may begin to self-manage their treatments through dose changes or even abandon certain therapies depending on the outcome of the assessment. For example, false genotype results for your warfarin drug response test could have significant unreasonable risk of illness, injury, or death to the patient due to thrombosis o

Changes to our health-related product.

At this time, we have suspended our health-related genetic tests to comply with the U.S. Food and Drug Administration's directive to discontinue new consumer access during our regulatory review process. In the future, you may be able to receive health-related results, dependent upon FDA marketing authorization.

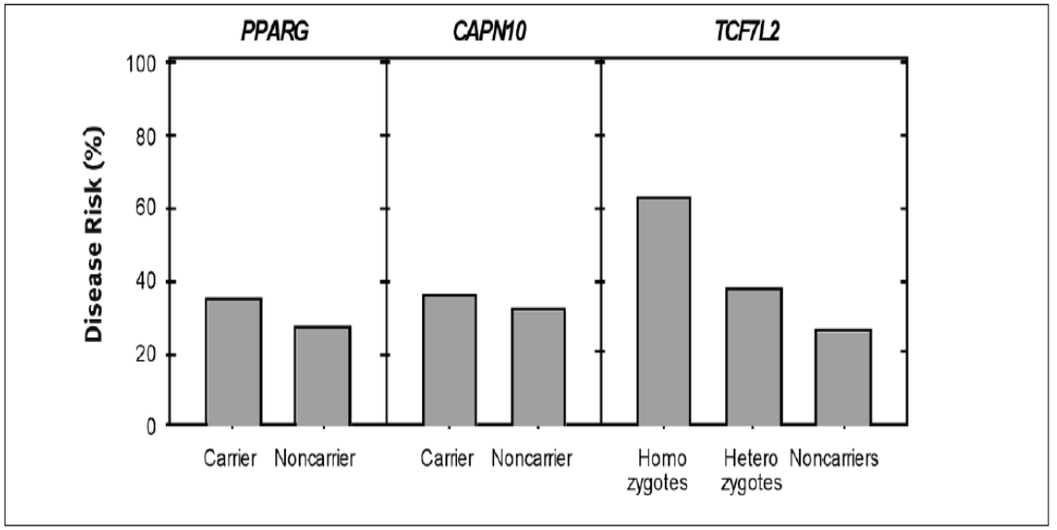
High vs. lower predictive value

Figure 1. Disease risks of carriers and noncarriers in genetic testing for monogenic disorders



Legend: The genetic variants tested are CAG repeats in 4p16.3 for Huntington disease,[10] *BRCA1/BRCA2* for breast cancer,[11] and *hMLH1/hMSH2* for colorectal cancer.[12]

Figure 2. Disease risks of carriers and noncarriers in single genetic testing for multifactorial disorders



Legend: Data on the odds ratios of the genetic variants are obtained from the literature (*PPARG*[30], *CAPN10*[31], *TCF7L2*[32]). For the calculation of the disease risks from the published odds ratios, we assumed a lifetime risk of type 2 diabetes of 33%.[33]

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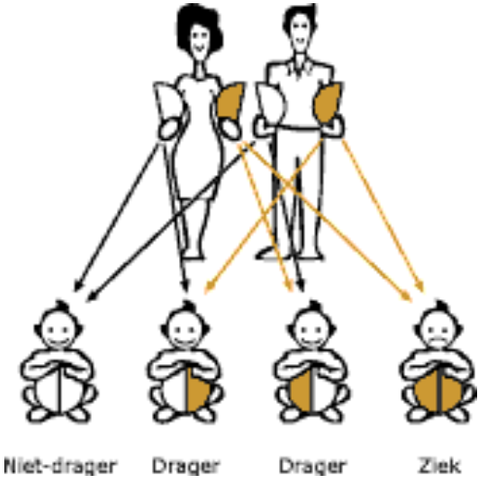
Screening (op maat)

- **Iedereen** op bepaalde leeftijd
- Systematisch indelen risicogroepen (**selectieve preventie**?), naar analogie van man/vrouw ook etniciteit of familieanamnese betrekken?
- Individuen die vragen stellen naar aanleiding van hun familieanamnese (**geïndiceerde preventie**?) zoals “mijn eerstegraads familielid had op jonge leeftijd Dx, kan ik dat ook krijgen?”

- Wat hoort in basispakket?
  Geen gezondheidszorg, alleen ziekenzorg?
  Wel presymptomatisch onderzoek familieleden? Wel PGx?

Rond geboorte - Verzekerde zorg?

- Preconceptiedragerschapsscreening
  - Zijn beide partners drager van een ziekte met verborgen erfelijkheid?
  - Zwanger worden en dit risico vermijden?
  - Reageerbuisbevruchting, embryoselectie



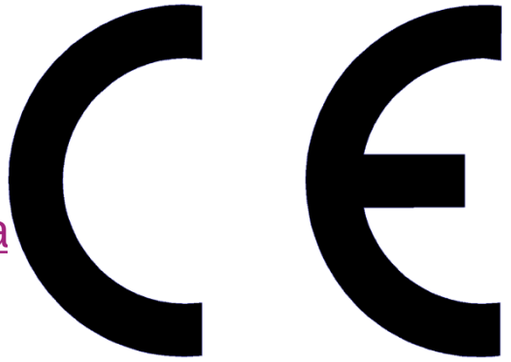
- Prenatale screening
  - NIPT voor chromosomale afwijkingen zoals Down syndroom
  - Afbreken zwangerschap als aangedaan
  - Goede counseling om geïnformeerde keuze te garanderen

Breder

- Welke test wordt vergoed in de gezondheidszorg?
- Wie bepaalt wat voldoende “klinisch nut” heeft?
- Mag je een geneesmiddel niet meer voorschrijven zonder eerst een bepaalde test te doen? (compagnion diagnostic)

Goede test?

- IVD regulation van kracht in 2022
  http://ec.europa.eu/growth/sectors/medical-devices/regulatory-fra



- Genetische test in hogere risicocategorie
- “Notified bodies” nodig voor toezicht op testen

- Kanalisatieregeling om testen voor **hoog risico op ernstige ziekte** alleen in Universitair Medische Centra toe te laten?

- DTC bedrijven?
- “Postorderbedrijven”?

Anonimiteit?

- Spermadonor anomiem?
  - via DTC bedrijf opsporen halfbroers/ halfzussen
- Forensisch
  - Alle mannelijke inwoners van omgeving Zwaagwesteinde testen om de moordenaar van MV te identificeren
  - Via identiek Y-chromosoom van broer/zoon/neef
  - DNA voor daderprofiel
  - Nader gebruik van lichaamsmateriaal uit research of zorg (minister VWS Schippers mei 2017)
- Mijn DNA=50% van het DNA van mijn kinderen, broers, zussen

Samenvattend

- Er kan veel in theorie
  - Maar kijk uit voor hype en hoop
- Er moet nog veel meer naar de praktijk van de gezondheidszorg
  - (translationeel onderzoek)
- Juridisch: vergoeding, toelating, individu/familie, anonimiteit