

## *Farmacogenetica – analysetechnieken*

PUOZ labdag 22-11-2016

Dr. Jesse Swen, ziekenhuisapotheker  
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Sectiehoofd Laboratorium  
Klinische Farmacie & Toxicologie  
Leids Universitair Medisch Centrum



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## Is Your Medicine Right for You Metabolism?

More genetic tests aim to help predict how people might respond to many common medications

By MELINDA BECK

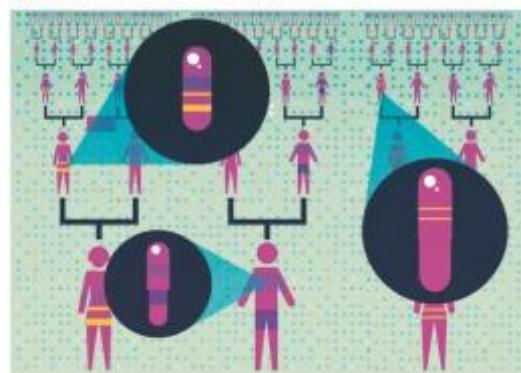
Updated March 14, 2016 3:05 p.m. ET

People can respond to drugs very differently. A medication that brings relief for some patients might show no benefit at all in others, or even cause harmful side effects.



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### The right drug for you

Personalized prescribing is gaining momentum, but is there enough evidence for it to become standard clinical practice?

BY LISA EHR

**F**or ten-year-old Isabella, getting her medicine was not going to work. Given the standard drug regimen, there were strong odds that she would have to take a break from school and make life sick, requiring a break in her therapy that would last the summer at least.

That's when her doctor, Dr. Michael

metabolizing those drugs, Isabella, for instance, has only one of the most common enzymes that break down certain drugs and eliminate them from the body. This means that she will make life sick, requiring a break in her therapy that would last the summer at least.

Testing Isabella's plasma for her TPMT status is one of the most common enzymatic tests done in hospitals and clinics. If her enzyme levels are normal, as is usually done, it predict the likelihood of adverse reactions. The results will be presented to her doctor immediately. The choice of which drug to use and at what dose



## 'Geef elke Nederlander een medisch dna-paspoort'

Dna-paspoort kan ernstige bijwerkingen voorkomen

Geef elke Nederlander een medisch dna-paspoort. Dat kan veel ernstige bijwerkingen tijdens behandelingen en zelfs sterfgevallen voorkomen. Dat

i farmacogenetica Ron van Schaik, in het Erasmus MC in -Jan Guchelaar in het Leidse LUMC.

Medical News & Perspectives

### Getting Pharmacogenomics Into the Clinic

Jennifer Abbasi

W

hat if there were a way to figure out if a patient would respond to a medication before it was prescribed? Or to predict bleeding risk from an anticoagulant?

In recent years, advances in genetic testing have made this kind of precision medicine possible, both within certain categories, like physician admissions in growing fields, say experts in the growing field of pharmacogenomics.

"While we've made tremendous rapid advances in terms of basic science and technology, when it comes to some gene-drug pairs, clinical implementation unfortunately hasn't been lagging behind," said Dr. A. Marcella Pergolizzi, MTS, associate professor and director of the Center for Pharmacogenetics and Pharmacogenomics Research at the University of Illinois at Chicago College

of Pharmacy.

Knowing this information can help clinicians choose the right medication for each patient.

A patient who has a genetic variant associated with slow metabolism of the drug diclofenac, for example, might experience major bleeding on standard doses. In contrast, a patient with a variant associated with fast metabolism might not get any benefit from the standard dose, yet remain at greater risk of a major stroke or thromboembolism.

How Does Pharmacogenomics Work?

"Genetic variability affects essentially every single drug," says Dr. Daniel R. Fierman, director of the Center for Personalized Therapeutics at the University of Michigan.

"For example, if you're taking a statin drug to reduce your cholesterol level, it may not work for everyone, although 80% of them—80%—agreed their drug response may be influenced by genetic variations."

Genetic variability can also influence a patient's response to cancer treatments, for instance, or to immunotherapy.

"It's likely that a time will come in the near future where patients will start to demand the use of such information during care—that they will ask of their physicians, 'Have you considered my genetics?' before accepting a prescription,'" said Peter H. O'Donnell, MD, associate director

of the National Institute of Child Health and Human Development.

In the results of a nationwide survey by the American Medical Association and Medco released in 2012, only 10% of more than 10,000 responding physicians had ordered a pharmacogenomic test in the past year, although 80%, although only 38% of them—38%—agreed their drug responses may be influenced by genetic variations.

Genetic testing can be expensive, however. For instance, a pharmacogenomic test, or PGx, is recommended to become routine in genetic profiling for breast cancer.

"Eds is likely that a time will come in the near future where patients will start to demand the use of such information during care—that they will ask of their physicians, 'Have you considered my genetics?' before accepting a prescription,'" said Peter H. O'Donnell, MD, associate director



DR. J.J. SWEN, ZIEKENHUISAPOTHEKER  
1 minuut en 33 seconden

Hart van Nederland.nu

Gezondheid van Nederland

## 'DNA-paspoort voor alle Nederlanders'

Gepubliceerd: 30 april 09:04

Update: 30 april 21:20

Alle Nederlanders moeten een DNA-paspoort krijgen. Daarin staan de genen die bepalen of iemand afwijkend op een medicijn of behandeling reageert. Verschillende artsen en hoogleraren van het Erasmus MC en het LUMC pleiten daarvoor.

# Personalised = trendy



# 'Most drugs don't work'

Effective (%).....

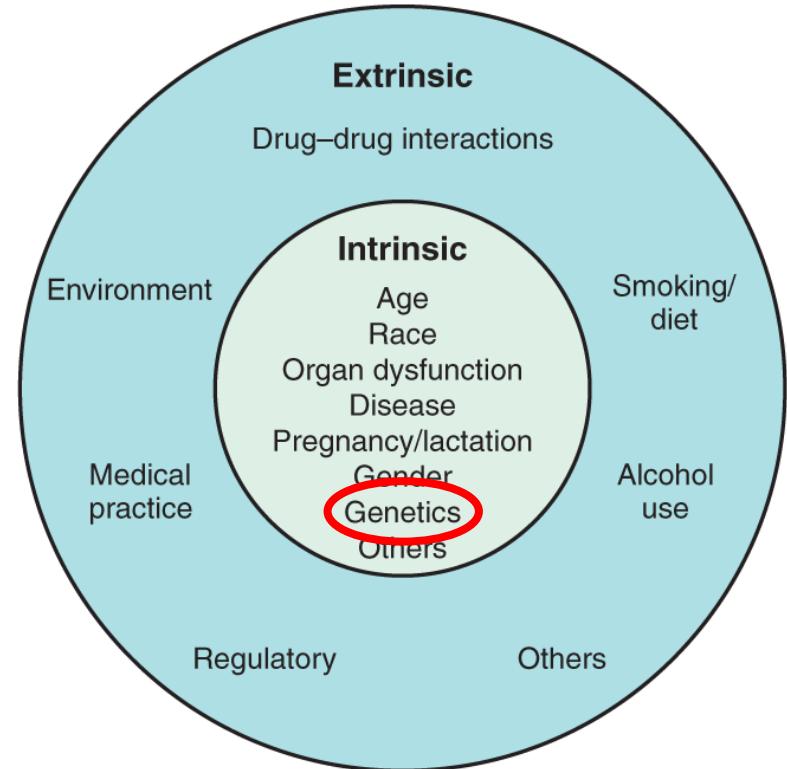
- Alzheimer 30
- Depression (SSRI) 62
- Asthma 60
- Diabetes mellitus 57
- Migraine (acute) 52
- Migraine (profyl.) 50
- Cardiac dysrhythmia 60
- Tumors 25
- Schizophrenia 60
- Reumatoid arthritis 50
- Reumat. art. (Cox-2) 80
- Hepatitis C 47



# How is this possible?

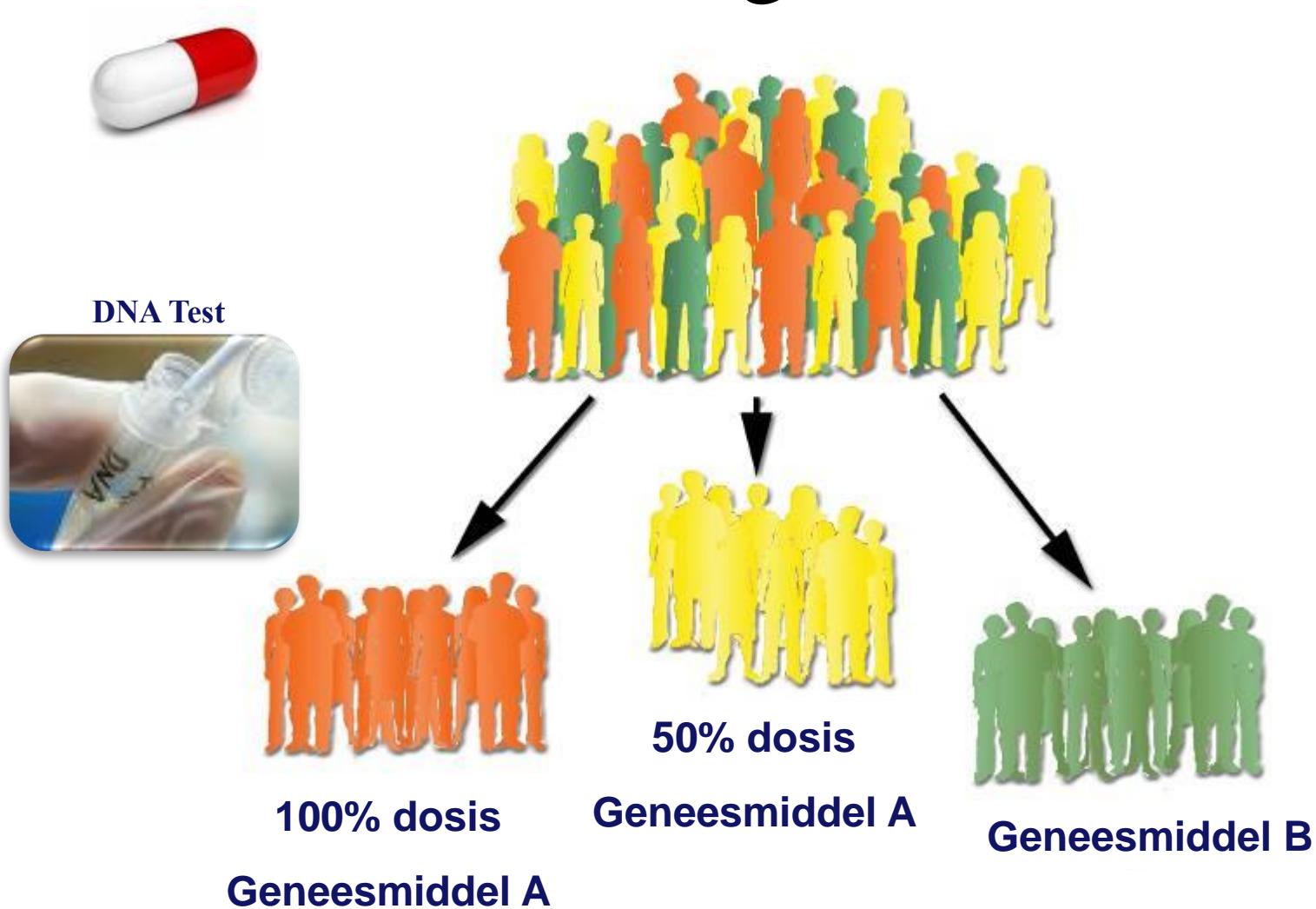
- Non-adherence
- Wrong drug-use
- Interacting co-medication
- Organ dysfunction
- Different stage of the disease
- Environmental factors
  - food, alcohol

....



**Heritable (genetic) differences in drug sensitivity**

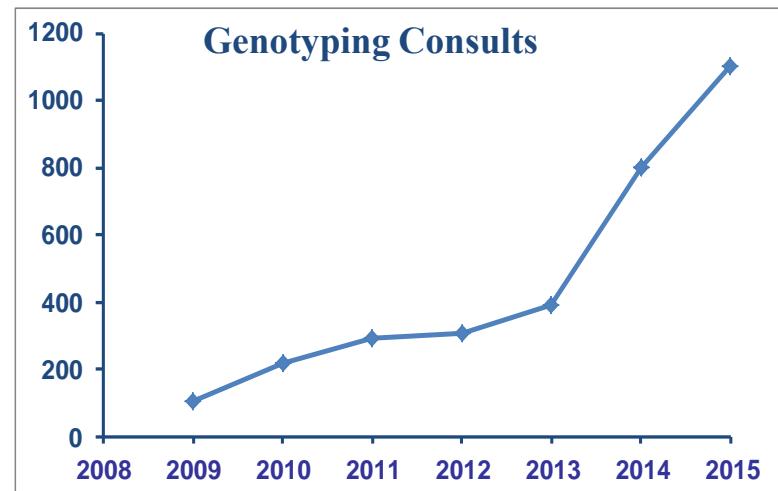
# Farmacogenetica



# Pharmacogenetics @ LUMC

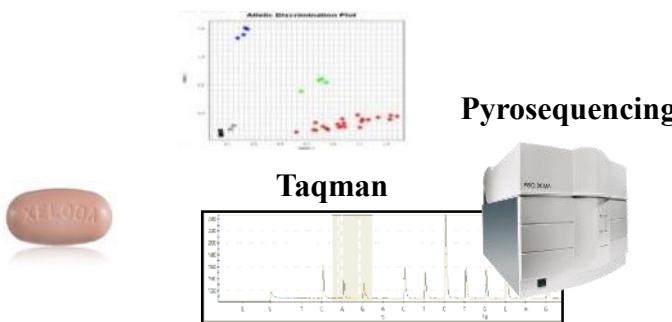
## Prospective test:

- Oncology: patients receiving CAP or 5-FU tested for *DPYD* (rs3918290, rs55886062, rs67376798, rs56038477)
- Nephrology: kidney transplant patients tested for *CYP3A5* (rs776746, rs10264272).
- Psychiatry: patients with a therapy resistant depression, referred to LUMC for ECT, tested for *CYP2D6*, and *CYP2C19*.
- PGx consultation service for outpatients



On request: VKORC1, CYP2C9, SLCO1B1, UGT1A1, THYMS, ABCB1, NAT1, NAT2, CYP3A4, CYP2B6 other....

## Workflow example: Individualization of fluoropyrimidine therapy



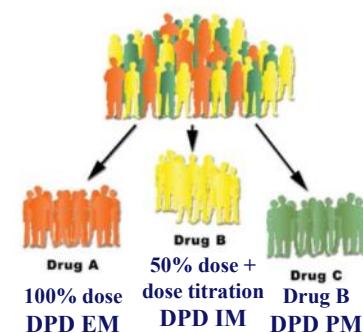
Rx  
fluoropyrimidine

Genotyping with 2  
independent techniques

Technical approval

Interpretation and  
Consult: Hospital  
Pharmacist

Individualized Dose  
Recommendation



# Van monster naar DNA

Monstername

DNA isolatie

“QC”

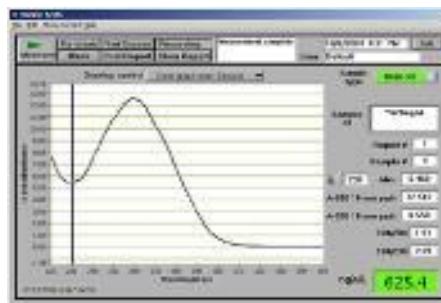
DNA voor analyse



2 ml speeksel



4 ml EDTA bloed



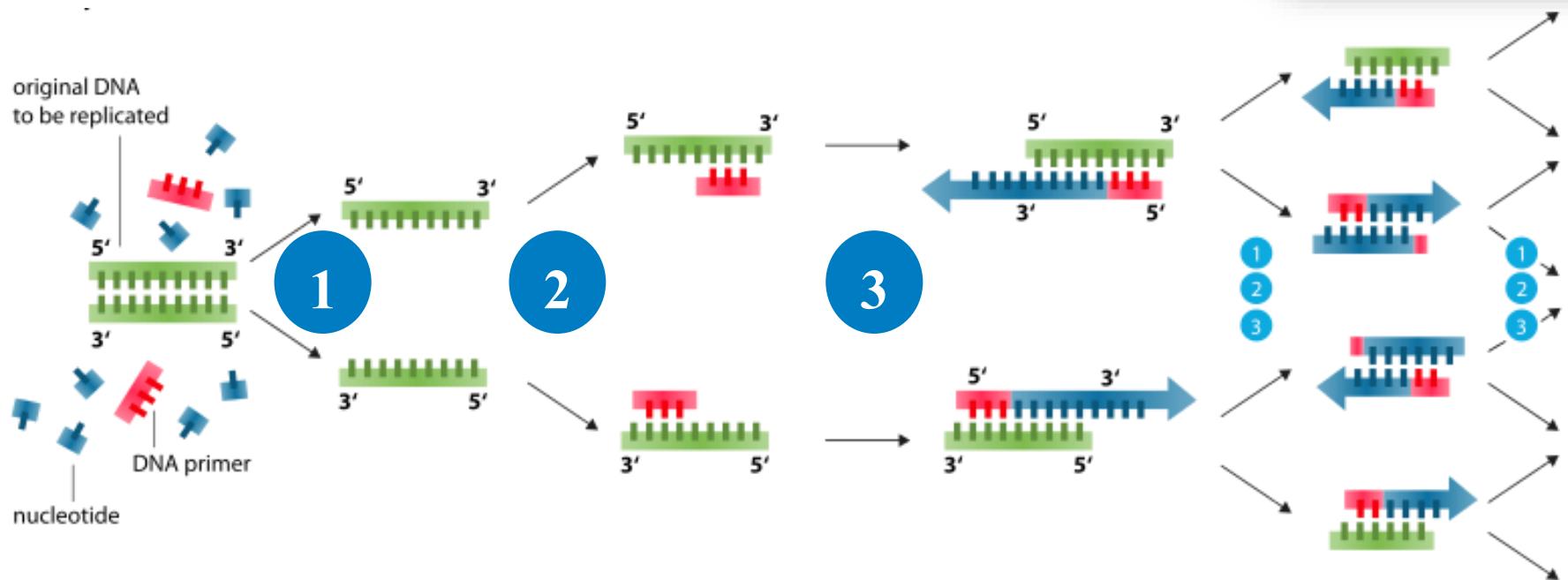
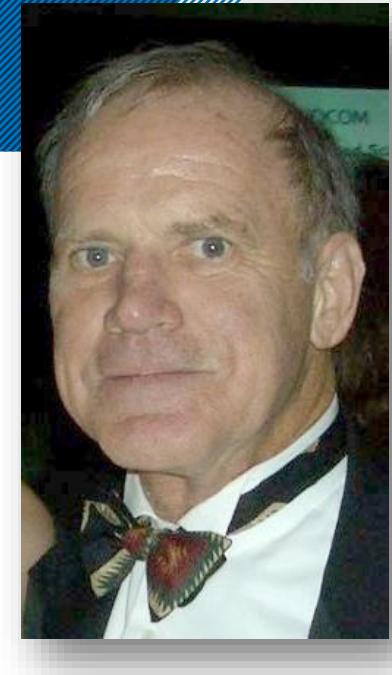
260/280 ratio → 1,5 - 2



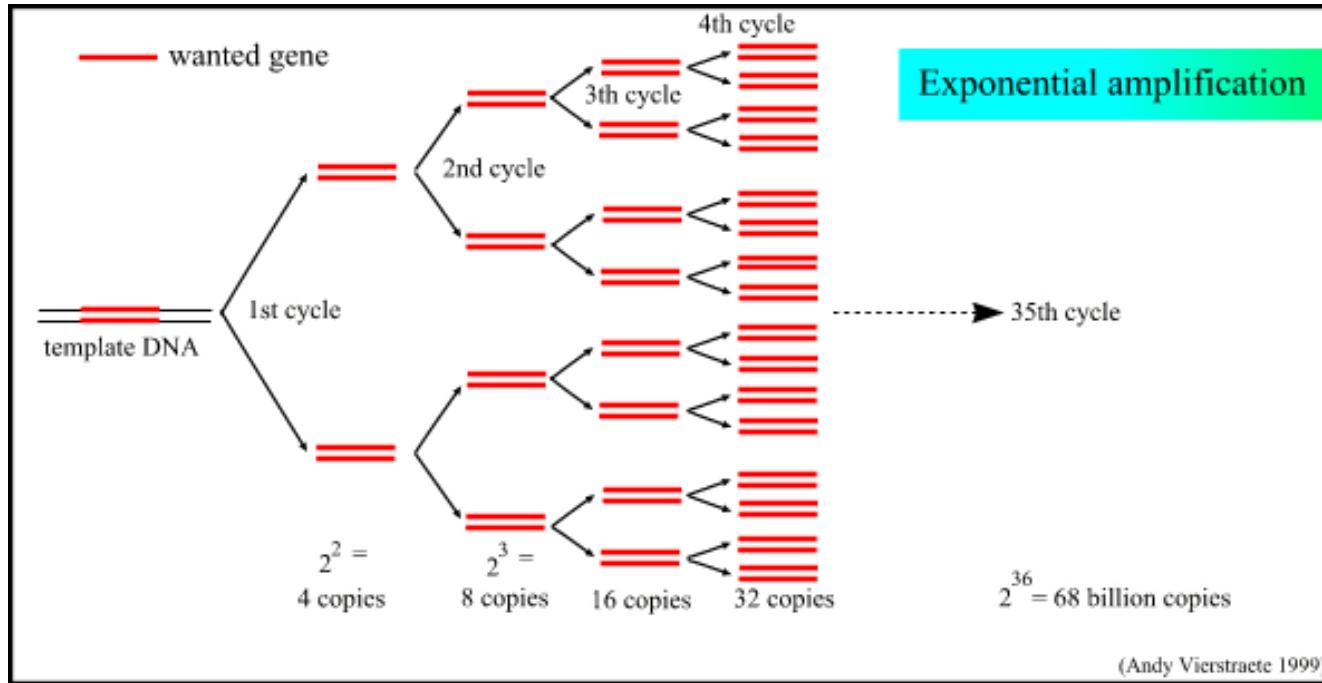
# Polymerase Chain Reaction

Invented in 1983 by Kary Mullis, Nobelprice in 1993

1. Denaturation (90 - 96°C)
2. Annealing of primers (45 - 65°C)
3. Elongation by thermostable polymerase (72°C)



# Polymerase Chain Reaction



1983



1993



2003



## Beschikbare methoden

- PCR gel electroforese
- Real-time PCR genotyping assays
- Pyrosequencing
- Genotyping SNP Array
- High Resolution Melting
- Sanger sequencing
- Next Generation Sequencing

# Techniek 1 Real-time PCR: TaqMan

## PacMan



- Real-time PCR
- Addition of labeled probes
- Easy to perform; pre-designed assays
- Fluorescence

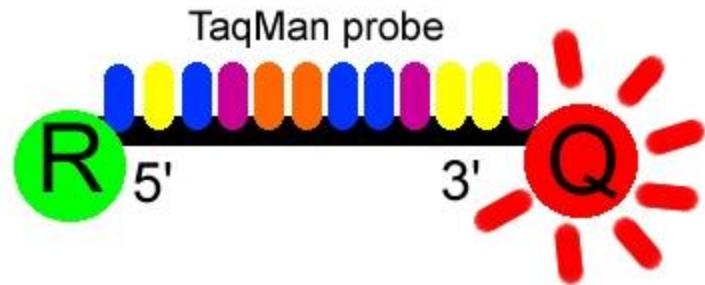


LightCycler® 480 System

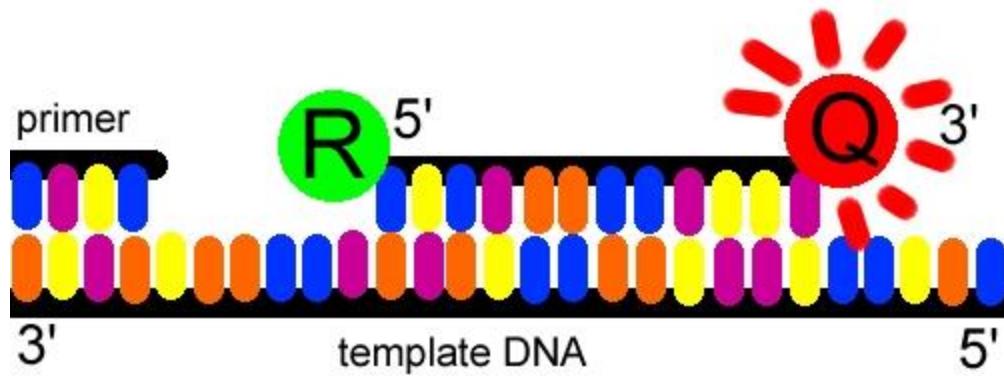
ViiA™ 7 Real-Time PCR System

QuantStudio 12K Flex RT PCR System

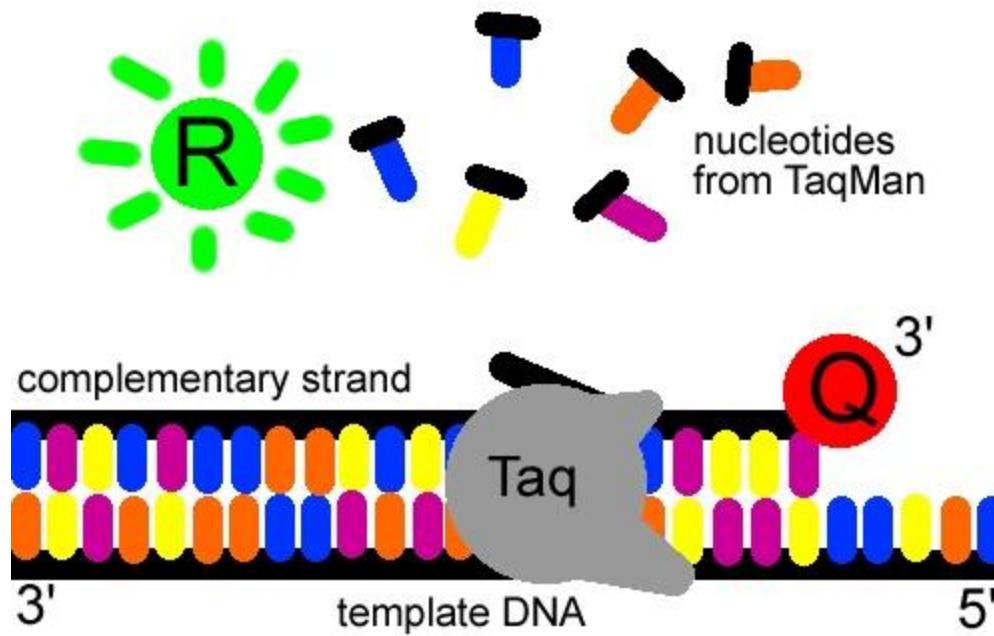
# Principle: probe based SNP detection



# Annealing



# Cleavage

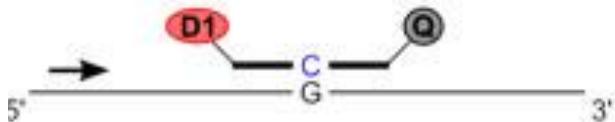


# SNP detectie; verschillende probes

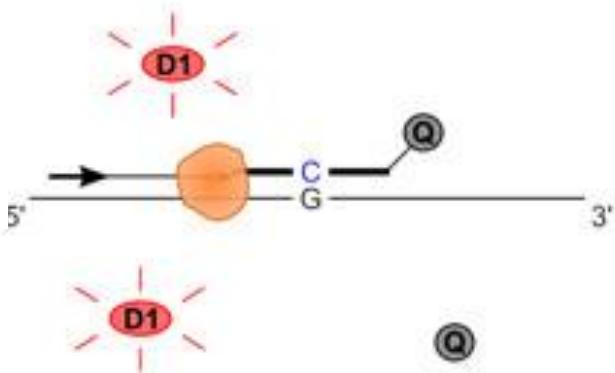
Perfect match TaqMan® probe

Single mismatch TaqMan® probe

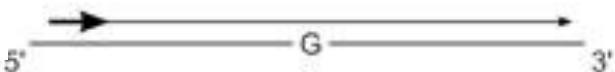
Hybridization



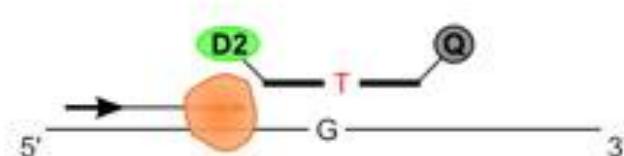
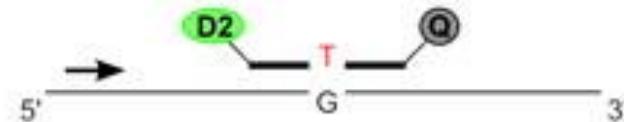
Extension



Completed



Probe cleavage: signal



Probe displacement: no signal

D1 : Dye 1

D2 : Dye 2

Q : Quencher



: DNA polymerase

→ : Forward primer

# Hoe ziet dat er in de praktijk uit?

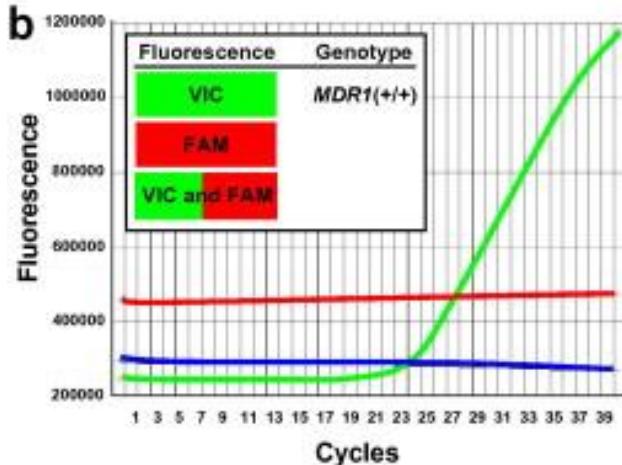
## a normal allele *MDR1*(+)



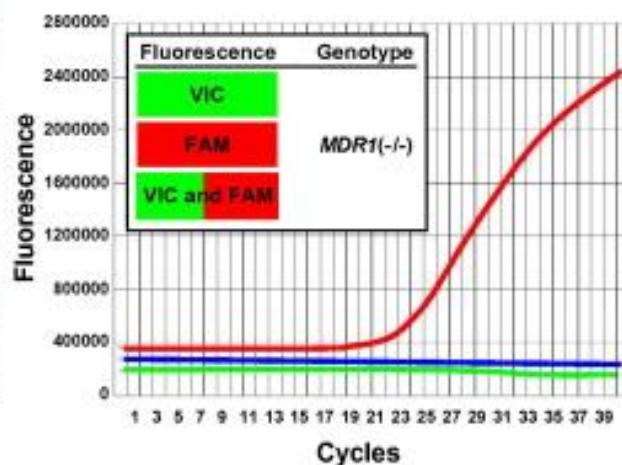
## nt230[del4] mutant allele *MDR1*(-)



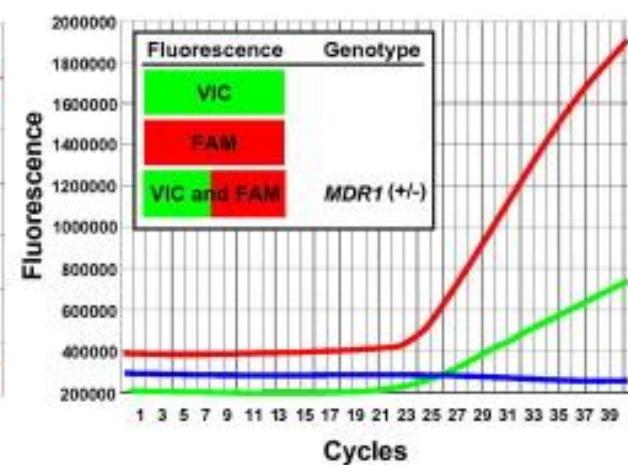
**WT/WT**



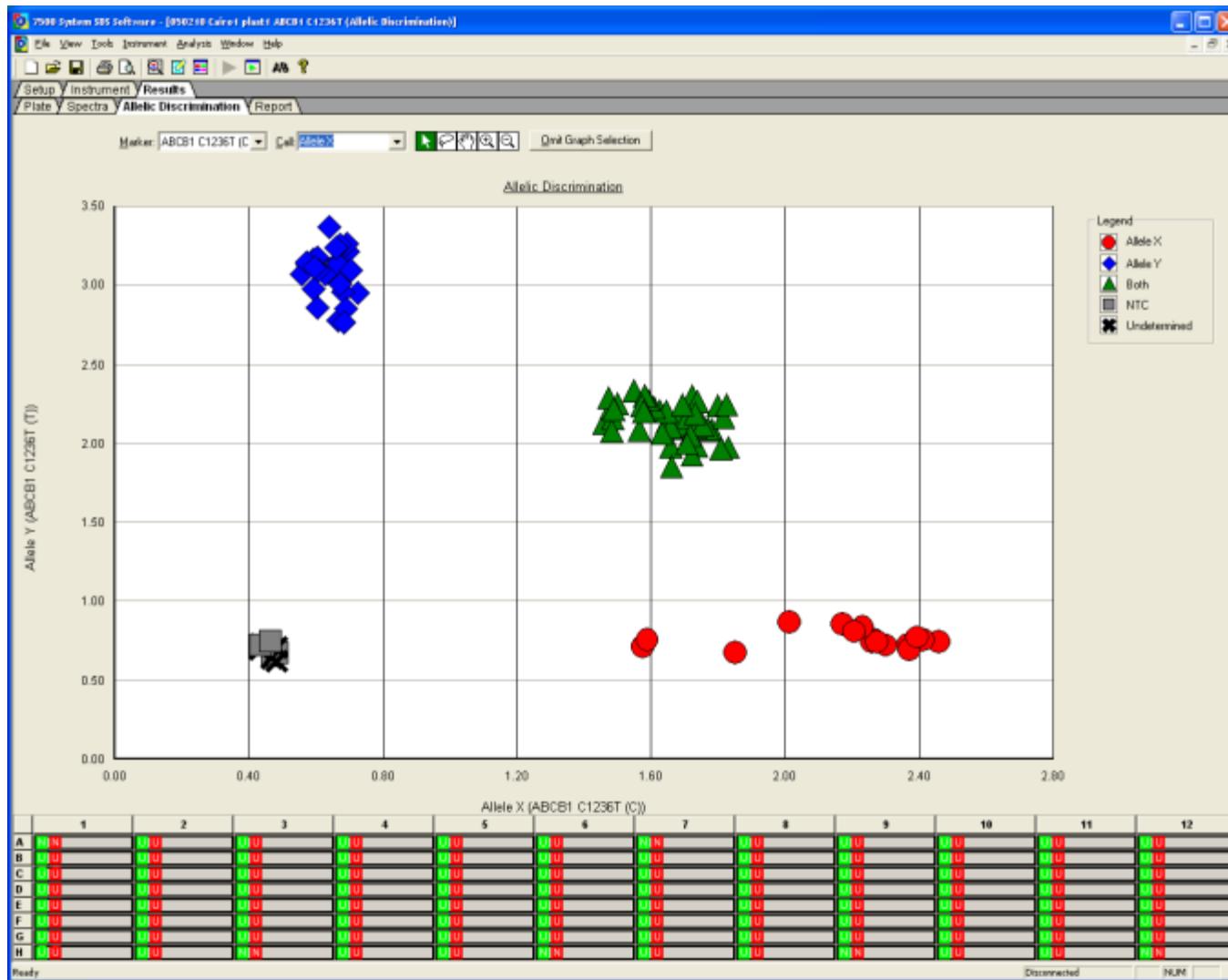
**MUT/MUT**



**WT/MUT**



# Een typisch resultaat

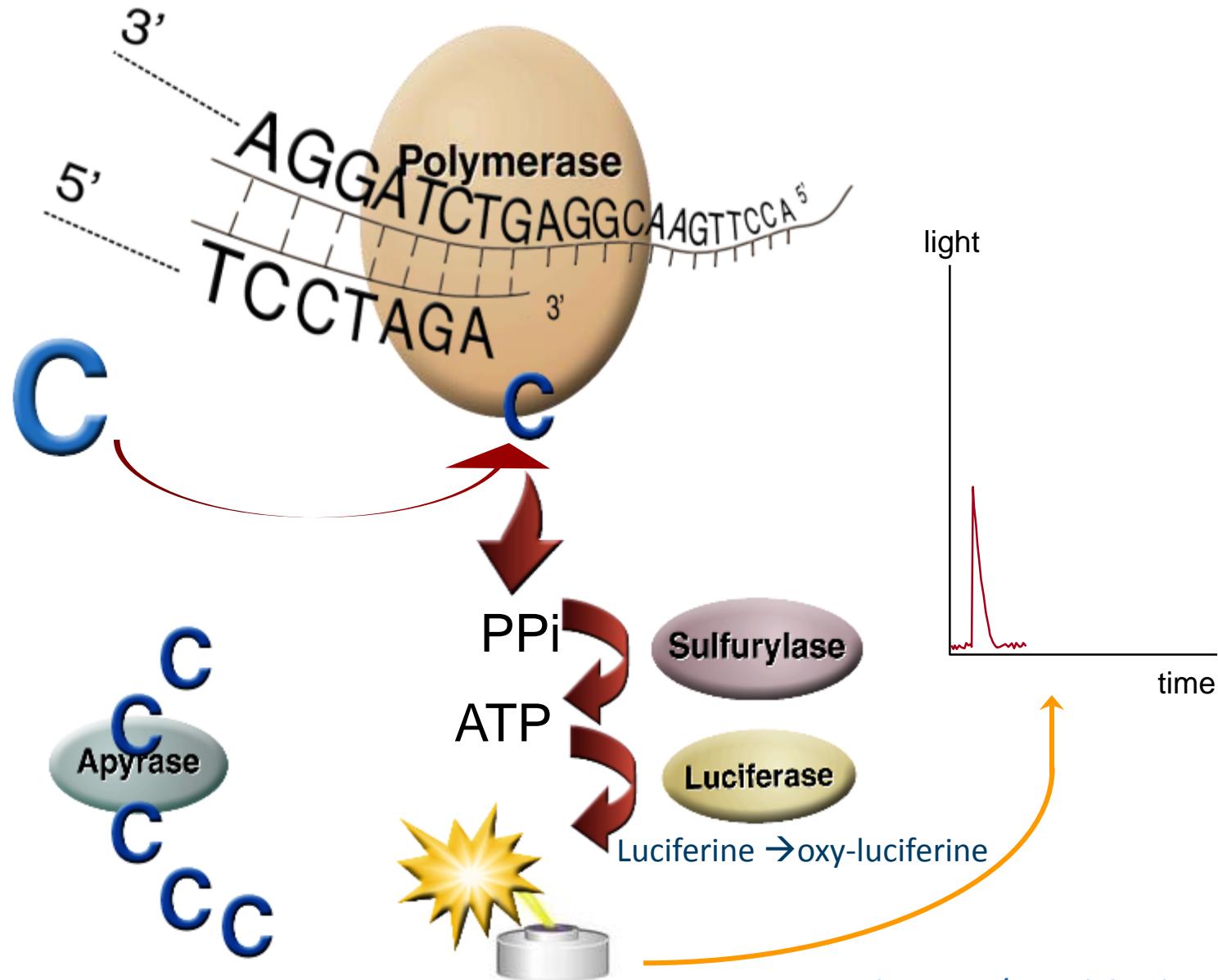


## Techniek 2: Pyrosequencing



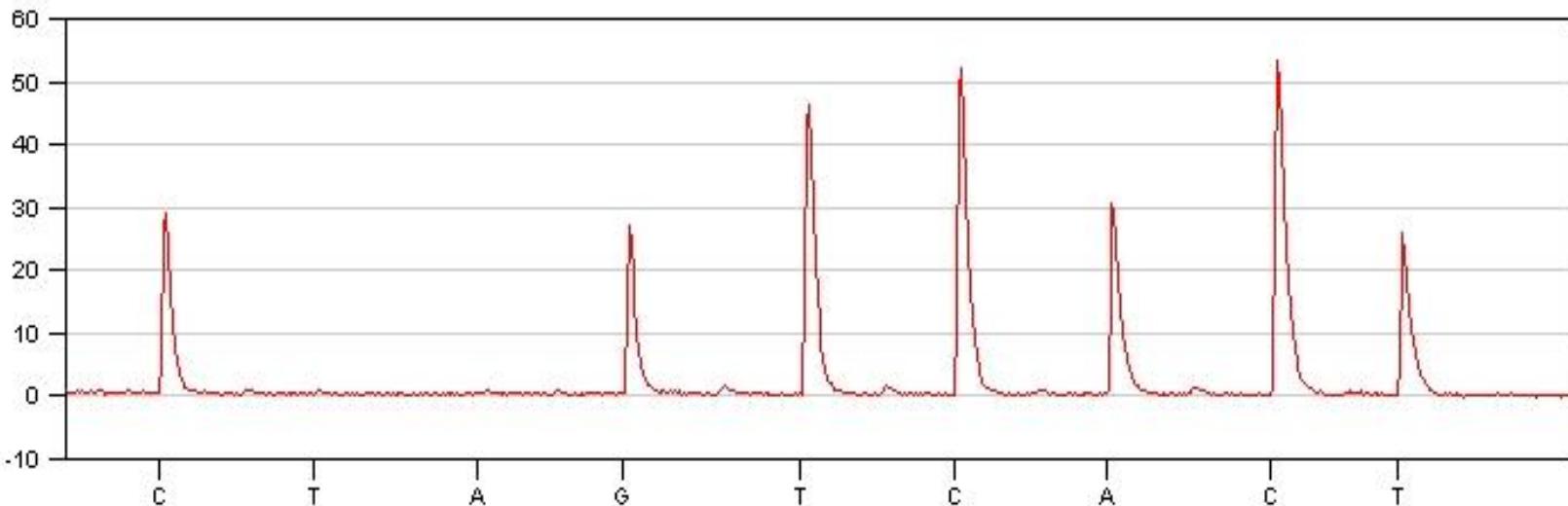
- “Sequencing by synthesis” principle
- Ontwikkeld in 1996
- 4 enzymen
- Chemiluminescentie,
- Reads van ~300-500 nucleotiden

# Principle



- Each peak represents the incorporation of 1 nucleotide.
- 1 nucleotide is added at a time.

**3'-----GCAAGGTGGA-----5'**



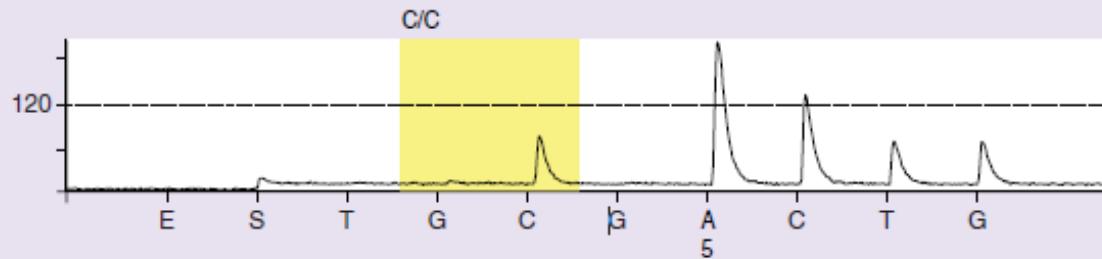
# Quality Control— plasmide controles



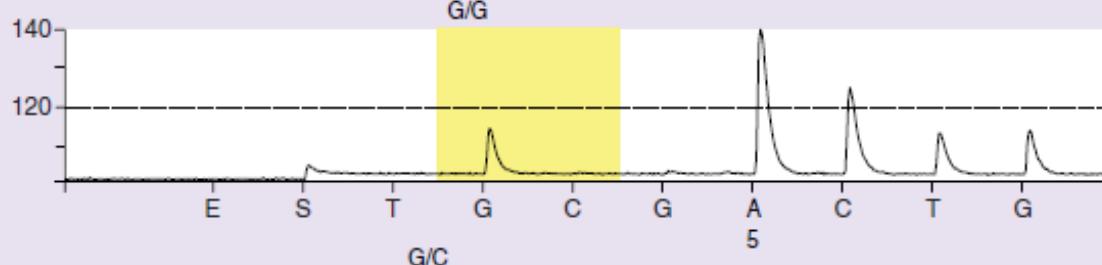
Figure 1. Example of validation report of *TPMT\*2*.

A

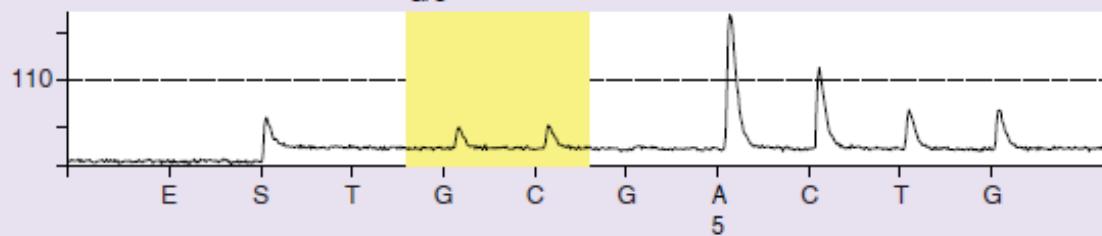
Plasmid 29  
Entry: *TPMT\*2* rs1800462  
Position 1: C/C (passed)



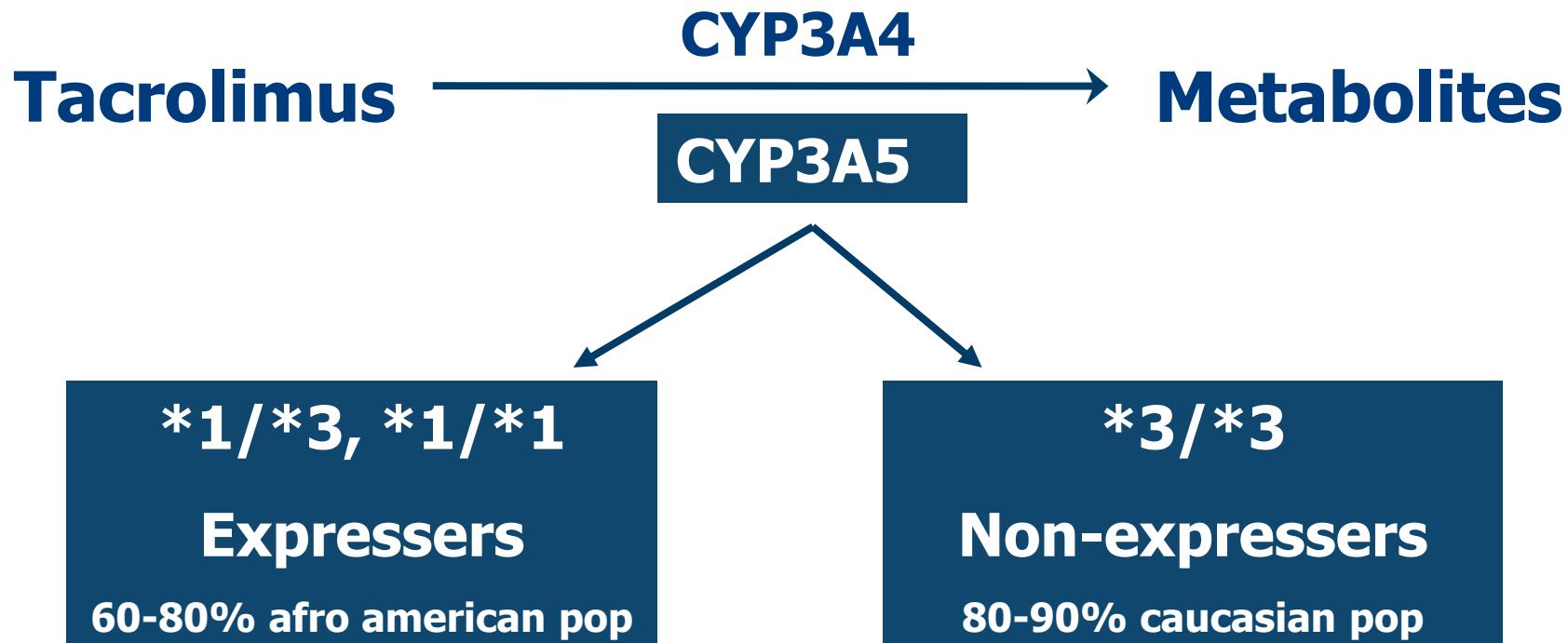
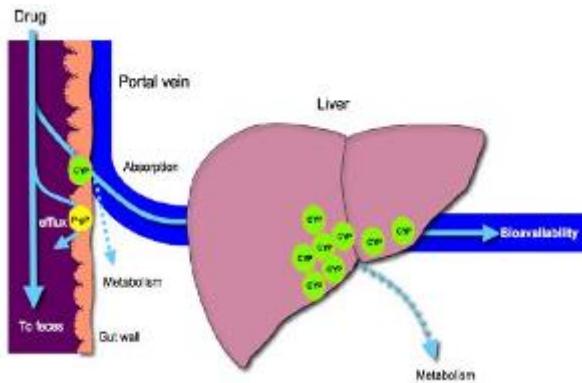
Plasmid 30  
Entry: *TPMT\*2* rs1800462  
Position 1: G/G (passed)



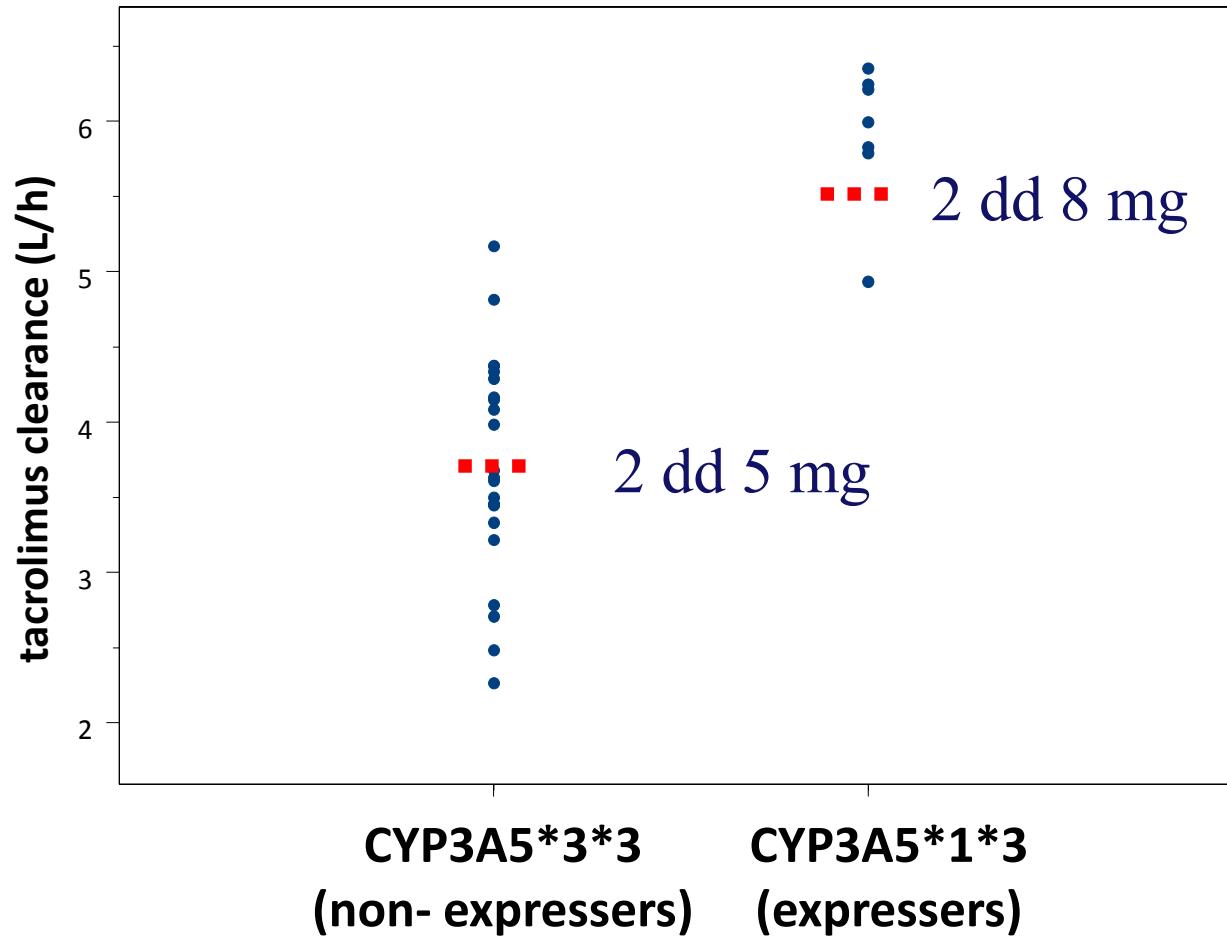
Plasmid 29 + 30  
Entry: *TPMT\*2* rs1800462  
Position 1: G/C (passed)



# Example 1:tacrolimus



## Adult kidney transplant patients



# Case: Mind the Mix

20 yr old female, referred for PGx test prior to kidney transplantation

PGx tests :      *CYP3A5\*3 (rs776746)*  
                      *CYP3A5\*6 (rs10264272)*

*Conflicting genotype results.....*

nature publishing group

PERSPECTIVES

## LETTERS TO THE EDITOR

### Pharmacogenetics in Transplant Patients: Mind the Mix

MH ten Brink<sup>1</sup>,  
T van der Straaten<sup>1</sup>,  
H Bouwsma<sup>2</sup>, R Baak-Pablo<sup>1</sup>,  
HJ Guchelaar<sup>1</sup> and JJ Swen<sup>1</sup>

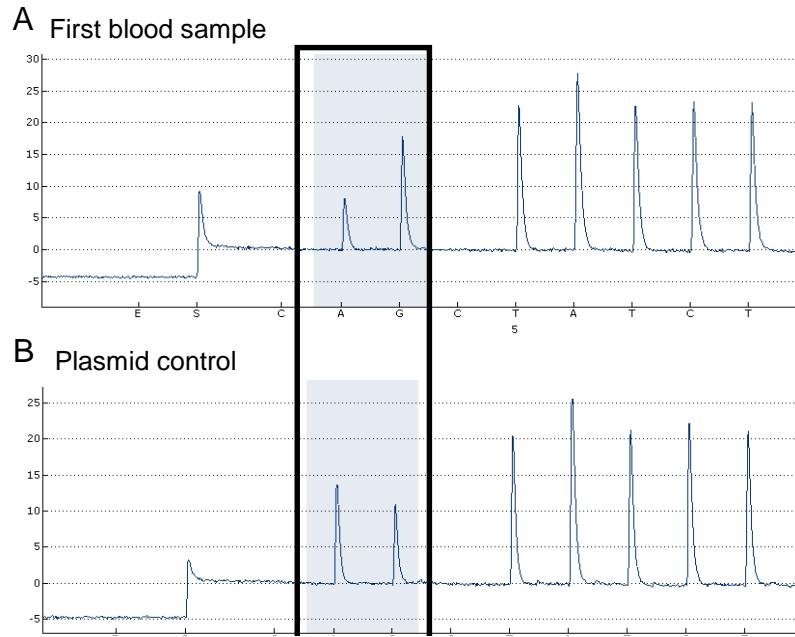
**To the Editor:** Several consortia have published guidelines to aid clinicians with the interpretation of pharmacogenetic test results,<sup>1,2</sup> and an increasing number of medical centers are implementing prospective genotyping.<sup>3</sup> Among these, there are many highly specialized care centers with complex patient populations. These patients may present unexpected

challenges, as demonstrated by this case.

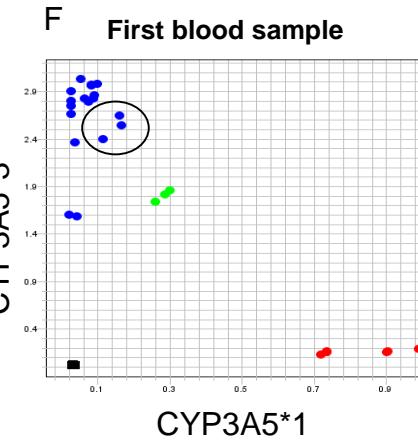
A 20-year-old women was admitted for a living-related kidney transplant. A standard quadruple immunosuppressive regimen was prescribed (basiliximab induction, tacrolimus, mycophenolate, and prednisolone). Tacrolimus is metabolized by cytochrome P450 (CYP)3A4 and

# First blood sample: conflicting results

PSQ



CY3A5\*1/\*3  
→ 1.5x normal dose

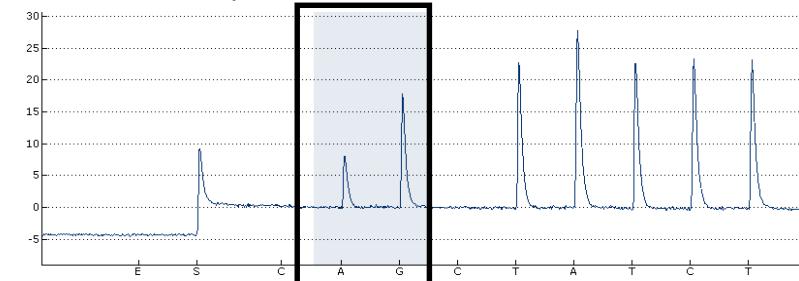


TaqMan

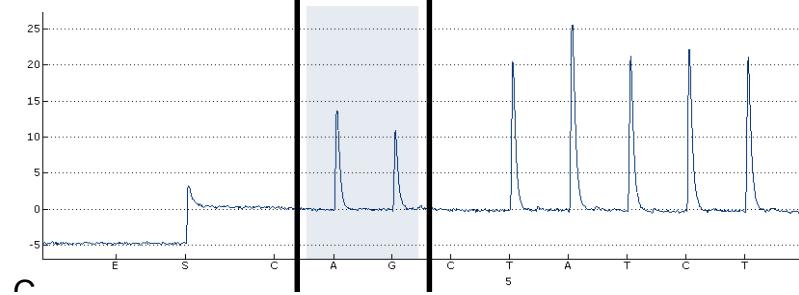
CYP3A5\*3/\*3  
→ normal dose

## Second blood sample

A First blood sample



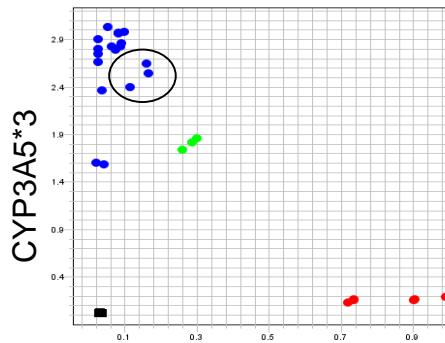
B Plasmid control



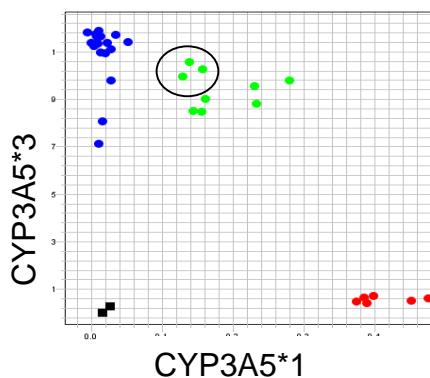
**CYP3A5\*1/\*3**

→ 1.5x normal dose

F First blood sample



G Second blood sample



**CY3A5\*1/\*3**

→ 1.5x normal dose

## Consulted attending nephrologist:

- Hematopoietic stem cell transplantation
- Mixed hematopoietic chimerism
- 28% autologous, 72% donor

Saliva results of patient and donor

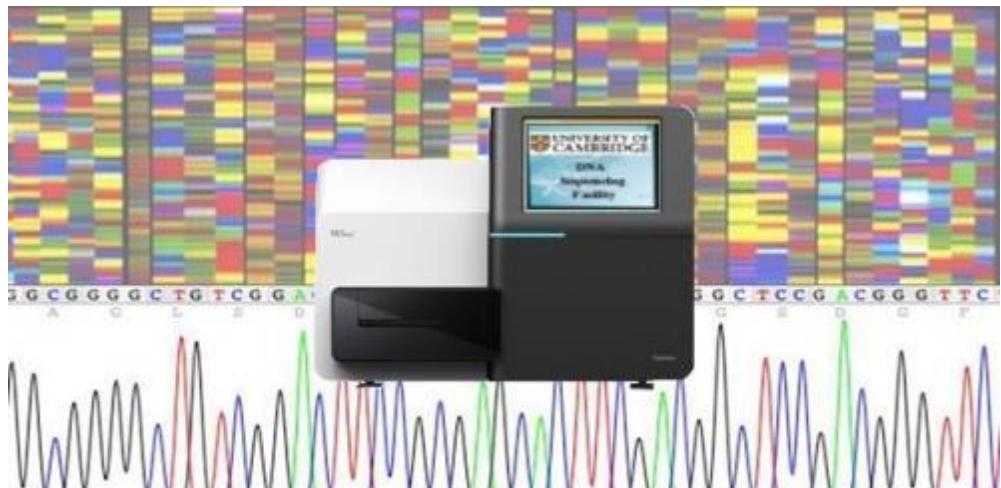
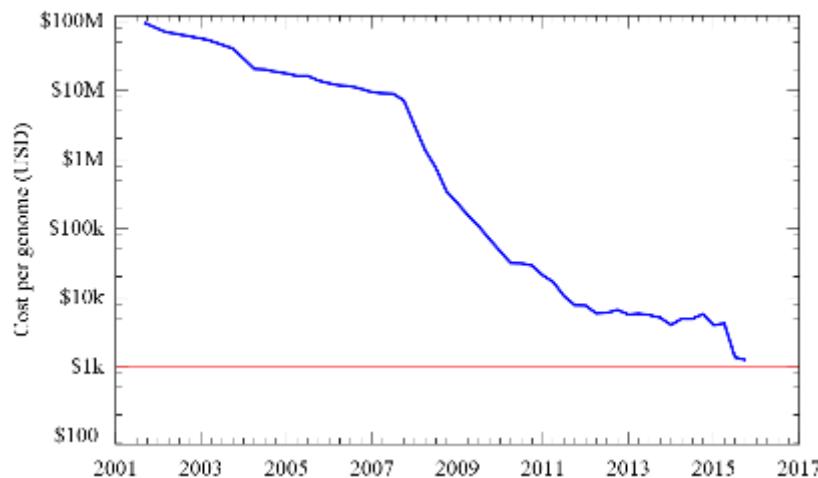
- Patient: *CYP3A5\*1/\*3*
- Donor: *CYP3A5\*3/\*3*

Patient tacrolimus trough level: 5.5 µg/L,  
AUC: 110 µg/hr\*I → 2dd 8 mg of tacrolimus

# WAAR GAAT HET VELD NAAR TOE?



## Cost to sequence a human genome



**For \$999, Veritas Genetics Will Put Your Genome on a Smartphone App**

Getting your entire genome decoded is now more affordable than ever. Will consumers buy it?

by Antonio Regalado March 4, 2016

MIT  
Technology  
Review

**Price of sequencing your genome falls to \$999**

By Gary Robbins | 2:17 p.m. March 4, 2016



Announced at Future of Genomic Medicine Conference  
4 March 2016

U-T  
San Diego

Common Medical Test/Scan/Med	Cost*
Head CT scan	\$1,200
Abdominal CT scan	\$1,420
MRI scan	\$2,611
Echocardiogram	\$1,300
10 "Most Important" Lab Tests*	\$ 319
Crestor 1 year supply from Costco	\$3,222
Xarelto 1 year supply from Costco	\$4,444
3 Pharmacogenetic tests Quest^	\$ 975
<b>Whole Genome Sequence</b>	<b>\$ 999</b>

\* A "package" significantly discounted via DirectLabs.com

+ vary widely, these are average US charges from centers w/ data available on the web

^ 3 SNPs which account for 0.0000000001% of the genome

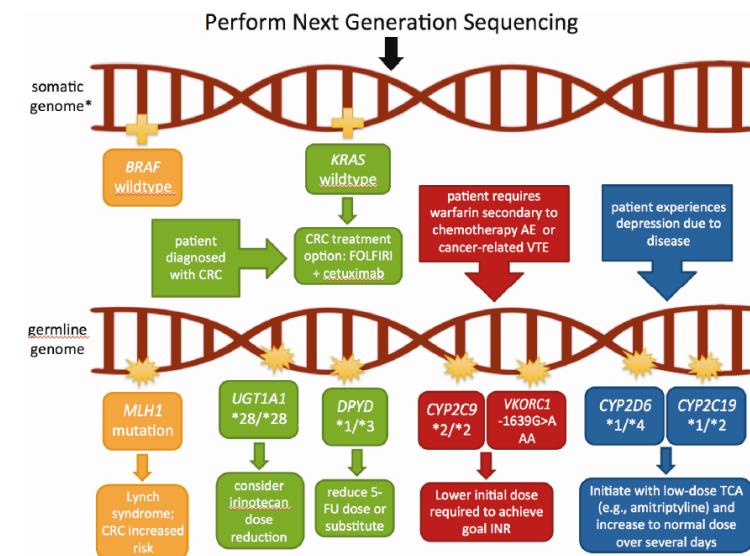
# Complimentary pharmacogenetic information

- WES, WGS data in clinical genetics
- WGS data in oncology



Tumor Genome

Germline Genome



# Dutch PGx Working Group (DPWG) guidelines ( $N=84$ )

## CYP2D6

- Amitriptyline
- Aripiprazol
- Atomoxetine
- Carvedilol
- Citalopram
- Clomipramine
- Clozapine
- Codeine
- Doxepine
- Flecainide
- Flupentixol
- Haloperidol
- Imipramine
- Metoprolol
- Mirtazapine
- Nortriptyline
- Olanzapine
- Oxycodeine
- Paroxetine
- Propafenon
- Risperidone
- Tamoxifen
- Tramadol
- Venlafaxine
- Zuclopentixol
- **DPYD**
- Capecitabine / 5-FU

## CYP2C9

- Acenocoumarol
- Phenprocoumon
- Phenytoin
- Glibenclamide
- Glicazide
- Glimepride
- Tolbutamide
- **UGT1A1**
- Irinotecan
- **VKORC1**
- Acenocoumarol
- Phenprocoumon
- **CYP3A5**
- Tacrolimus

## CYP2C19

- Citalopram
- Clopidogrel
- Imipramine
- Lansoprazol
- Moclobemide
- (es)Omeprazol
- Pantoprazol
- Rabeprazol
- Sertraline
- Voriconazol
- **TPMT**
- Azathioprine
- Mercaptopurine
- Thioguanine

Patient is linked to CYP2D6 status in health record

### CYP2D6 PM



Clomipramine is in drug database linked to CYP2D6PM and advice



Physician prescribes clomipramine

Physician gets advice on his screen

*Swen et al, Clin Pharmacol Ther 2008;83(5):781*

*Swen et al, Clin Pharmacol Ther 2011;89(5):662-73*



**A**

Pon  
Patientnummer 3760 63  
Alle klinische medicatie  
Poliklinische medicatie Klinische medicatie  
Reguliere medicatie  
Status: Actief  
PANTOPRAZOL TABLET M5R 40MG  
PARACETAMOL TABLET 500MG

Pon  
Patientnummer 3760 63  
NORTRIPTYLINE TABLET 25MG (ORAAL), 1 x per dag 100 mg  
Let op: er zijn afgeleide contraindicationen. [details](#)

Medicatie opdracht  
Geneesmiddel NORTRIPTYLINE TABLET 25MG  
Toedieningsweg ORAAL  
Geneesmiddel vrijekeutel  
Periode 04-05-2011 13:15 tot --:--  
Aantal 0 STUK Chronicus

Doseerschema  
Doseering Eenheid Duur Interv Notitie  
1d100 MG

Geneesmiddel waarschuwingen  alleen relevante  alle waarschuwingen  
Contra-indicatie: NORTRIPTYLINE TABLET 25MG - CYP2D6 POOR METABOLISME  
Teksten  
Kan het reactievermogen verminderen  
Pas op met alcohol  
 Bewaar als VMO  TNO gestopt  Eigen beheer  
Zoeken Herstel Detail

Details  
Arts akkoord Apotheek akkoord Geparkeerd TNO gestopt Afwijkende toediening onder voorbehoud Actief gepland gestopt Adhoc Geen bewaking

Gegevens van patiënt 3760 Onderhoud... Allergieën J.Y.M. Medicatielijst voor patiënt... [nieuwe zonde...](#)

Geboren 02-09-1947 M Aldeing HU/2/150/

Opnameperiode: <geen opname filter>

Stopdatum S... Motivatie Motivate in VCMO WC...

8:15 --:-- :-- 8:14 --:-- :--

Controle op contra-indicatie aard [00006561] 16564/00226157/CYP2D6 POOR METABOLISME  
Teksten  
CYP2D6-polymerisme leidt tot een verlaagde metabole capaciteit van plasmaconcentratie van nortriptyline. Verlaag de dosering tot 40% van de normale dosering en monitore de concentratie van nortriptyline voor de behoudende dosering.

**B**

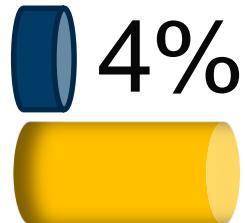
16:45

Zoeken Info

# Enquête arts en apotheker

Heeft u in de afgelopen 6 maanden een farmacogenetische test aangevraagd of aanbevolen?

Ja



~400 huisartsen  
~667 apothekers

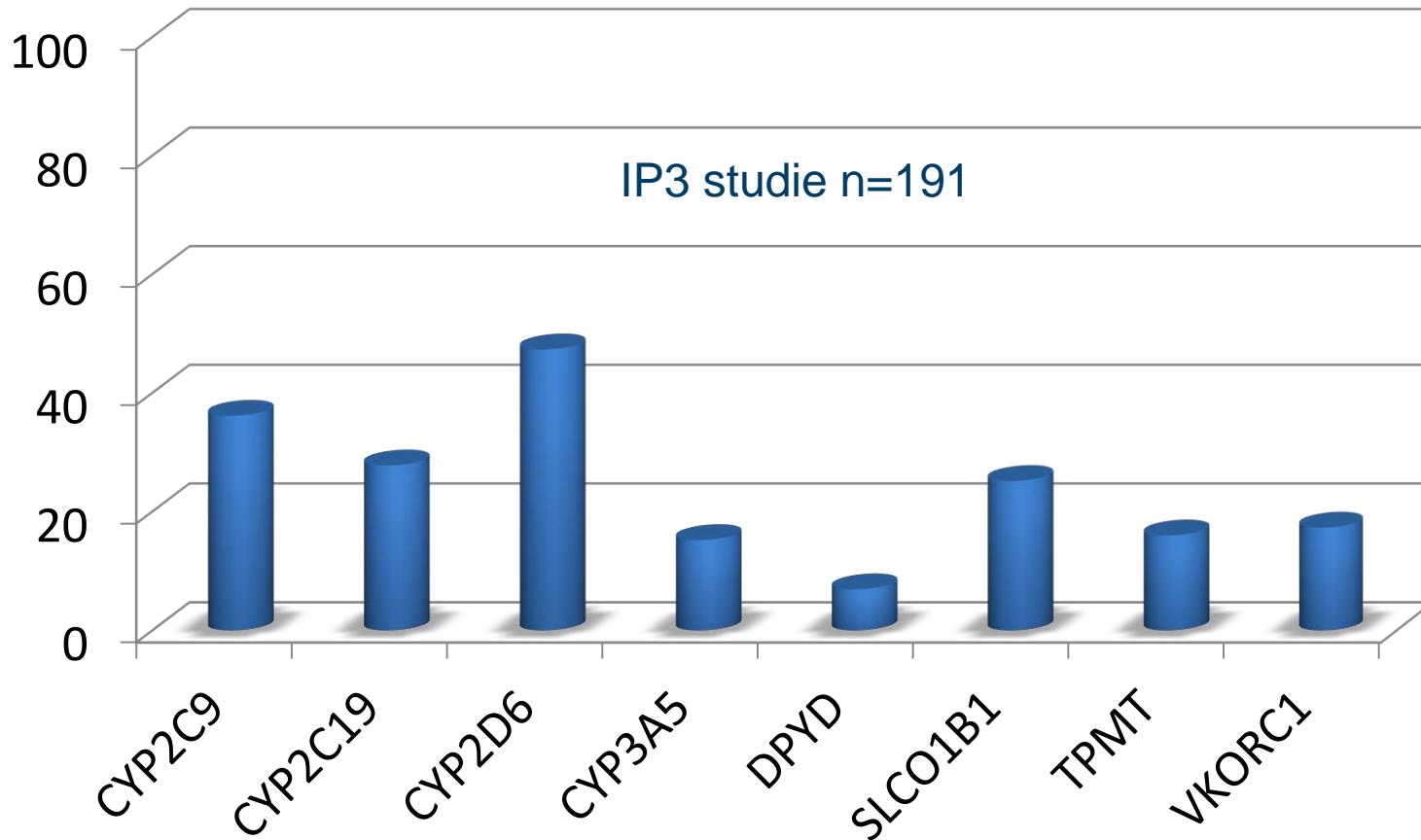
# Implementing PGx in Primary Care Project (IP<sup>3</sup>)



- 200 patients
  - Primary care, vicinity Leiden
  - Apply PGx in own practice
- Focus on primary care drug with recommendation  
*CYP2D6*: TCAs, atomoxetine, venlafaxine
  - *CYP2C9*: acenocoumarol, fenprocoumon
  - *CYP2C19*: (es)citalopram, imipramine,
  - *SLCO1B1*: simvastatine, atorvastatine
- Genotyping
  - Panel of 8 genes: *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *SLCO1B1*, *TPMT*, *VKORC1*



# Hoe vaak komt farmacogenetische variatie voor?



→ ~95% van de patiënten heeft ten minste 1  
**actionable** genotype, 10% heeft er  $\geq 4$



# U-PGx | Ubiquitous Pharmacogenomics

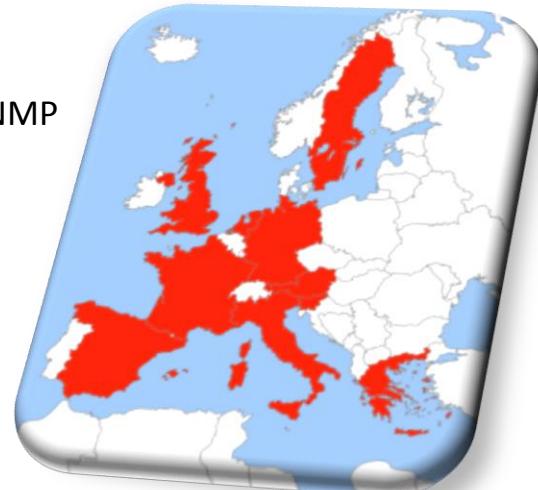


- €15 million, HORIZON2020, 10 EU countries
- Implement pre-emptive PGx testing in a real world clinical setting across 7 EU sites
- Evaluate **patient outcome** and **cost effectiveness** using solid **scientific methodology**
- Start 1-1-2016, 5 yr, n=8,000
- Consortium members:

- H-J Guchelaar (Coordinator),  
JJ Swen, M Kriek, LUMC
- M Pirmohamed, R Turner, UOL
- J Stingl, FDMD
- M Ingelman-Sundberg, KI
- M Karlsson, S Jönsson, PBUU
- M Schwab, E Schaeffeler, IKP
- VHM Deneer STZHM
- M Samwald, G Sunder-Plassmann, MUWV
- M van Rhenen, KC Cheung, KNMP
- C Mitropoulou, GHXF
- D Steinberger, BIOL
- CL Davila Fajardo, SAS
- G Patrinos, UPAT
- V Dolžan, ULMF
- A Cambon-Thomsen, UPS
- G Toffoli, E Cecchin, CROA



WWW.UPGX.EU



## Boodschap:

- Geneesmiddelrespons is erfelijk.
- Real-time PCR meest gebruikte techniek.
- Technologie en bewijs ontwikkelen zich razendsnel; het aantal mensen met een bekend genotype neemt toe.
- Farmacogenetica is:
  - niet 'iets zeldzaams'
  - niet 'iets academisch', 'voor de toekomst' etc.
- Arts en apotheker kunnen komende week nog een patiënt tegenkomen waarvoor een farmacogenetische test nuttig zou zijn.

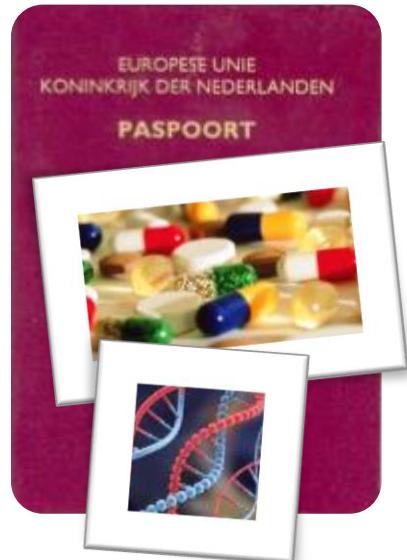


# Dank voor uw aandacht!



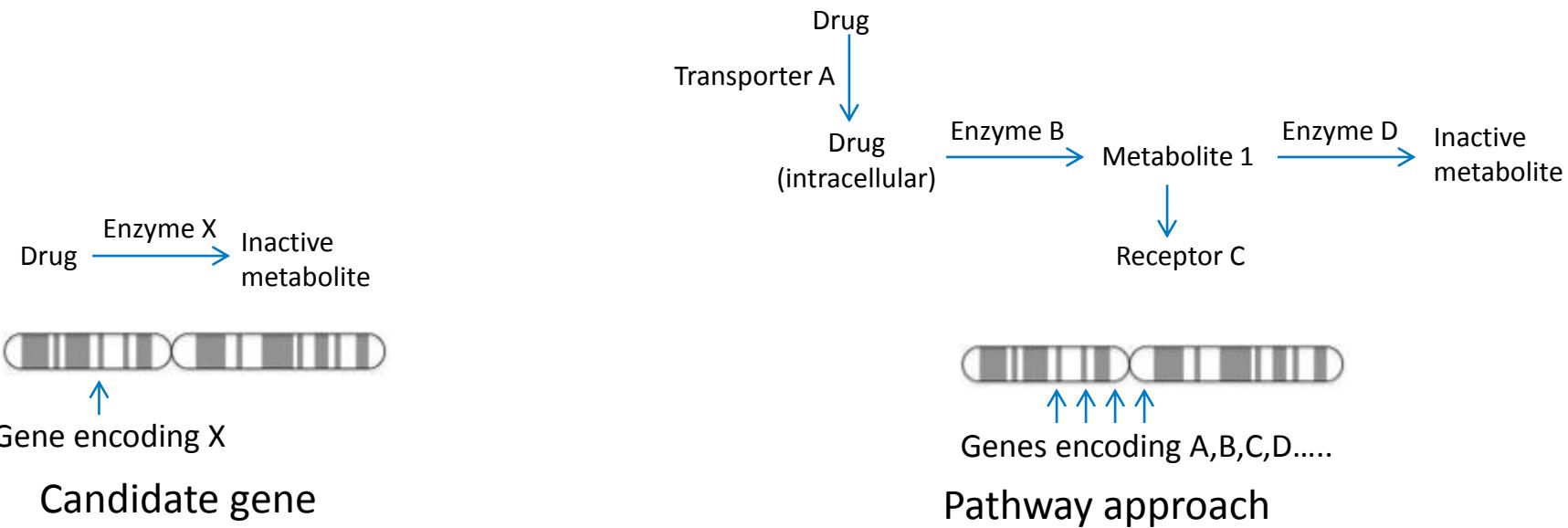
Email: [J.J.Swen@lumc.nl](mailto:J.J.Swen@lumc.nl)

# Wat betekent dit voor mij?



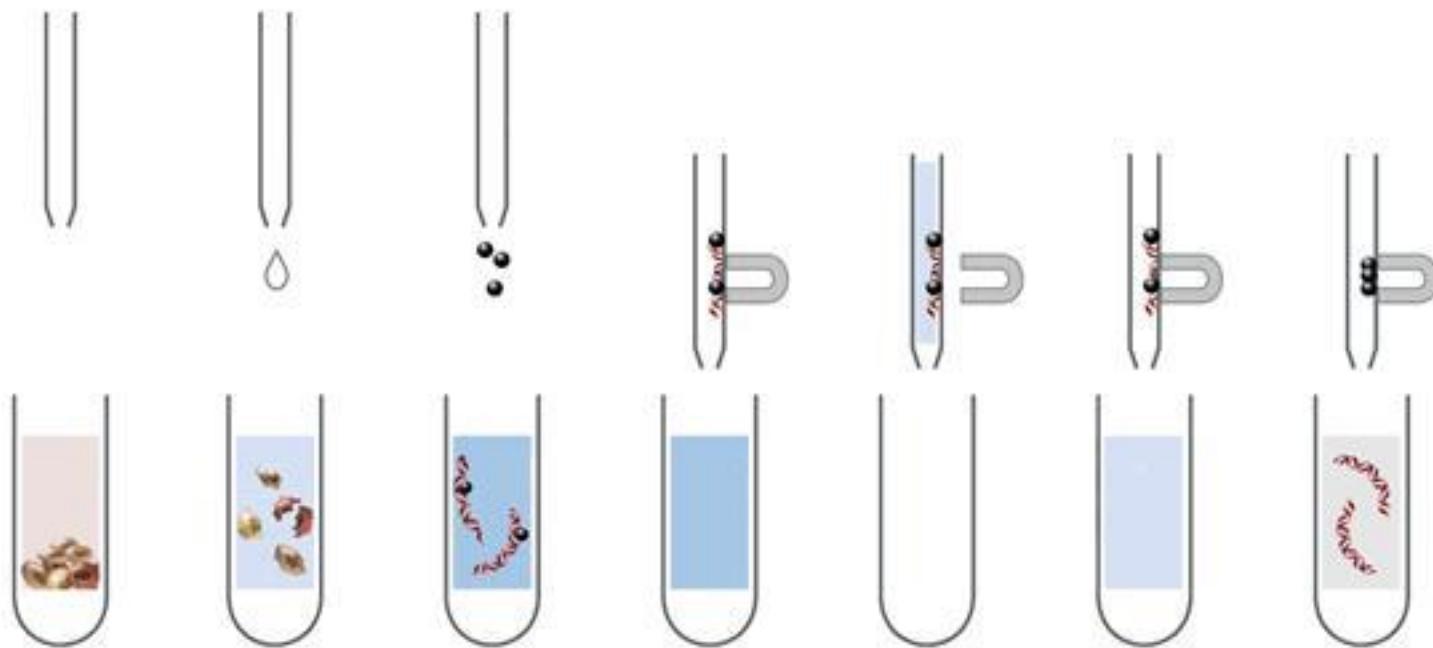
Genotype	Fenotype	Geneesmiddel	Actie
<b>CYP2D6*2/*5</b>	IM	Codeine Metoprolol Nortriptyline	Alternatief 50% dosis 60% dosis
<b>CYP2C9*1/*1</b>	EM	Acenocoumarol	Normale dosis
<b>CYP2C19*1/*2</b>	IM	Citalopram Clopidogrel	Max 20 mg Alternatief
<b>SLCO1B1TT</b>	Norm act	Simvastatine	Normale dosis
<b>TPMT*1/*1</b>	EM	Azathioprine	Normale dosis

# Ontwikkelingen in onderzoek



## Hypothesis free approaches





Sample material

Cell disruption and protein digestion by the addition of Lysis Buffer and Proteinase K.

NA binding to the surface of Magnetic Glass Particles. (DNA is removed by DNase digestion)

Magnetic separation of the nucleic acid-bead complex

Removal of cellular debris by extensive washing steps

Magnetic separation of the nucleic acid-bead complex

Nucleic acid elution at high temperatures during the removal of the Magnetic Glass Particles