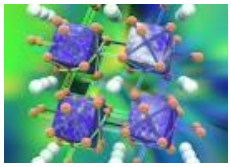




Personalized Medicine in China

Hong-Hao Zhou, MD & Professor
Institute of Clinical Pharmacology
Pharmacogenetics Research Institute
Central South University

Genetic variations of human individuals

- The human genome contains approximately 3 billion of base pairs
- That code over thirty thousand genes

Individual DNA sequence: 99.9% identical

0.1%

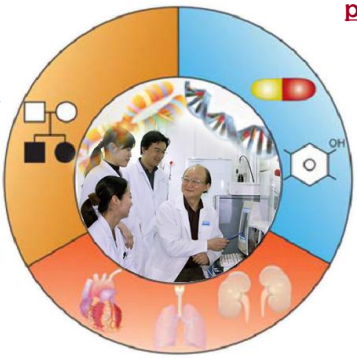
3M variations

- Disease-related
- Drug response difference-related

Genome-based personalized medicine

Early prediction of disease risk

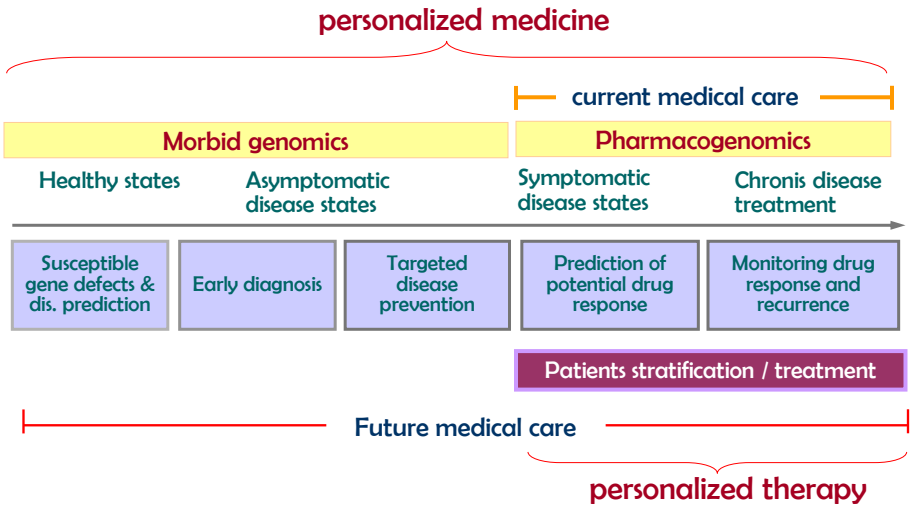
- **Rare disease:**
  - Identifying Causative genes for Mendelian disorders
  - Pre-conception and pre-implantation genetics diagnosis
- **Common diseases**
  - Risk prediction
  - Risk stratification
  - Early behaviour changes/risk reduction



Pharmacogenetics-personalized therapy

- Testing for disease-specific or enriched drug targets
- Estimating drug efficacy and dosing
- Prediction and avoiding side effects

Personalized medicine and personalized therapy



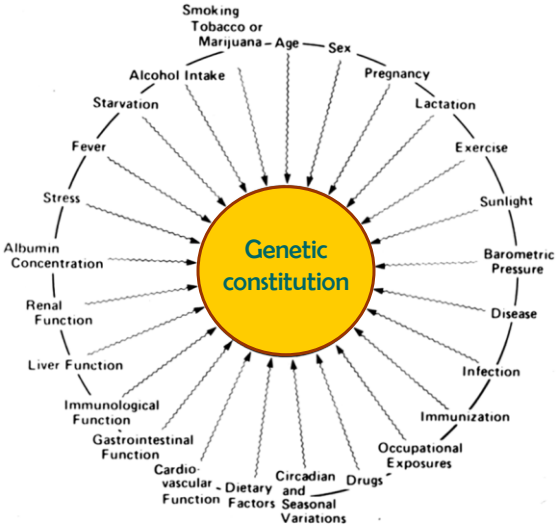
Contradicts of clinical trail and application of drugs

Clinical trials:

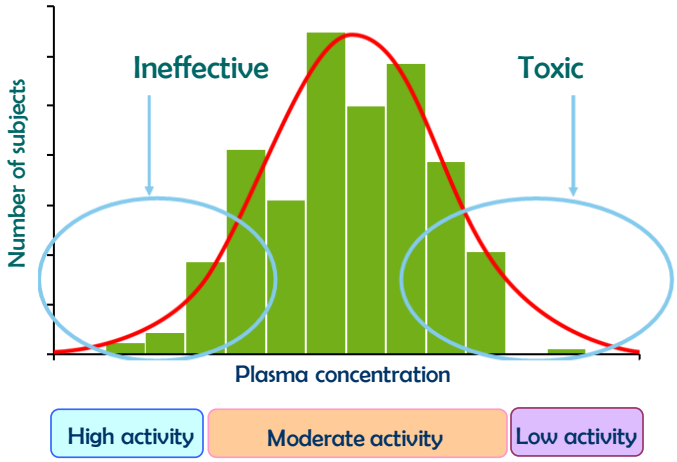
populations

Clinical medication:

Individual

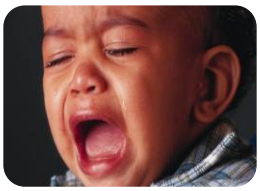


Variation of DMEs activity



Drug quantities in body after taking same doses

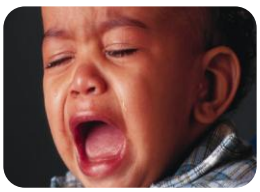
Genetic variation of drug receptors induce Interindividual difference in drug effect and safety



Asthma ends

β2 adrenergic receptor agonist
Albuterol
Salmeterol

Both have acute asthma



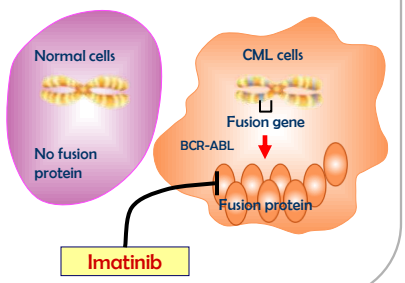
Death ?

Variation in β2 adrenergic receptor (Arg/Arg16)

Personalized medication application 1: Testing for disease-specific or enriched drug targets

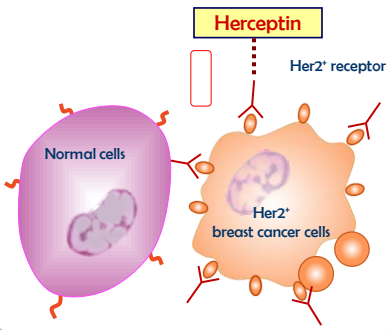
Specific targets

- Imatinib, BCR-ABL tyrosine kinase inhibitor, used in the treatment of bcr-abl fusion gene-positive chronic myeloid leukemia



Overexpression targets

- Herceptin, humanized Her-2 monoclonal antibody, high affinity to Her-2, used in the treatment of Her-2 receptor overexpressed breast cancer.

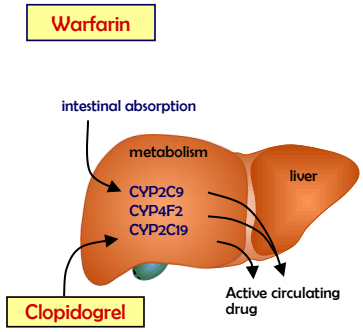


Salari K, Eur Heart J, 2012: 33, 1564–70

Personalized medication application 2: prediction of drug effect and dosage

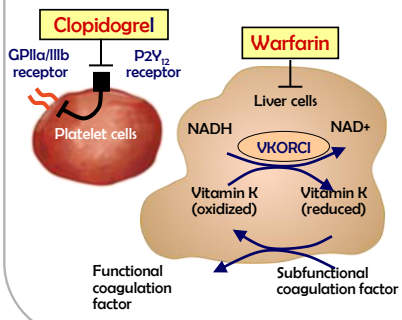
Pharmacokinetics

- Clopidogrel (prodrug): CYP2C19 polymorphism
- Warfarin: CYP2C9 polymorphism



Pharmacodynamics

- Platelet membrane glycoprotein (GP)IIα/IIIβ and membrane protein P2Y12 receptor polymorphism: clopidogrel
- VitK epoxidation reductase polymorphism: warfarin



Salari K, Eur Heart J, 2012: 33, 1564–70

Personalized medication application 2: prediction of drug effect and dosage

-Tamoxifen: selective estrogen receptor regulator for breast cancer

Tamoxifen

↓ CYP2D6

4-OH-N-desmethyl-amoxifen (Endoxifen)

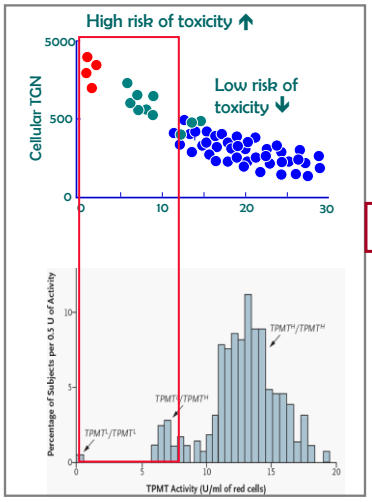
activity: 100X

- Poor metabolizer: significant increase in the recurrence of breast cancer, lower survival rate
- When combined with CYP2D6 inhibitor, plasma concentration of highly active metabolite desmethyltamoxifen decrease by 65-75%

- EMA Pharmacovigilance Working Party (PhVWP) warns:
- CYP2D6 poor metabolizer may have lower response to tamoxifen
- CYP2D6 inhibitor combination should be avoided

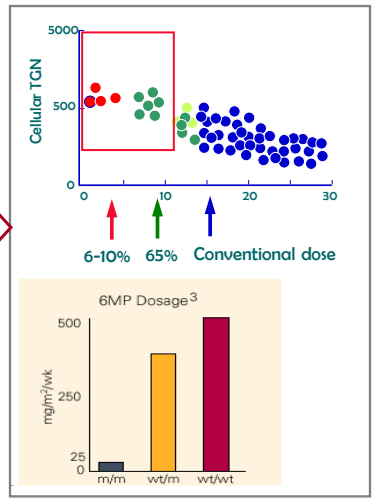
Personalized medication application 2: prediction of drug effect and dosage

Conventional dosage of 6-MP for ALL

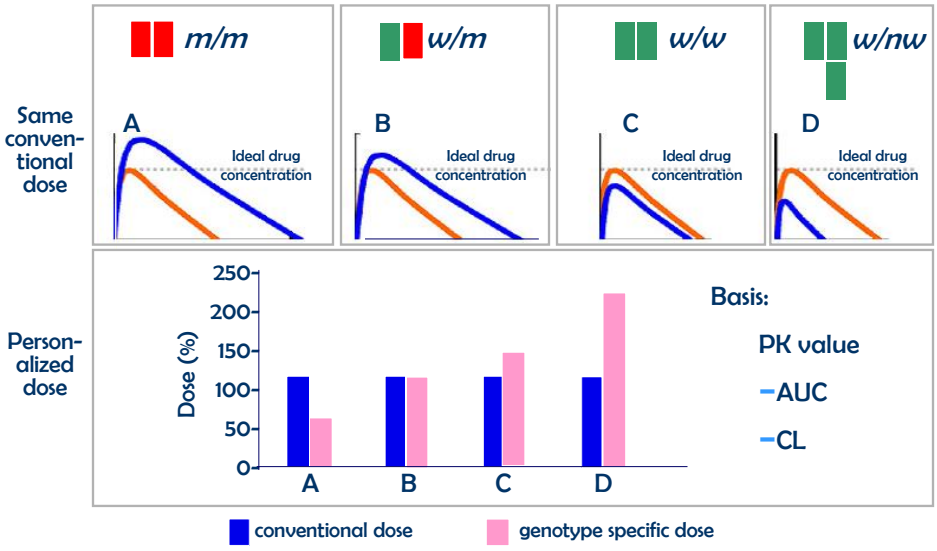


Gene test

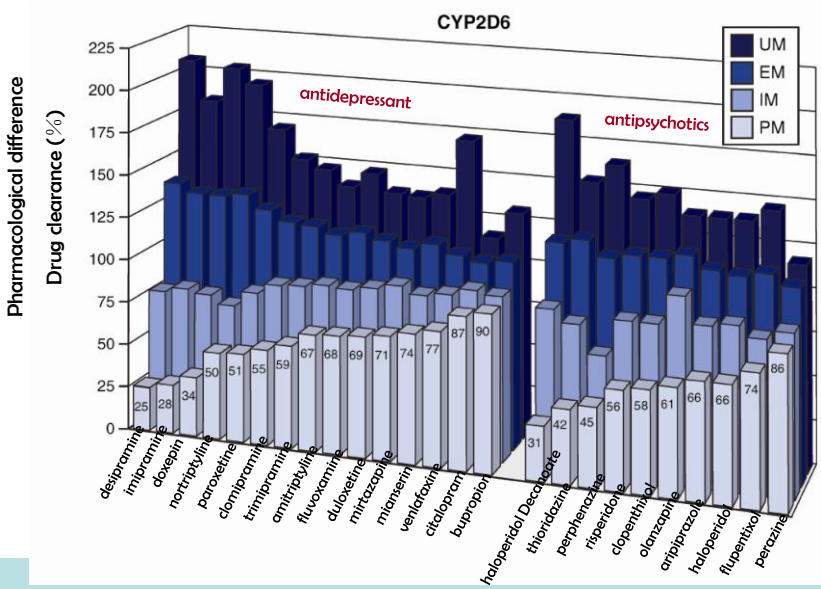
Dosage of 6-MP based on TPMT genotype for ALL



Personalized medication application 2: prediction of drug effect and dosage-Single factor

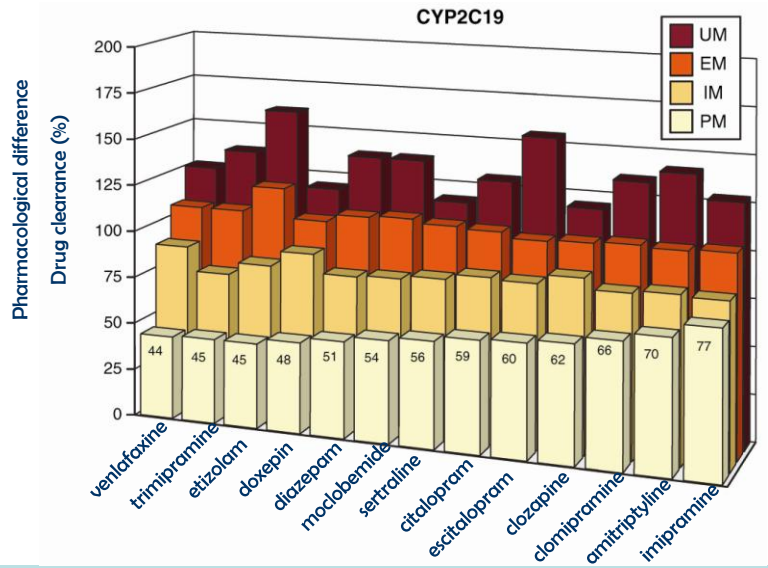


Personalized medication application 2: prediction of drug effect and dosage-Single factor



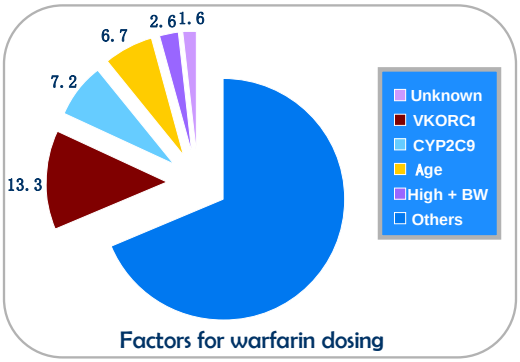
Molecular Psychiatry (2012), 1 -- 15

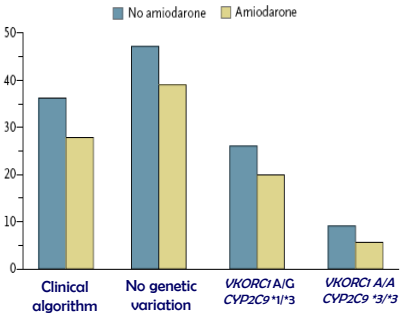
Personalized medication application 2: prediction of drug effect and dosage-Single factor



Molecular Psychiatry (2012), 1 -- 15

Personalized medication application 2: prediction of drug effect and dosage-Multiple factor





- Pharmacogenetic dosing algorithm (international multiple center containing 9 contries and 4043 subjects

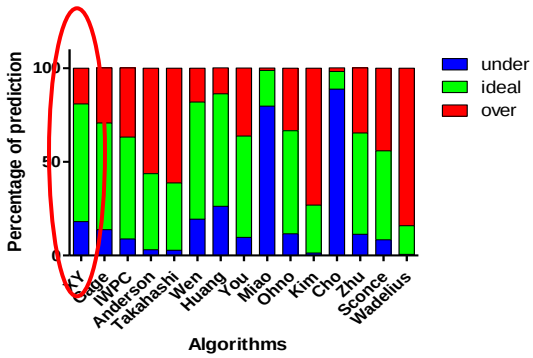
Warfarin dose（mg/d）= [5.6044 – 0.2614(age) + 0.0087(height in cm) + 0.0128 (weight in kg) – *0.8677(VKORC1-1639 A/G)* –*1.6974(VKORC1-1639 A/A)* – 0.4854(VKORC1 genotype unknown) – *0.5211(CYP2C9*1/*2)* – *0.9357(CYP2C9*1/*3)* – 1.0616(*CYP2C9*2/*2*) – 1.9206 (*CYP2C9*2/*3*) –2.3312 (*CYP2C9*3/*3*) – 0.2188 (*CYP2C9* genotype unknows) – 0.1092(Asian race) – 0.2760(Blacks) – 0.1032(Mixed race) + 1.1816(Enzyme induces) – 0.5503(Amidarone)]^2/7

The International Warfarin Pharmacogenetics Consortium, N Engl J Med 2009;360:753-64.

Personalized medication application 2: prediction of drug effect and dosage-Single factor

Xiang-Ya PGx dosing algorithm

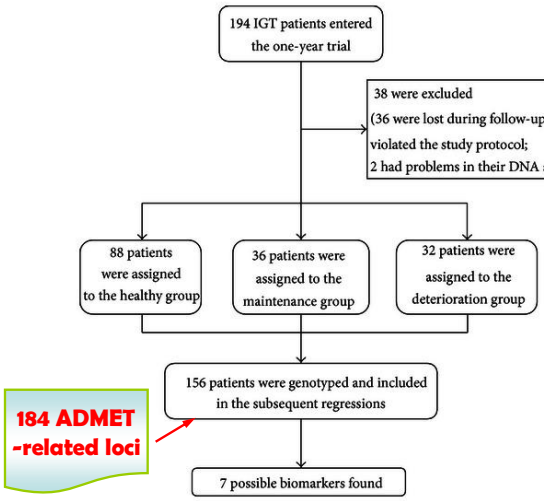
Warfarin stable dose (mg/d) = [2.140 – 0.370×(*VKORC1-1639G>A*) – 0.332×(*CYP2C9*3*) + 0.324×(BSA) – 0.004×(age in decades) – 0.231×(number of increasing INR drugs) + 0.105×(smoking habit) – 0.135×(preoperative stroke history) – 0.108×(hypertension)]^2



The dosing calculated via Xiang Ya dosing algorithm is more close to the actual required for the individual patient from South Central area of China

Personalized medication application 2: prediction of drug effect and dosage

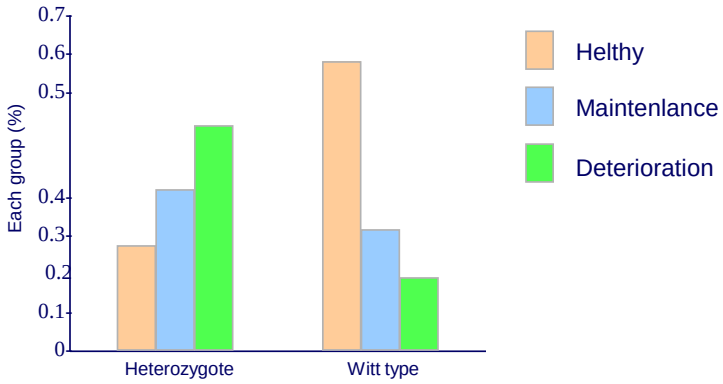
PGx research of TCM Tianqi hypoglycemic capsule



SNP	Gene	Allele	MAF	p	FDR P
rs1142345	**TPMT**	**A/G**	**0.229**	**0.001**	**0.038**
rs2306168	SLCO2B1	T/C	0.305	0.003	0.114
rs2231142	ABCG2	A/C	0.260	0.021	0.798
rs717620	ABCC2	C/T	0.230	0.023	0.874
rs1799931	NAT2	A/G	0.153	0.024	0.912
rs4244285	CYP2C19	A/G	0.344	0.027	1
rs4124874	UGT1A1	G/T	0.337	0.044	1

Li X, Evidence-Based Complementary and Alternative Medicine, 2013
http://dx.doi.org/10.1155/2013/327629

Personalized medication application 2: prediction of drug effect and dosage



Percentage of each group in rs1142345 heterozygote (AG) and wild-type (AA) patients.

Li X, Evidence-Based Complementary and Alternative Medicine, 2013
http://dx.doi.org/10.1155/2013/327629

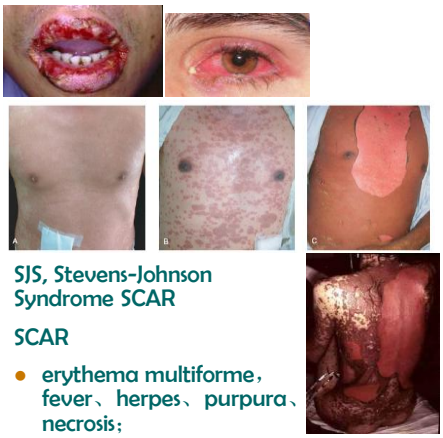
Personalized medication application 3: prediction and prevention of drug toxicity

*HLA-B*5801* and surrogate SNP rs9263726 are associated with allopurin-SCAR in mainland Chinese

- Allopurinol
  - Primary and secondary gout patients, hyperuricemia
  - HLA-B*5801

    Allele positive: 85/90 (94.4%)
  - Rs9263726 A

    Allele positive: 82/90 (91.1%)



SJS, Stevens-Johnson Syndrome SCAR

SCAR

- erythema multiforme，fever、herpes、purpura、necrosis；
- Mortality 30-40%

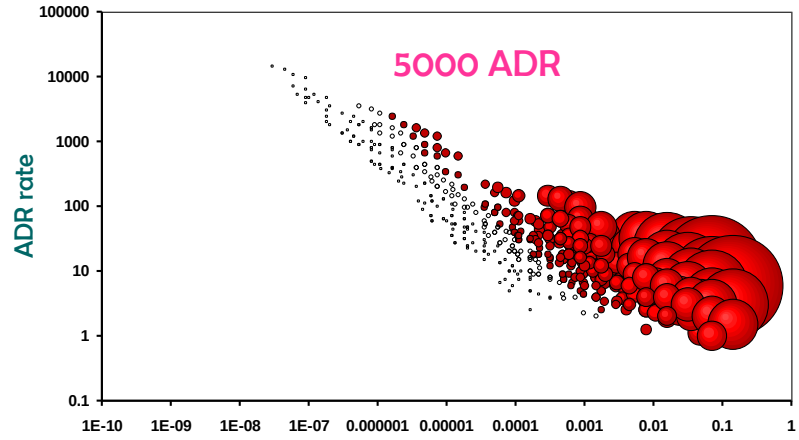
Personalized medication application 3: prediction and prevention of drug toxicity

*HLA-B*5801* and surrogate SNP rs9263726 are associated with allopurin-SCAR in mainland Chinese

Patients	Allele positive /total (%)	OR (95% CI)	Sensitivity /specificity	*P*
HLA-B*5801				
Allopurinol-SCAR	85/90 (94.4%)	399.5 (74.6~2139.4)	94.4% /95.9%	$7.10X10^{-26}$
Allopurinol-Tolerated	2/49 (4.1%)			
Rs9263726 A				
Allopurinol-SCAR	82/90 (91.1%)	240.9 (49.1~1181.7)	91.1% /95.9%	$7.10X10^{-26}$
Allopurinol-Tolerated	2/49 (4.1%)			

Personalized medication application 3: prediction and prevention of drug toxicity

Adverse drug reaction rate is related to mutant gene frequency



- 59% drugs with ADRs are metabolized by polymorphic DMEs

Guidance for Industry

Pharmacogenomic Data Submissions

(2005)

Pharmacogenomic data must be submitted to the IND under if ANY of the following apply (these required pharmacogenomic information may influence FDA's decision to these drugs):

1. The test results are used for making decisions pertaining to a specific clinical trial, or in an animal trial used to support safety.
2. A sponsor is using the test results to support scientific arguments pertaining to, for example, the pharmacologic mechanism of action, the selection of drug dosing and dosing schedule, or the safety and effectiveness of a drug.
3. Test results constitute a known valid biomarker for physiologic, pathophysiologic, pharmacologic, toxicologic, or clinical states or outcomes in humans, or the test is a known valid biomarker for a safety outcome in animal studies (CYP2D6).

Pharmacogenomic information of most other drugs, which are not part of the investigational or marketing submissions, are not required but such data would be welcome on a voluntary basis.

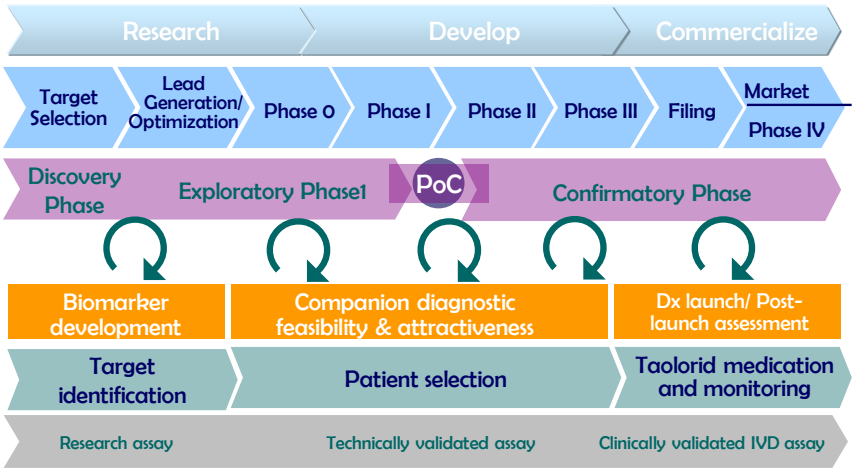
Genetic variations in FDA approved drug labels

	Biomarkers	Drugs or representative drugs
1	*CCR-5*	maraviroc (anti-retroviral agents)
2	*EGFR* expression	cetuximab、panitumumab、gefitinib
3	Her2/neu overexpression	herceptin
4	Philadelphia chromosome positive reaction	dasatinib
5	*C* protein deletion (inherited or acquired)	warfarin
6	*TPMT* variation	azathioprine
7	*UGT1A1* variation	irinotecan
8	*HLA-B*1502* allele	carbamazepine
9	*UCD*	valproic acid
10	*CYP2C9* mutant	warfarin
11	*VKORC1* variation	warfarin
12	familial hyerlipoproteinemia LDL receptor deletion or mutant	atorvastatin
13	*G6PD* deletion	rasburicase
14	*HLA-B*5701* allele	abacavir

Genetic variations in FDA approved drug labels

	Biomarkers	Drugs or representative drugs
15	C-KIT expression	imatinib mesylate
16	*PML/RAR(α)* expression (retinoic acid receptor effective/ineffective)	retinoic acid
17	*UGT1A1* variation	nilotinib
18	*CYP2C19* mutant	voriconazole
19	*CYP2C9* mutant	celecoxib
20	*CYP2D6* variation	tomoxetine
21	*CYP2D6* and other variation	fluoxertine hydrochloride
22	gap gene deletion on the long arm of chromosome 5	lenalidomide
23	*DPD* deletion	capecitabine
24	*EGFR* expression	erlotinib
25	*EGFR* expression	gefitinib (head and neck cancer)
26	*G6PD* deletion	primaquine
27	*NAT* variation	isoniazide，busulfan
28	Philadelphia chromosome positive reaction	busulfan

Biomarker and drug co-development



Examples of parallel development projects

Anticarcinogen used in subpopulation with specific gene mutant, approved by FDA in 2011

Xalkori (crizotinib)

- tyrosine kinase inhibitor, used in the treatment of anaplastic lymphoma kinase (ALK)-positive locally advanced or metastatic non-small cell lung cancer (NSCLC).
- FDA also approved Vysis ALK Break-Apart FISH kit from Abbott, ALK-positive NSCLC patients detected by this kit can be treated with Xalkori.

Zelboraf (vemurafenib)

- BRAF inhibitor, used in the treatment of advanced metastatic or unresectable melanoma.
- FDA also approved the first diagnostic reagent used for detection of cobas 4800 BRAFV600 mutant。

Application and promotion of personalized medicine in Central South University

- Mid 1990: Firstly proposed gene-directed telorid therapy in China
- 2004-4: Personalized Medicine Consultant Center
- 2006-2: Personalized Medicine Genetic Analysis Center
- 2010-9: Xiangya Medical Laboratory, CSU
- 2013-8: National Health and Family Planning Commission Personalized Medicine Testing and Training Base

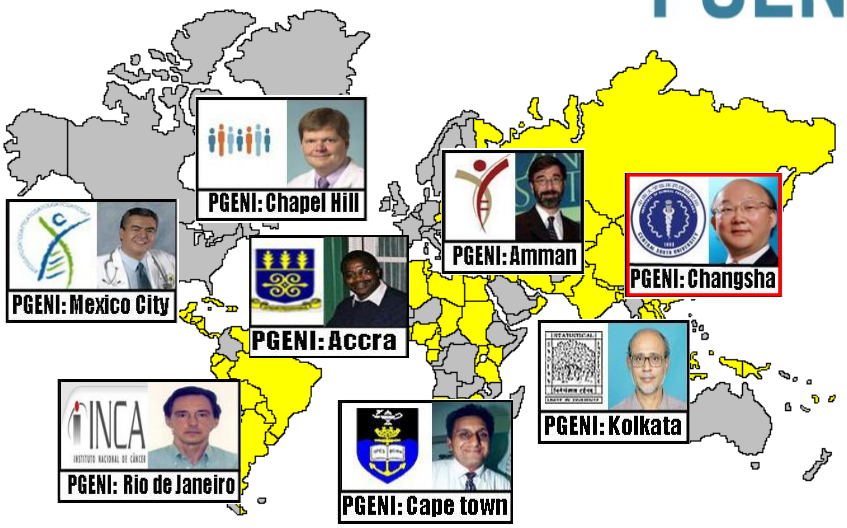
Xiangya Medical Laboratory

- Founded on the basis of 30-year clinical scientific research and in combination with the strong technical force of the 3 affiliated hospitals of CSU, XYML is a testing organization with third-party arbitration qualification.

PGENI local centers

Research centers with high reputation in pharmacogenomics study and application in the world

PGENI



Personalized medicine clinical service by Xiangya Medical Laboratory

Pharmacogenomics (Personalized Medicine) Translational Medicine Chain in CSU

Basic research	Clinical service	Preliminary product	Product development
Discovery and confirmation of genetic mutation, experimental study (small sample ➡ large sample) clinical research	Construction of genetic testing technology: consulting, training and genetic testing service of personalized therapy in clinic	Construction and standardization of detect methods of drug related gene mutations, and earlier stage of test kits.	Later stage of test kits for drug related gene mutation detection, i.e. product submission and production
Institute of Clinical Pharmacology	Xiangya Medical Laboratory	Hunan personalized medicine industry cultivating base	Changsha, Wuhan, Shanghai bio-industry company
30 years fundamental research: •300 SCI paper •57 genes •81 gene locus		212 mature testing products, ready for preliminary product development	11 mature preliminary products approved or submitted by industry company

The world's first personalized medication gene chip

http://app1.sfda.gov.cn/datasearch/face3/base.jsp

Registration number	National food and drug administration 2012 no.3401324
Production unit	Hunan Honghao Gene Bio-technology limited company
Product name	CYP2D6*10、CYP2C9*3、ADRB1(1165G>C)、AGTR1(1166A>C)、 ACE(I/D)testing kit(gene chip)
Product standard	YZB/PRC 4686-2012
Product performance, structure and component	Gene chip, wash buffer A, wash buffer B, positive control, negative control, hybridization solution, PCR reaction buffer 1, PCR reaction buffer 2, PCR reaction buffer 3, PCR reaction buffer 4, PCR reaction buffer 5, enzyme 1, enzyme 2, locate reference. Period of validity: A: 2-8℃； B: -20℃， 6 months. Accessory: registration product standard, instruction
Expire date	2016.10.28
Approval date	2012.10.29
Scope of application	Test 5 polymorphism：CYP2D6*10(CYP2D6*1/*1、CYP2D6*1/*10、CYP2D6*10/*10)、CYP2C9*3(CYP2C9*1/*1、CYP2C9*1/*3、CYP2C9*3/*3)、ADRB1(1165G/G、1165G/C、1165C/C)、AGTR1(1166A/A 、1166A/C、1166C/C)、ACE(II、ID、DD)。
Specifications	20 runs/kit

Personalized Medicine in Jiao Tong Univ. Shanghai

The genetic mutation databases of important P450 genes have been constructed for the Chinese population

Sequencing raw data

Alleles and genotypes data

Analysis results data

Primers and conditions data

news feature

A great leap forward

After helping to sequence the human genome, Chinese scientists are debating how best to continu… toward world p… David C… reports

news feature

ing has produced a homogeneous genetic make-up within each population, and the lack of emigration or immigration means that it is easy to construct large family pedigrees. These features have made Iceland and Finland hotspots for mapping the chromosomal location of genes predisposing to disease. "We have several Icelands and several Finlands," says Lin He of Shanghai Jiao Tong University, who coordinates a bank for blood, tumour and cerebrospinal fluid samples collected from populations across China.

Xu Zhi-Hong, president of Peking University. "This is our challenge."

It is a challenge that both the government and researchers are taking on. In 1998, the government set up a programme alongside the HGP sequencing effort, which has estab-

Nature
1 March 2001:10–12

news feature

Tong University, meanwhile, He claims to have compiled the world's largest sample bank related to neuropsychiatric disease, in a joint project with the Shanghai Institutes of Biological Sciences.

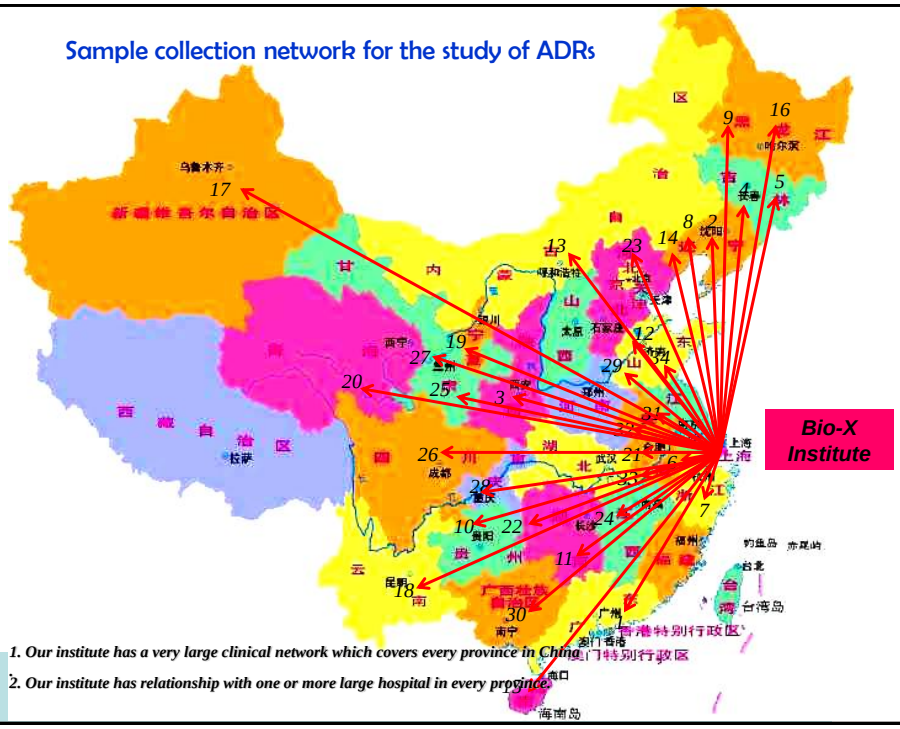
Rising stars

Studies of samples taken from Chinese populations are already yielding advances. The first success came in 1998, when a team led by Jia-hui Xia of the National Laboratory of Medical Genetics of China in Changsha, Hunan province, identified a gene for a form of neurological deafness[2]. Then, last year, researchers led by He mapped the gene for brachydactyly type A-1 — a disease in which joints of the figures are missing, mis-

And the pace looks set to pick up. Last month, researchers led by Yan Shen of the

JiaoTong University have compiled the world's largest sample band related to neuropsychiatric disease in a joint project with the Shanghai Institutes of Biological Sciences.

Sample collection network for the study of ADRs

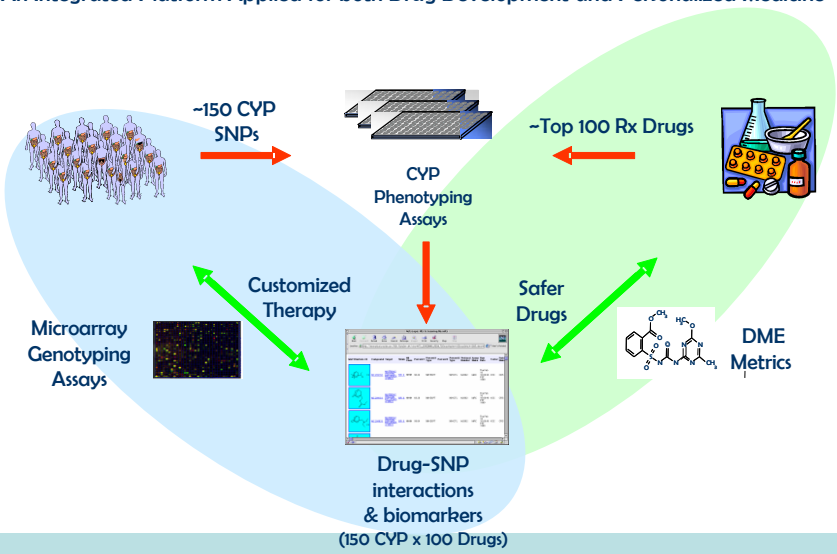


1. Our institute has a very large clinical network which covers every province in China

2. Our institute has relationship with one or more large hospital in every province

Personalized Medicine in Northwest Univ. Xi'an

An Integrated Platform Applied for both Drug Development and Personalized Medicine



Personalized Medicine in Northwest Univ. Xi'an

More CYP and CYP SNP Reagents

CYP	Companies	Lifegen	Cypex	Invitrogen		BD Gentest	
	Host	S. cerevisiae	E.coli	Baculovirus-insect cells	E.coli	Baculovirus-insect cells	Human Lymphoblast
	SNP ID						
1A2	Prototype	Yes	Yes	Yes	Yes	Yes	Yes
2A6	Prototype	Yes	Yes			Yes	
2C9	Prototype	Yes	Yes	Yes	Yes	Yes	Yes
	R144C		Yes	Yes		Yes	Yes
	I359L			Yes		Yes	
2D6	Prototype	Yes	Yes	Yes	Yes	Yes	Yes
	R296C	Yes	Yes				
	S486T	Yes	Yes				
	P34S	Yes	Yes			Yes	
	N166D	Yes					
	L91M	Yes					
	H94R	Yes					
	H324P	Yes					
	G42R	Yes					
	G169R	Yes					
	T107I	Yes					
	R28C	Yes					
	A85V	Yes					
	I297L	Yes					
	R343G	Yes					
	I369T	Yes					
	V7M	Yes					
	Q151E	Yes					
	V136M	Yes					
	V338M	Yes					
	R440H	Yes					
	E410K	Yes					
	A237S	Yes					
	V11M	Yes					
	R201H	Yes					
	R26H	Yes					
	G373S	Yes					
	R173C	Yes					
	L213P	Yes					
	E418Q	Yes					
	P325L	Yes					
	G212E	Yes					
	L213S	Yes					
	L421P	Yes					

CYP	Companies	Lifegen	Cypex	Invitrogen		BD Gentest	
	Host	S. cerevisiae	E.coli	Baculovirus-insect cells	E.coli	Baculovirus-insect cells	Human Lymphoblast
	SNP ID						
2C19	Prototype	Yes	Yes	Yes	Yes	Yes	
	I331V	Yes					
	E92D, I331V	Yes					
	R433W, I331V	Yes					
	R132Q, I331V	Yes					
	W120R, I331V	Yes					
	R144H, I331V	Yes					
	P227L, I331V	Yes					
	R150H, I331V	Yes					
	R410C, I331V	Yes					
	L17P, I331V	Yes					
	I19L, I331V	Yes					
	R442C	Yes					
	R329H	Yes					
	S51G	Yes					
	D360N	Yes					
	A161P	Yes					
	M74T	Yes					
	F168L	Yes					
	W212C	Yes					
	E122A	Yes					
3A4	Prototype	Yes	Yes	Yes	Yes	Yes	Yes
	S222P	Yes					
	M445T	Yes					
	I118V	Yes					
	P218R	Yes					
	G56D	Yes					
	R130Q	Yes					
	V170I	Yes					
	D174H	Yes					
	T363M	Yes					
	L373F	Yes					
	P416L	Yes					
	L15P	Yes					
	R162Q	Yes					
	T185S	Yes					
	F189S	Yes					
	L293P	Yes					
	P467S	Yes					

Personalized Medicine in Northwest Univ. Xi'an

Lifegen Cypro-screen ™

A Broad Range of Cytochrome P450s and Over Hundred CYP SNPs

High-throughput Analysis Service for Your Drug Candidates

1. CYP Reaction Phenotyping

- CYP isoform identificationin
- Evaluate the drug metabolic differences caused by CYP SNPs

2. CYP Mediated Drug-Drug Interaction by High-throughput Fluorometric Assay and LC-MS

- CYP-based inhibition assay
- CYP SNPs-based inhibition assay

Enzymes Available

12 CYP prototype :
1A1 1A2 2A6 2A13 2B6 2C8 2C9 2C19 2D6 2E1 3A4 3A5

10 SNPs of 2C9
R144C R150H L19I H251R E272G R335W I359L I359T D360E P489S

20 SNPs of 2C19:
A161P L17P I19L S51G D360N I331V E92D E122A F168L W120R W212C M74T R132Q R144H R150H R329H R410C R433W R442C P227L

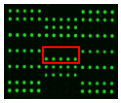
55 SNPs of 2D6:
V7M V11M V136M V374M V338M R25W R26H R28C R173C R201H R329L R365H R296C R343G R380H P34S P325L P430L G42R G169R G212E G479R L91M H94R T107I F120I A237S A122S A85V A90V A300G Q151E W152G E155K E156A N166D S168A S486T S311L L213P L213S L231P M279K I297L H324P E334A I369T G373S E410K E418Q H478Y P345-S486T R296C-S486T

19 SNPs of 3A4:
S222P M445T I118V P218R G56D R130Q V170I D174H T363M L373F P416L L15P R162Q T185S F189S L293P P467S

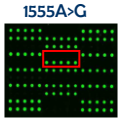
Personalized Medicine in Tsinghua University Univ. Beijing

Mutation Screening for Neonatal Deafness

Used for the detection of 9 hot mutations of four genes, Mitochondrial 12S rRNA, GJB2, GJB3 and PDS, which are in conjunction with hereditary hearing loss. Especially, the 1555 A>G and 1494 C>T mutations at Mitochondrial 12S rRNA could be detected. Exposure to aminoglycoside antibiotics such as streptomycin can lead to severe-to-profound hearing loss when patient carry 12S rRNA the mutations.



1555A>G



Kit

SFDA

BioMixer

SlideWasher

LuxScan

Software

Human Mutation, 29:306-14, 2008

The Screening of the Deaf in Beijing

6 北京日报 2011年3月4日 星期五

两万多聋人免费查病因

基因检测可查出是否遗传下一代 医生建议健全人也可进行检测

今天是全国“爱耳日”，本市高危人群致聋基因筛查项目宣告启动，两万多名特殊病人证聋人可申请免费检查筛查，持证聋人可提出申请，区县残联将为其安排来点点进行检测。

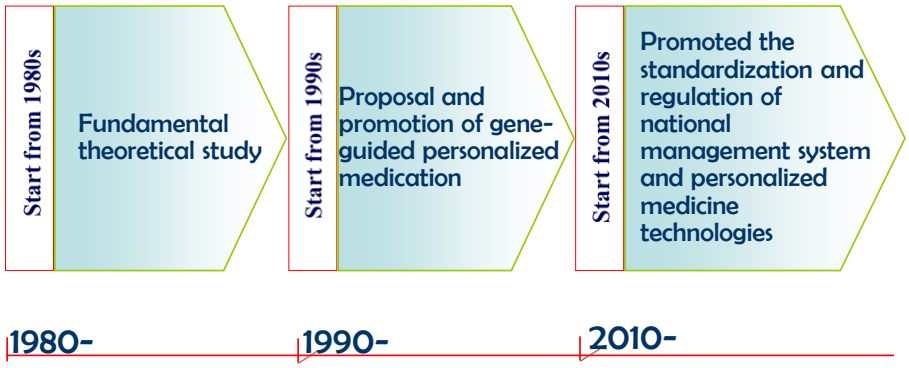
抽血两三毫升可验致聋原因

三色卡告诉聋人致聋原因

健全人也可进行检测

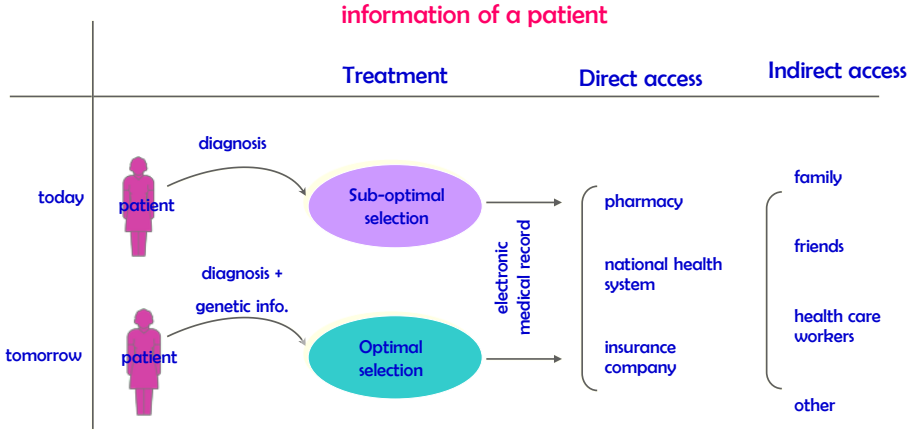
本市每年至少150名新生儿听力障碍

Three stages of the development of personalized medicine in China



Ethics, law and social issue of personalized medication

People who have access to diagnostic, genetic and medication information of a patient



Direct access: systems that automatically acquire diagnostic and medication information, such as electronic medical record.

Indirect access: people who intentionally or unintentionally acquire medication information of a patient.

Ethical, legal and social issues of personalized medication

(1) Protect individual's genetic privacy

- Individual's genetic information should not be acquired without consent.
- Individual's decision of not to know his genetic information for any reason should be respected.
- Procedure and regulation of rational acquisition, transport and reservation of individual's genetic information should be assigned by gene testing units.

(2) Respect individual's right to knowledge

- The aim, purpose, procedure, outcome and risk of genetic test should be informed to, and agreed by, the participant.
- Testing outcomes with individual's consent should be given to participant veritably. Risk evaluation of disease and positive significance in medication should be explained to participant scientifically, comprehensively and positively, to help patient understand his genetic information correctly and positively.

(3) Against genetic discrimination

- Individual's genetic information might give rise to genetic discrimination. To protect people against genetic discrimination, firstly the privacy of an individual's genetic information should be protected, secondly advanced legal system and social moral system should be established.

Although genetic testing in personalized medication is not aimed for genetic disease detection and prediction, potential risk in society, psychology and economy might rise when gene mutation, potential disease susceptibility and prognosis information are known. Therefore, informed consent form should be signed by participant before sample collection, except for somatic mutation detection.

National missions undertaken by CSU

Guided by the official letter of approving the regulation and management of personalized medicine testing by the general office of the Ministry of Health, ([2013]No195), the bureau of medical policy and management, National Health and Family Planning Commission has convened the first conference of personalized medicine committee in Beijing on May 13 2013.

- As a main member of the personalized medicine committee, the Ministry of Health, take part in the standardized management of personalized medicine molecular testing laboratory in China;
- Set the standard of personalized molecular testing laboratory;
- Draw up the guidelines of personalized medication-related gene (DMEs, transporters, receptors) molecular testing projects;
- National Health and Family Planning Commission Personalized Medicine Testing and Training Base (exclusive);
- National Pilot Standard Laboratory of Personalized Medicine Molecular Testing (one of the three).


Acknowledgment
Thank you for your attention