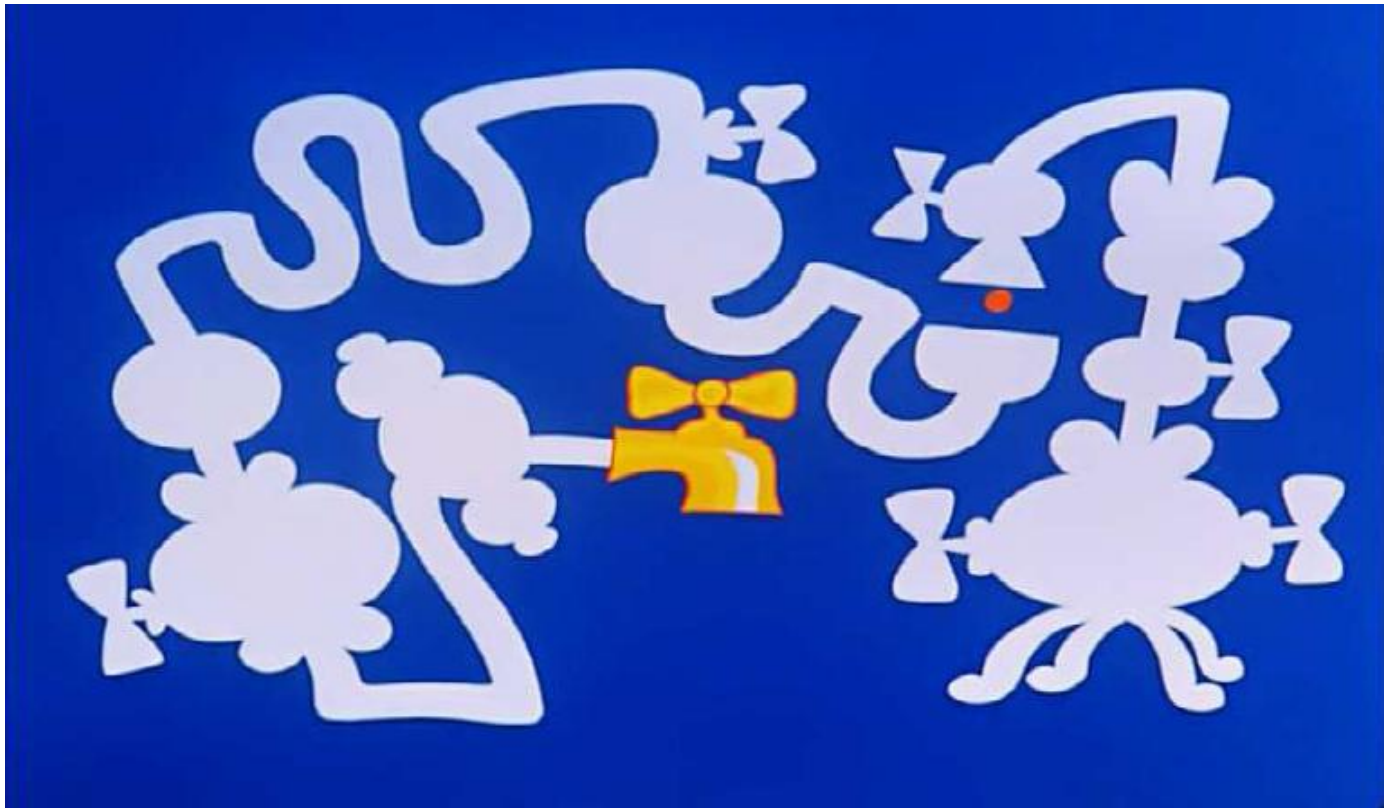
La risposta individuale ai PPI: le nuove acquisizioni in tema di farmacogenomica



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2) Polimorfismo epatico


3) PPIs


4) Interazioni PPIs


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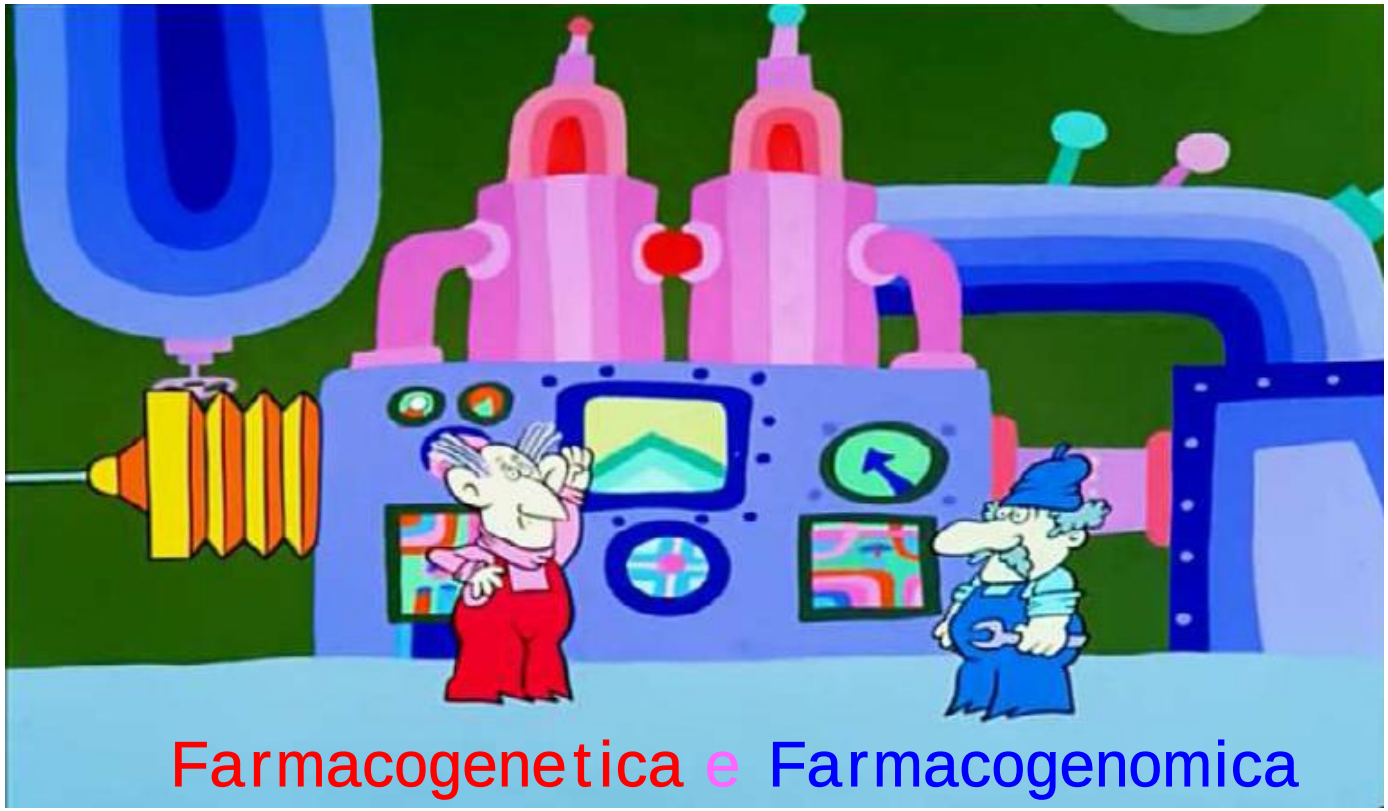
Introduzione alla farmacogenomica



- applicazione della Genetica alla Farmacologia
- variabilità genetica degli effetti dei farmaci [Evans and Relling, 2004]
- ereditarietà di geni specifici sugli effetti dei farmaci → Farmacogenetica (dal fenotipo al genotipo)
- effetti dell'intero genoma sugli effetti dei farmaci → Farmacogenomica/ Personalized Medicine (dal genotipo al fenotipo)

Farmacogenomica:

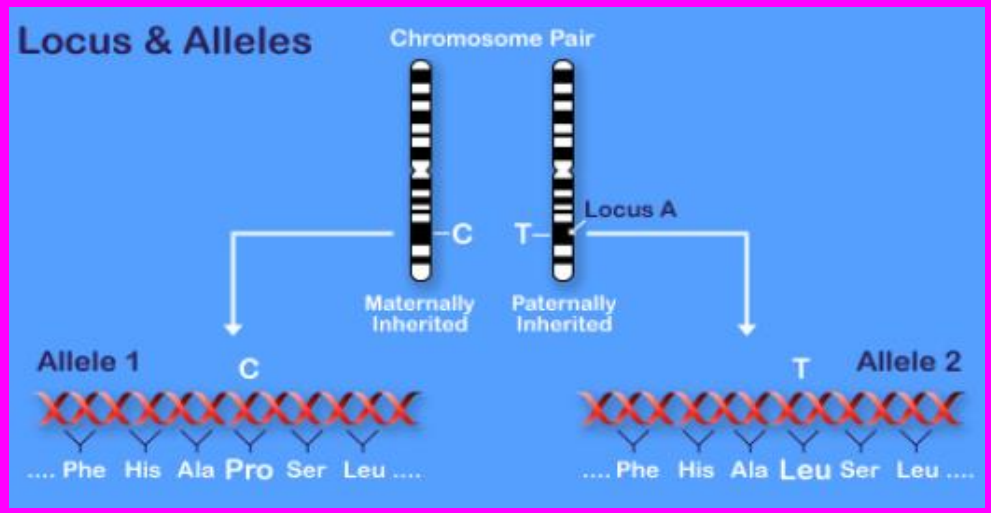
- nasce 50 anni fa
- 20 anni fa nasce single nucleotide polymorphism (SNP)

• variazioni nel genoma di una specie (1:1200 basi ogni 2 uomini)

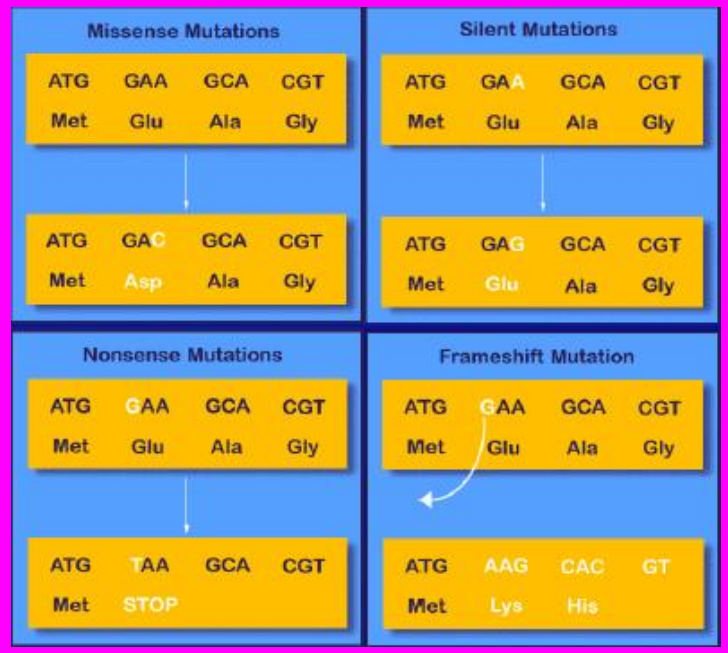
• differenze nelle proprietà individuali osservabili e variazioni del corredo enzimatico

• equivalente alle variazioni metaboliche di popolazione e alla variabilità di metabolizzazione dei farmaci

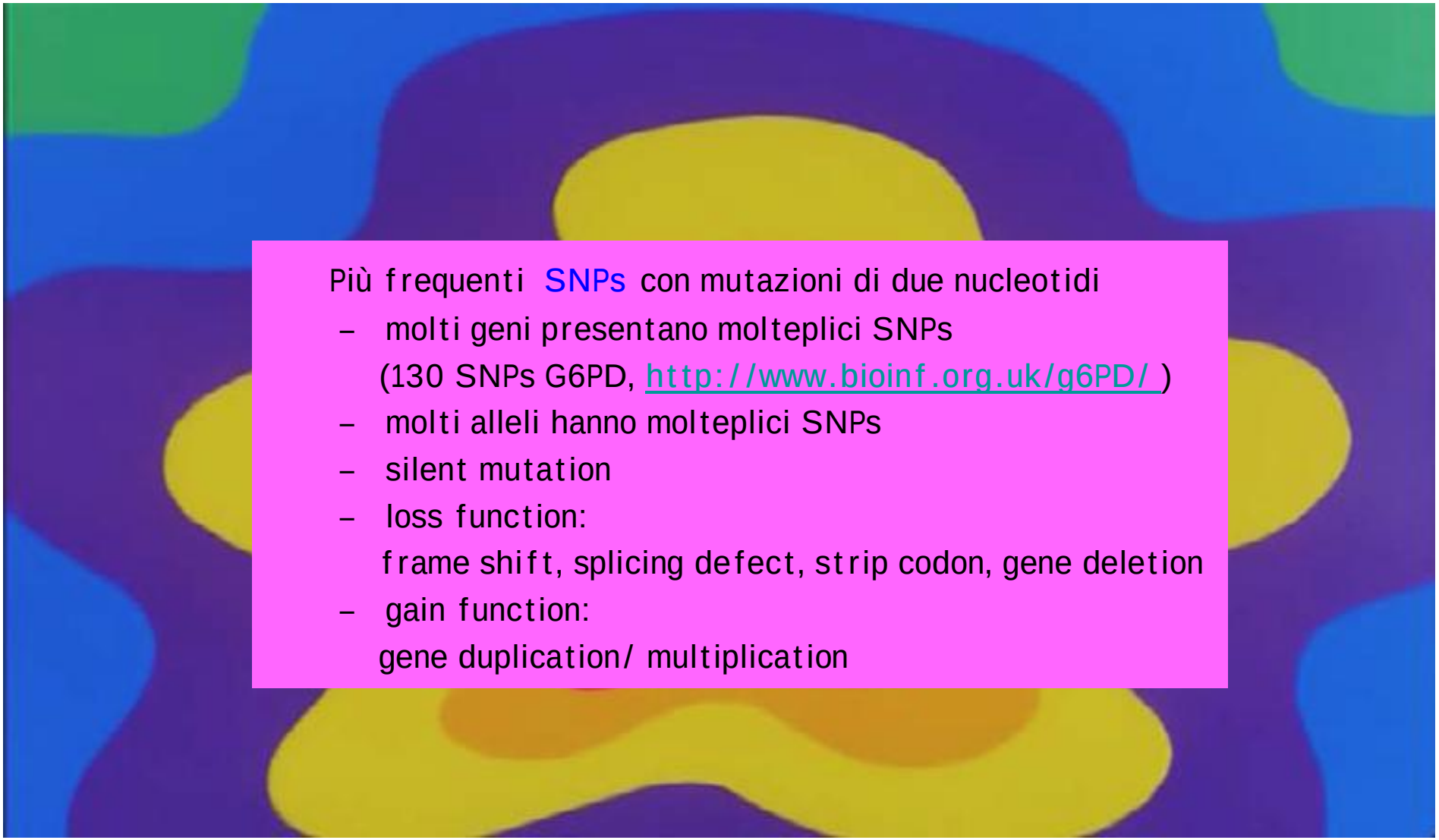
• minimo allele in comune che si osserva nell'1% o più della popolazione (mutazioni sono differenze rare che si osservano in molto meno dell'1% della popolazione)



Alleli: forme alternative di un locus genetico su un singolo cromosoma Genotipo: varianti genetiche ad uno o più loci di un individuo. Le due copie cromosomiche di un sito polimorfico, per esempio A/A o A/G o G/G (SNPs)



SNPs



Più frequenti SNPs con mutazioni di due nucleotidi

- molti geni presentano molteplici SNPs
  (130 SNPs G6PD, http://www.bioinf.org.uk/g6PD/)
- molti alleli hanno molteplici SNPs
- silent mutation
- loss function:
  frame shift, splicing defect, strip codon, gene deletion
- gain function:
  gene duplication/ multiplication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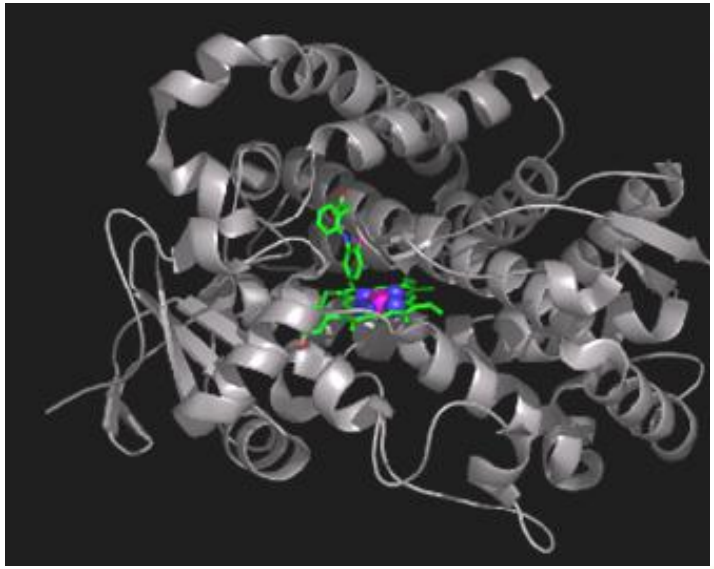
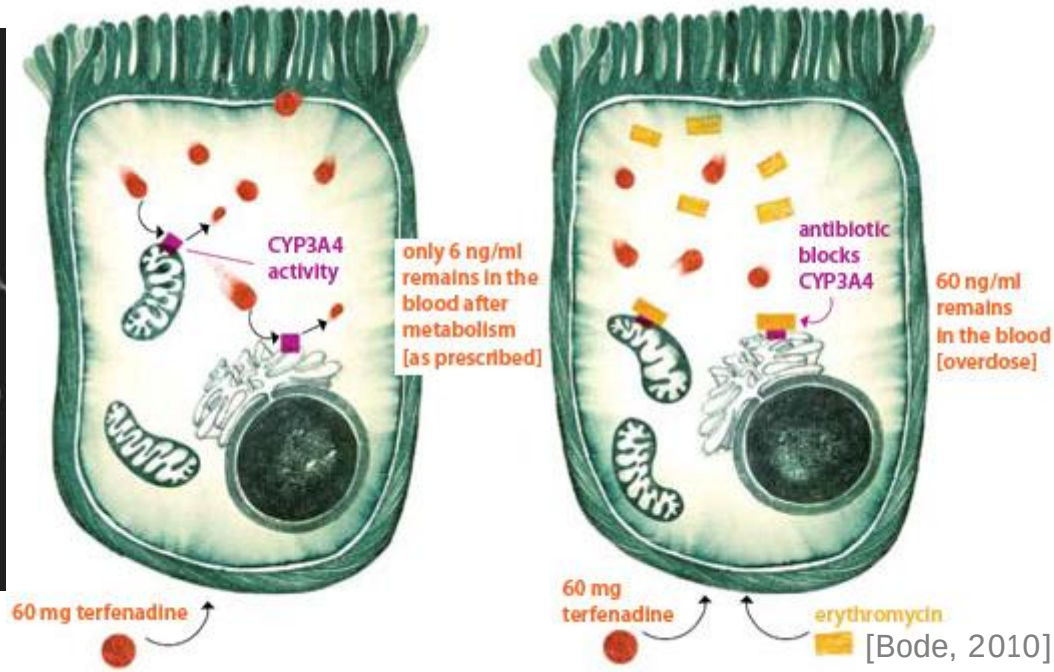
SNPs del sistema microsomiale epatico





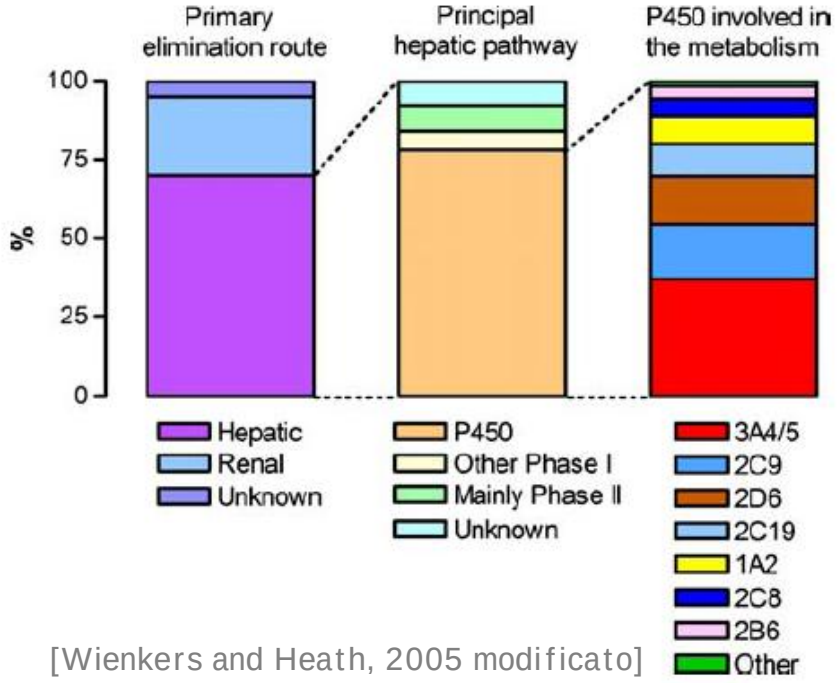
[Bode, 2010]

Enzimi-citocromo P450

- metabolismo ossidativo di molti farmaci e composti endogeni (bilirubina, colesterolo, ormoni steroidei, vitamina D, autoregolazione vascolare barriera emato-encefalica)
- contengono un anello eme e un sito di legame per substrato e/o farmaco
- rendono i farmaci più idrosolubili e più facilmente eliminabili
- polimorfismi genetici causano effetti collaterali avversi, variabili risposte al farmaco, accumulo di farmaci tossici e metaboliti tossici dei farmaci
- presenti nella frazione microsomiale e membrana interna mitocondriale epatica, in minore entità nel tratto gastroenterico, polmoni, cervello e reni
- metabolismo di primo passaggio nel fegato (farmaci assunti per os e rettale)

Metabolismo dei farmaci e citocromo P450

Enzyme reaction	Metabolic reaction	Examples of enzymes
Phase I reactions		
Oxidation	Introduces hydroxyl, epoxide and ketone groups	Alcohol and aldehyde dehydrogenases
	Shortens alkyl side chains	Amine oxidases
	Converts alcohols to aldehydes and acids	Cytochromes P450
Reduction	Introduces hydrogen into ketones and nitro groups	Nitro- and azo-reductases
Hydrolysis	Breaks down esters to alcohols and acids	Esterases
Phase II reactions		
Acetylation	Adds acetate to polar sites	Acetyltransferases
Amino acid conjugation	Adds amino acids to polar sites	Glutathione transferases
Glucuronidation	Adds sugars to polar sites	Glucuronyl transferases
Methylation	Adds methyl groups to polar sites	Methyltransferases
Sulphation	Adds inorganic sulphate to polar sites	Sulphotransferases



[Wienkers and Heath, 2005 modificato]

Human Cytocrome Allele Nomenclature Committee

Allele nomenclature for Cytochrome P450 enzymes: [Nelson D, 2008]

CYP1 family:
CYP1A1; CYP1A2; CYP1B1

CYP2 family:
CYP2A6; CYP2A13; CYP2B6; CYP2C8; CYP2C9; CYP2C19; CYP2D6; CYP2E1; CYP2F1; CYP2J2; CYP2R1; CYP2S1; CYP2W1

CYP3 family:
CYP3A4; CYP3A5; CYP3A7; CYP3A43

CYP4 family:
CYP4A11; CYP4A22; CYP4B1; CYP4F2

CYP>4 families:
CYP5A1
CYP8A1
CYP19A1
CYP21A2
CYP26A1

attivazione di procarcinogeni in carcinogeni (cancro del polmone associato a fumo di tabacco)

20% metabolismo

50% metabolismo

CYP 450 geni, enzimi e reazioni

- 57 geni differenti attivi
- 18 diverse famiglie
- Identificati attraverso la sigla CYP, un numero arabo indica la famiglia (>40% di omologia di sequenza), la lettera in maiuscolo la sottofamiglia (>55% di omologia di sequenza) ed un secondo numero arabo specifica il singolo gene (isoenzima)
- CYP1, CYP2 and CYP3 metabolismo dei farmaci
- CYP2A6, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP2E1 e CYP3A4 metabolismo farmaci più importanti

- Reazioni
  - Ossidazione alifatica
  - Idrossilazione aromatica
  - Formazione di sulfossidi
  - N-ossidazione e N-idrossilazione
  - N-/O-/S-dealchilazione
  - Dealogenazione ossidativa o riduttiva

GENETICA MOLECOLARE

- ricerca il difetto metabolico senza test farmacologico al paziente
- migliora il significato statistico dell'analisi farmacogenetica

Gli individui sono raggruppati in 4 categorie (fenotipi), determinati dal livello più elevato di funzionamento dell'allele *CYP*:

1) UM – metabolizzatore ultrarapido: copie multiple di un singolo gene *CYP*
2) EM – metabolizzatore rapido (wild-type): individuo con due alleli "normali" o almeno un allele funzionale
3) IM –metabolizzatore intermedio: allele funzionale dominante con funzione ridotta o due alleli parzialmente defettivi
4) PM – metabolizzatore lento: due alleli non funzionali

Profarmaci

•Il supereroe necessita dell'accesso alla cabina telefonica (enzima citocromo P450). Se Cyp è bloccato da un potente inibitore, non si ha alcuna attivazione

• Il profarmaco è sottoposto ad una tappa di attivazione prima dell'azione farmacologica sul bersaglio [Mannheimer and Eliasson, 2010]

Drug	Slow metabolizer phenotype	Fast metabolizer phenotype
Prodrug, needs metabolization to work (eg. codeine is metabolized by CYP 2D6 to morphine)	Poor efficacy Possible accumulation of prodrug	Good efficacy, rapid effect
Active drug, inactivated by metabolization (example is omeprazole)	Good efficacy Accumulation of active drug can produce adverse reactions May need lower dose	Poor efficacy Need greater dose or slow release formulation

Variabilità di risposta ai farmaci

- Metabolizzatori lenti hanno più effetti collaterali avversi per alti livelli di farmaci non metabolizzati
- metabolizzatori ultrarapidi più frequentemente non responsivi ai farmaci al loro normale range di dosaggio.
- CYP2D6, CYP2C9 e CYP2C19 tipi di citocromo P450 più studiati

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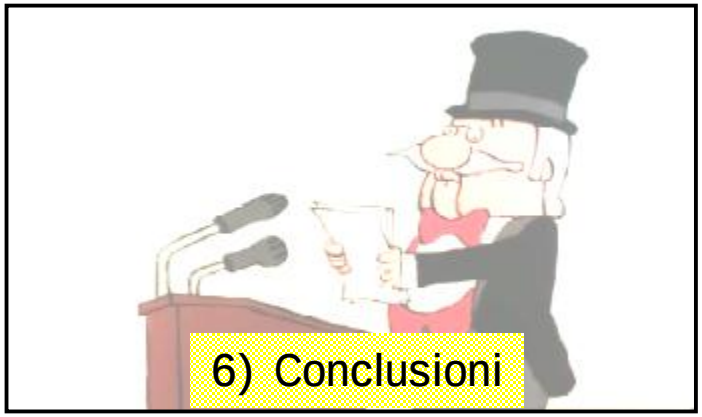


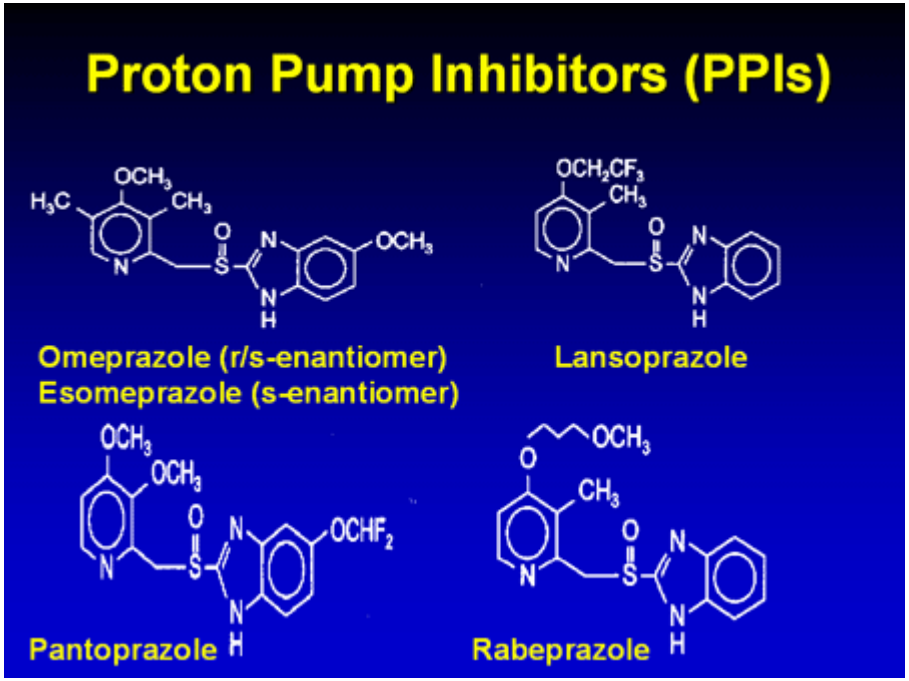
6) Conclusioni

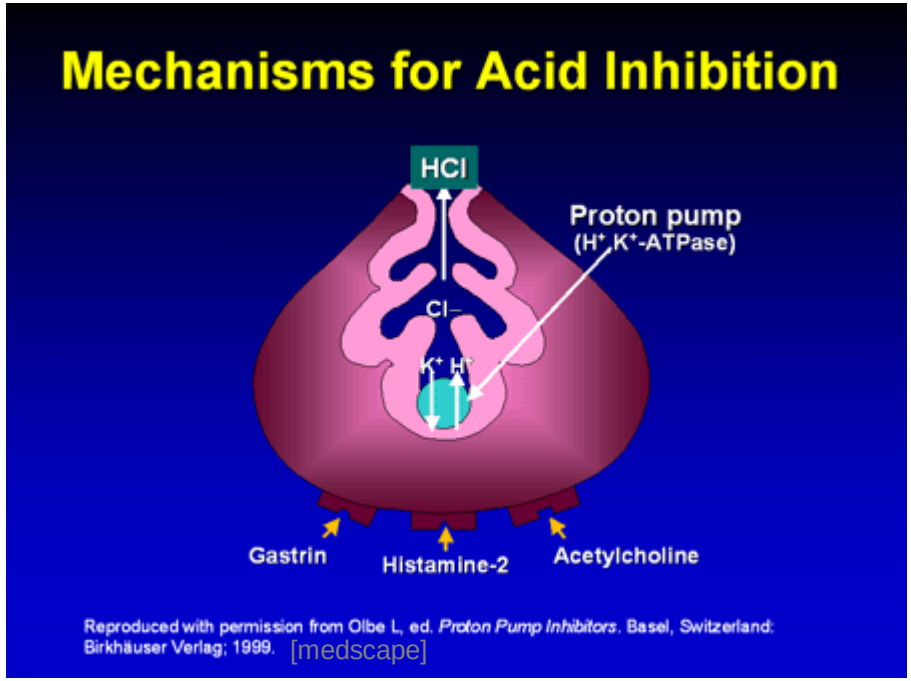
Farmacogenomica dei PPI

Farmacogenomica dei PPI

Farmacogenomica dei PPI

Proton pump inhibitors (PPIs)





- Derivati benzimidazolici, inibitori selettivi ed irreversibili della pompa gastrica H+K+-ATPase
- omeprazolo, primo della famiglia PPIs comprendente pantoprazolo, lansoprazolo, rabeprazolo e più nuovo, esomeprazolo, *S-isomero* dell'omeprazolo
- biotrasformazione epatica dal sistema CYP 450; principali isoenzimi CYP2C19 e CYP3A4
- omeprazolo e esomeprazolo (lansoprazolo e rabeprazolo) metabolizzati predominantemente da CYP2C19 (80% della clearance) con saturazione enzimatica dose-dipendente; affinità 10 volte più bassa per il CYP3A4, enzima alternativo attivo ad alte concentrazioni di omeprazolo
- pantoprazolo bassa affinità per CYP2C19 e CYP3A4 metabolizzato da solfotransferasi, non saturabile, esterno al sistema CYP

Allele	Protein	Nucleotide changes		Trivial name	Effect	Enzyme activity		References
		cDNA	Gene* *To be considered CYP2C19*1A, the genomic reference sequence must be changed to -1041G, 99C and 80357A			In vivo	In vitro	[Nelson D, 2008]
CYP2C19*1A	CYP2C19.1A	None	None		None	Normal	Normal	Rettie et al. 1994
CYP2C19*1B	CYP2C19.1B	99C>T; 991A>G	99C>T; 80181A>G		I331V	Normal		Xiaodong et al. 1997
CYP2C19*1C	CYP2C19.1B	991A>G	80181A>G		I331V	Normal		Blaisdell et al. 2002
CYP2C19*1d		99C>T; 481G>A; 990C>T; 991A>G	99C>T; 80181A>G; 80680C>T; 80181A>G	m1; m1A	Splicing defect; I331V	None		de Morais et al. 1994a
CYP2C19*1E		99C>T; 27C>C; 481G>A; 990C>T; 991A>G	99C>T; 124490C>C; 80181A>G; 80190C>T; 80181A>G	m1B	RFXP; splicing defect; I331V	None		Roy et al. 1999b
CYP2C19*2C (also called CYP2C19*2)		99C>T; 481G>C; 681C>A; 990C>T; 991A>G	-985T>C; 99C>T; 11122G>A; 12682A>G; 12834G>C; 13916G>A; 19120A>G; 57740C>G; 70926T>A; 80180C>T; 80181A>G		A101P; splicing defect; I331V			Fukushima-Uesaka et al. 2005
CYP2C19*2D		99C>T; 481G>A; 990C>T; 991A>G; 12130>A	-985T>C; 99C>T; 12682A>G; 12834G>A; 57740C>G; 80180C>T; 80181A>G; 87270>A		splicing defect; E405K			Lee et al. 2002
CYP2C19*3A		636G>A; 801A>G; 1251A>C	27948G>A; 80681A>G; 87153A>C	m3	W212X; I331V	None		de Morais et al. 1994b
CYP2C19*3B (also called CYP2C19*3)		636G>A; 801A>G; 1078G>A; 1251A>C	-889T>G; 12915T>G; 13122G>A; 13268G>A; 13356T>C; 27948G>A; 28811A>G; 80458A>G; 80748G>A; 87153A>C		W212X; D360N; I331V	None		Fukushima-Uesaka et al. 2005
CYP2C19*4A		1A>G; 99C>T; 991A>G	1A>G; 99C>T; 80181A>G	m4	GTG initiation codon; M1A;	None		Ferguson et al. 1998
CYP2C19*4B		1A>G; 99C>T; 991A>G	-806C>T; 1A>G; 99C>T; 80181A>G		GTG initiation codon; I331V	None		Scott et al. 2011; Hachiya et al. 2010
CYP2C19*5A	CYP2C19.5A	319TC>T	80603C>T	m4	R433W	None	None	Xiaodong et al. 1997; Roy et al. 1999a
CYP2C19*5B	CYP2C19.5B	99C>T; 991A>G; 319TC>T	99C>T; 80181A>G; 80603C>T		I331V; R433W	None		Roy et al. 1999a
CYP2C19*6	CYP2C19.6	99C>T; 399G>A; 991A>G	99C>T; 127485G>A; 80581A>G	m5	R132Q; I331V	None	None	Roy et al. 1999b
CYP2C19*7			39294T>A		Splicing defect	None		Roy et al. 1999
CYP2C19*8	CYP2C19.8	358T>C	127181T>C		W120R	None	Decr	Roy et al. 1999
CYP2C19*9	CYP2C19.9	99C>T; 481G>A; 991A>G	99C>T; 127846G>A; 80581A>G		K140E; I331V		(Decr)	Blaisdell et al. 2002
CYP2C19*10	CYP2C19.10	99C>T; 480C>T; 991A>G	99C>T; 80580C>T; 80141A>G		P227L; I331V		Decr	Blaisdell et al. 2002
CYP2C19*11	CYP2C19.11	99C>T; 449G>A; 991A>G	99C>T; 128003G>A; 80141A>G		R150H; I331V			Blaisdell et al. 2002
CYP2C19*12	CYP2C19.12	99C>T; 991A>G; 1475A>C	99C>T; 80141A>G; 80598A>C		I331V; Y408C; 26 extra aa		Unstable	Blaisdell et al. 2002
CYP2C19*13	CYP2C19.13	991A>G; 1210C>T	80181A>G; 87090C>T		I331V; R410C			Blaisdell et al. 2002
CYP2C19*14	CYP2C19.14	282C>C; 99C>T; 991A>G	60T>G; 99C>T; 80141A>G		L17P; I331V			Blaisdell et al. 2002
CYP2C19*15	CYP2C19.15	55A>C; 991A>G	55A>C; 80141A>G		I19L; I331V			Blaisdell et al. 2002
CYP2C19*16	CYP2C19.16	1204C>T Existence of the 1375-1377 polymorphism 681G>A on the same allele can not be excluded	80660C>T		R402C			Mizuno et al. 2004
CYP2C19*17	CYP2C19.17B	99C>T; 991A>G	-3402C>T; -806C>T; 99C>T; 80181A>G		I331V	Incr	Incr. transcr.	Sim et al. 2006; Sanford et al. 2007; Hachiya et al. 2010
CYP2C19*18	CYP2C19.18	99C>T; 686G>A; 991A>G	99C>T; 80360>A; 80141A>G; 87108T>C		R229K; I331V			Fukushima-Uesaka et al. 2005
CYP2C19*19	CYP2C19.19	99C>T; 151A>G; 991A>G	99C>T; 151A>G; 80181A>G; 87108T>C		S51G; I331V			Fukushima-Uesaka et al. 2005
CYP2C19*20	See CYP2C19*5B							
CYP2C19*21	See CYP2C19*2C							
CYP2C19*22	CYP2C19.22	517G>C; 991A>G	17689G>C; 80181A>G		R185P; I331V			Mehlotra et al. 2009
CYP2C19*23	CYP2C19.23	99C>T; 1710>C; 990A>G	99C>T; 124450>C; 80181A>G		490R; I331V			Zhou et al. 2009
CYP2C19*24	CYP2C19.24	99C>T; 991A>G; 1084G>A; 1197A>G	99C>T; 80181A>G; 80174G>A; 87158A>G		I331V; R315Q			Zhou et al. 2009
CYP2C19*25	CYP2C19.25	99C>T; 991A>G; 1344C>G	99C>T; 80181A>G; 99089C>G		I331V; F448L			Zhou et al. 2009
CYP2C19*26	CYP2C19.26	99C>T; 766G>A; 990A>G	99C>T; 19139G>A; 80181A>G		D216N; I331V			Lee et al. 2009
CYP2C19*27	CYP2C19.27B	990A>G	-3943G>A; 80181A>G		I331V		Decr. expr.	Drögemöller et al. 2010
CYP2C19*28 (Preliminary)	CYP2C19.28	55A>C; 991A>G; 1120G>A	-3099C>T; -2030C>A; -349T>C; 55A>C; 80654A>G; 80190G>A		I19L; I331V; V374I			Drögemöller et al. 2010
Additional SNPs, where the haplotype has not yet been determined								

Varianti alleliche *CYP2C19*

- Membro di CYP 450 preposto al metabolismo di antiepilettici, antiulcera, antiaggreganti piastrinici ed alcuni inbitori della pompa dei protoni
- descritte da Human Cytocrome Allele Nomenclature Committee (http://www.cypalleles.ki.se) più di 25 varianti alleliche di CYP2C19
- gli alleli wild-type del gene *CYP2C19* sono *CYP2C19*1A* e *CYP2C19*1B*
- *CYP2C19*2* e **3* spiegano il 95% dei casi di PM phenotypes
- *CYP2C19*2*, variante allelica defettiva più comune prodotta da un aberrante splicing nell'esone quinto del gene
- *CYP2C19*3*, altro allele defettivo comune presente nell' 1% degli alleli metabolizzatori lenti dei Caucasici e in oltre il 25% degli alleli defettivi della popolazione Orientale
- ci sono sostanziali differenze nella prevalenza dei polimorfismi di *CYP2C19* fra i vari gruppi di popolazione

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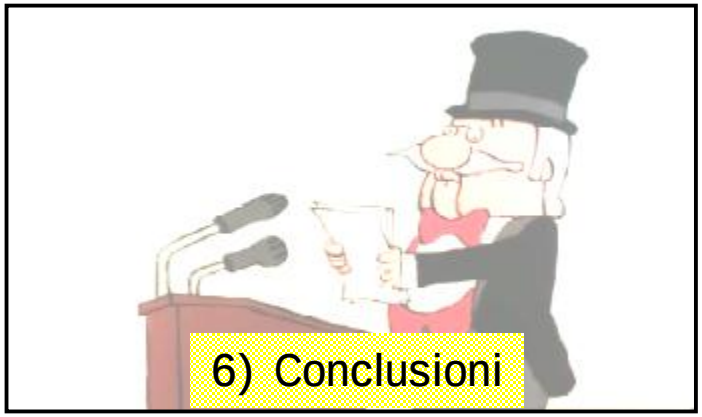

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PPI e interazioni farmacologiche

Table 2 Common drug substrates and clinically important inhibitors of CYP2C19

CYP2C19 substrates	CYP2C19 inhibitors	CYP2C19 inducers
Proton-pump inhibitors: omeprazole, esomeprazole, lansoprazole, rabeprazole, and pantoprazole	Omeprazole, esomeprazole, lansoprazole, rabeprazole	Rifampicin
Antiprotease: Nelfinavir		
Antiplatelet: clopidogrel, ticlopidine	Ticlopidine, clopidogrel	
Antifungal	Voriconazole	
Anticonvulsivant: phenytoin, diazepam	Cimetidine	Carbamazepine
Anticancer: cyclophosphamide, tamoxifene		
Antidepressants: amitriptyline, citalopram, clomipramine, sertraline	Fluvoxamine	

[Verstuyft et al., 2009 mod.]

Table 5 Allele frequencies of CYP2C19*2 and *3 polymorphisms in various ethnic populations [Verstuyft et al., 2009]

Population	Subject, n	CYP2C19 *1	*2	*3	Study
Caucasians					
Caucasians, Germany	328	84	15.9	0.3	Aynacioglu et al.[156]
Caucasians, Italy	360	88.9	11.1	0	Scordo et al.[157]
Caucasians, Turkey	404	84	15.9	0.15	Aynacioglu et al.[156]
Caucasians, European-American	210	87	13	0	Ozawa et al.;[158] Goldstein et al.[159]
Caucasians, European-American	546	86.4	12.7	0.9	Luo et al.[160]
Non-oriental					
African American	216	75	25	0	Goldstein et al.[159]
African American	472	81	18.2	0.8	Luo et al.[160]
Bolivian	778	92.2	7.8	0.1	Bravo-Villalta et al.[161]
Ethiopian	114	86.4	13.6	0	Persson et al.[162]
Mexican Americans	692	90.2	9.7	0.1	Luo et al.[160]
Palestinian	200	91.3	5.8	3	Sameer et al.[163]
Saudi Arabian	194	85	15	0	Ozawa et al.[158]
Native Canadian Indians	115	80.9	19.1	0	Nowak et al.[164]
Asians					
Burmese	127	66	30	4	Tassaneeyakul et al.[165]
Chinese	27	50.0	45.5	4.5	Yamada et al.[166]
Chinese Han	400	69.75	24.67	3.27	Chen et al.[167]
Filipinos	104	54	39	7	Goldstein et al.[159]
Iranian	400	86	14	0	Zand et al.[168]
Indian-North	200	70	30	0	Lamba et al.[169]
Indian-Tamilian	112	60	38	2	Adithan et al.[170]
Japanese	30	61.8	27.4	10.8	Takakubo et al.[171]
Japanese	106	67	23	10	Ozawa et al.[158]
Korean	206	67.5	20.9	11.6	Herrlin et al.[173]
Korean	377	64.2	28.3	7.6	Lee et al.[172]
Thai	774	68	29	3	Tassaneeyakul et al.[165]
Southeast Asians	160	63.1	31.2	5.7	Luo et al.[160]
Vietnamese	165	68.8	26.4	4.9	Lee et al.[172]
Vietnamese	90	62	24	14	Yamada SJ et al.[166]

- CYP2C19-PM in 2-3% Caucasici, 4% Africani, 10-25% Asiatici e 70% Vanuatuans
- linee guida 2008 American Heart Association profilassi PPI in terapia antiaggregante post-infarto miocardico..ma
- PPIs alterano assorbimento farmacologico modificando pH gastrico
- PPIs via metabolica di clopidogrel e inibitori CYP2C19
- CYP2C19-PM double hit su clopidogrel e omeprazolo

- Alta frequenza suicidio e Duplicazione/Multiduplicazione gene CYP2D6
- metabolismo ultrarapido Antidepressivi [Zackrisson et al., 2010]
- interazione Antidepressivi-PPIs e intossicazione CYP2C19-dipendente

- nuovo allele CYP2C19*17 metabolizzazione ultrarapida omeprazolo, mefenitoina, antidepressivi e attivazione ultrarapida clopidogrel; più comune tra Caucasici e Etiopi (16%)
- CYP2C19*17 riclassifica il 30% dei metabolizzatori rapidi in ultrarapidi
- nuovo allele afunzionale CYP2C19*4 in linkage disequilibrium con *17
- nuovo aplotipo CYP2C19*4B altera l'interpretazione della genotipizzazione di CYP2C19 quando testato per *17 (Scott et al. 2011)

Circulation
JOURNAL OF THE AMERICAN HEART ASSOCIATION

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ACCF/ACG/AHA 2010 Expert Consensus Document on the Concomitant Use of Proton Pump Inhibitors and Thienopyridines: A Focused Update of the ACCF/ACG/AHA 2008 Expert Consensus Document on Reducing the Gastrointestinal Risks of Antiplatelet Therapy and NSAID Use: A Report of the American College of Cardiology Foundation Task Force on Expert Consensus Documents

Writing Committee Members, Neena S. Abraham, Mark A. Hlatky, Elliott M. Antman, Deepak L. Bhatt, David J. Bjorkman, Craig B. Clark, Curt D. Furberg, David A. Johnson, Charles J. Kahi, Loren Laine, Kenneth W. Mahaffey, Eamonn M. Quigley, James Scheiman, Laurence S. Sperling and Gordon F. Tomaselli

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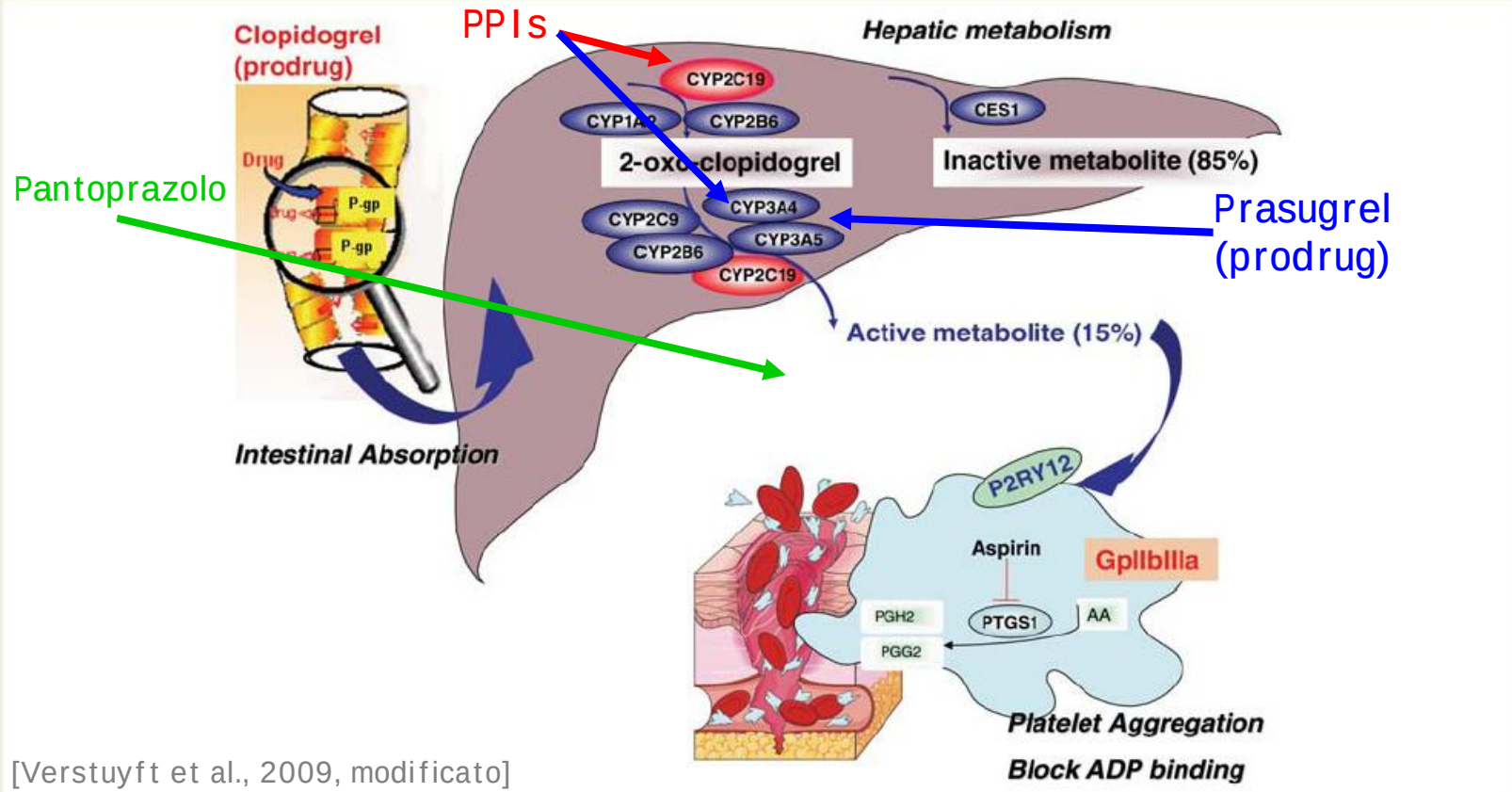
 Print ISSN: 0009-7322. Online ISSN: 1524-4539

- Tailored therapy: basata su tests di pharmacogenomic profiling per CYP2C19 e funzionalità piastrinica (thienopyridine, PPIs, H2RAs)
- evidenze empiriche aberranti (Abraham et al. 2010)

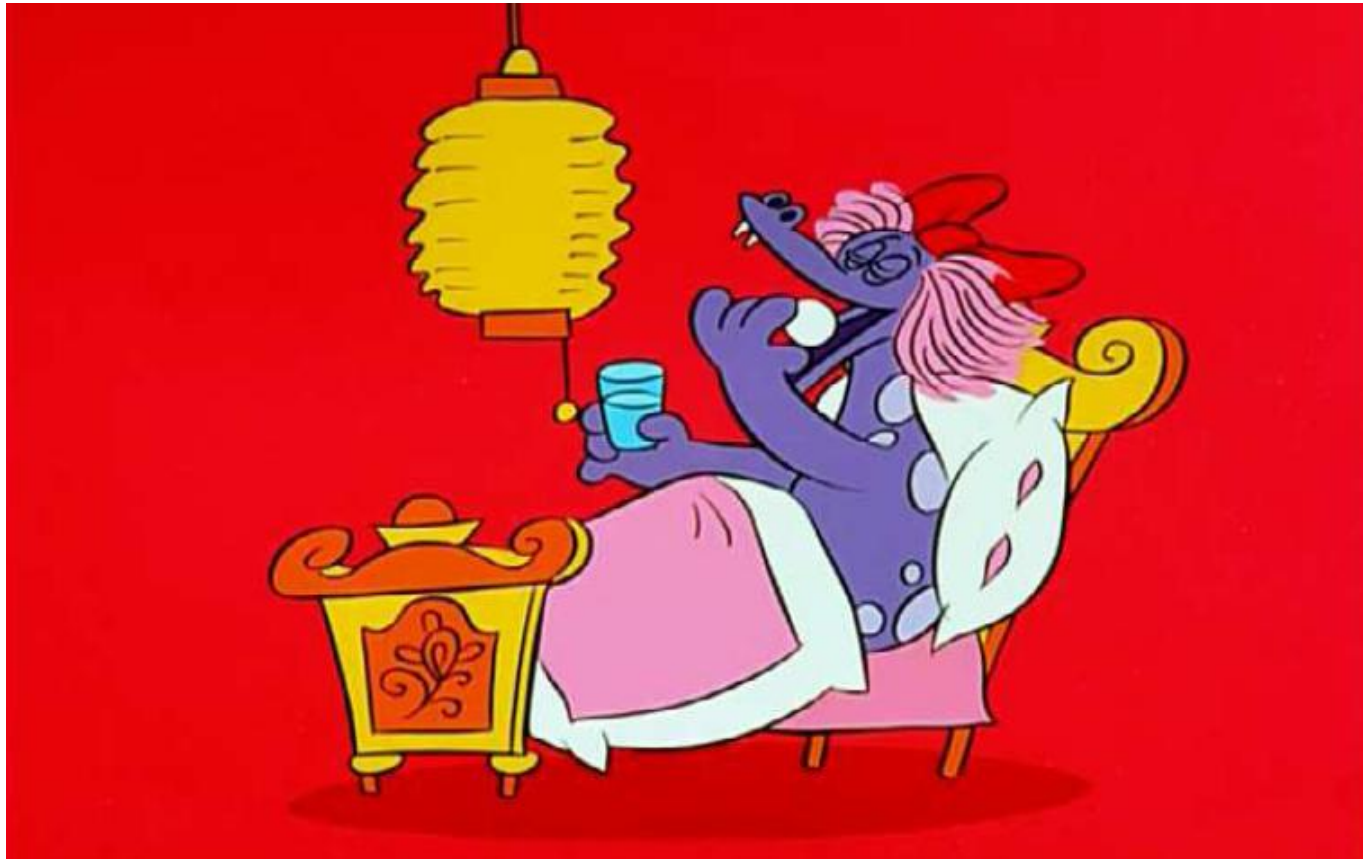
CYP2C19, PPIs e thienopyridine



[Verstuyft et al., 2009, modificato]

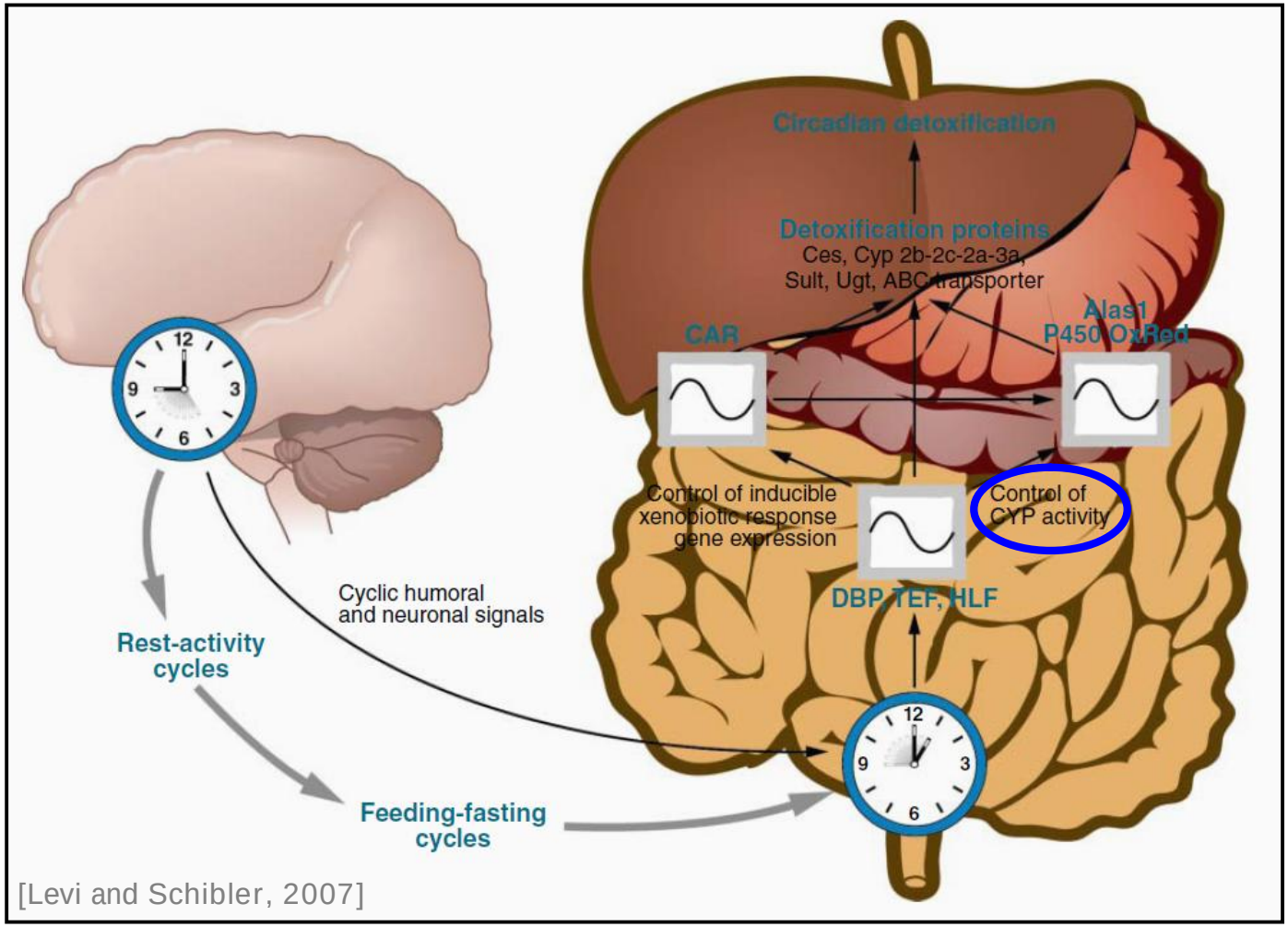
- Pantoprazolo Clopidogrel-non correlato [Pharmacogenetics and Genomics 2010]
- Prasugrel, thienopyridine di III generazione, antagonista irreversibile di P2Y12 ADPR
  - idrolizzato da esterasi a thiolactone inattivo, poi attivato da Cyps epatici
  - via epatica maggiore CYP3A4 e CYP2B6, minore CYP2C9 e CYP2C19 ma..

CYP3A4, CYP3A5-genetic polymorphisms e PPIs



- >> farmaci (clarithromycin), bassa frequenza allelica, variabilità interindividuali limitate e popolazione-specifica (4% popolazioni bianche, più alta frequenza popolazioni di colore)
- sex-dependent drug-metabolizing P450, ↓ 1.5- 2 volte > [Zanger et al., 2008]
- GH-dependente [Waxman and O'Connor, 2006], geni-sex-polimorfismo [Schrader et al., 2007]
- ritmi circadiani [Levi and Schibler, 2007]
- regolazione trascrizionale negativa da inflammatory signaling pathways [Univer et al., 2002]

CYP3A4, CYP3A5-genetic polymorphisms e PPIs



[Levi and Schibler, 2007]

• Pacemaker centrale nucleo soprachiasmatico ipotalamico, sincronizza gli oscillatori cellulari circadiani periferici

• orologi molecolari fegato, rene e piccolo intestino regolano fattori di trascrizione PARbZip modulanti enzimi detossificanti

PPIs inibitori CYP2C19: nuove concezioni

MOL PHARM

ULAR PHARMACOLOGY

Regulation of CYP2C19 Expression by Estrogen Receptor α: Implications for Estrogen-Dependent Inhibition of Drug Metabolism

Jessica Mwinyi, Isa Cavaco, Rasmus Steen Pedersen, Anna Persson, Sabrina Burkhardt, Souren Mkrtchian, and Magnus Ingelman-Sundberg

Section of Pharmacogenetics, Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden (J.M., I.C., R.S.P., A.P., S.B., S.M., M.I.-S.); and Centre for Molecular and Structural Biomedicine, IBB-Institute for Biotechnology and Bioengineering, University of Algarve, Faro, Portugal (I.C.)

Received April 10, 2010; accepted July 30, 2010

ABSTRACT

Cytochrome P4502C19 (CYP2C19) is an important drug-metabolizing enzyme involved in the biotransformation of, for example, proton pump inhibitors and antidepressants. Several in vivo studies have shown that the CYP2C19 activity is inhibited by oral contraceptives, which can cause important drug interactions. The underlying molecular mechanism has been suggested to be competitive inhibition. However, the results presented here indicate that estradiol derivatives down-regulate CYP2C19 expression via estrogen receptor (ER) α, which interacts with the newly identified ER-binding half site [estrogen response element (ERE)] at the position $-151/-147$ in the *CYP2C19* promoter. In gene reporter experiments in Huh-7 hepatoma cells, the activity of the luciferase construct carrying a 1.6-kb long *CYP2C19* promoter fragment cotransfected with ERα was down-regulated upon treatment with 17β-estradiol (EE) or 17α-ethinylestradiol (ETE) at half-maximum concentrations of 10^{-7} and 10^{-8} M, respectively. Mutations introduced into the ERE half site $-151/-147$ significantly inhibited these ligand-dependent effects. Electrophoretic mobility shift assays and quantitative chromatin immunoprecipitation experiments revealed that estrogen receptor α binds to this element. A significant suppression of *CYP2C19* transcription by female sex steroids was confirmed by reverse transcription polymerase chain reaction after hormonal treatment of human hepatocytes. Inhibition experiments using a stable human embryonic kidney 293 CYP2C19 cell line revealed competitive inhibition at much higher concentrations of EE and ETE compared with those required for transcriptional inhibition. These results indicate that both EE and ETE inhibit CYP2C19 expression via an ERα-dependent regulatory pathway, thus providing a new insight into the molecular mechanism behind the inhibitory effect of oral contraceptives on CYP2C19 activity.

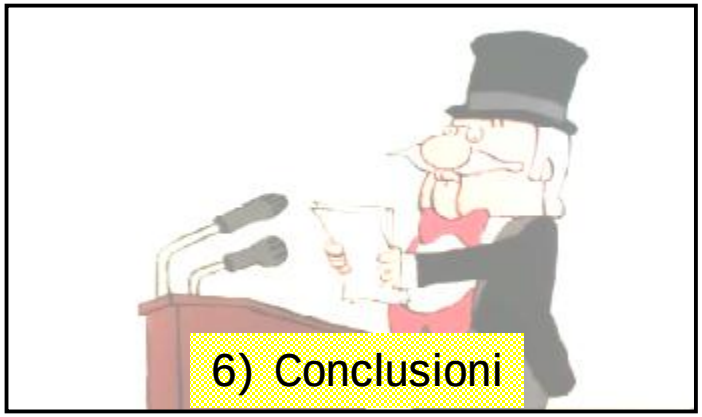
1) Introduzione

2) Polimorfismo epatico

3) PPIs

4) Interazioni PPIs

5) Teranostica



6) Conclusioni

Teranostica

DNA Microarray Technology in the Clinical Environment: *The AmpliChip CYP450 Test for CYP2D6 and CYP2C19 Genotyping*

By Jose de Leon, MD, Margaret T. Susce, RN, MLT, Maria Johnson, RN, Mike Hardin, RN, Lorraine Maw, MA, Alison Shao, MSc, Antonette C.P. Allen, MSc, Francis A. Chiafari, MSc, Grantland Hillman, MSc, and D. Michele Nikoloff, PhD

ABSTRACT

Introduction: An important technological advance in genetic testing is the DNA microarray, which allows for the simultaneous testing of thousands of DNA sequences. The AmpliChip CYP450 Test employs this microarray technology for cytochrome P450 (CYP) 2D6 and CYP2C19 genotyping. Isoenzymes encoded by these genes are responsible for the metabolism of many widely prescribed drugs. The objectives of this study were to identify CYP2D6 and CYP2C19 alleles and phenotypes in a psychiatric patient population in Kentucky, and to describe practical issues associated with DNA microarray technology.

FOCUS POINTS

- Recruiting included a total of 4,532 psychiatric patients from three state hospitals in Kentucky. The AmpliChip CYP450 Test provided successful genotyping results in 94% of the cytochrome P450 (CYP) 2D6 patients (4,265/4,532) and in 98% of the CYP2C19 patients (4,450/4,532).
- The prevalences of CYP2D6 poor metabolizers (7.6%), CYP2D6 ultrarapid metabolizers (1.5%), and CYP2C19 poor metabolizers (2.0%) suggest that the sample may be similar to what would be expected in a general population of similar racial background.
- Describing CYP2D6 activity with a numeric system reduces the complexity of predicting phenotypic variation in CYP2D6 and CYP2C19 enzyme activity. Our proposed numeric system is a preliminary, rough approximation that serves as a useful teaching tool and predicts risperidone levels.

Dr. de Leon is the medical director of the Mental Health Research Center at Eastern State Hospital in Lexington, Kentucky; professor of psychiatry at the College of Medicine and of Pharmacy, Practice & Sciences at the University of Kentucky College of Pharmacy in Lexington; member of the Psychiatry and Neurosciences Research Group (CTS-549); and visiting professor at the Institute of Neurosciences at the Medical School of the University of Granada in Spain. Ms. Susce is clinical research coordinator and Ms. Maw is editing/research associate at the Mental Health Research Center at Eastern State Hospital. Ms. Johnson and Mr. Hardin were nurse researchers at the Mental Health Research Center at Eastern State Hospital at the time of this study. Ms. Shao is assistant clinical supervisor and lead forensic analyst, Ms. Allen is lab manager, and Mr. Chiafari is molecular technology director, all at BRT Laboratories, Inc., Baltimore, Maryland. Mr. Hillman is bioinformatics manager and Dr. Nikoloff is a research leader at Roche Molecular Diagnostics, Inc., in Pleasanton, California.

Faculty Disclosure: Roche Molecular Systems, Inc., markets the US Food and Drug Administration-approved AmpliChip CYP450 Test, which identifies cytochrome P450 (CYP) 2D6 and CYP2C19 gene variations. Dr. de Leon is a co-investigator in a National Institutes of Health Small Business Innovation Research Grant awarded to Genomas, Inc. Since October 1, 2005, Dr. de Leon has received two researcher initiated grants, one from Roche Molecular Systems for this study and one from Eli Lilly (the latter as co-investigator) and was on the advisory board of Roche Molecular Systems (2006). He personally develops his presentations for lecturing and has never lectured using any pharmaceutical company presentations. Since October 1, 2005, his lectures have been supported six times by Roche Molecular Systems (six times in 2006), twice by Eli Lilly (2006), once by Janssen (2006), and once by Bristol-Myers Squibb (2006). Mr. Hillman and Dr. Nikoloff are Roche employees. Mses. Susce, Johnson, Maw, Shao, and Allen and Messrs. Hardin and Chiafari do not have an affiliation with or financial interest in any organization that might pose a conflict of interest. None of the authors have any conflict of interest regarding the tools used to collect DNA samples described in the article.

Funding/Support: Subject recruitment was supported by a researcher-initiated grant from Roche Molecular Systems (75% of direct costs), a NARSAD Independent Investigator Award to Jose de Leon, MD (6% of direct costs), and internal resources (19% of direct costs). Funding for genotyping in this study was provided by Roche Molecular Systems.

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Reprint requests: Jose de Leon, MD, Eastern State Hospital, Mental Health Research Center, 627 West Fourth St., Lexington, KY 40508; Tel: 859-246-[illegible]; Email: joseleon@uky.edu.

Methods: A total of 4,532 psychiatric patients were recruited from three state hospitals in Kentucky. Whole blood, buccal swabs, or saliva samples were genotyped with the AmpliChip CYP450 Test to derive a predicted phenotype.

Results: In this cohort, the overall prevalence of CYP2D6 poor metabolizers was 7.6% (95% CI 7%, 8.3%), 8.2% in the Caucasians (95% CI 7.4%, 9.1%) and 1.8% in the African Americans (95% CI 0.9%, 3.5%). The overall prevalence of CYP2D6 ultrarapid metabolizers was 1.5% (95% CI 1.2%, 1.9%), 1.5% in the Caucasians (95% CI 1.1%, 1.9%) and 2.0% in the African Americans (95% CI 1.1%, 3.7%). The overall prevalence of CYP2C19 poor metabolizers was 2.0% (95% CI 1.8%, 2.7%), 2.2% in Caucasians (95% CI 1.6%, 2.5%) and 4.0% in African Americans (95% CI 2.6%, 6.1%).

Conclusion: We also propose a numeric system for expression of CYP2D6 and CYP2C19 enzyme activity to aid clinicians in determining treatment strategy for patients receiving therapeutics that are metabolized by the CYP2D6 or CYP2C19 gene products.

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Unraveling human complexity and disease with systems biology and personalized medicine

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Drug-dosing chip

Hoffmann–La Roche AG (Basel, Switzerland) states on its website that it aims to "determine disease predisposition, provide information that can act upon to prevent or delay the onset of illness, and even monitor treatments". The company currently produces the AmpliChip®, a DNA chip-based diagnostic test that aids in individualized drug dosing. According to Roche's company website, CYP450 is the world's first pharmacogenetic microarray-based test approved for clinical use. The AmpliChip CYP450 test provides comprehensive coverage of gene variations, including deletions and duplications, for both *CYP2D6* and *CYP2C19* genes, which play a major role in the metabolism of an estimated 25% of all prescription drugs [74]. It is intended to be an aid for physicians in individualizing treatment selection and dosing for drugs metabolized through these genes. This is yet another example of a currently available personalized medicine approach that allows for a more quantified approach to medicine. This should ultimately encourage more patient drug compliance and, if used on a broad scale, has implications with respect to increasing the efficacy and safety of pharmaceuticals, as adverse drug reactions are a major problem in the current system.

Teranostica

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Rapid Identification of the Hepatic Cytochrome P450 2C19 Activity Using a Novel and Noninvasive [^{13}C]Pantoprazole Breath Test

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Received October 22, 2008; accepted January 8, 2009

ABSTRACT

We tested the hypothesis that the stable isotope [^{13}C]pantoprazole is *O*-demethylated by cytochrome P450 CYP2C19 and that the $^{13}CO_2$ produced and exhaled in breath as a result can serve as a safe, rapid, and noninvasive phenotyping marker of CYP2C19 activity in vivo. Healthy volunteers who had been genotyped for the *CYP2C19*2*, *CYP2C19*3*, and *CYP2C19*17* alleles were administered a single oral dose of [^{13}C]pantoprazole sodium-sesquihydrate (100 mg) with 2.1 g of sodium bicarbonate. Exhaled $^{13}CO_2$ and $^{12}CO_2$ were measured by IR spectroscopy before (baseline) and 2.5 to 120 min after dosing. Ratios of $^{13}CO_2/^{12}CO_2$ after [^{13}C]pantoprazole relative to $^{13}CO_2/^{12}CO_2$ at baseline were expressed as change over baseline (DOB). Maximal DOB, DOB_{15} to DOB_{120}, and area under the DOB versus time curve (AUC_{0-120} and $AUC_{0-\infty}$) were significantly different among three genotype groups (*CYP2C19*1/*1*, $n = 10$; *CYP2C19*1/*2* or *CYP2C19*1/*3*, $n = 10$; and *CYP2C19*2/*2*, $n = 5$) with predicted extensive metabolizers (EMs), intermediate metabolizers (IMs), and poor metabolizers (PMs) of CYP2C19, respectively (Kruskal-Wallis test, $p < 0.01$); linear regression analysis indicated a gene-dose effect relationship (r^2 ranged between 0.236 and 0.522; all $p < 0.05$). These breath test indices were significantly lower in PMs than IMs ($p < 0.05$) or EMs ($p < 0.01$) of CYP2C19. [^{13}C]Pantoprazole plasma exposure showed significant inverse correlation with breath test indices in the respective subjects (Pearson $r = -0.74$; $p = 0.038$). These feasibility data suggest that the [^{13}C]pantoprazole breath test is a reliable, rapid, and noninvasive probe of CYP2C19 and seems to be a useful tool to optimize drug therapy metabolized by CYP2C19.

Influence of Different Proton Pump Inhibitors on Activity of Cytochrome P450 Assessed by [^{13}C]-Aminopyrine Breath Test

Chise Kodaira, MD, Shinya Uchida, PhD, Mihoko Yamade, MD, Masafumi Nishino, MD, Mutsuhiro Ikuma, MD, PhD, Noriyuki Namiki, PhD, Mitsushige Sugimoto, MD, PhD, Hiroshi Watanabe, MD, PhD, Akira Hishida, MD, PhD, and Takahisa Furuta, MD, PhD

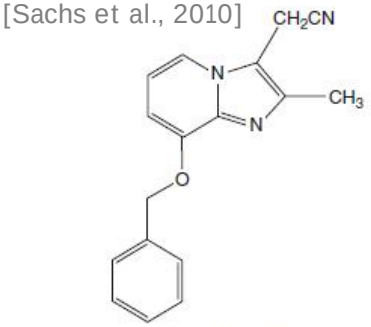
Aminopyrine is metabolized by cytochrome P450 (CYP) in the liver. The investigators evaluated influences of different PPIs on CYP activity as assessed by the [^{13}C]-aminopyrine breath test ([^{13}C]-ABT). Subjects were 15 healthy volunteers with different CYP2C19 status (5 rapid metabolizers [RMs], 5 intermediate metabolizers [IMs], and 5 poor metabolizers [PMs]). Breath samples were collected before and every 15 to 30 minutes for 3 hours after oral ingestion of [^{13}C]-aminopyrine 100 mg on day 8 of each of the following regimens: control; omeprazole 20 mg and 80 mg, lansoprazole 30 mg, and rabeprazole 20 mg. Changes in carbon isotope ratios in carbon dioxide ($^{13}CO_2/^{12}CO_2$) in breath samples were measured by infrared spectrometry and expressed as delta-over-baseline (DOB) ratios (‰). Mean areas under the curve of DOB from 0 to 3 h ($AUC_{0\text{-}3h}$ of DOB) were significantly decreased by omeprazole 20 mg and lansoprazole 30 mg but not by rabeprazole 20 mg. Conversely, higher PPI dose (ie, omeprazole 80 mg) seemed to further decrease $AUC_{0\text{-}3h}$ of DOB in RMs but increased it in PMs. Omeprazole and lansoprazole at the standard doses inhibit CYP activity but rabeprazole does not, whereas high-dose omeprazole seems to induce CYPs.

Keywords: *[^{13}C]-aminopyrine breath test; cytochrome P450; proton pump inhibitor; CYP2C19*

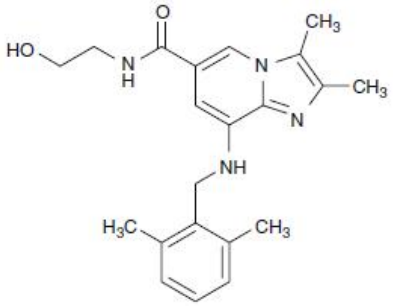
Journal of Clinical Pharmacology, XXXX;XX:xxx-xxx

Nuovi farmaci

[Sachs et al., 2010]



SCH28080



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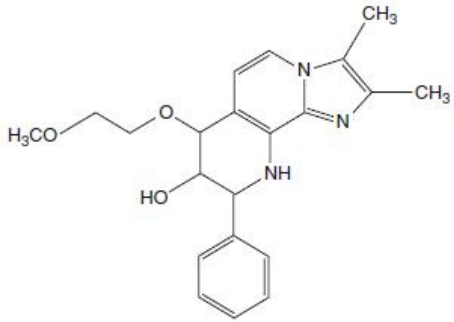
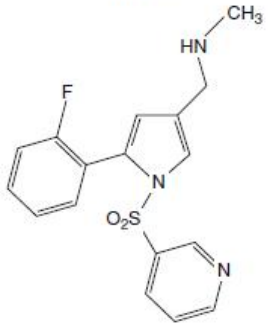
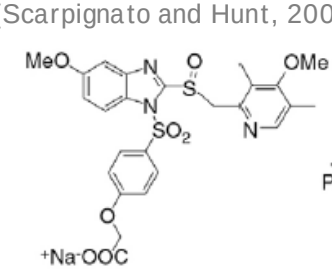


Soraprazan



TAK-438

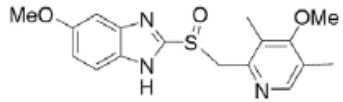
[Scarpignato and Hunt, 2008]



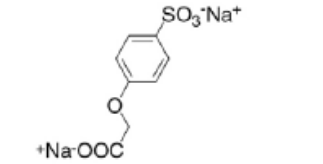
AGN201904-Z

Sodium 2-(4-(5-methoxy-2-((4-methoxy-3,5-dimethylpyridin-2-yl)methylsulfinyl)-1H-benzo[d]imidazol-1-ylsulfonyl)phenoxy)acetate
[CAS Registry No: 651728-41-5]

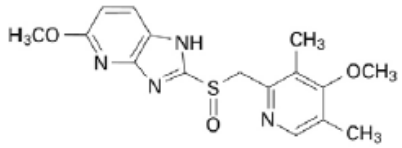
Absorption
Plasma hydrolysis



Omeprazole
+
Sulfonate



Sodium 2-(4-sulfonatophenoxy)acetate



Tenatoprazole (TU-199)
(RS)-5-methoxy-2-[[(4-methoxy-3,5-dimethylpyrid-2-yl)methyl]sulfinyl]1H-imidazo[4,5b] pyridine
[CAS Registry No:113712-98-4]

Table 1 *Potassium-competitive acid blockers* *Proton pump inhibitors*

P-CABs and PPIs: main differences in the mechanism of action (from Scarpignato *et al.* [2•]).

P-CABs	PPIs
Acts directly on the H^+,K^+-ATPase enzyme	*Requires transformation* to the active form
Superconcentrates in parietal cell acid space (*100 000-fold* higher than those in plasma)	*Concentrate* in parietal cell acid space (*1000-fold* higher than those in plasma)
P-CABs binds *competitively* to the potassium binding site of H^+,K^+-ATPase	Sulfenamide binds *covalently* to H^+,K^+-ATPase
Duration of effect related to half-life of drug in plasma	Duration of effect related to half-life of the sulfenamide–enzyme complex
Full effect from *first* dose	Full effect after *repeated* doses

Helicobacter Pylori Genomics

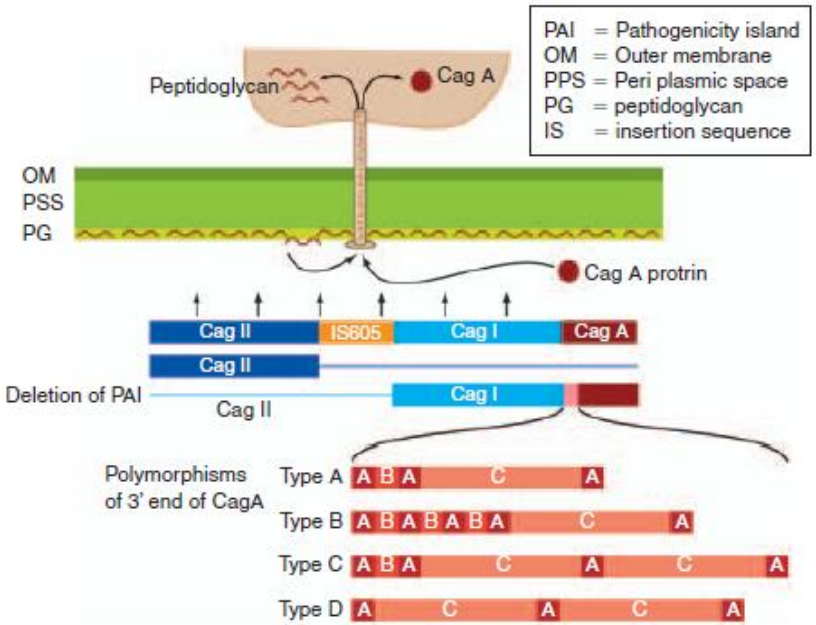


Figure 92.6 This diagram illustrates the PAI and its deletions and the polymorphisms found with in the *cagA* gene based upon the variation in the number of repeats of sequences A, B and C. The diagram also illustrates the inoculation of both bacterial peptidoglycan from the cell wall of *Helicobacter pylori* and the cagA protein into a gastric cell leading to disruption of intercellular signaling. The materials are transferred into the eukaryotic cell by a Type IV secretion system which is coded for by genes on the pathogenicity island.

TABLE 92.2 Current diagnostic and management uses of genomic and proteomic information

Methods	Diagnosis
PCR	HP risk markers identified
Microarray	HP few studies, NSAID no studies. Will be of use in understanding pathogenesis and identifying markers
Immunoproteomic	Early results encouraging. Will be of use in understanding pathogenesis and identifying markers
SNP	HP/NSAIDS risk factors identified
Serology	HP/NSAIDS – specific tests for colonization by HP; no specific tests for PUD; early studies indicating specific PUD-associated proteins require verification
	Management
SNP	HP markers identified of use in tailoring treatment. NSAIDS provision risk marker identified requires verification

[Genomic and Personalized Medicine, 2009]

Found 249 studies with search of: Proton pump inhibitors

Hide studies that are not seeking new volunteers.
Hide studies with unknown recruitment status.

Display Options

Rank	Status	Study
1	Unknown †	Endoscopic Fundoplication Versus Proton Pump Inhibitors for GERD Treatment Condition: Gastroesophageal Reflux Disease (GERD) Interventions: Procedure: Transoral Incisionless Fundoplication; Drug: Proton Pump Inhibitors; active control
2	Not yet recruiting	Cohort Study of Clopidogrel and Proton Pump Inhibitors Conditions: Coronary Heart Disease; Acute Coronary Syndrome; Drug Interactions; Clopidogrel; Proton Pump Inhibitors; Gastrointestinal Hemorrhage Intervention:
3	Completed	The Effect of Proton Pump Inhibitors on Calcium and Bone Metabolism Condition: Calcium Metabolism Disorders Intervention: Drug: Rabeprazole or Esomeprazole or Lanzoprazole
4	Recruiting	Proton Pump Inhibitor Therapy and Bone Density in Premature Infants Conditions: Osteopenia; Prematurity Intervention:
5	Recruiting	High Dose Versus Standard Dose Proton Pump Inhibitor (PPI) in High-risk Bleeding Peptic Ulcers After Combined Endoscopic Treatment Conditions: Endoscopy; Peptic Ulcer; Bleeding; Proton Pump Inhibitors Interventions: Drug: High dose pantoprazole infusion; Drug: Standard dose pantoprazole infusion
6	Completed	A Drug-drug Interaction Study of Oral 1250 mg of Vatalinib Administered Under Fasting and Fed Conditions With a Proton-pump Inhibitor in Healthy Sterile or Postmenopausal Female Volunteers Condition: Healthy Intervention: Drug: Vatalinib
7	Recruiting	Fundic Gland Polyps and Proton Pump Inhibitor (PPI) Drugs Condition: Fundic Gland Polyp Intervention:
8	Enrolling by invitation	Risk of Cancer Among Pantoprazole Users Condition: Esophagitis Intervention: Other: Does not apply

1) Introduzione

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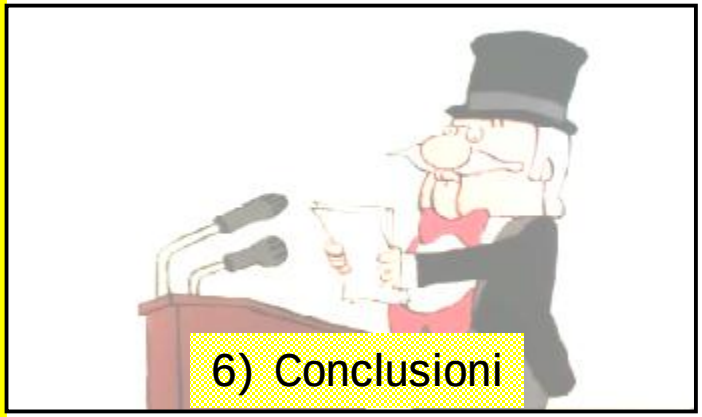
4) Interazioni PPIs

5) Teranostica



6) Conclusioni

Conclusioni

1) Introduzione

2) Polimorfismo epatico

3) PPIs

4) Interazioni PPIs

5) Teranostica

6) Conclusioni

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[Zagreb Film]