Molecular Genetic assays in cancer pharmacogenetics

By

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The aims of this technology :

1.To built a genetic profile for each patient.

2. To provide doctors and researchers an effective tool to select correct medicine prescription for cancer and other diseases .

3. To reduce the coast of cancer treatment.

4. To avoid the toxicity of drugs or to reduce it and to maximize the efficiency of treatment.

5. To reduce the mortality due to cancer.
6. and...
We are actually talking about Personalized Medicine

What are molecular genetic technologies used in cancer pharmacogenetics?

Table 1: Examples of molecular diagnostic technologies used for personalized medicine..... 44 assays

Polymerase chain reaction (PCR)-based methods Cold-PCR Digital PCR
DirectLinear™ analysis Quantitative fluorescent PCR Real-time PCR
Reverse transcriptase (RT) PCR Restriction fragment length polymorphism
Scorpions™ (DxS Ltd): closed-tube platform for the efficient homogeneous detection of PCR
Amplicons Single-strand conformational polymorphism Non-PCR methods
Arrayed primer extension Enzyme mutation detection Fluorescence resonance energy transfer (FRET) based assays: Invader assay Locked nucleic acid (LNA) technology Peptide nucleic acid (PNA) technology
Transcription-mediated amplification Gene chip and microfluidic microarrays
Nanodiagnostics
Nanoparticle-based integration of diagnostics with therapeutics
Nanotechnology-based refinement of diagnostics for pharmacogenetics
Toxicogenomics
Single nucleotide polymorphism genotyping DNA methylation studies
Gene expression based tests DNA sequencing Multiplex DNA sequencing
Sequencing in microfabricated high-density picoliter reactors
Whole genome sequencing Cytogenetics Comparative genomic hybridization (CGH)
Fluorescent in situ hybridization Proteomic-based methods
Fluorescent in situ protein detection
Protein/peptide arrays for identification of multiple biomarkers in blood and tissue samples
Protein biochip technology Toxicoproteomics MicroRNA-based diagnostics
Molecular imaging Functional MRI with nanoparticle contrast FDG-PET
Optical imaging

Allelic Polymorphism

Single Nucleotide Polymorphisms-SNPs
300,000- 1,000,000 SNPs

Table 2: Technologies for SNP analysis.....31 technologies

Digital Genetic Analysis

DNA chips and microarrays

DNA sequencing

Electrochemical DNA detection

Solution-borne ferrocene-modified DNAs

Redox-active intercalators

Surface-bound molecular beacon-like DNA

Fluorescence-detected 5¢-exonuclease assays

Hybridization assays

REFLPs

Allele-specific oligomer hybridization

Array hybridization assays, e.g., MASDA (mutiplexed allele-specific diagnostic assay)

Hybridization with PNA probes

Invader assay

Mass spectrometry (MS)

Matrix Assisted Laser Desorption Ionization Time of Flight MS (MALDI-TOF MS)

Competitive Oligonucleotide Single Base Extension

Nanoparticle probes

Oligomer-specific ligation assays

PCR-based methods

PCR-CTPP (confronting two-pair primers)

Degenerate oligonucleotide primed (DOP)-PCR

TaqMan real-time PCR

Smart amplification process version 2

Peptide nucleic acid (PNA) probes

Primer extension

Pyrosequencing

Single base extension-tag array on glass slides (SBE-TAGS)

Single molecular fluorescence technology

Triplex Assay (Genetic Technologies, Inc.)

WAVE System's Temperature Modulated Heteroduplex Analysis method

Zinc finger proteins

Molecular genetic assays in diagnosis of risk factor

Effect of MDR1 Gene Expression Related with C3435T Polymorphism in Iraqi Acute Myeloid Leukemia patients

Abdul Hussein M. AL-Faisal [1] and Kifah Jabbar Alyaqubi[2]

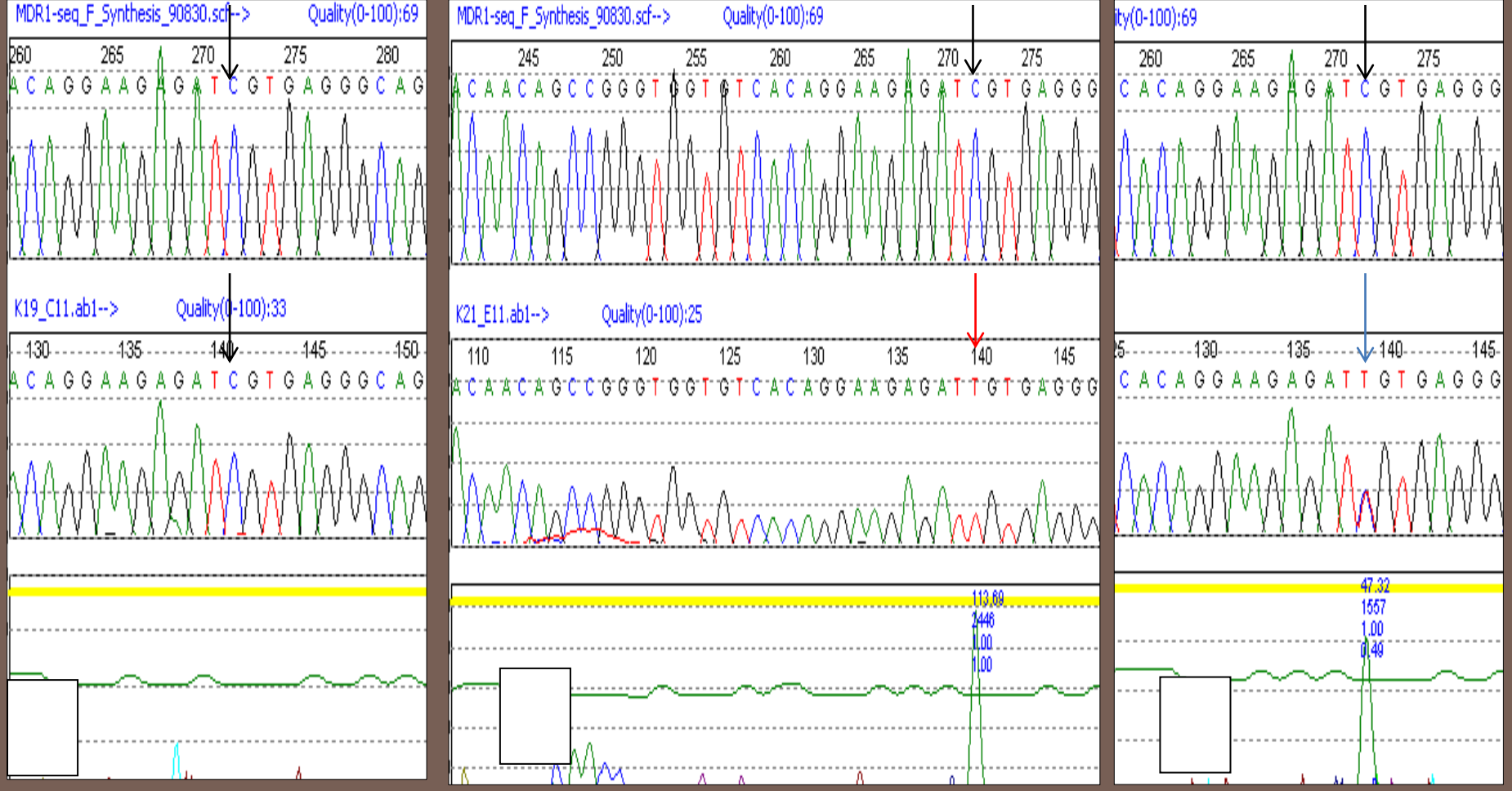


Figure 1: Electrograph show DNA sequencing for (A) wild type C3435T wt/wt(C/C) (B) homozygous mt/mt (T/T) (C) heterozygous wt/mt (C/T). upper arrow represented references MDR1 (wild type) and lower row the sample

Genotype C3435T	Control n=10	AML n=31	P-value
CC	2(20)	6(19.35)	0.844 NS
CT	5(50)	15(48.38)	0.749 NS
TT	3(30)	10(32.25)	0.802 NS
P-value	0.0038 **	0.014 **	
Alleles frequency			
C	9(45)	27(43.5)	0.752 NS
T	11(55)	35(56.5)	0.955 NS
P-value	0.044 *	0.052 *	

No.(%) (P<0.05), (P<0.01)**, NS (no significant)*

Genotype C3435T	AML n=31	Control n=10	χ^2	OR	(95%CI)
CC	6(19.35)	2(20)	0.991	CC vs CT	1.0
CT	15(48.38)	5(50)		CT vs TT	0.90.73-1.101
TT	10(32.25)	3(30)		CC vs TT	0.90.73-1.101
Allele frequency					
C	9(45)	27(43.5)	0.909		
T	11(55)	35(56.5)			

Table 2: Estimation of risk developing in AML association with MDR1C3435T Genotype

1. ++ CC & TT are protective genotypes against AML --- CT genotype with high risk to have AML

Genotype results showed there was significant difference in genotype and allele frequency with heterozygous CT (50%: p=0.0038<0.01) and mutant T-allele (55%: p=0.044<0.05) respectively for MDR1 SNP C3435T in normal Iraqi population.

2. ORs and (95%CI) revealed no relative risk associated with MDR1 C3435T polymorphism to development AML.

3. According to the clinical outcome,

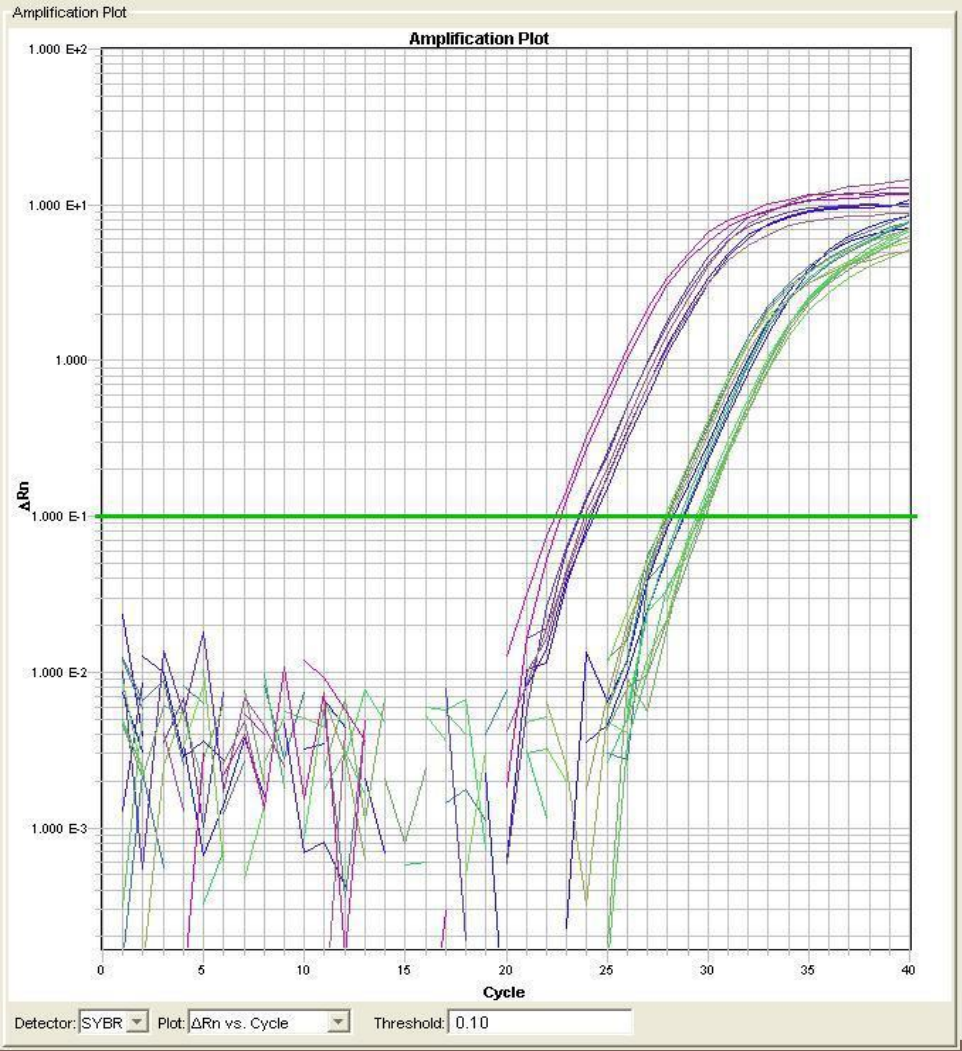
--there were (54.83%) patients showed NR to chemotherapy at presentation,

--While (45.16%) patients were showed CR.

4. According to the clinical outcome status,

--the percentage of patients with MDR1 3435CT was higher than those with 3435CC/TT among NR AML, while in CR group was showed high with homozygous TT.

complete remission (CR)
Not response (NR)



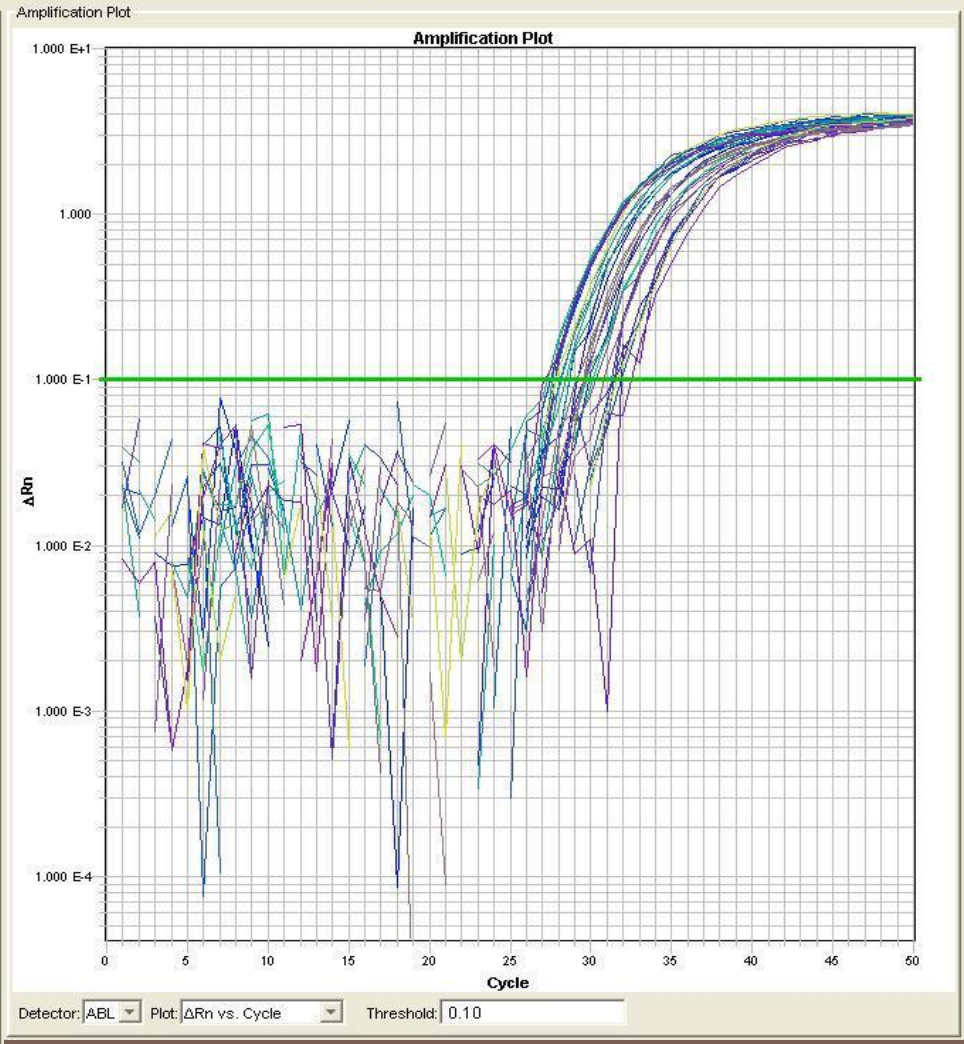


Figure (4-7) Amplification plot of MDR1 (red arrow) and ABL (green arrow) A) Shows samples with high level of MDR1 compare with low level of ABL. B) Shows samples with different level of expression for ABL and MDR1

Relationship between MDR1 Gene Expression and MDR1 C1236T Genotype with AML Clinical Outcomes

Genotype	MDR1 Fold Change of NR AML	MDR1 Fold Change of CR AML
	n=17	n=14
CC n=6	0.45 ± 0.02 (3)	0.37 ± 0.02 (3)
CT n=17	3.32 ± 0.11 (10)	0.30 ± 0.02 (7)
TT n=8	3.01 ± 0.08 (4)	0.41 ± 0.01 (4)
p-value	0.013 ** 0.317	NS

Increasing of MDR1 Gene expression cause NR to drug

Genotype C3435T	AML NR	AML CR	p-value
CC	0.21	0.29	0.439 NS
CT	3.10	0.17	0.0025 **
TT	3.01	0.50	0.0126 **

Table 3: MDR1 expression related with C3435T SNP in AML clinical outcome

AML clinical outcome the statistical analysis showed :

----highly significant differences in MDR1 gene expression dependent in 3435CT/TT genotype in non-responding patients (3.10 p=0.0025<0.01) (3.01 p=0.126**<0.01) respectively,**

----while CC genotype appeared non-significant with clinical outcome

In conclusion

---healthy Iraq populations and AML patients have predominantly CT genotype and mutant-T allele frequency for MDR1 C3435T polymorphism.

---MDR1 3435CT/TT genotype in regard with MDR1gene expression in de novo AML patients associated with poor prognosis,

---while CC genotype was protective Carrier.

Molecular genetic assays in evaluation of cancer drug efficiency

The effectiveness of any drug must associate with:

--its good absorption,

--correct metabolism,

--specific target and

--un accumulated metabolites

-This make any drug as very effective weapon against specific disease.

-But the reality is some think quite different from that.

-This due to the differences of patients response to drug.

-Some patients have good response to drug therapy, others are either with mild to poor response or resist the drug.

- On the other hand, some patients are reflect a kind of toxicity when they use a kind of drug.

-- Statistically, 30 to 50 % of patients have poor response or resist the drug in addition to 5% reflect high drug toxicity.

-- This will coast the community a lot of money.

If we look to in deep we will find that the drug effectiveness leads by enzymes which are the mirror copies of genes.

This mean that response/resist and toxicity to drug depend not just on drug but on genes(enzymes) that metabolite the drugs.

This mean that response / resistance and toxicity to drug depends on individual genetic variations.

So what are the sources of genetic variations??

-Sources of variations in individuals

A. Crossing Over

B. Dominance & Recessive

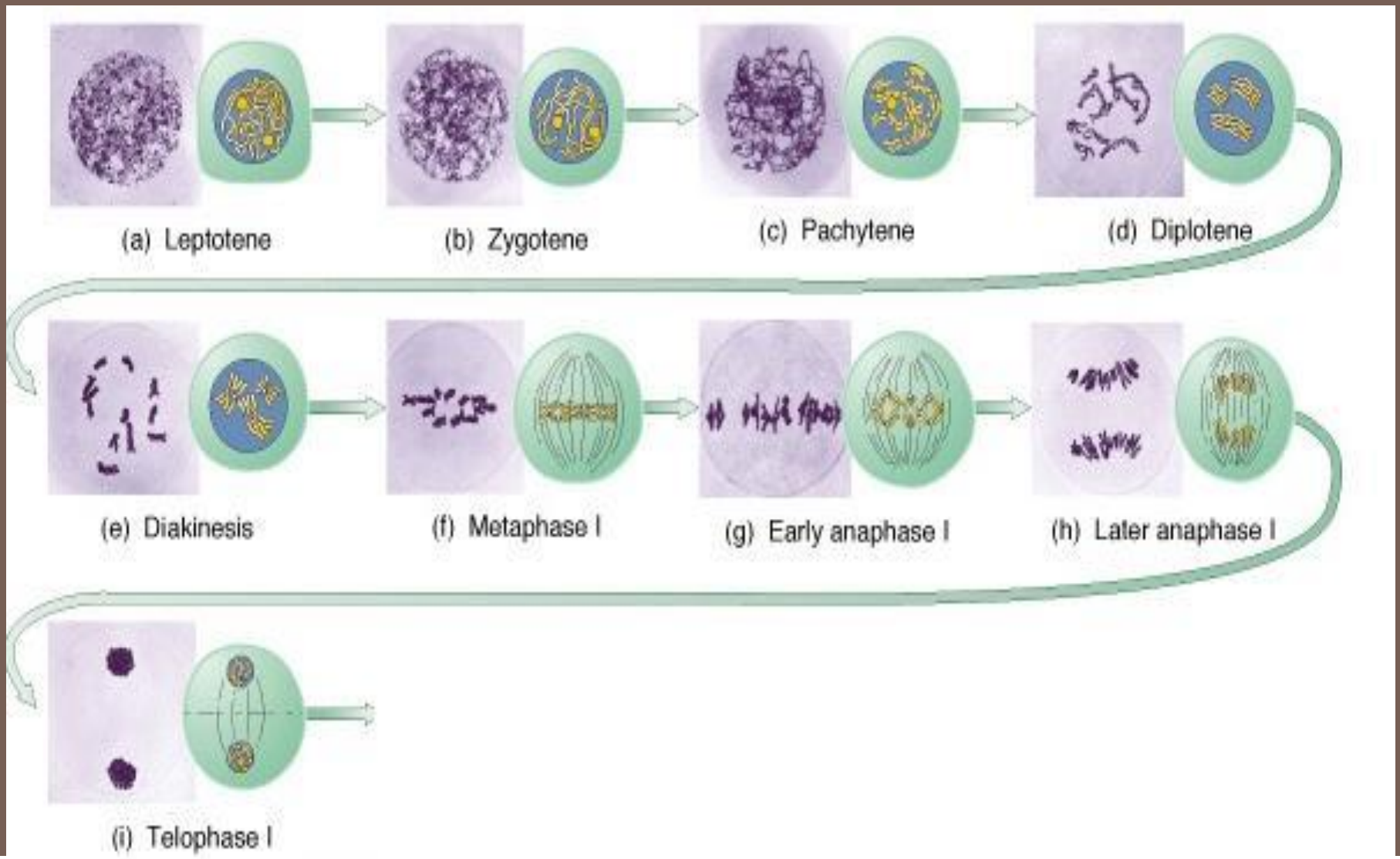
C. Allelic Polymorphism

D. Hormonal Influence

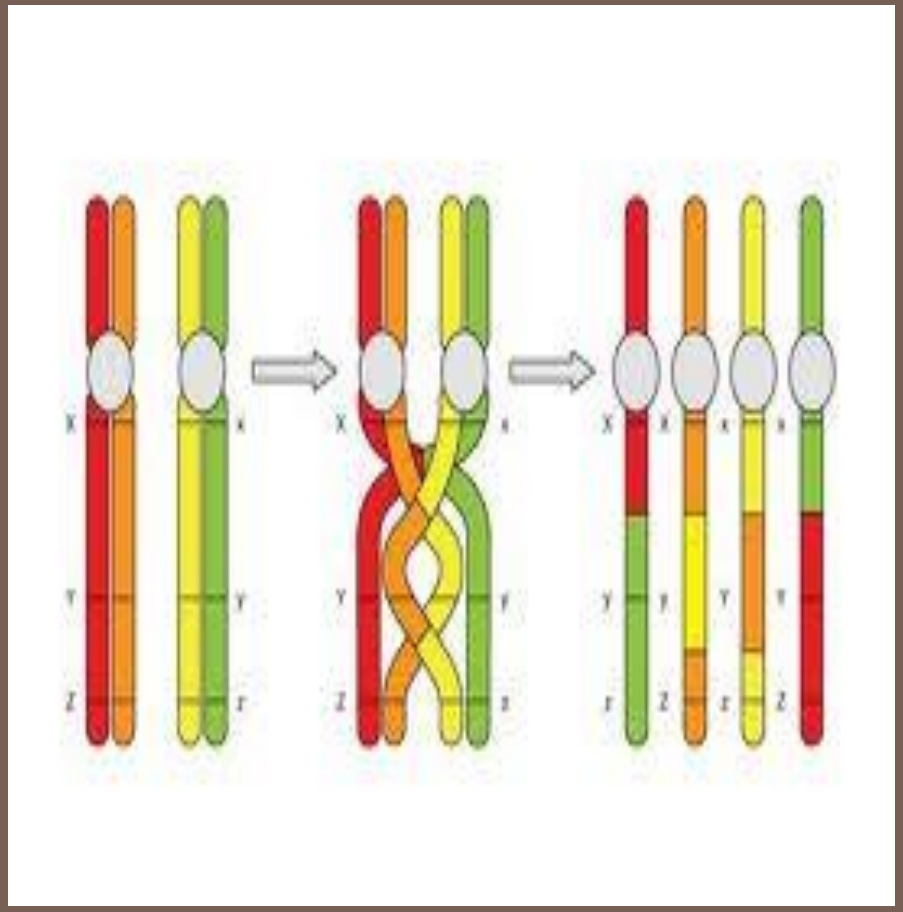
E. Chromosome X inactivation

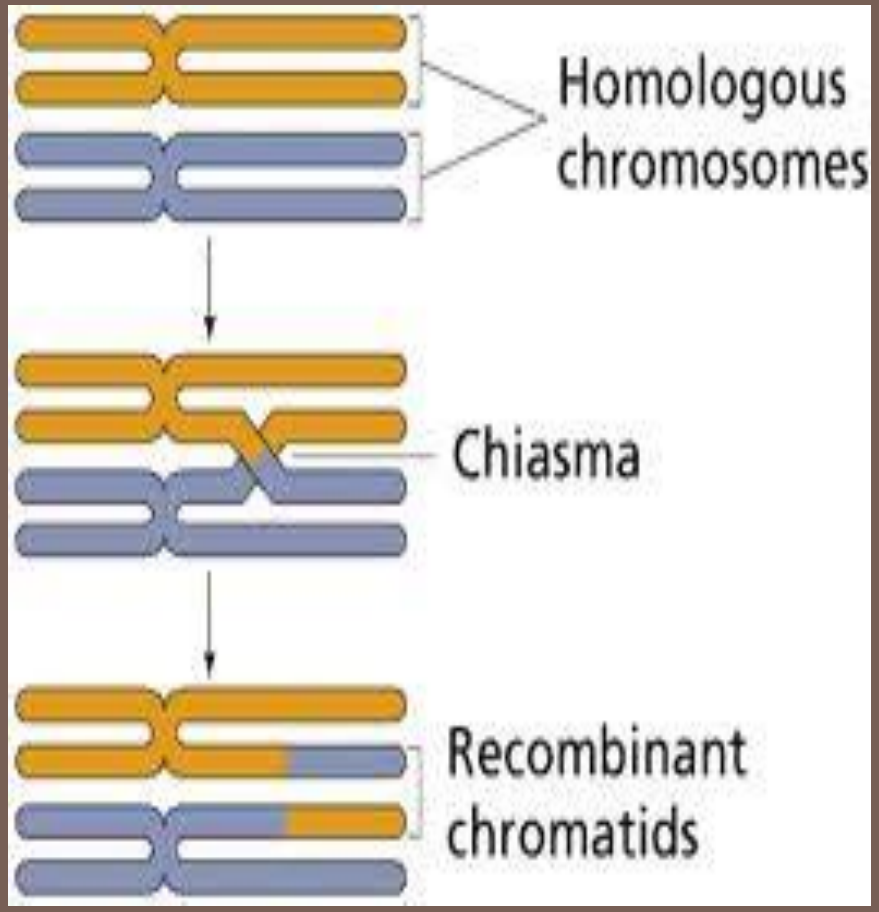
F. Race

Meiosis I division in Sex or Germ Cells



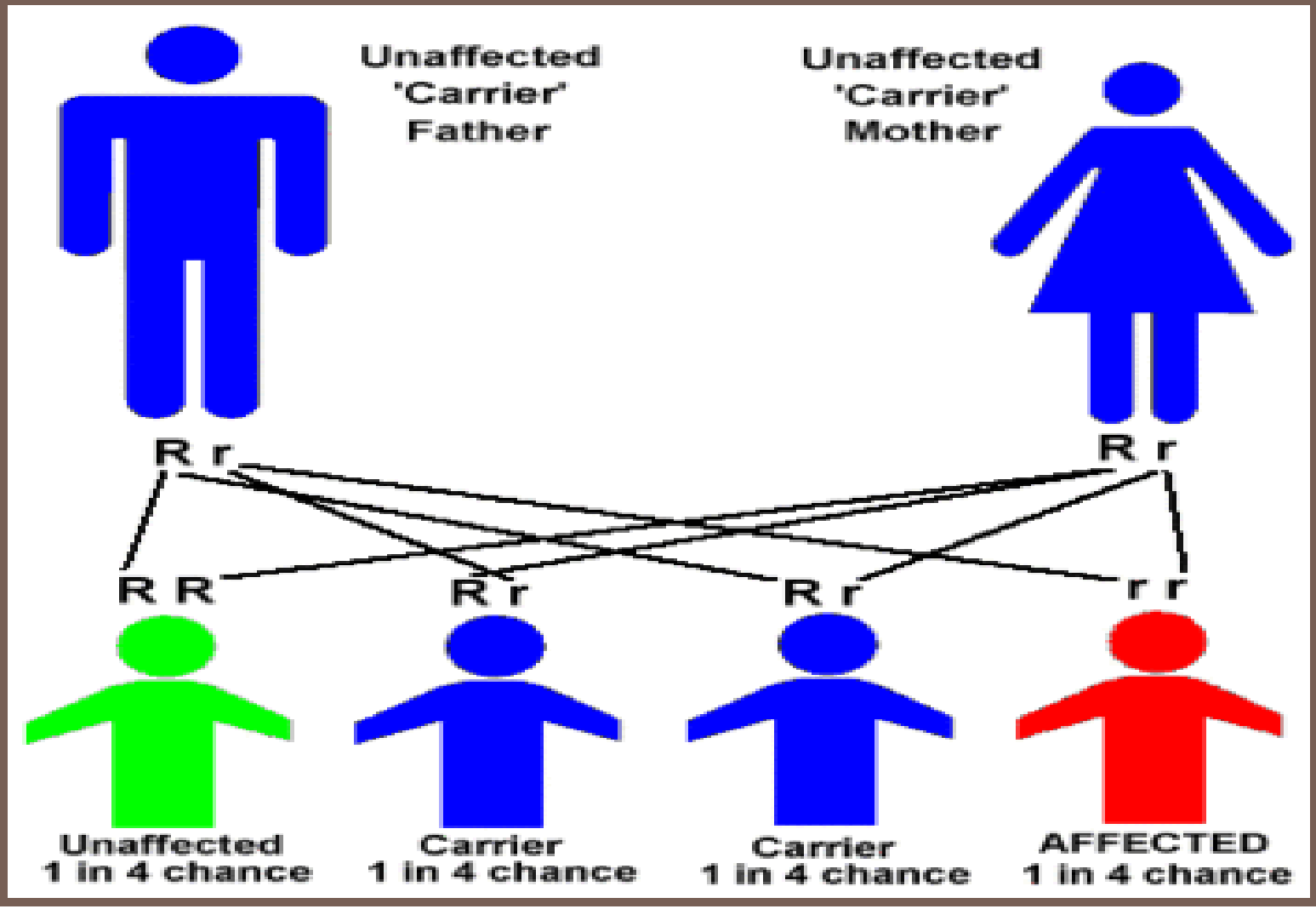
Crossing Over





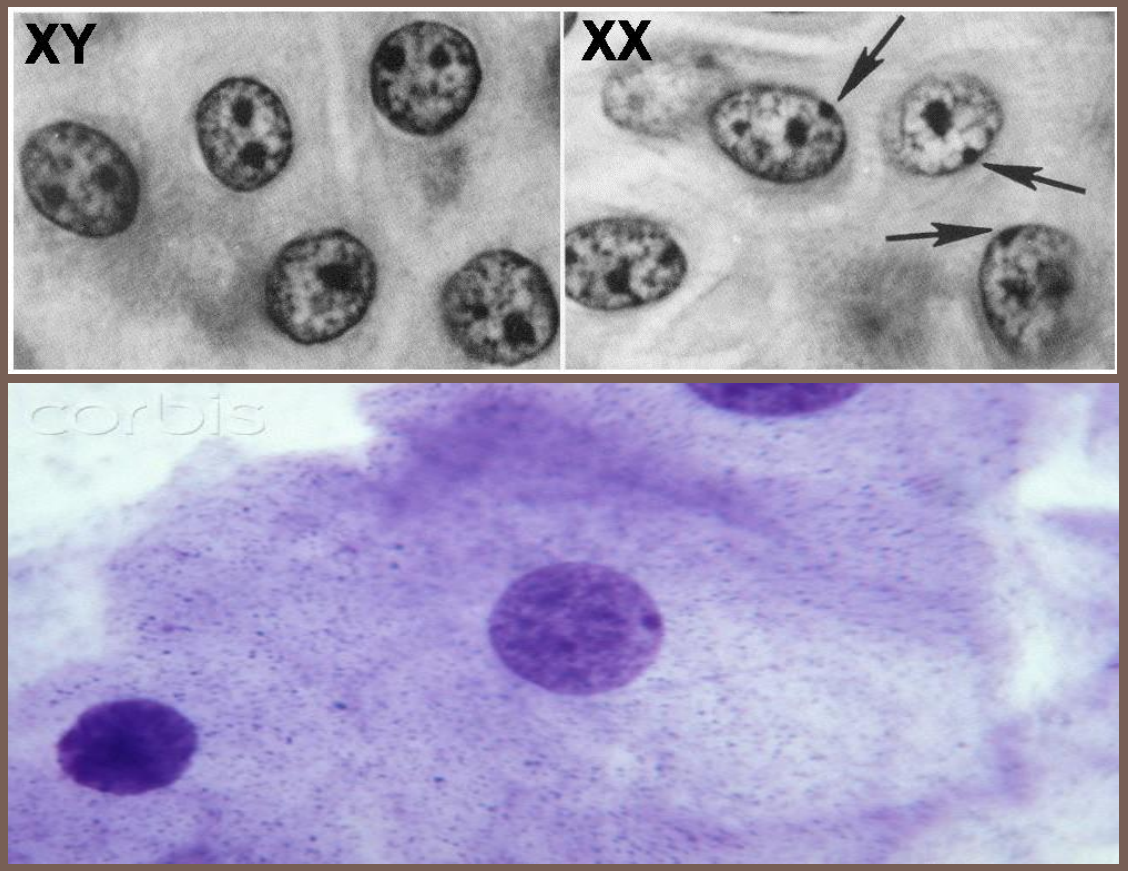
B. Dominance & Recessive

T



D. Hormonal Influence

E. Chromosome X inactivation



The most important genetic source for drug response-resistance and toxicity is the

Allelic Polymorphism

Single Nucleotide Polymorphisms-SNPs

--Enzymes: CYP450, CYP2D6, thiopurine S-methyltransferase (TPMT)

--Drugs: 6-mercaptopurine,

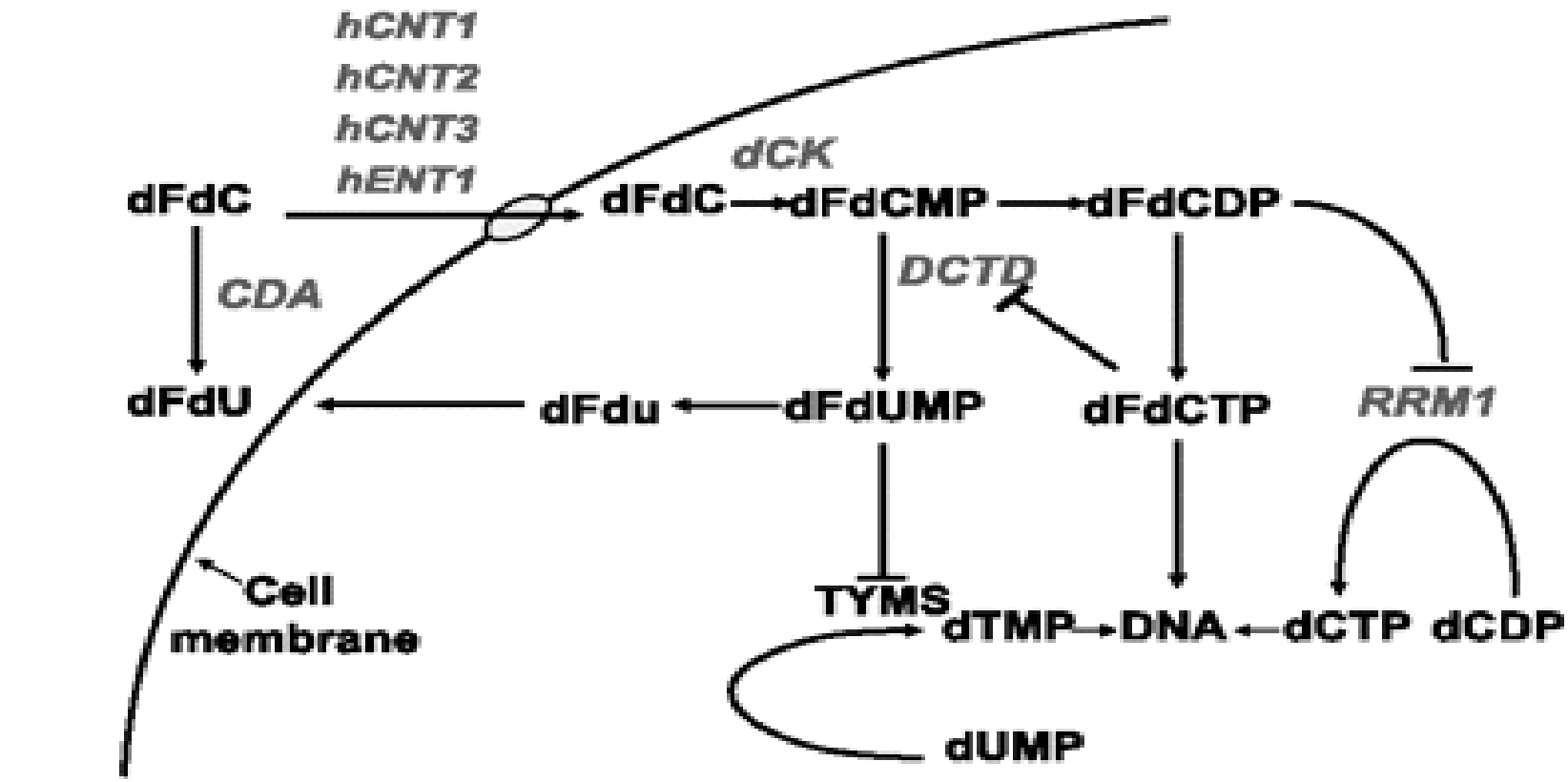
6-thioguanine,azathioprine,Thiopurine

autoimmune disease, inflammatory bowel disease, anticancer

Vit B12…no absorption cause malignant anemia

Iressa, Herceptin ….Lung cancer

Schematic description of gemci tabine (*dFdC*) transportation and metabolism..



Single Nucleotide Polymorphisms of Gemcitabine Metabolic Genes and Pancreatic Cancer Survival and Drug Toxicity

Table : Genotype and tumor response to preoperative treatment

Genotype	≤50%*	>50%	*OR (95% CI)†	*Pn* (%)*n* (%)
***dCK* C-1205T**				
TT	31 (73.8)	11 (26.2)	1.0	
CT/CC	37 (53.6)	32 (46.4)	2.73 (1.15-6.45)	0.022
***dCK* A9846G**				
GG	31 (75.6)	10 (24.4)	1.0	
AG/AA	37 (53.6)	32 (46.4)	2.96 (1.23-7.13)	0.015*h*
***CNT3* A25G**				
AA	42 (70.0)	18 (30.0)	1.0	
AG/GG	24 (49.0)	25 (51.0)	2.733 (1.21-6.17)	0.016*h*
***CNT3* C-69T**				
CC	55 (68.8)	25 (31.2)	1.0	
CT/TT	14 (43.8)	18 (56.3)	3.08 (1.30-7.31)	0.011.

Thank you