Genetic and Epigenetic Changes as Prospective Pain Targets

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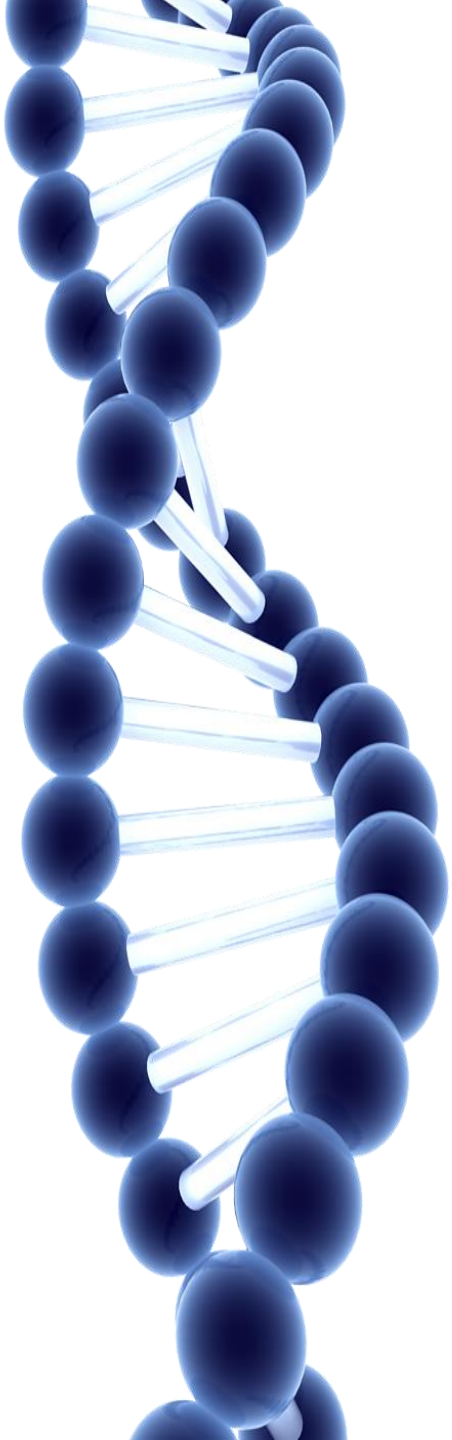
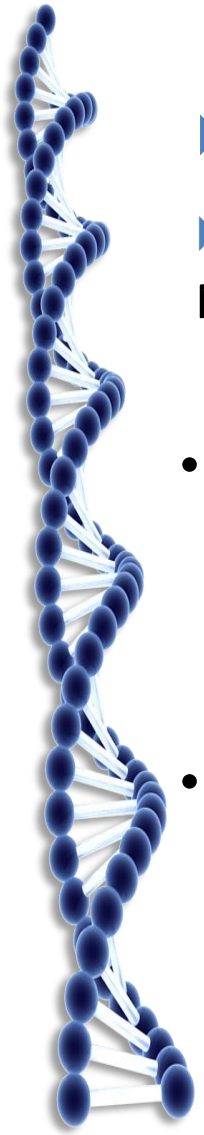
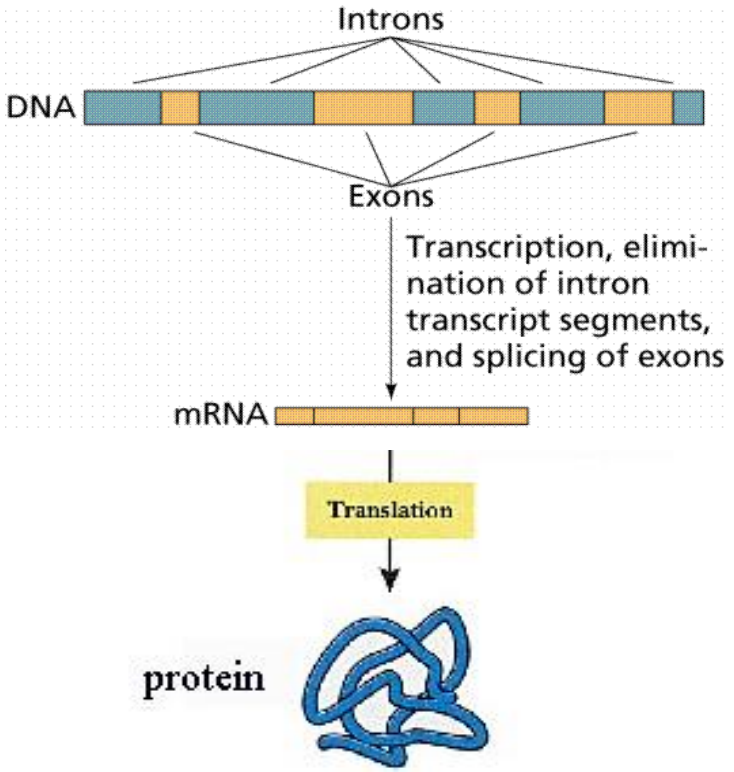


- *Human Genome Project, HapMap Project,The 1000 Genomes Project…*
- Genetic polymorphisms - genome variations with a frequency of >1%



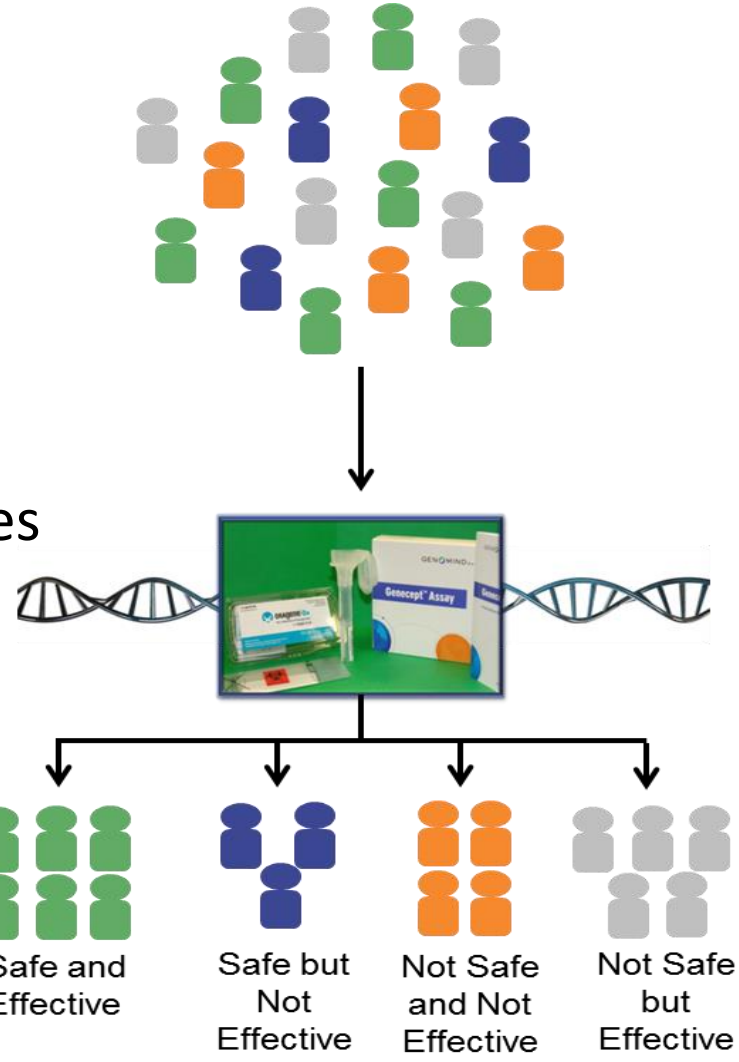
  - SNPs (single nucleotide polymorphisms)
  - 1/300bp, ≈ 15 million SNPs (The 1000 Genomes Project Consortium, Nature 526;68-74, 2015)

- Coding SNPs: Changes in amino acid → inter-individual variation in physiological response
- SNPs in TF binding sites, microRNA binding sites: ↑/↓ mRNA & protein (drug target) expression or degradation

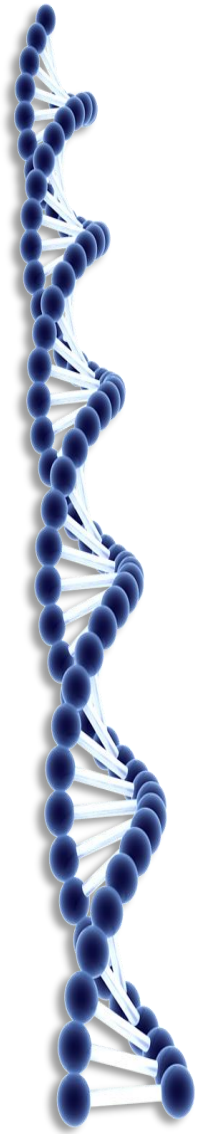


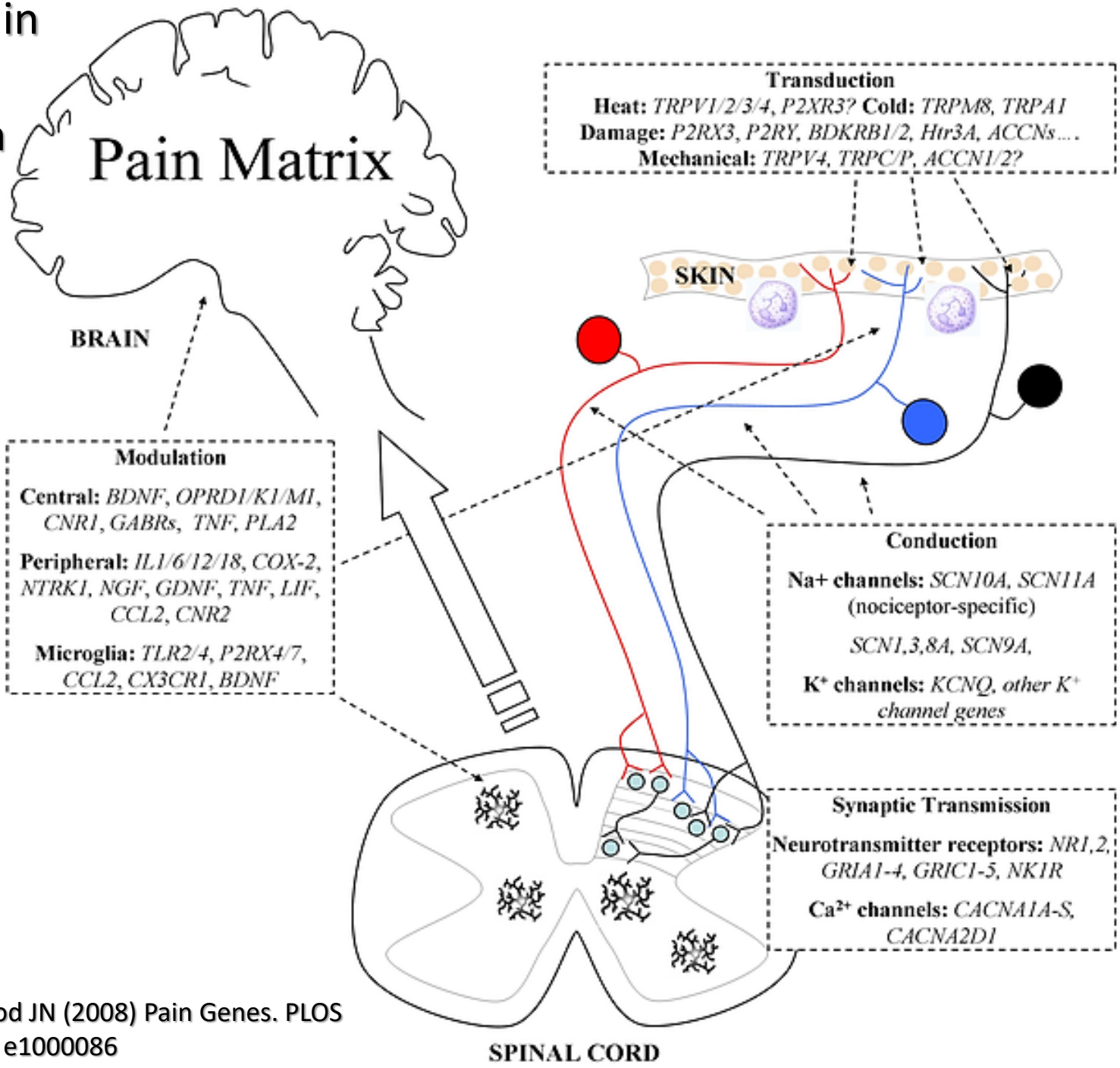
Clinical significance of SNPs

- Risk factors, predisposition to complex diseases - 50,000-250,000 SNPs (Genome Wide Association Studies, GWAS)
- Early Diagnosis
- Prognostic/Outcome Markers
- PHARMACOGENOMICS - Individual differences in the therapy responses - Personalized medicine



Genes Involved in Pain Perception and Modulation



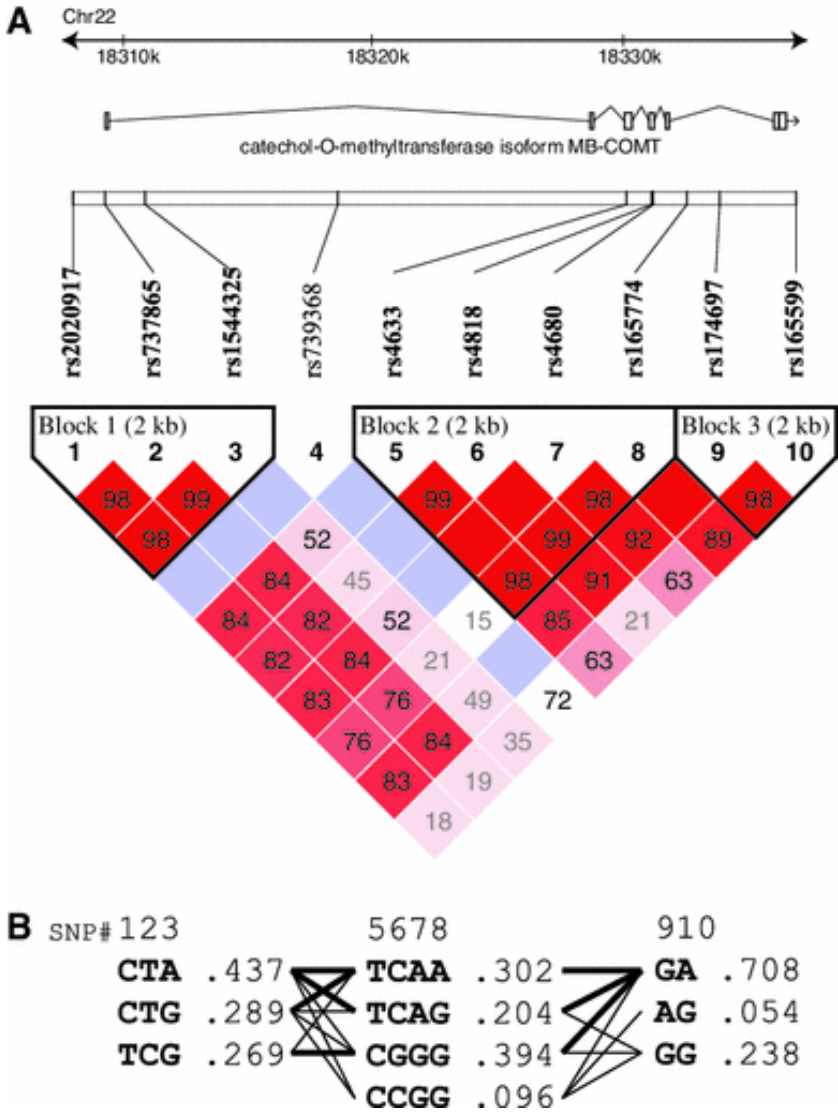


Foulkes T, Wood JN (2008) Pain Genes. PLOS Genetics 4(7): e1000086

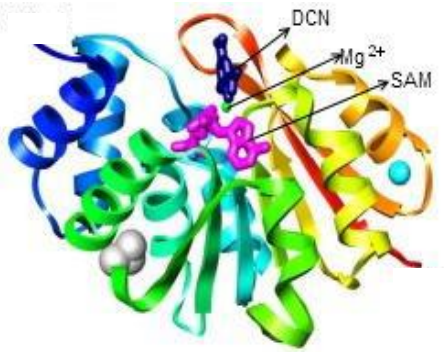
Pain Genetic vs. Pain Genomics

- SNPs in coding region
- SNPs in noncoding region
- Candidate Genes

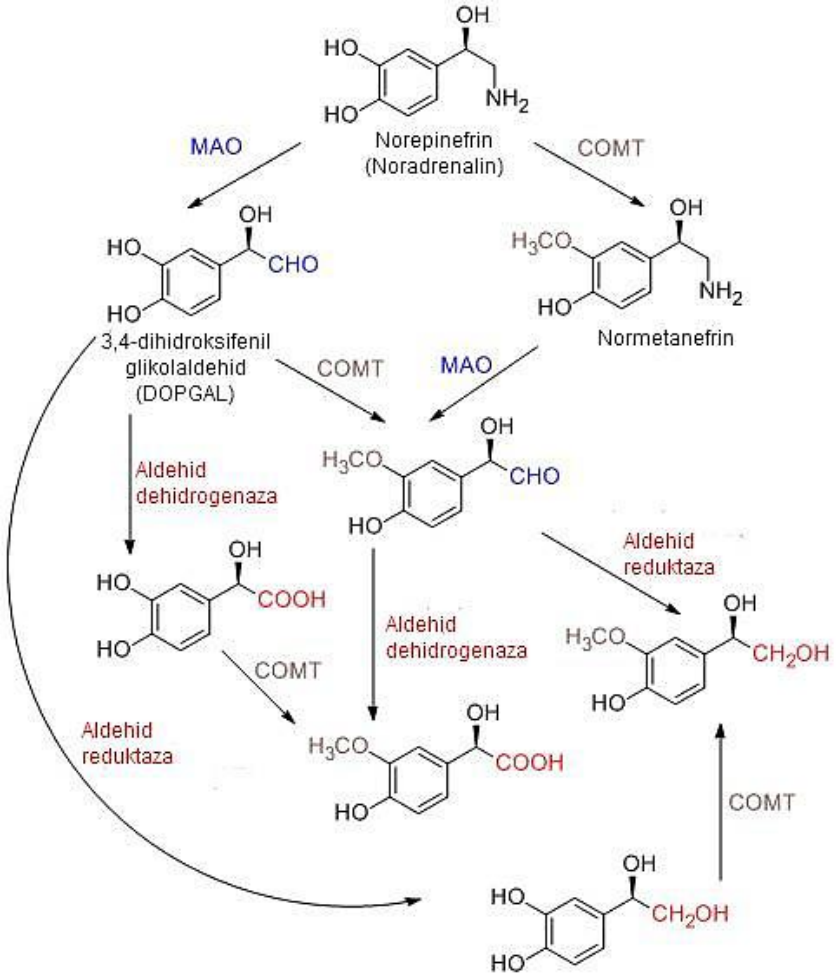
- Haplotypes
- Epistasis (gene-gene interactions)



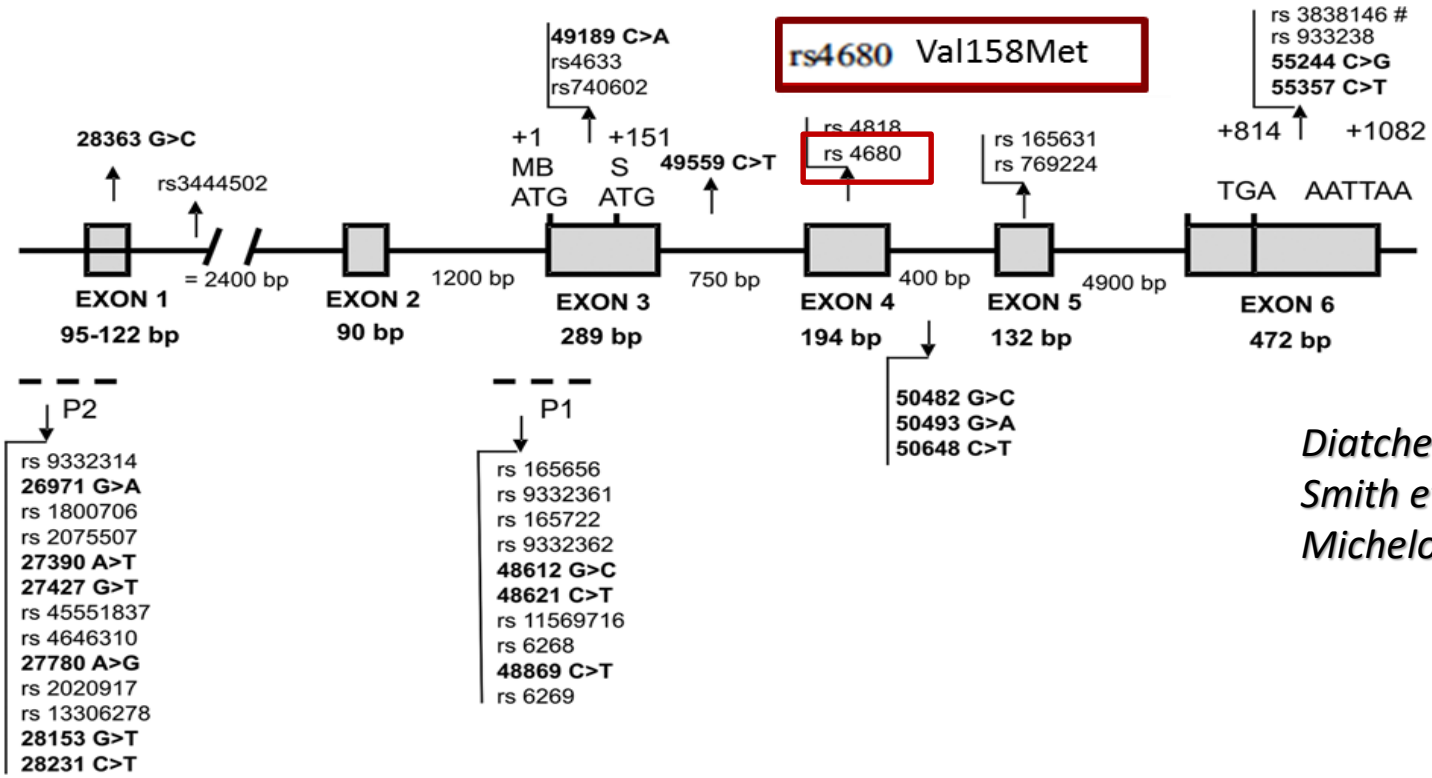
COMT: Catechol-O-Methyltransferase



- Metabolizes catecholamines in CNS and other tissues
  - Noradrenaline, adrenaline, dopamine
- Regulates neurotransmission - breaks down catecholamine neurotransmitters, thereby stopping or reducing transmission of signals
- **Pain,** Mood, Inflammation



COMT: > 900 SNP variants



Diatchenko et al., 2005
Smith et al., 2011
Michelotti et al., 2014

- Val158Met: widely studied
  - Met/Met: 4-fold ↓ in activity
  - Met/Met (26%) individuals versus Val/Val (25%)
    - higher sensory & affective pain ratings
    - Lower morphine doses
- Increased risk of chronic pain with Met individuals (fibromyalgia, TMD)

Genetic basis for individual variations in pain perception and the development of a chronic pain condition

Diatchenko et al Human Mol Gen 14, 135, 2005

Luda Diatchenko[1,8,*], Gary D. Slade[2], Andrea G. Nackley[1], Konakporn Bhalang[3], Asgeir Sigurdsson[1], Inna Belfer[4,7], David Goldman[4], Ke Xu[4], Svetlana A. Shabalina[5], Dmitry Shagin[6], Mitchell B. Max[7], Sergei S. Makarov[8] and William Maixner[1]

Table 1. Variation in pain sensitivity (summed *z*-score) among tested SNPs and diplotypes of the *COMT* gene

SNPs	Genotype	Number of subjects	Genotypes frequencies	Mean (SD) *z*-score	ANOVA[a] R^2
rs6269	G/G	31	0.153	−4.7 (9.1)	0.061
	A/G	97	0.480	−0.7 (10.5)	
	A/A	74	0.366	3.0 (10.7)	
rs4633	C/C	52	0.257	−2.0 (10.5)	0.013
	C/T	98	0.485	0.4 (10.9)	
	T/T	52	0.257	1.3 (10.2)	
rs4818	G/G	28	0.139	−5.2 (8.0)	0.07
	G/C	100	0.495	−0.9 (10.5)	
	C/C	74	0.366	3.2 (10.7)	
rs4680 Val158Met	G/G	51	0.252	−2.1 (10.3)	0.017
	A/G	102	0.505	0.2 (10.9)	
	A/A	51	0.253	−0.2 (10.3)	
Haplotype combination	ATCA_ACCG	15	0.081	8.9 (11.4)	0.107
	ATCA_ATCA	49	0.263	1.7 (10.3)	
	ATCA_GCGG	80	0.430	−1.3 (10.2)	
	GCGG_ACCG	14	0.075	1.5 (12.3)	
	GCGG_GCGG	28	0.151	−5.2 (8.0)	

- rs4648, rs4633, rs6269 relevant SNP for TMD
- Haplotypes: multiple SNPs in gene - Differentiates less (GCGG) and high (ATCA/ACCG) pain sensitivity haplotype group
- TMD incidence higher in high pain sensitivity group (65%) than in low pain sensitivity haplotype group (28%)

Journal of Oral & Facial Pain and Headache 2016;30(4):302-10

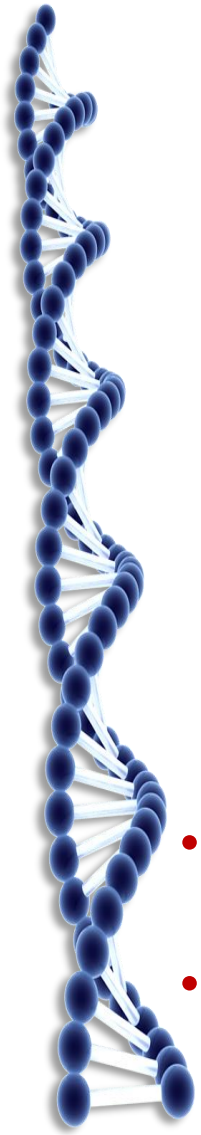
Genetic Polymorphisms of Catechol-O-Methyltransferase: Association with Temporomandibular Disorders and Postoperative Pain

Irena Mladenovic, Gordana Supic, Ruzica Kozomara, Slobodan Dodic, Nedeljka Ivkovic, Bojana Milicevic, Ivana Simic, Zvonko Magic

J Endod 2018;44:717-21

Pulp Sensitivity: Influence of Sex, Psychosocial Variables, COMT Gene, and Chronic Facial Pain

Irena Mladenovic, Jelena Krunic, Gordana Supic, Ruzica Kozomara, Dejan Bokonjic, Nikola Stojanovic, Zvonko Magic



COMT SNP		TMD	Controls	*p*	OR* (95% CI)	*p*
rs4680	wt (GG)	17	23	NS	Ref.	
	het (AG)	55	52		1.407 [0.670-2.952]	NS
	mut (AA)	18	17		1.427 [0.567-3.597]	NS
rs6269	wt (GG)	1	5	NS	Ref.	
	het (AG)	57	60		4.316 [0.483-38.535]	0.190
	mut (AA)	32	27		5.320 [0.578-48.928]	0.140
rs165774	wt (GG)	30	47	**0.006**	Ref.	
	het (AG)	49	43	**#0.015**	1.769 [0.949-3.298]	0.072
	mut (AA)	11	2		**9.448 [1.913-46.652]**	**0.006**
					#2.088 [1.138-3.829]	**#0.017**

*adjusted for age and sex ; #A allele carriers vs. GG genotype, (het/mut vs. wt)

- COMT rs165774: significant risk factor; No association for rs4680 and rs6269 (Mladenovic et al, J Oral Facial Pain Headache 2016;30:302-10)
- COMT rs165774 GG contribute to individual variation in electric and cold pulp sensitivity responses to electric stimuli and is associated with female sex ($p < .01$) (Mladenovic et al, J Endod 2018;44:717-21)

Epistasis of several genes in COMT pathway

PAIN® 155 (2014) 2390–2399

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www.elsevier.com/locate/pain

Epistasis between polymorphisms in COMT, ESR1, and GCH1 influences COMT enzyme activity and pain

Shad B. Smith [a], Ilkka Reenilä [b], Pekka T. Männistö [b], Gary D. Slade [a], William Maixner [a], Luda Diatchenko [a,c], Andrea G. Nackley [a,*]

GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence *Tegeder et al Nat Med 12, 1269, 2006*

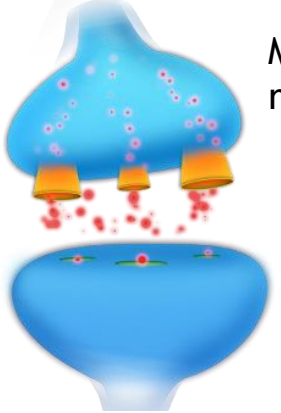
- GCH1: GTP cyclohydrolase 1 - cofactor for catecholamine synthesis
- ESR1: oestrogen receptor 1 - regulates COMT gene expression
- GTP cyclohydrolase - tetrahydrobiopterin (BH4) formation
- BH4: modulates peripheral neuropathic and inflammatory pain, cofactor in serotonin, catecholamine & NO production

COMT Met individuals : ↑ musculoskeletal pain

COMT Met individuals + GCH1 variant: increased pain threshold (↓ pain)

COMT Val individuals + ESR1 Variant: ↑ pain

Mu Opioid Receptor *OPRM1*



- Endogenous opioids (endorphins and encephalins) reduce pain sensation
- *OPRM1* - Neuroendocrine Functions - Stress response through hypothalamic-pituitary-adrenal axis

μ-Opioid Receptor Gene A118G Variants and Persistent Pain Symptoms among Men and Women Experiencing Motor Vehicle Collision

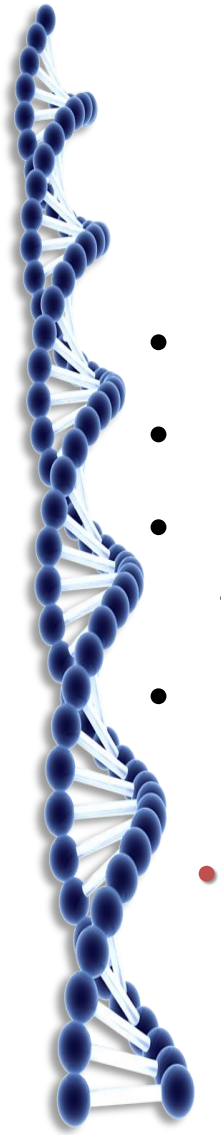
OPRM1 (A118G)

Linnstaedt et al J Pain 2015

In women with high pain prior to MVC[b] (n = 97)				In men with high pain prior to MVC[b] (n = 50)			
A118G allele	-1.727	0.507	0.001	A118G allele	1.742	0.914	0.057
Age	0.017	0.017	0.309	Age	0.065	0.031	0.034
Education	-0.182	0.168	0.276	Education	-0.530	-0..269	0.049
Income	-0.565	-0.840	<0.001	Income	0.100	0.224	0.656
Pain at the time of initial evaluation	0.226	0.093	0.015	Pain at the time of initial evaluation	0.646	0.133	<0.001

- OPRM1 A118G on persistent pain after motor vehicle accidents - Sex dependent differences
- Women with G allele – reduced pain after 6 weeks
- Men with G allele – increased pain after 6 weeks

Citokines and pain



IASP

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Cytokine biomarkers and chronic pain: Association of genes, transcription, and circulating proteins with temporomandibular disorders and widespread palpation tenderness

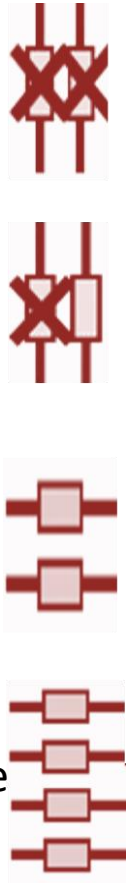
Gary D. Slade[a], Mathew S. Conrad[a], Luda Diatchenko[a], Naim U. Rashid[b], Sheng Zhong[a], Shad Smith[a], Jesse Rhodes[a], Alex Medvedev[b], Sergei Makarov[c], William Maixner[a], Andrea G. Nackley[a,*]

- Cytokines contribute to TMD & widespread pain
- MCP-1 & IL-8 limit tissue damage; initiate remodeling
- Persistent ↑ MCP-1 & IL8: tissue pathology & nociceptor function changes
- Proinflammatory cytokines concentrations correlate with ↑ pain sensitivity
  - Significant epistasis between IL-8 SNPs and TGFB1 SNPs

CYP2D6 SNPs

- Cytochrome P450 family - crucial role in the metabolism of 70% of drugs - β-blockers, antipsychotics, antidepressants, cardiovascular drugs and opioids, codeine, tramadol, oxycodone;
- CYP2D6 >100 different SNPs

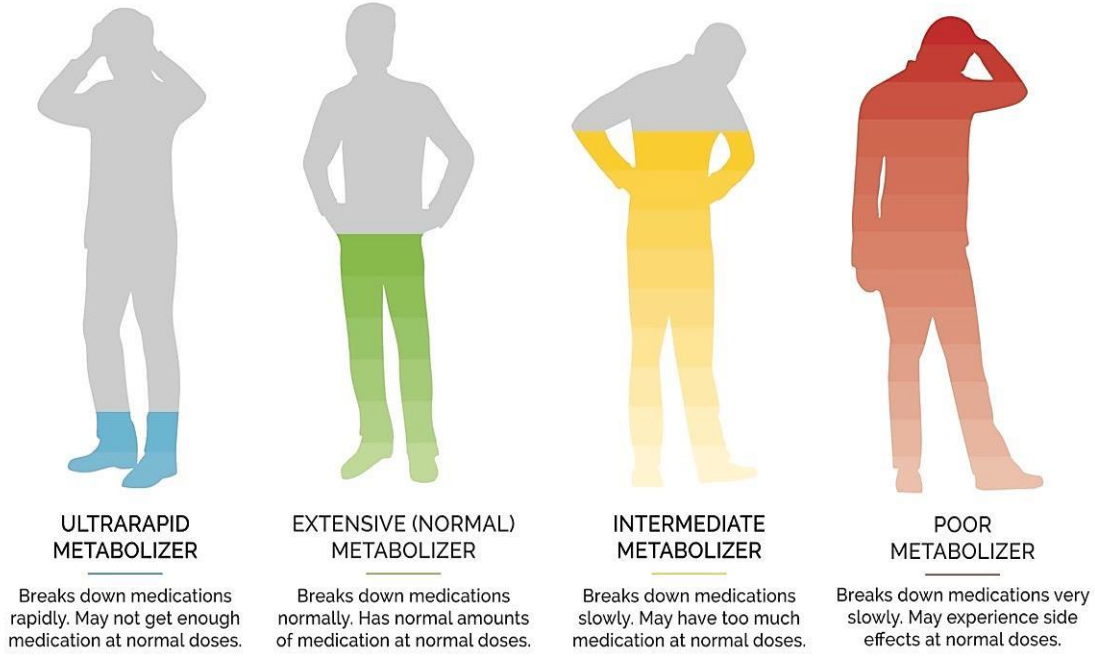
1. **Extensive metabolizers (EMs)** - regular metabolic capacity; two active alleles ****1,*2***
2. **Intermediate metabolizers (IMs)** - intermediate metabolic capacity; heterozygous for a defective allele or two alleles with reduced activity ***Aa**, AA* **- *10, *17, *29, *41**
3. **Poor metabolizers (PMs)** - lack a functional enzyme; two mutant alleles - ***aa *3, *4, *5, *6, *7, *8, *12***
4. **Ultra-rapid metabolizers (UMs)** - higher metabolic capacity than EM; multiple copies of functional alleles - ***AAAA*** of the two active alleles - ****1,*2***



Designation	Characteristic mutation(s)
*CYP2D6*1*	Wild type
*CYP2D6*2*	$G_{1749}C$, $C_{2938}T$, $G_{4268}C$ substitutions
*CYP2D6*3*	A_{2637} deletion
*CYP2D6*4*	$G_{1934}A$ substitution
*CYP2D6*5*	Gene deletion
*CYP2D6*6*	T_{1795} deletion
*CYP2D6*7*	$A_{3023}C$ substitution
*CYP2D6*8*	$G_{1846}T$ substitution
*CYP2D6*9*	(A_{2701}–A_{2703}) or (G_{2702}–A_{2704}) deletion
*CYP2D6*10*	$C_{188}T$, $G_{1749}C$, $G_{4268}C$ substitutions
*CYP2D6*11*	$G_{971}C$ substitution
*CYP2D6*12*	$G_{212}A$ substitution
*CYP2D6*13*	Hybrid: *2D7* exon 1, *2D6* exons 2–9
*CYP2D6*14*	$G_{1846}A$ substitution
*CYP2D6*15*	T_{226} insertion
*CYP2D6*16*	Hybrid: *2D7* exons 1–7, *2D6* exons 8–9
*CYP2D6*1* × 2	Gene duplication
*CYP2D6*2* × 2	Gene duplication
*CYP2D6*4* × 2	Gene duplication

*3, *4, *5, *6, *41 more common in Caucasians
*17 in Africans
*10 in Asians

CYP2D6



- EM - 70-90% Caucasians
- IM - 1-13% Caucasians
- PM - 1-10% Caucasians
- UM - 1-2% Caucasians

- Crews K, et al. 2011. CPIC guidelines for codeine therapy in the context of CYP2D6 genotype. Clin Pharmacol Ther. 91(2):321-6.

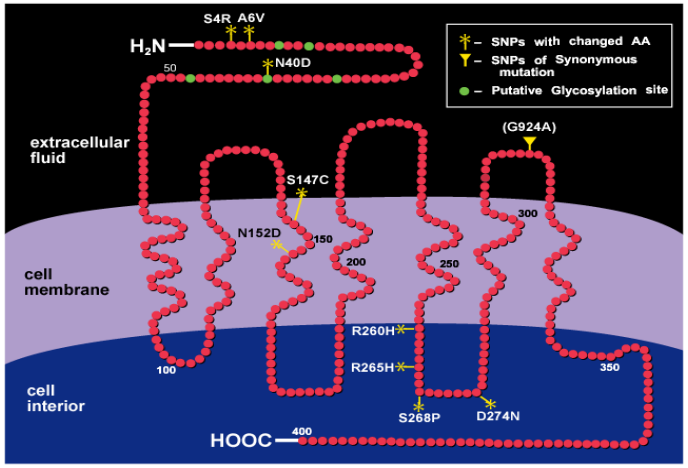
Codeine (pro-drug) —*CYP2D6*→ **Morphine** (active)

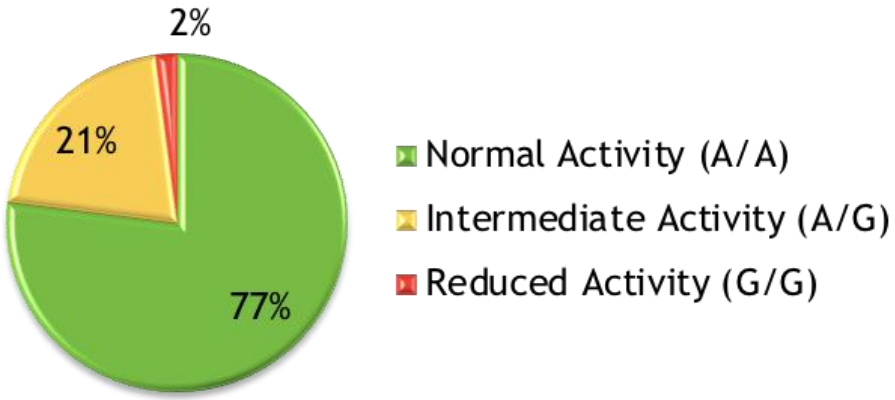
Information for Healthcare Professionals: Use of Codeine Products in Nursing Mothers

Update: The issues described in this communication have been addressed in product labeling (see Drugs@FDA)

FDA Alert: [8/17/2007] FDA has important new information about a very rare, but serious, side effect in nursing infants whose mothers are taking codeine and are ultra-rapid metabolizers of codeine. These babies may be at increased risk for morphine overdose.

OPRM1 (A118G)



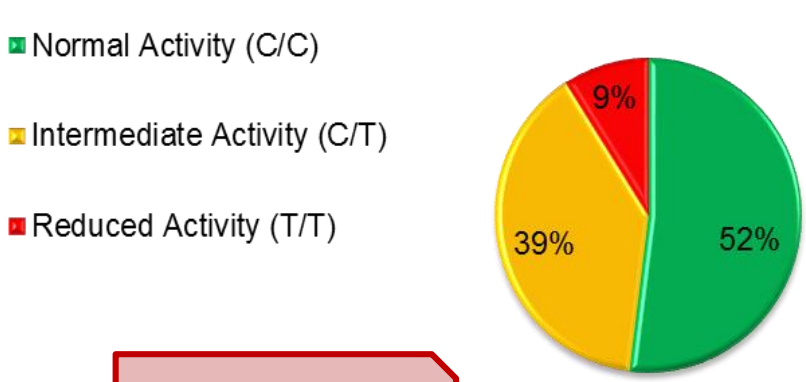


Reyes-Gibby, et al. Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain: OPRM1 and COMT gene. Pain 2007;130 (1-2)25-30.

- Patients who are carriers of the G allele for the A118G SNP show a reduced analgesic response with opioid medications such as morphine, codeine, and oxycodone

MTHFR

- Folate plays a critical role in the formation of SAM, an important precursor to neurotransmitter synthesis
- Methylenetetrahydrofolate reductase (*MTHFR*) SNPs - A1298C
- C677T

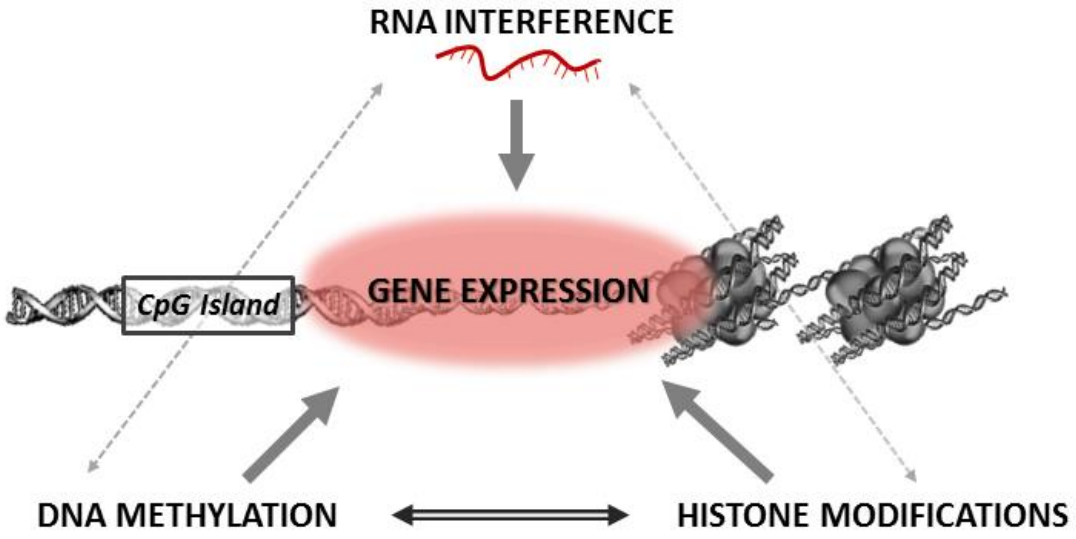


- Migraine - *Christensen et al, Cephalalgia 2016, 36, 624–639*
- Chronic pain - *Zorina-Lichtenwalter et al. Neuroscience 2016, 338, 36–62*
- Fibromialgia - *Inanir et al, Gene 2015, 564, 188–192.*

EPIGENETICS – heritableand potentially reversible changes in gene expression that occur without a change in the DNA sequence.

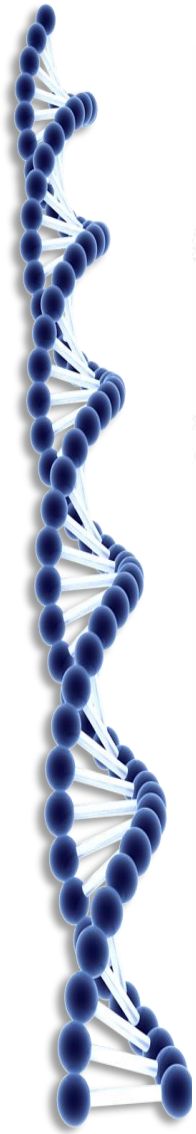
Mechanisms of epigenetic control:

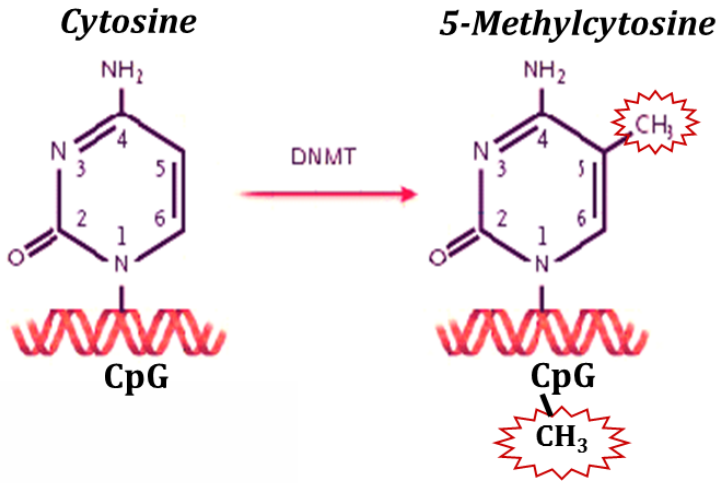
- **DNA Methylation**
- **Histone modifications**: Histone variants, *"histone code"*
- **RNA silencing**: Regulatory non-coding RNAs (siRNA, miRNA, …)

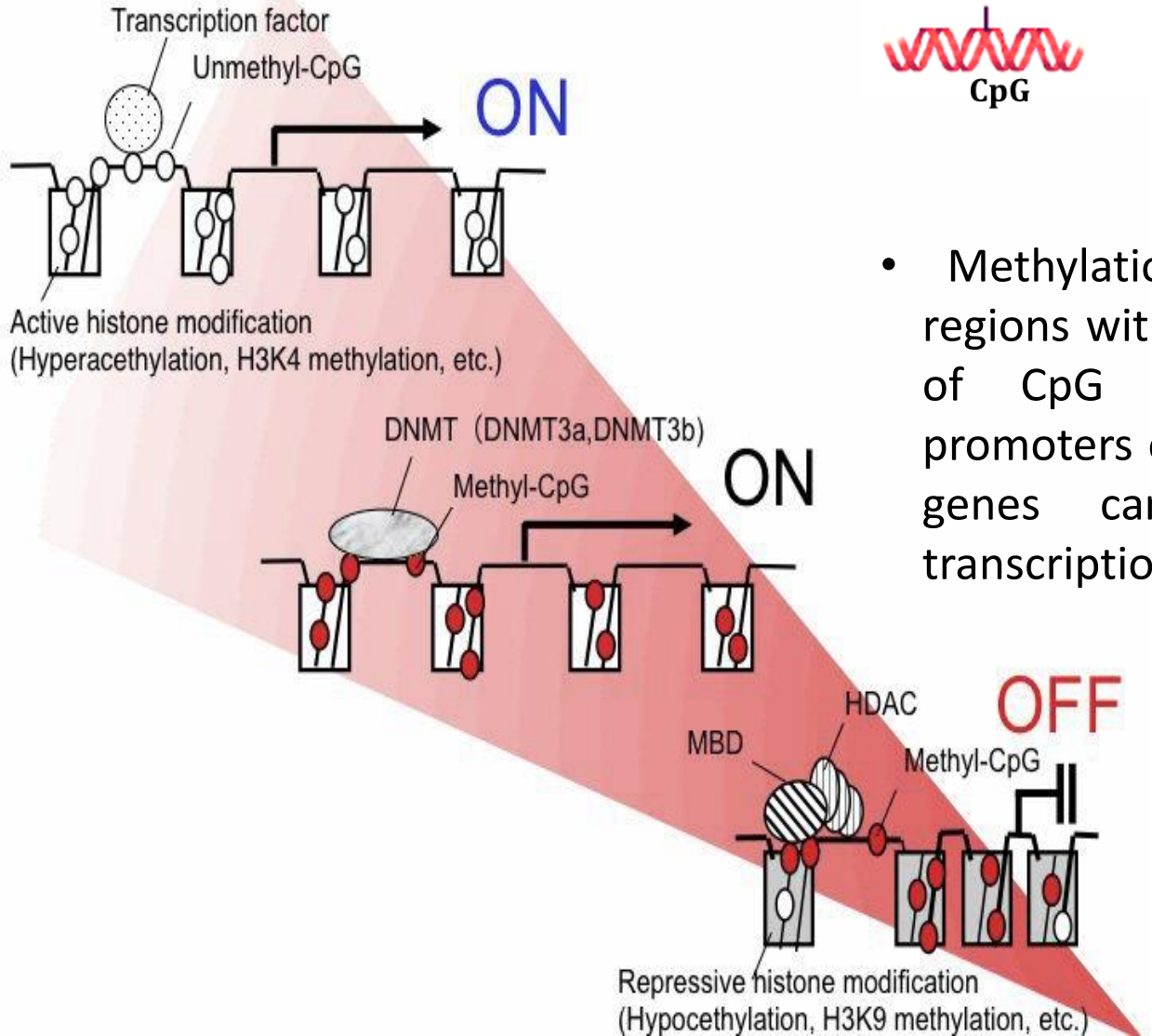


Supic et al, 2016

DNA Methylation



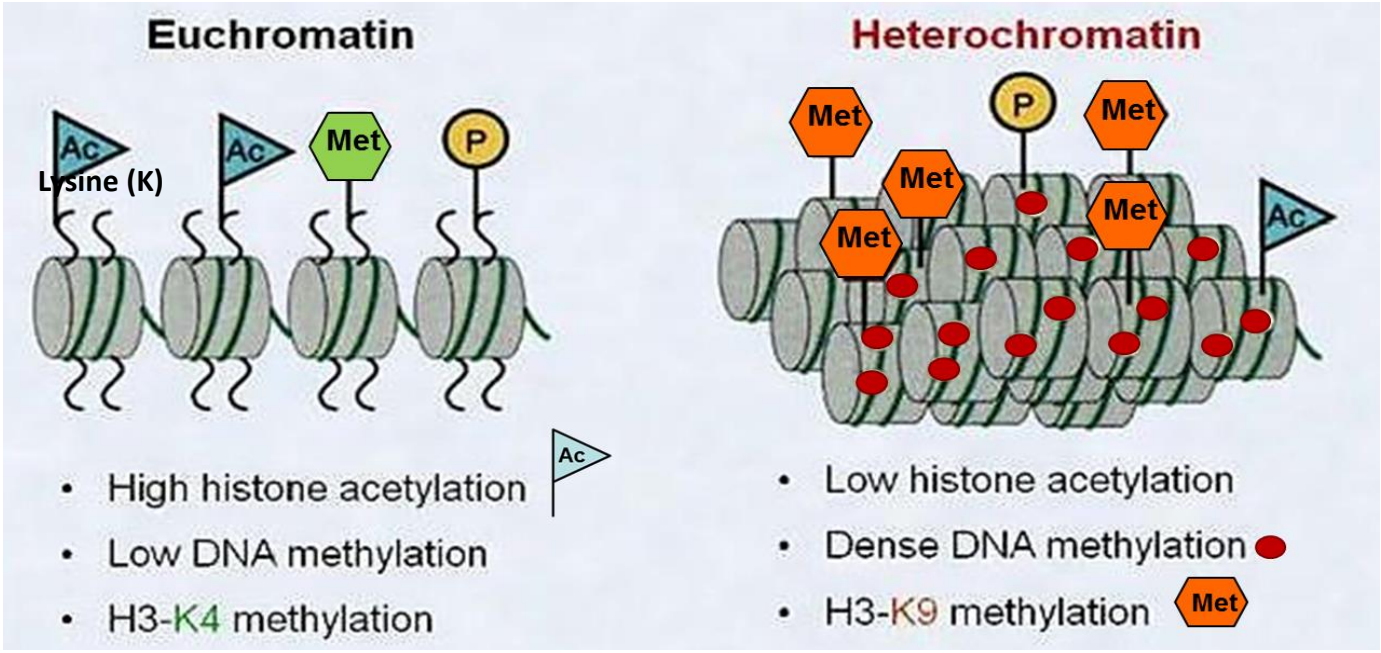




- Methylation of CpG islands, regions with high concentration of CpG sites, within the promoters of more than 60% of genes can lead to their transcriptional silencing.

Histone modifications - Histone code

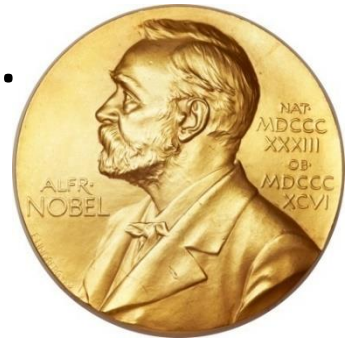
- **Histones** - dynamic role in the regulation of chromatin and gene activity
- Acetylation, methylation, phosphorylation, ubiquitination…
- **Histone acetylation** neutralizes the positive charge on histone tails, chromatin is accessible to the transcriptional factors. Histone deacetylases (HDAC) deacetylize lysine (K) in histones, nucleosomes are tightly compacted.
- **Histone methylation** influences the chemical characteristics of histones and their affinity to DNA



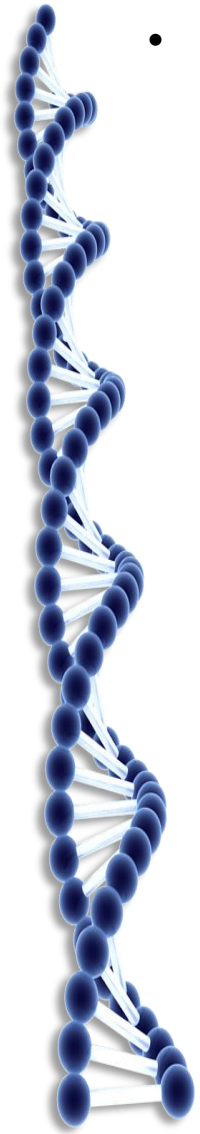
Modified from Vertino, Stewart, 2003

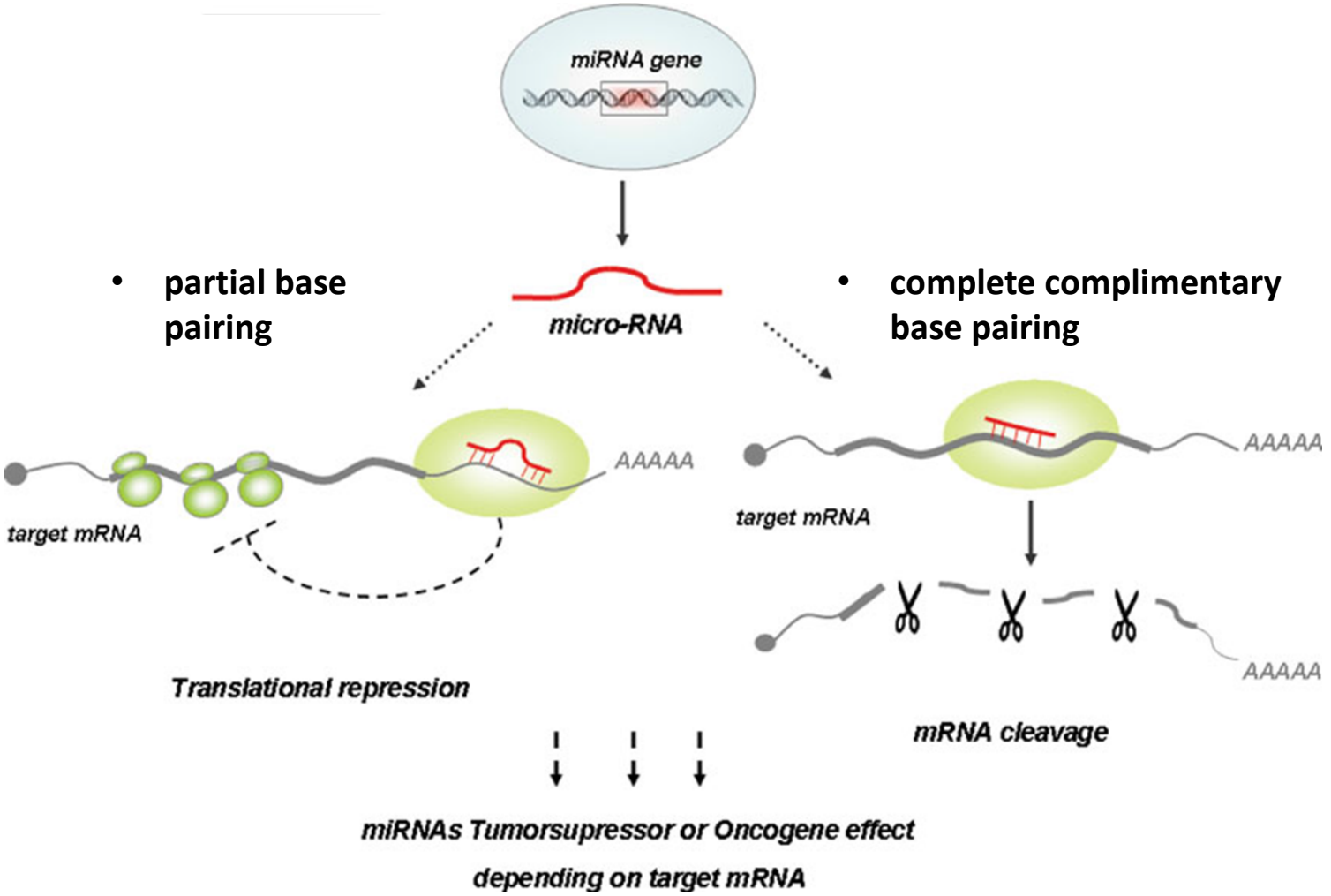
RNA interference – RNA silencing

2006.



- Non-coding RNAs (siRNA, miRNA, stRNA, shRNA, ALU, LTR, LINE)
- Micro-RNAs (miRNAs) >2000, 18-22 nt, posttranscriptional regulation

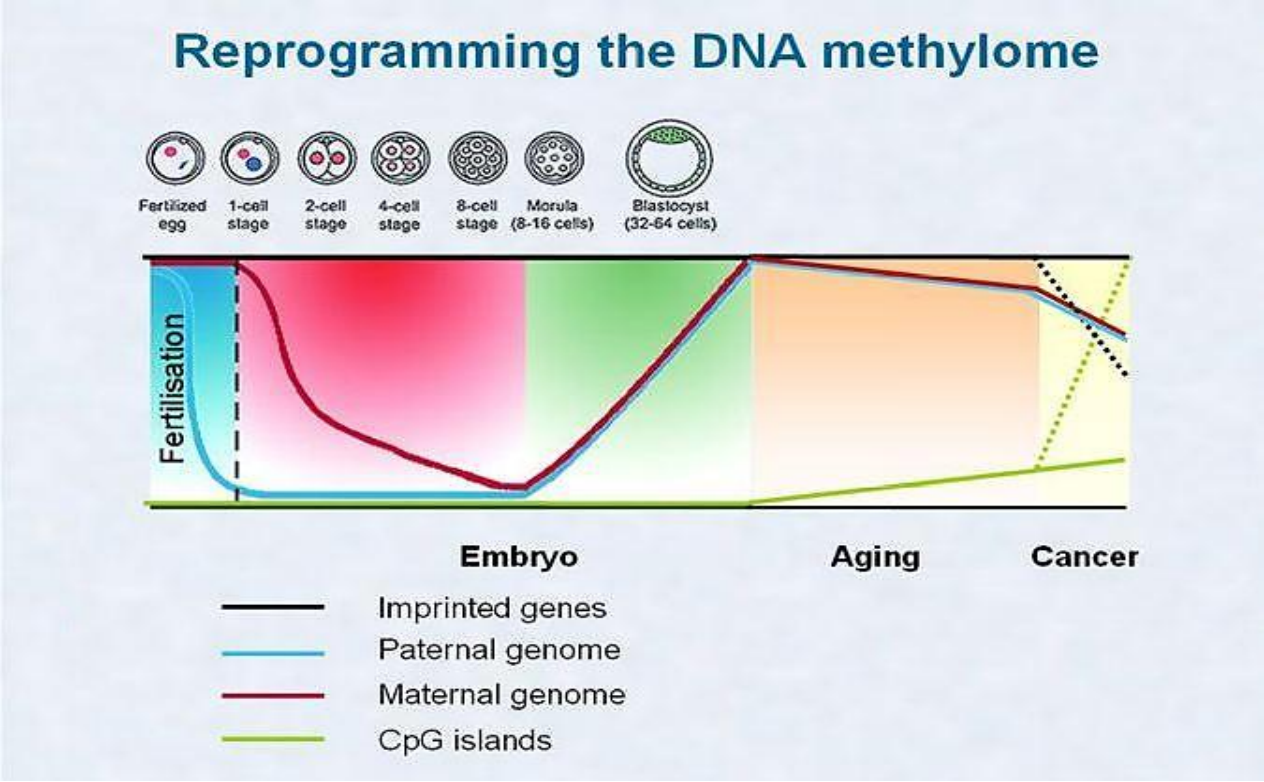




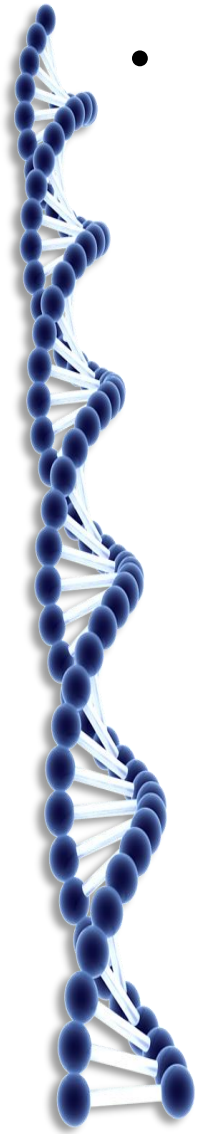
Supic et al, 2013

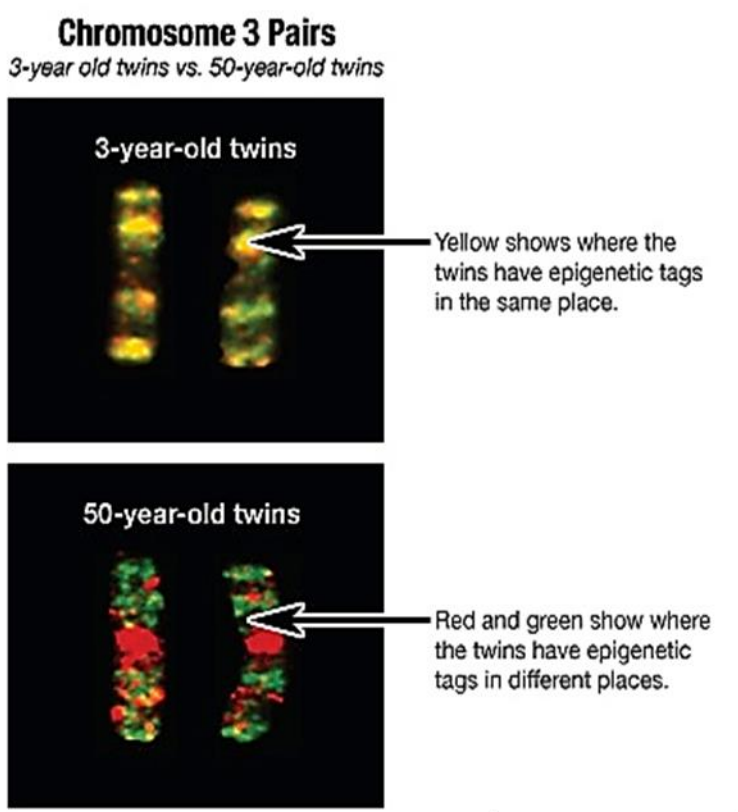
The Roles of Epigenetic Programming in Normal Cells

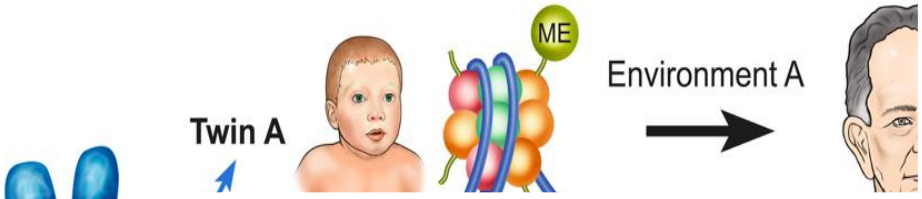
- Cellular Differentiation
- Maintaining of genome stability
- Embryonic development
- Genomic imprinting
- X -inactivation
- Aging
- Cancer
- ***PAIN …***

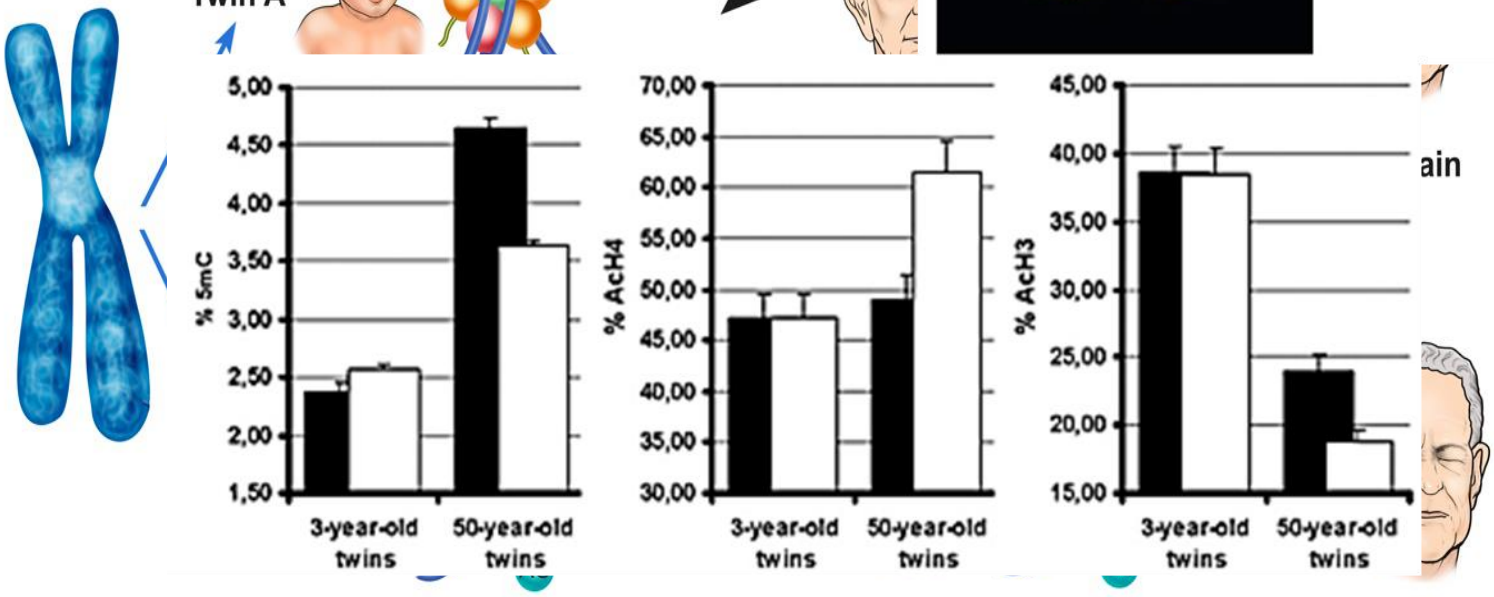


- Monozygotic twins - identical genome and epigenome at birth
- Environmental factors through development alter epigenome -> phenotype differences (No pain/ pain)





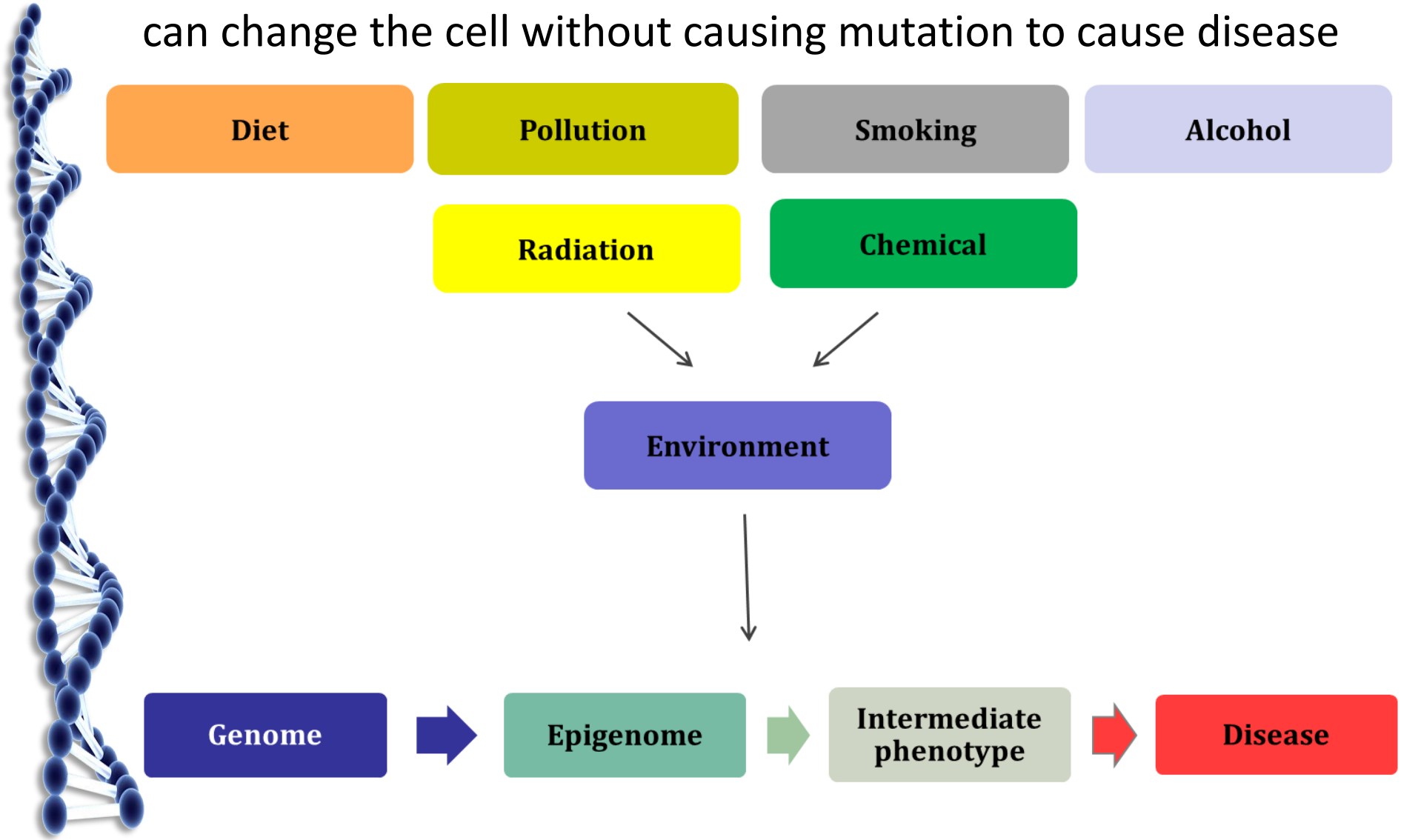




PNAS 2005;102:10604

Environment influence epigenetic changes

- Epigenetics provides the missing link on how the environment can change the cell without causing mutation to cause disease

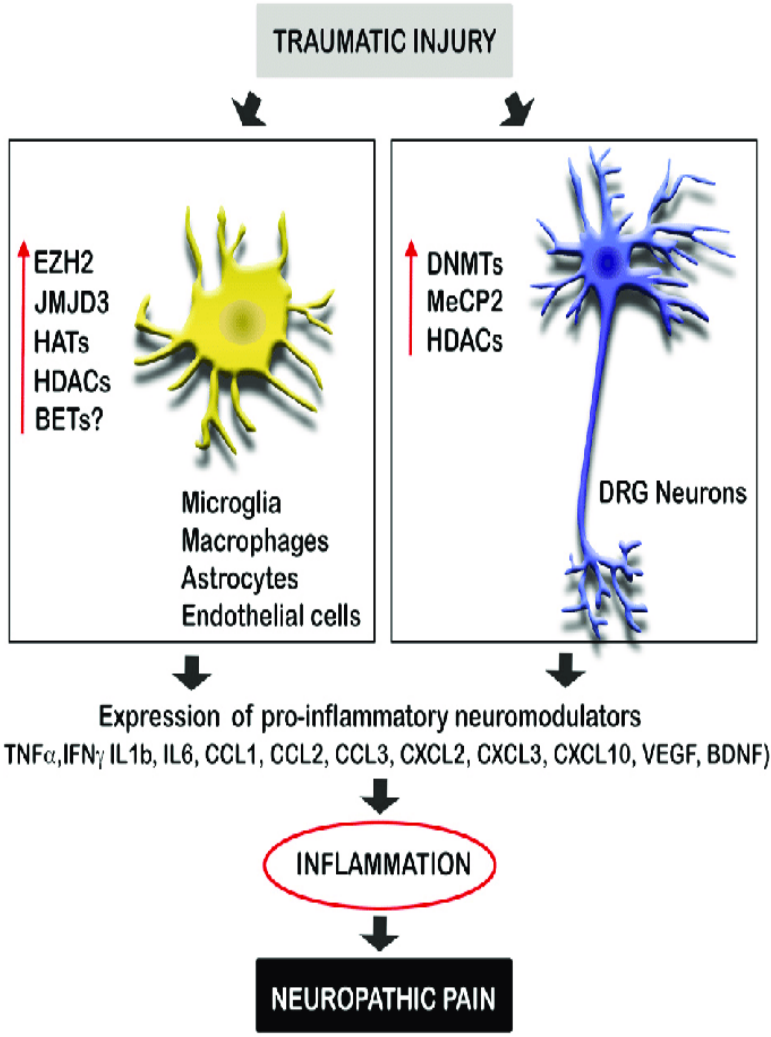


Epigenetics and the Transition from Acute to Chronic Pain

Pain Medicine 2012; 13: 1474–1490

Thomas Buchheit, MD, Thomas Van de Ven, MD, PhD, and Andrew Shaw, MB, FRCA, FCCM

- Inflammatory cytokines - under epigenetic control
- Glucocorticoid receptor (affects pain sensitivity and inflammation) - modulated through DNA methylation
- Glutamic acid decarboxylase (pain regulatory enzyme) - downregulated by hypoacetylation in inflammatory and nerve injury pain states
- Opioid receptor regulated by HDAC, with implications for terapeutic pain modulation with HDAC inhibitors – demonstrated in animal models



Differential methylation of the *TRPA1* promoter in pain sensitivity

NATURE COMMUNICATIONS | 5:2978 | DOI: 10.1038, 2014

J.T. Bell[1,2], A.K. Loomis[3], L.M. Butcher[4], F. Gao[5], B. Zhang[3], C.L. Hyde[3], J. Sun[5], H. Wu[5], K. Ward[1], J. Harris[1], S. Scollen[6], M.N. Davies[1,7], L.C. Schalkwyk[7], J. Mill[7,8], The MuTHER Consortium*, F.M.K. Williams[1], N. Li[5], P. Deloukas[9,10,11], S. Beck[4], S.B. McMahon[12], J. Wang[5,11,13,14], S.L. John[3], T.D. Spector[1],

TRPA1: ligand-gated ion channel - sensor for pain, cold, stretch; chemicals: mustard, wasabi, horse radish

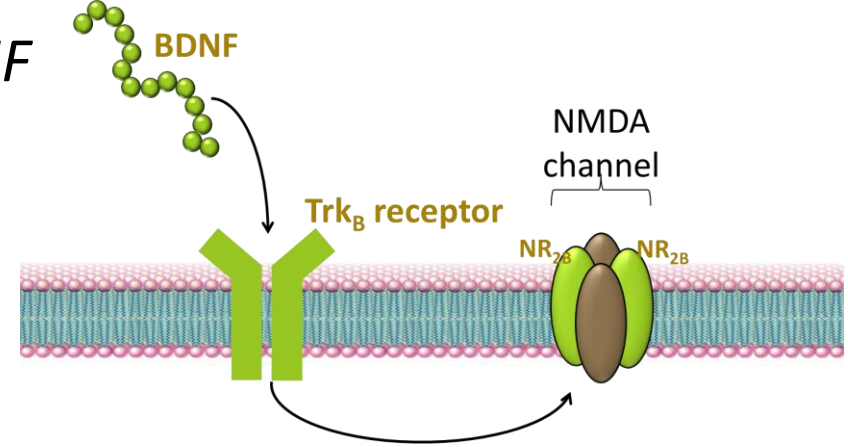
- 50 monozygotic twins - 50 controls
- Heat pain: low vs high sensitivity
- Methylation rate TRPA1 related to pain sensitivity

Table 1 | Meta-analysis DMRs for pain sensitivity at FDR 5%.

				Discovery ($n = 25$ MZ pairs)*				Follow-up ($n = 50$ individuals)*				Meta-analysis*		
Chr	DMR	Nearest gene (kb)†	% Repeat‡	β	s.e. (β)	*P*-value	MZ diff (r)§	β	s.e. (β)	*P*-value	R^2	β	s.e. (β)	*P*-value
8	73,151,000-	*TRPA1* (0.1)	0.00	−0.23	0.04	2.6×10^{-6}	−0.68	−0.40	0.07	1.3×10^{-6}	0.39	−0.28	0.04	1.2×10^{-13}

Brain-derived neurotrophic factor *BDNF*

- critical role in synaptic plasticity and modulatory role in pain transduction
- Val66Met SNP influences pain processing



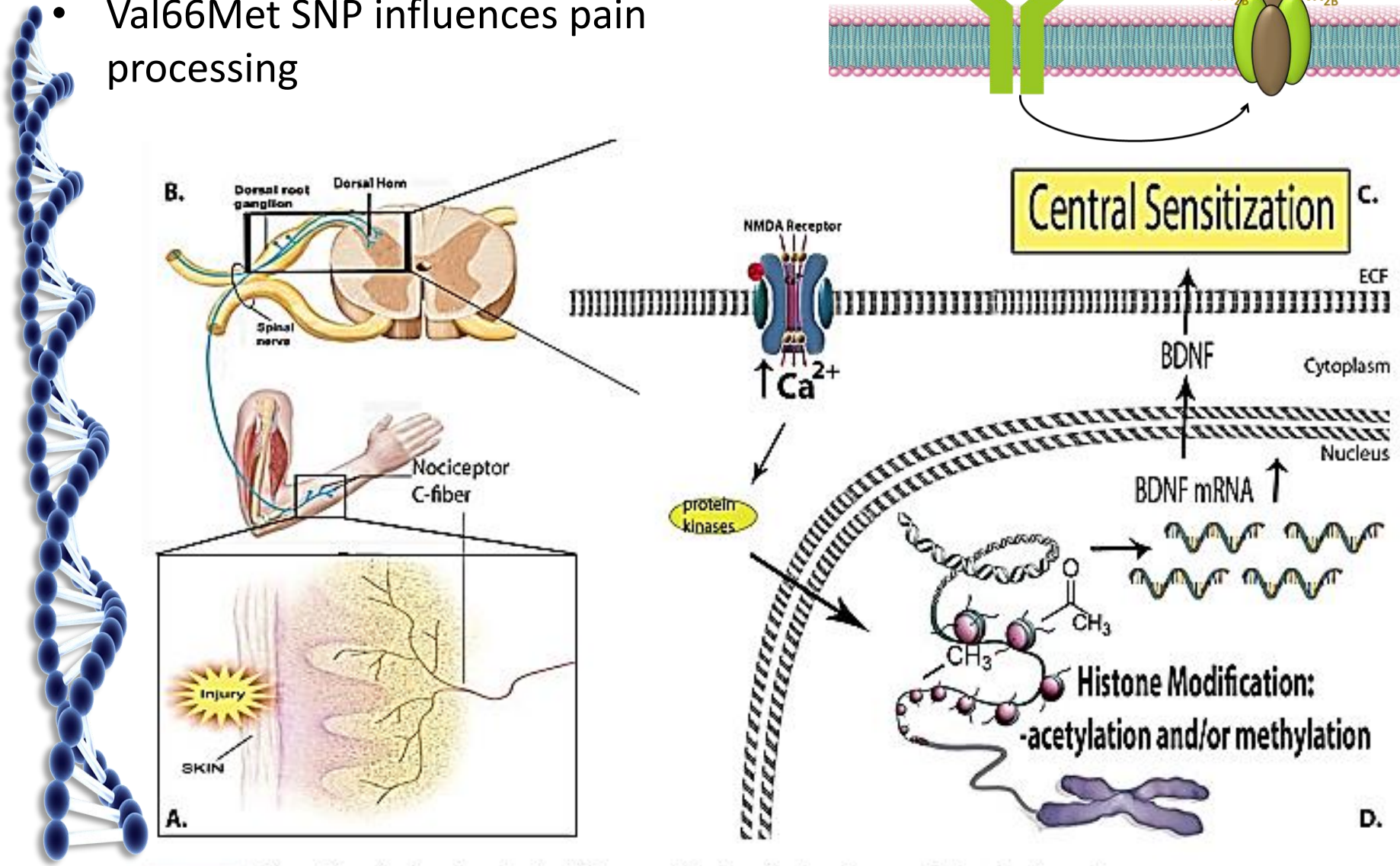
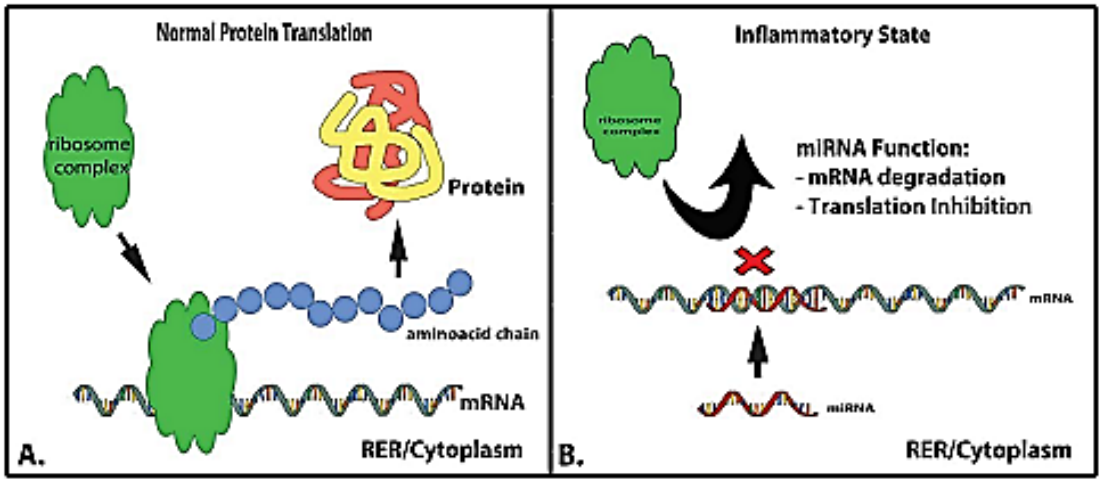


Figure 3 Epigenetic pain alteration due to Histone modification in dorsal horn and dorsal root ganglion

- RNA interference target TRPV1 channels, BDNF, cytokines such as TNF-α, and pain-related cation channels (P2X3)



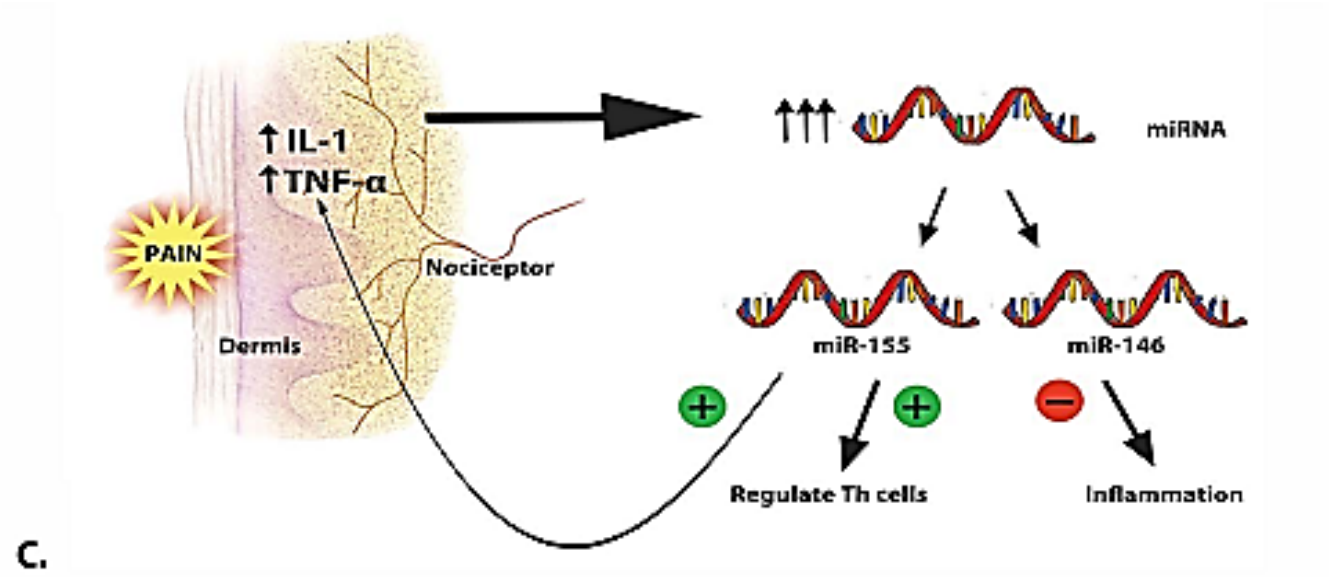
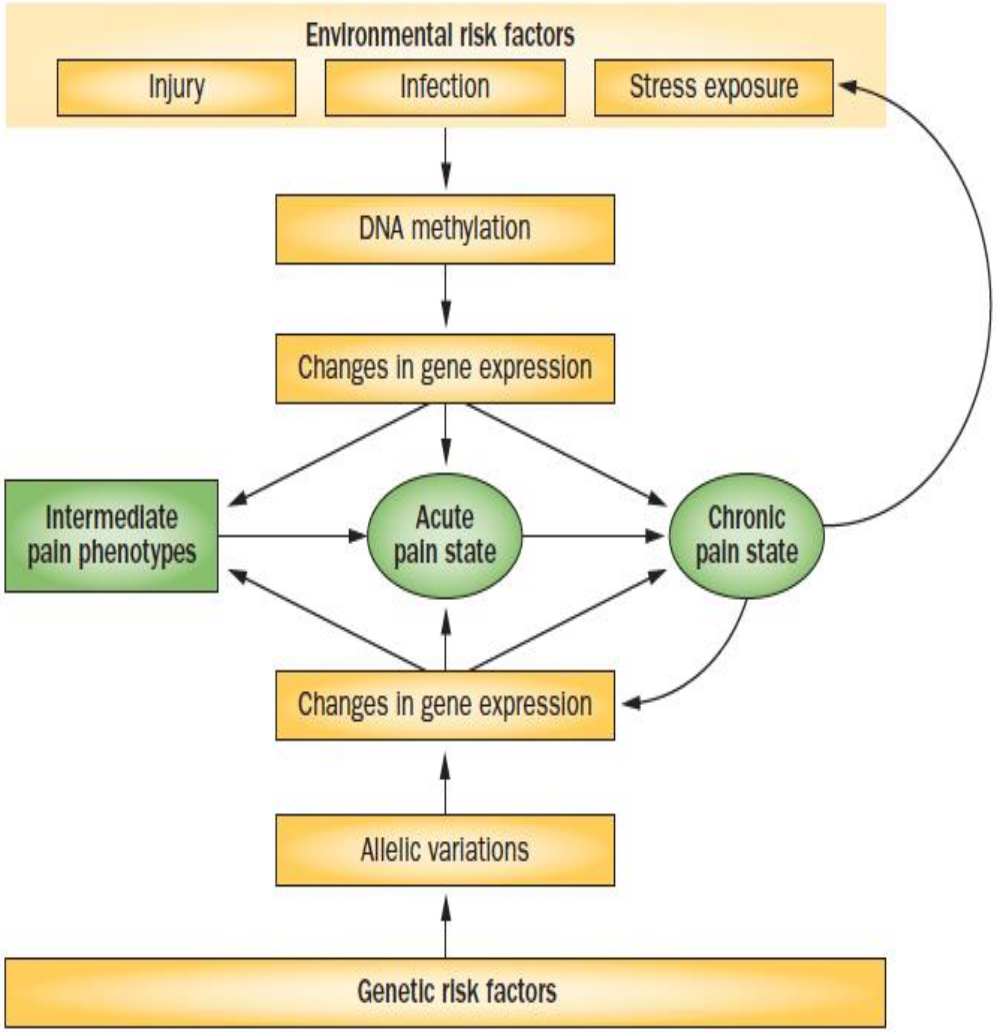


Figure 4 Epigenetic control of miRNA in an inflammatory state. A) Normal protein translation. B) Function of miRNA: mRNA degradation and inhibition of protein translation. C) Functions of miR-146 and miR-155 in rheumatoid arthritis; miR-146 decreases inflammation, whereas miR-155 modifies Th cell response and induces TNF-α mediated nociception. Abbreviations: RER- Rough Endoplasmatic Reticulum; Il-1-Interleukin 1; TNF-α-Tumor necrosis factor α.

- Genetic and environment interactions contribute to pain development and response to treatment
- Epigenetics
- Epigenetically Active Drugs (TSA enhances μ-opioid receptor transcription, SAHA reduces the nociceptive response in animals)
- Bioactive dietary components

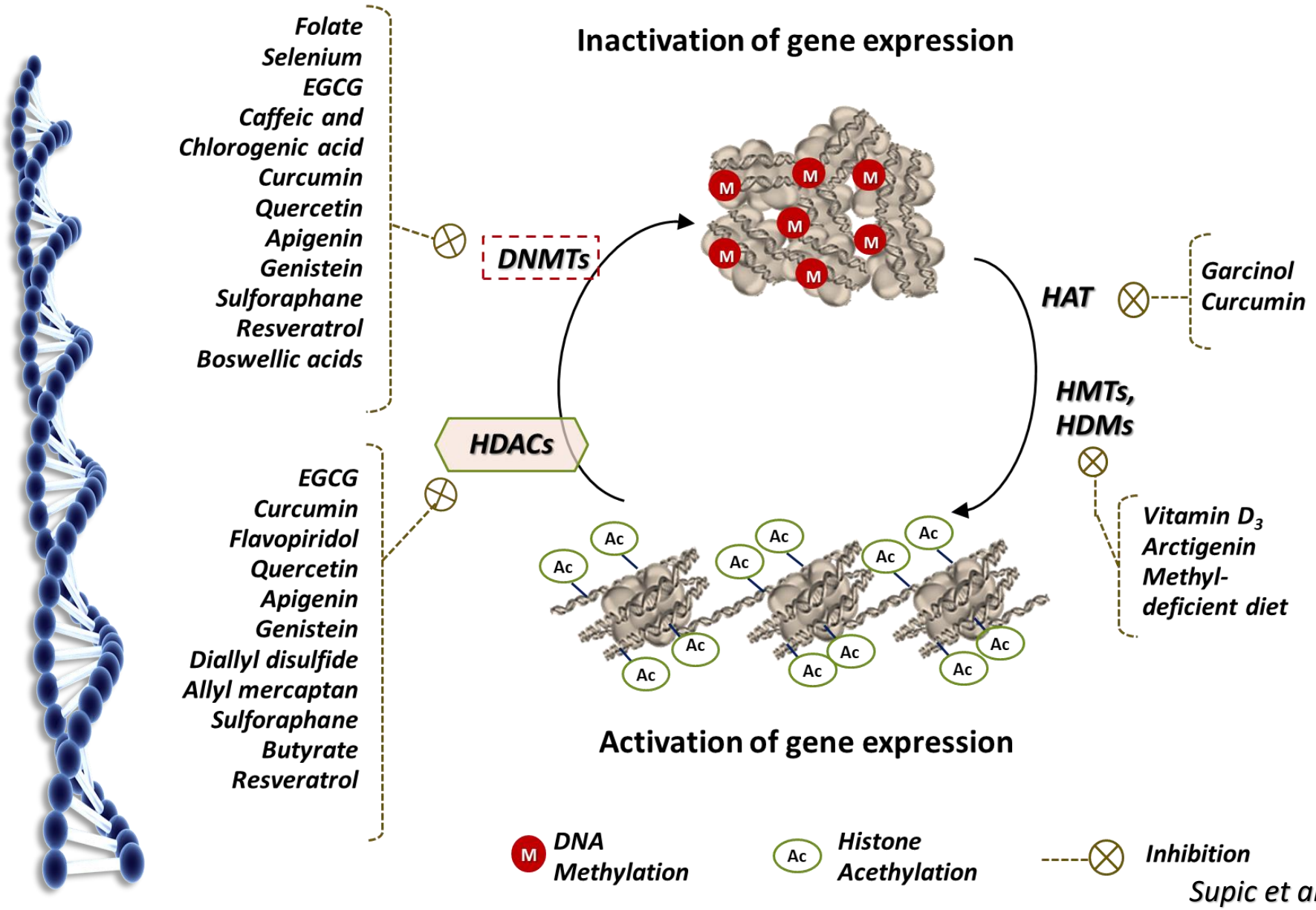


Diatchenko et al; Nat Rev Rheumatol 9, 340, 2013


If they ask you anything you don't know, just say it's due to epigenetics.

THANK YOU!

- Bioactive compounds with epigenetic effect on gene expression



Supic et al, 2016