Non-Mendelian Genetics

Epidemiology 217: Lecture 9

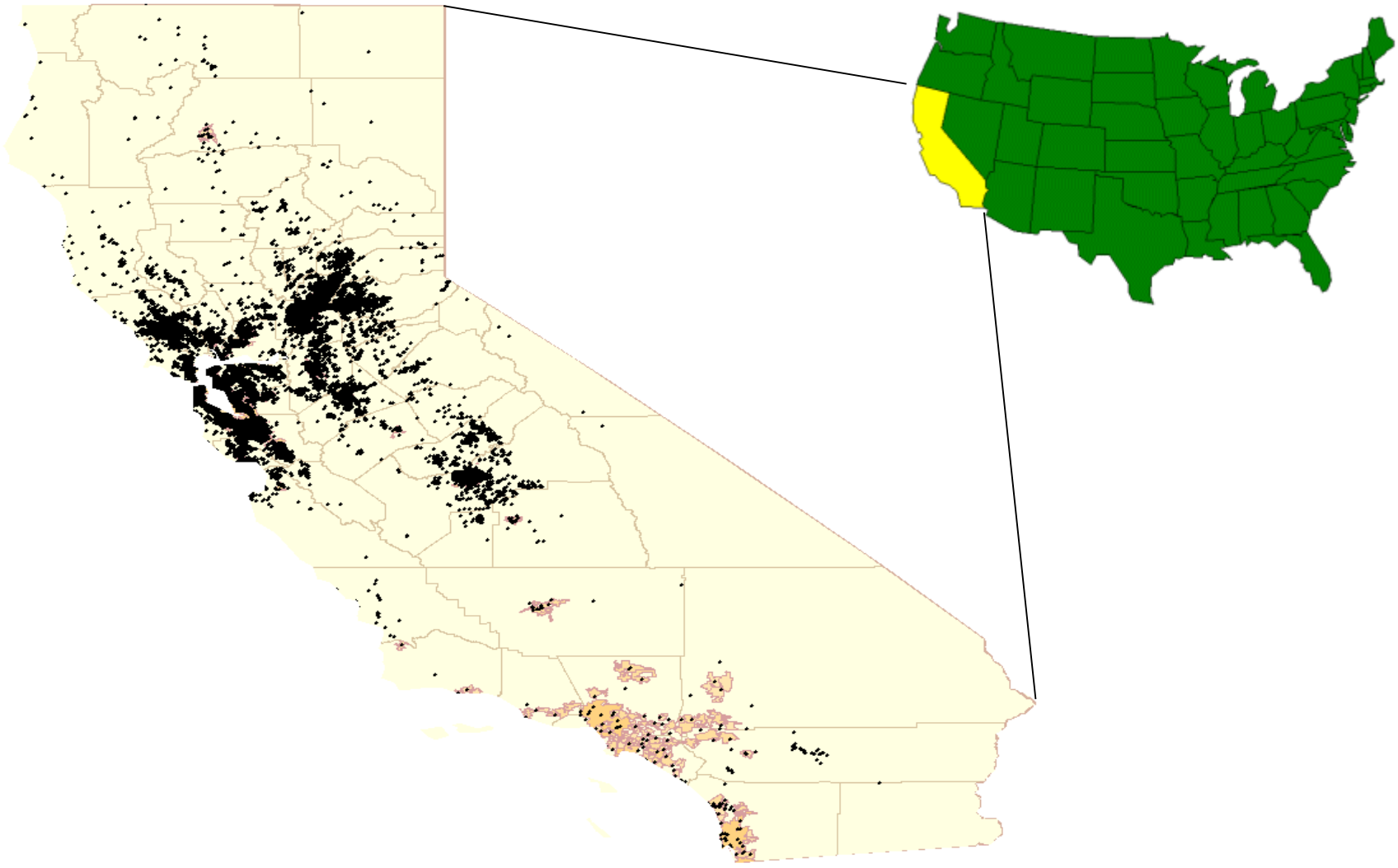
Eric Jorgenson, PhD

March 1st, 2016

Outline

- Epigenetics: DNA Methylation and Histone Modification
- Parent-of-origin Effects
- Mitochondrial DNA
- *De Novo* Variation

Kaiser Permanente Northern California Members



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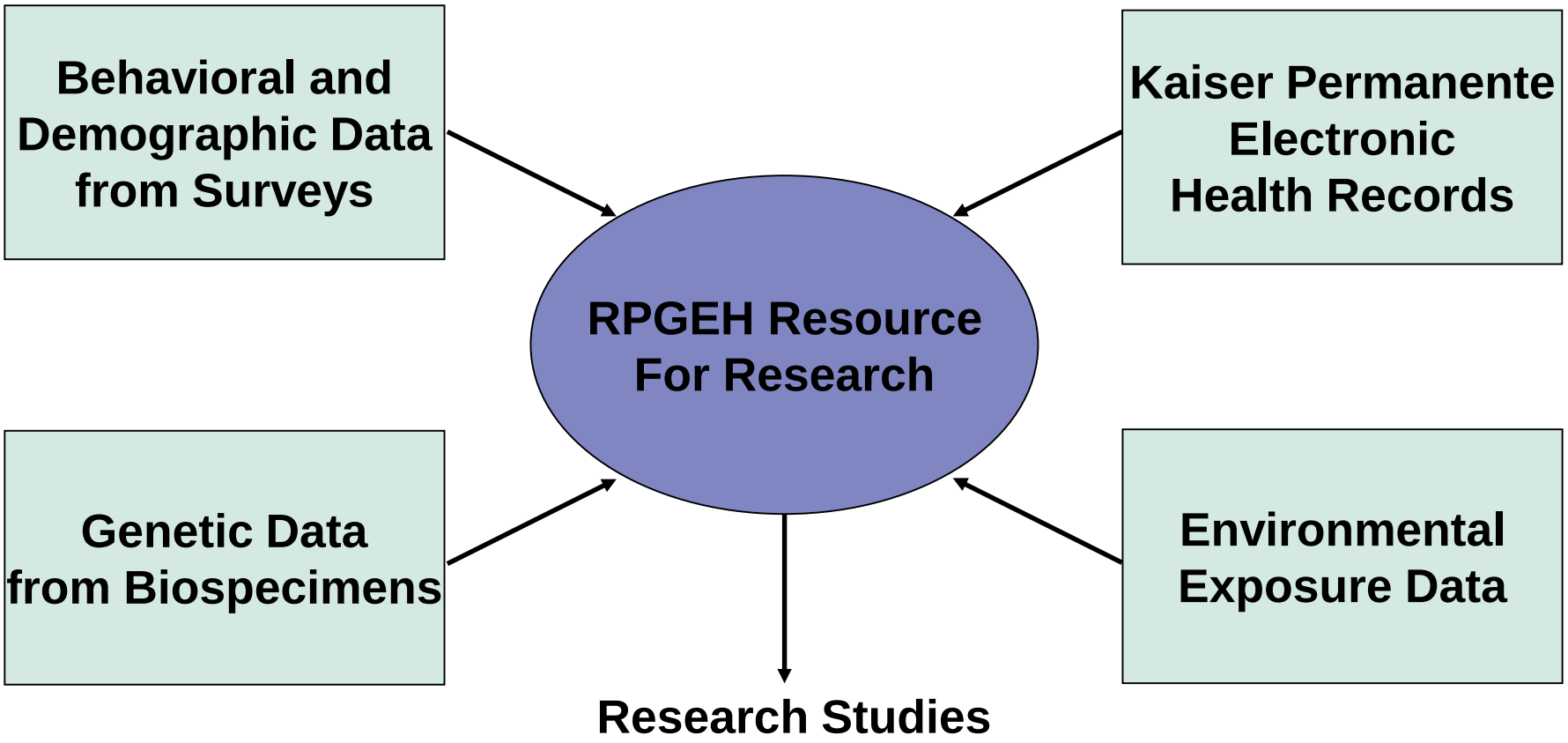
Research Program on Genes, Environment, and Health

430,000 Adult Members of Kaiser Permanente Northern California Provided Informed Consent



Co-directors: Catherine Schaefer and Neil Risch

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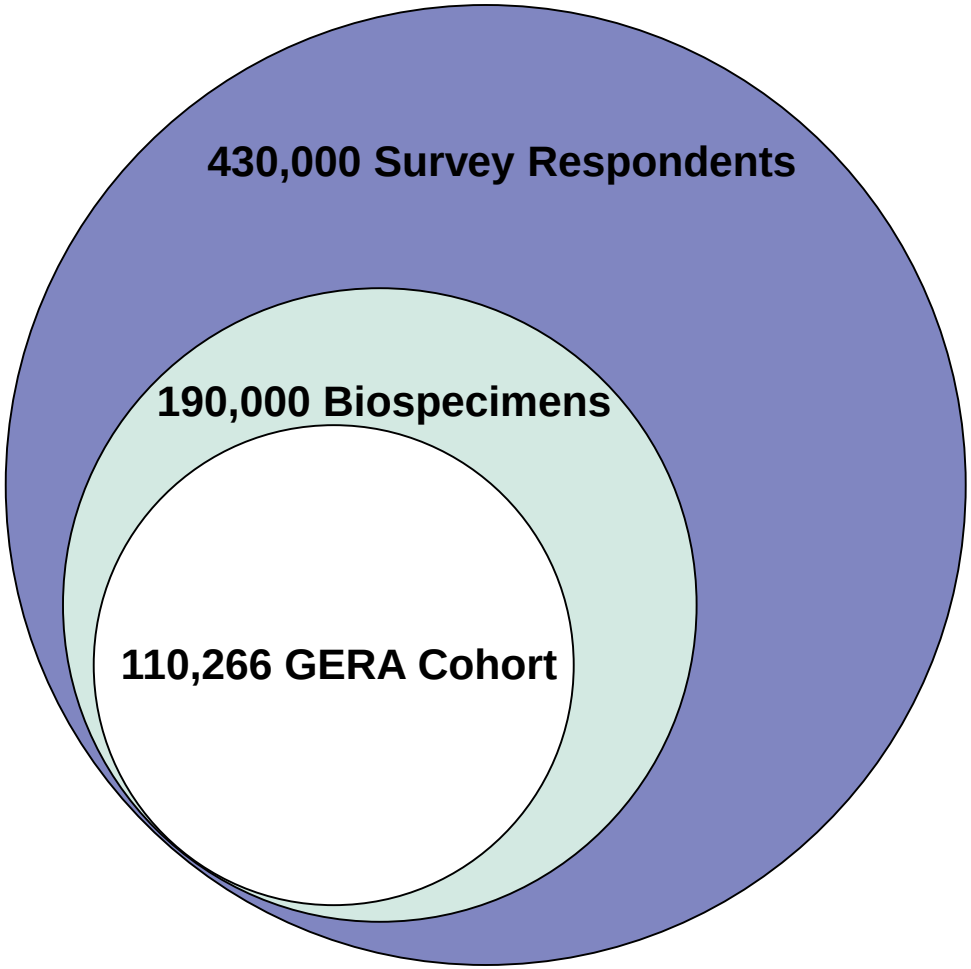
RPGEH Survey Respondents, Biospecimens, and GERA



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GERA Cohort Characteristics

	Number of Subjects	%
Age at Specimen Donation		
18-39	7,025	6.3
40-59	33,344	30.2
60-79	57,856	52.4
80+	12,041	10.9
Sex		
Women	63,883	57.9
Men	46,383	42.1
Race/Ethnicity		
African American	3,765	3.4
Asian	8,512	7.7
Hispanic	7,917	7.2
Non-Hispanic White	89,259	81.0
Total	110,266	100.0

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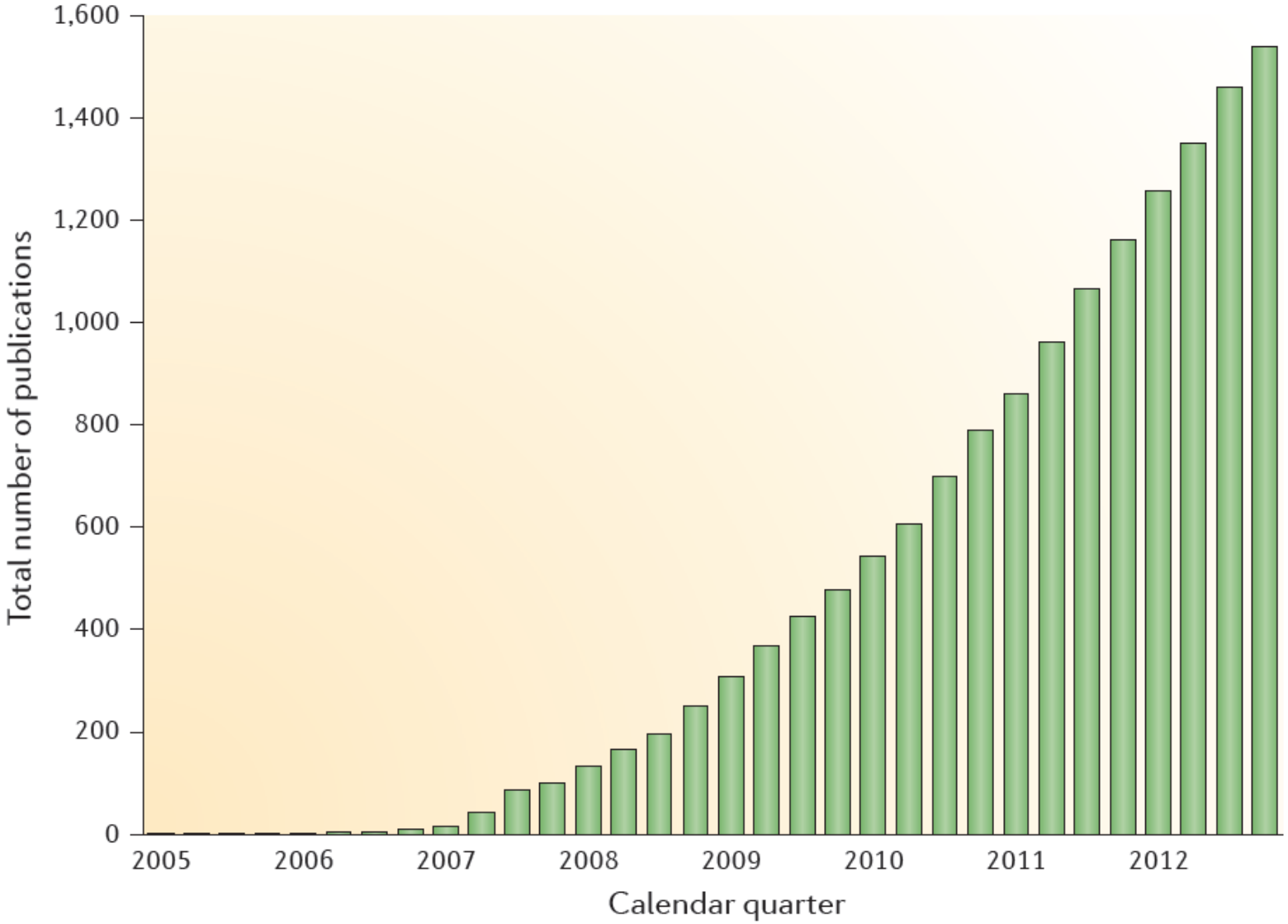
2,111 Published Genome-Wide Association Studies



http://www.genome.gov/gwastudies/

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15,396 SNPs Associated in GWAS

http://www.genome.gov/gwastudies/

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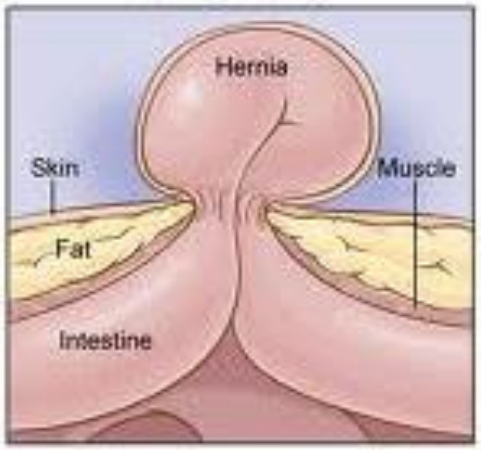
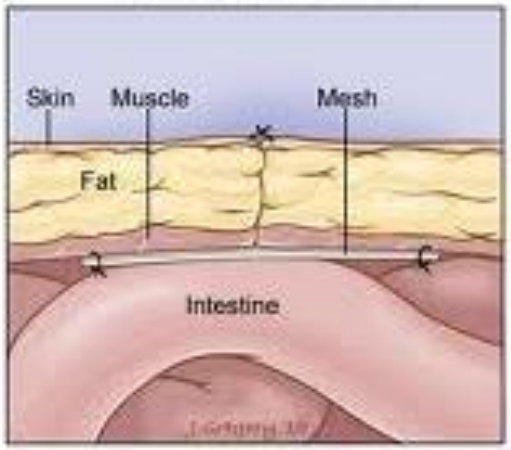
Hernia: A Condition with No GWAS Studies

Hernia



Repair Surgery



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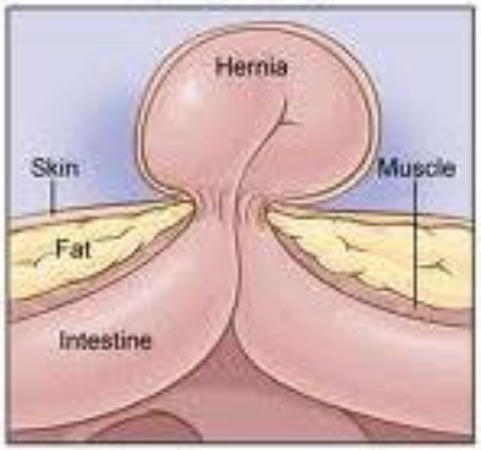
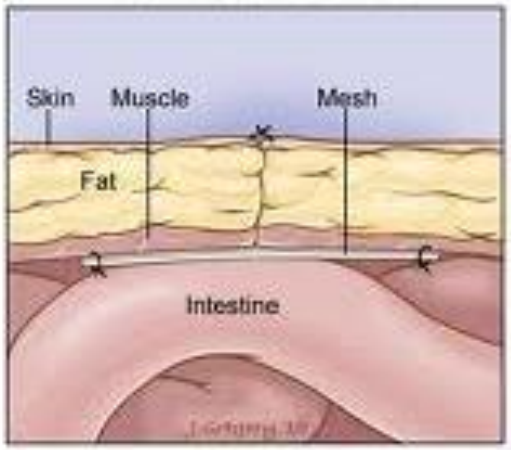
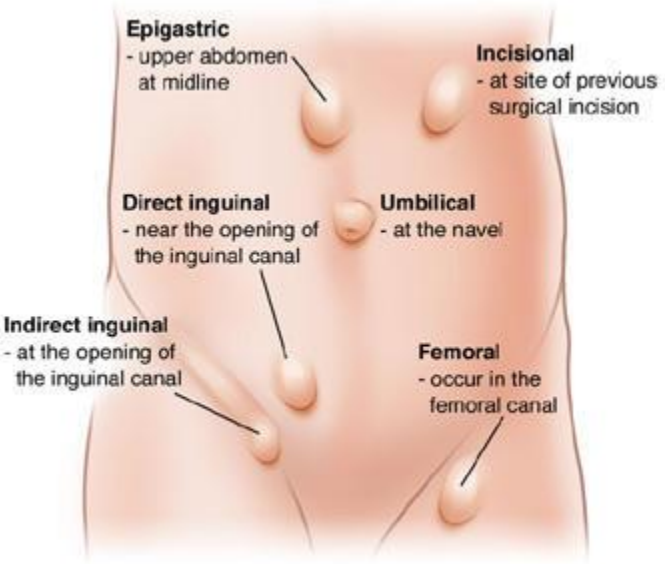
Hernias Are Classified by Anatomical Site

Hernia



Repair Surgery





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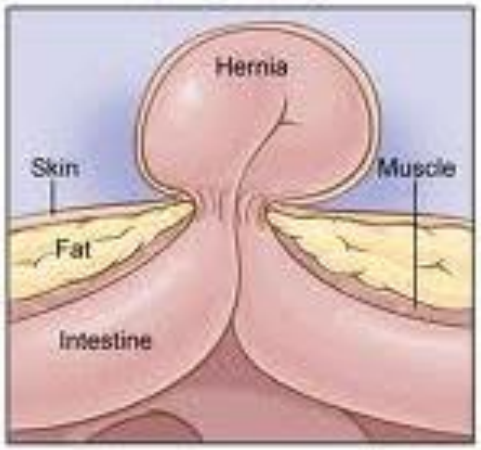
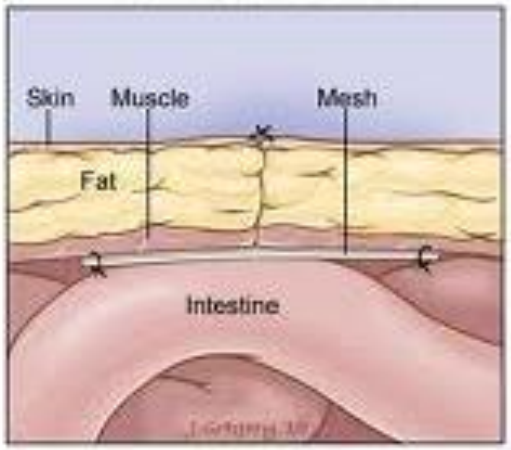
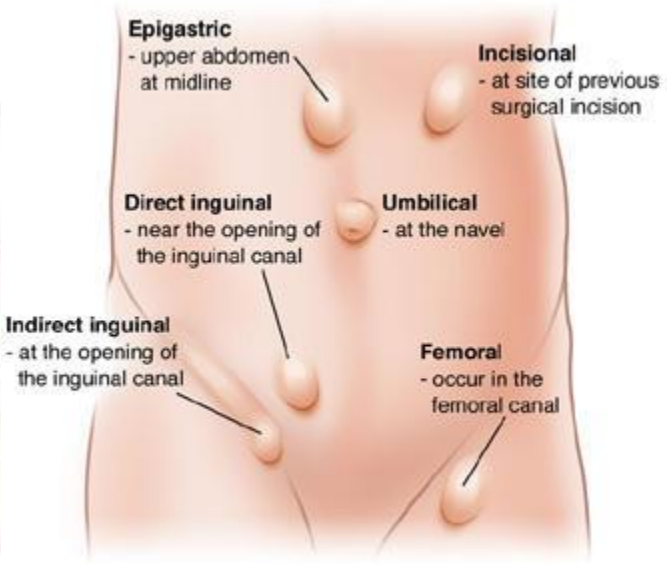
Inguinal Hernias Are Common in GERA

Hernia



Repair Surgery





GERA Cohort	Inguinal Hernia Cases						Controls	
Non-Hispanic whites	All (n=5,295)		Direct (n=2,335)		Indirect (n=2,647)		(n=67,510)	
Men	4,746	89.6%	2,193	93.9%	2,408	91.0%	25,011	37.1%
Women	549	10.4%	142	6.1%	239	9.0%	42,499	63.0%

Jorgenson *et al*. Nature Communications 2015

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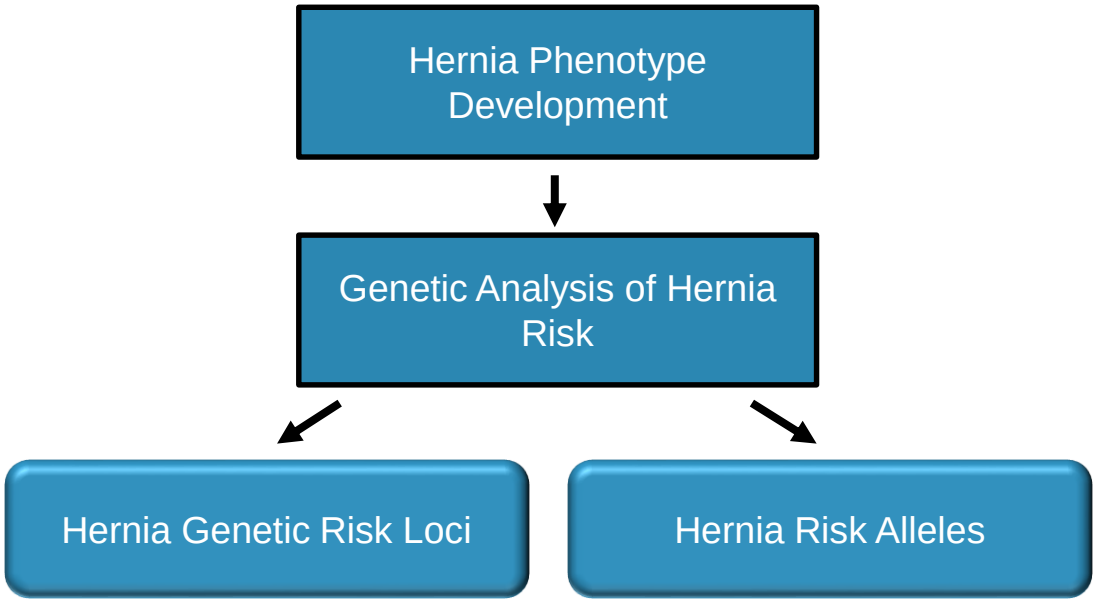
GWAS Can Identify Loci, not Genes



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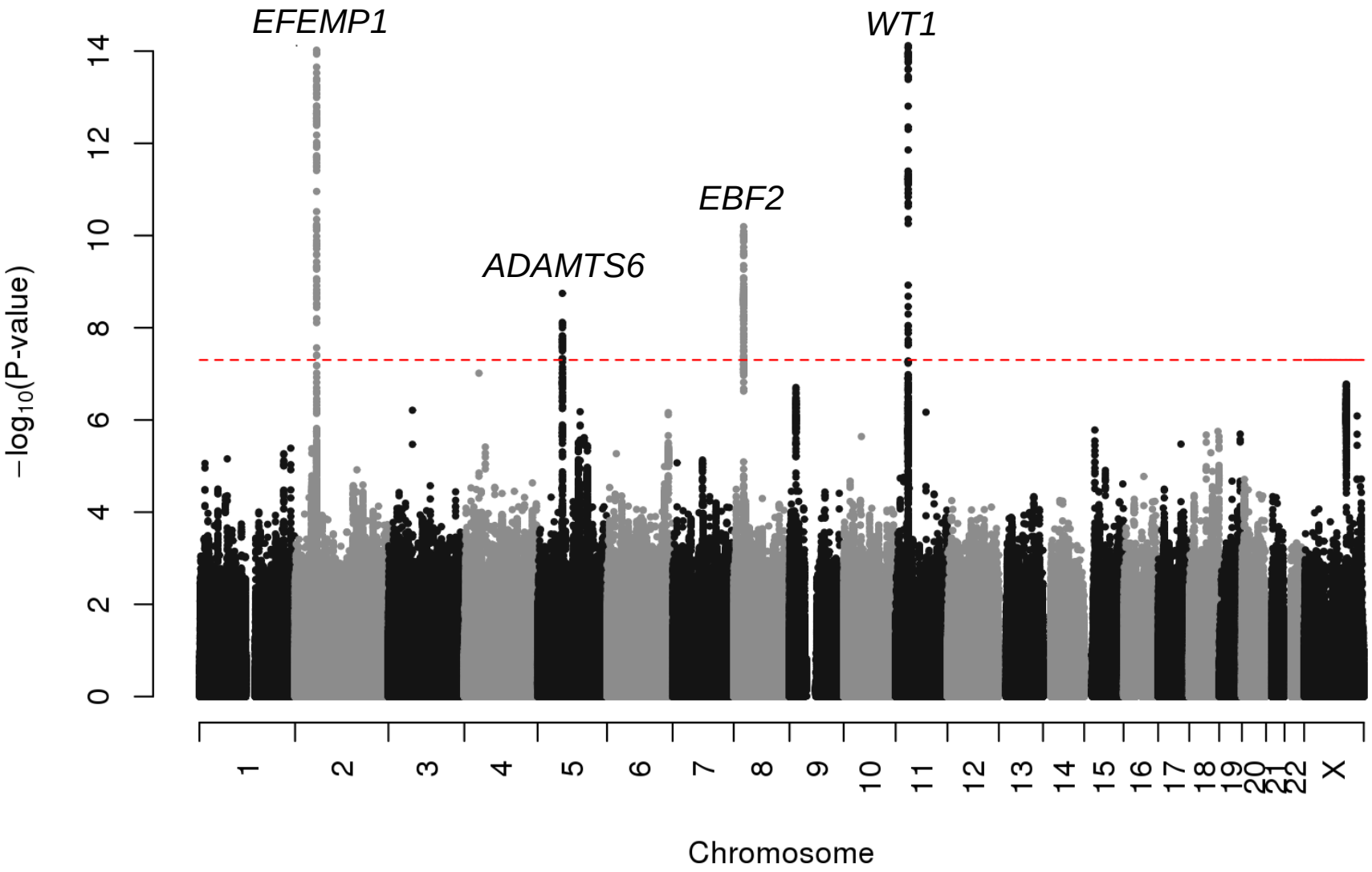
Manhattan Plot of Inguinal Hernia GWAS Results



Jorgenson *et al*. Nature Communications 2015

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Hernia Repair Replication Sample: 23&Me Subjects

"Have you ever had any of the following gastrointestinal surgeries (Hernia repair)?"

23&Me Subjects	Hernia Repair Cases (n=9,701)		Controls (n=82,743)	
Men	6,812	70.2%	41,611	50.2%
<30	246	3.6%	5,147	12.4%
31-45	1,087	16.0%	14,109	33.9%
46-60	1,605	23.6%	10,259	24.7%
>60	3,874	56.9%	12.096	29.1%
Women	1,889	29.8%	41,132	49.8%
<30	63	3.3%	4,252	10.3%
31-45	317	16.8%	11,099	27.0%
46-60	603	31.9%	11,431	27.8%
>60	906	48.0%	14,350	34.9%

Jorgenson *et al*. Nature Communications 2015

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Replication of Inguinal Hernia Significant Associations

Table 1 | SNP associations reaching genome-wide significance in the combined analysis of discovery and replication cohorts.

SNP	Chr.	Position	Gene	Risk allele	Discovery (5,295 cases, 67,510 controls)			Replication (9,701 cases, 82,743 controls)			Combined	
					RAF	OR (95% CI)	*P*-value	RAF	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs2009262	2	56,012,214	*EFEMP1*	T	0.78	1.23 (1.17–1.30)	3.66×10^{-15}	0.78	1.10 (1.06–1.15)	3.65×10^{-06}	1.15 (1.11–1.19)	1.45×10^{-17}
rs370763	5	64,355,060	*ADAMTS6*	A	0.65	1.14 (1.09–1.19)	9.70×10^{-09}	0.67	1.06 (1.02–1.09)	3.02×10^{-03}	1.09 (1.06–1.12)	3.73×10^{-9}
rs6991952	8	25,707,412	*EBF2*	G	0.43	1.14 (1.10–1.19)	1.17×10^{-10}	0.43	1.08 (1.05–1.12)	2.04×10^{-06}	1.11 (1.08–1.14)	6.68×10^{-15}
rs3809060	11	32,458,807	*WT1*	G	0.62	1.18 (1.13–1.23)	4.69×10^{-14}	0.63	1.07 (1.03–1.10)	1.69×10^{-04}	1.11 (1.08–1.14)	3.69×10^{-14}

Chr., chromosome; CI, confidence interval; RAF, risk allele frequency; SNP, single-nucleotide polymorphism.

Jorgenson *et al*. Nature Communications 2015

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Clinical Subtyping of Inguinal Hernia Risk SNPs

Table 2 | Sex-stratified analysis of direct and indirect inguinal hernia among GERA discovery cohort.

SNP	Inguinal Hernia Type	Men		Women		Combined					
		OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR_F	P_F	OR_R	P_R	I^2	P_{Het}
rs2009262	Direct	1.25 (1.16-1.36)	2.31×10^{-8}	1.42 (1.04-1.94)	0.03	1.26 (1.17-1.36)	2.81×10^{-9}	1.26 (1.17-1.36)	2.81×10^{-9}	0	0.46
	Indirect	1.21 (1.13-1.31)	4.48×10^{-7}	1.51 (1.18-1.93)	0.001	1.24 (1.15-1.33)	8.48×10^{-9}	1.31 (1.07-1.60)	0.009	62.4	0.1
rs370763	Direct	1.14 (1.06-1.22)	1.60×10^{-4}	1.22 (0.94-1.58)	0.133	1.14 (1.07-1.22)	6.38×10^{-5}	1.14 (1.07-1.22)	6.38×10^{-5}	0	0.65
	Indirect	1.15 (1.08-1.23)	2.86×10^{-5}	1.10 (0.91-1.34)	0.332	1.14 (1.08-1.22)	2.28×10^{-5}	1.14 (1.08-1.22)	2.28×10^{-5}	0	0.66
rs6991952	Direct	1.21 (1.14-1.29)	8.95×10^{-10}	1.03 (0.81-1.30)	0.814	1.20 (1.13-1.28)	2.38×10^{-9}	1.16 (1.01-1.34)	0.044	46	0.17
	Indirect	1.14 (1.08-1.21)	1.17×10^{-5}	1.07 (0.89-1.28)	0.461	1.14 (1.07-1.20)	1.20×10^{-5}	1.14 (1.07-1.20)	1.20×10^{-5}	0	0.46
rs3809060	Direct	1.21 (1.13-1.29)	2.71×10^{-8}	1.44 (1.11-1.86)	0.006	1.22 (1.14-1.30)	1.55×10^{-9}	1.26 (1.09-1.46)	0.003	44.7	0.18
	Indirect	1.17 (1.09-1.24)	2.16×10^{-6}	1.55 (1.26-1.89)	2.41×10^{-5}	1.20 (1.13-1.27)	8.14×10^{-9}	1.32 (1.00-1.74)	0.051	85.9	0.01

CI, confidence interval; GERA, Genetic Epidemiology Research in Adult Health and Aging; I^2, heterogeneity index; OR_F, odds ratio from fixed effects model; OR_R, odds ratio from random effects model; P_{Het}, *P* value for heterogeneity from Cochran's Q test; P_F, *P* value from fixed effects model; P_R, *P* value from random effects model; SNP, single-nucleotide polymorphism.

Jorgenson *et al*. Nature Communications 2015

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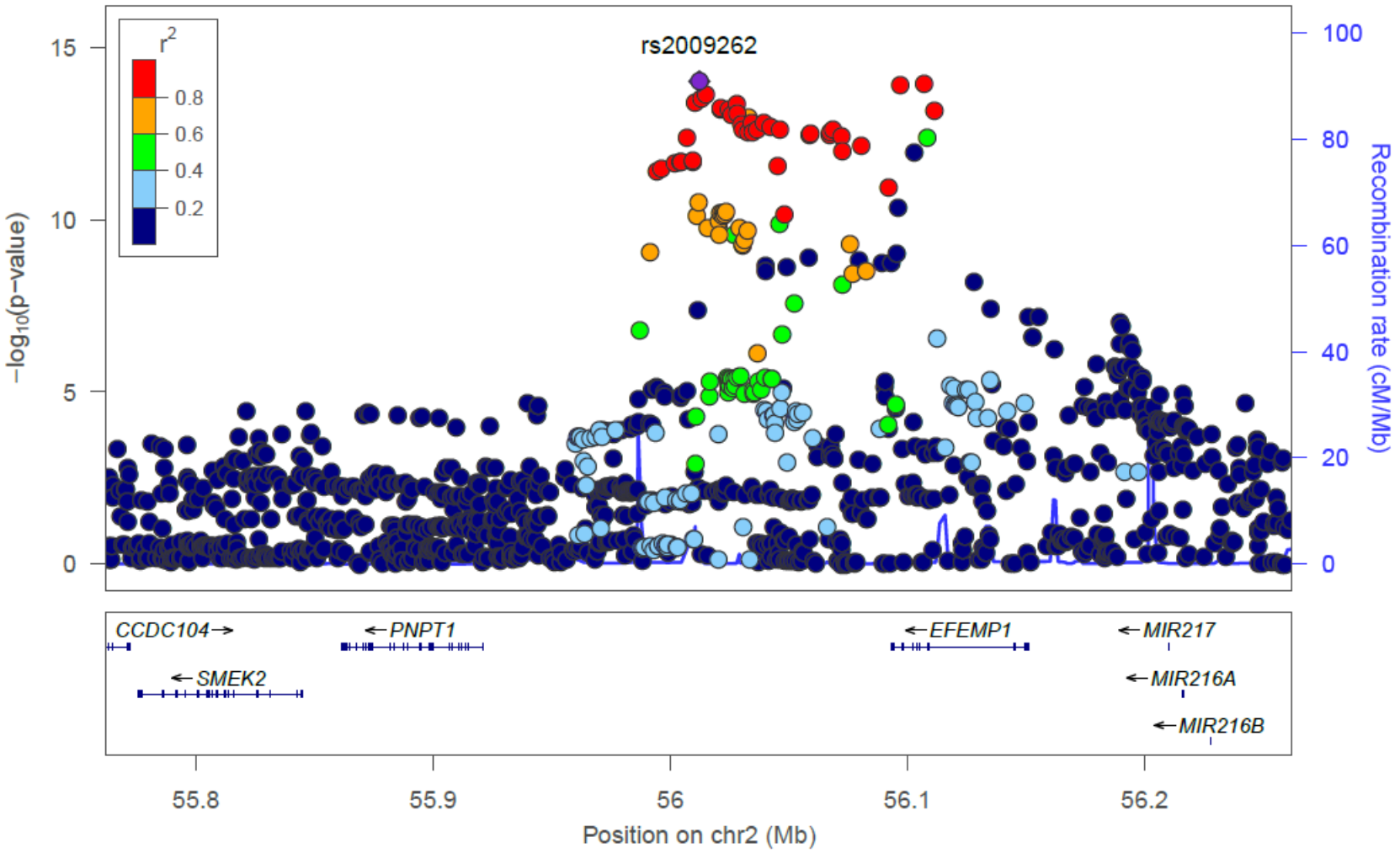
What Is in an Inguinal Hernia Risk Locus?



Jorgenson *et al*. Nature Communications 2015

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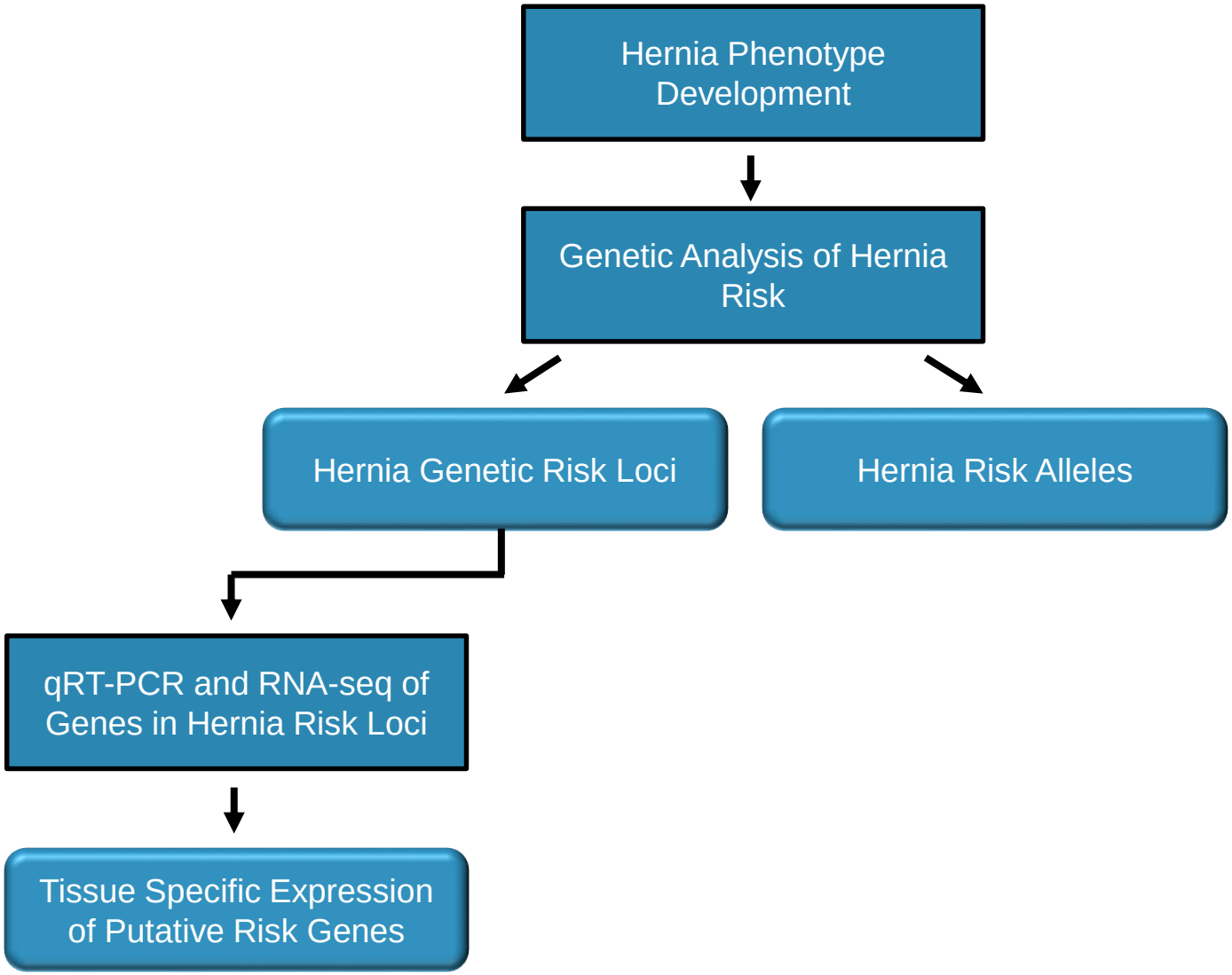
Are Genes in Risk Loci Expressed in Relevant Tissue?



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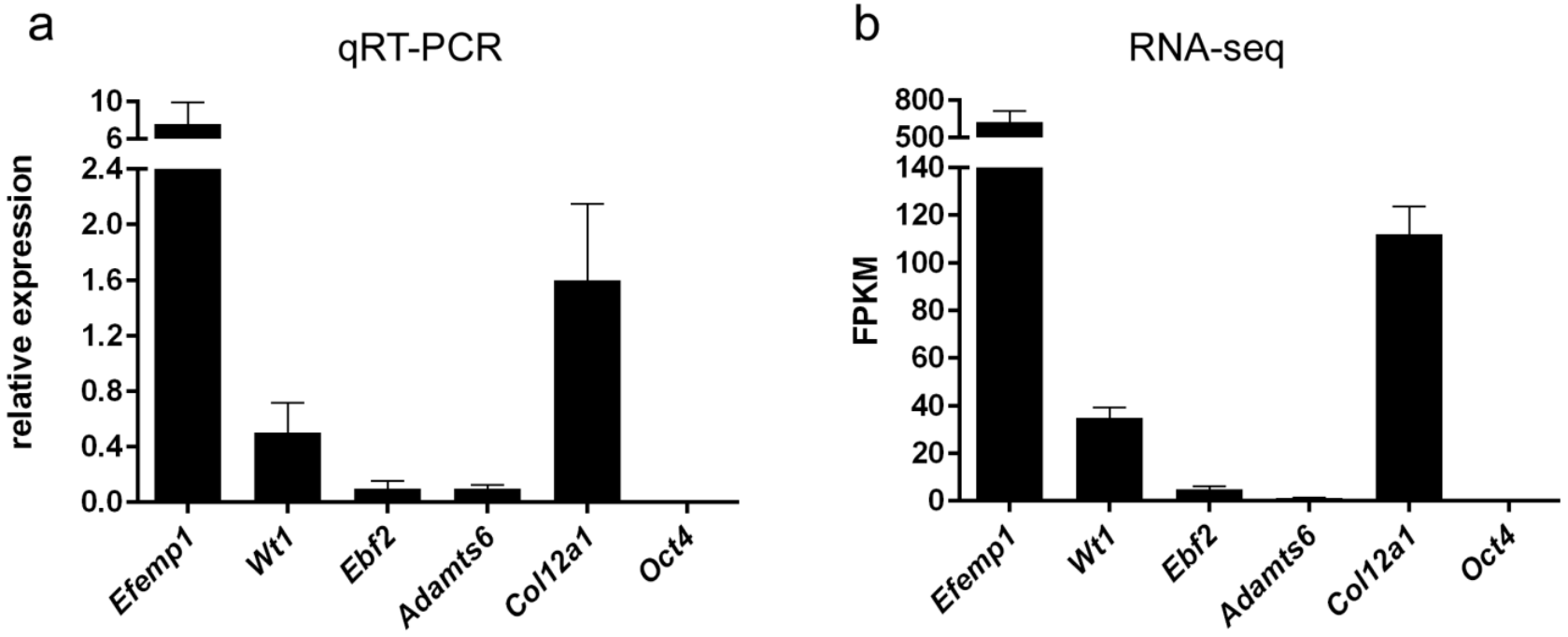
Genes at Inguinal Hernia Risk Loci are Expressed in Mouse Connective Tissue



Jorgenson *et al*. Nature Communications 2015

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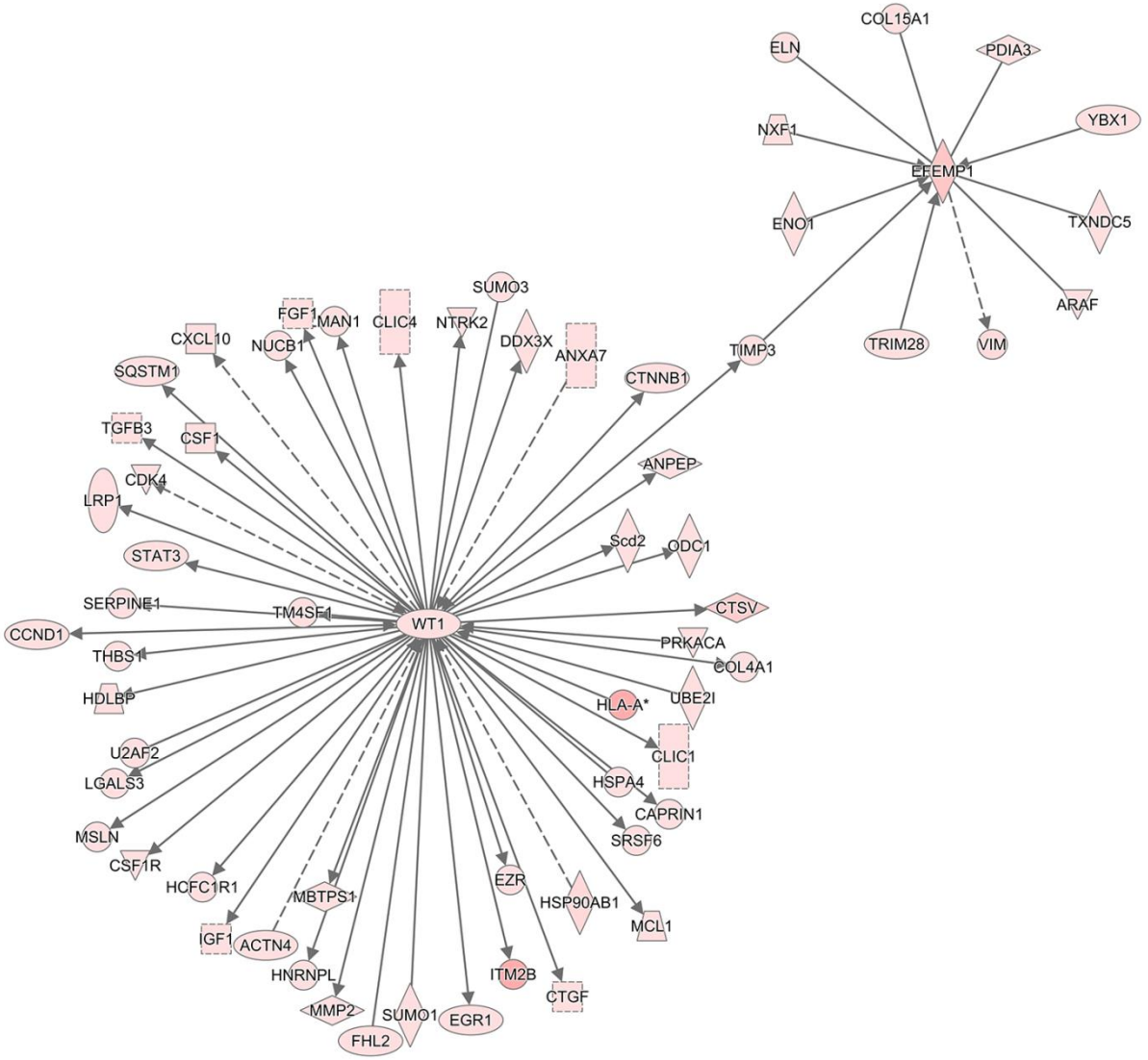
Expression Network Analysis Identifies a Pathway



Jorgenson *et al*. Nature Communications 2015

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What Have We Learned about Inguinal Hernia Genetics?

- **4 Inguinal Hernia Risk Loci**
  - Confirmed through replication in an independent cohort
  - Genes in the loci are expressed in equivalent mouse tissue
- **Hernia Loci Show Evidence of Pleiotropy**
  - *EFEMP1*: Height, Forced Vital Capacity, Multiple Cancers
  - *WT1*: Wilms' Tumor, Tuberculosis
  - *EBF2*: Prostate Cancer (different area in the gene)
  - *ADAMTS6*: Central Corneal Thickness, Osteosarcoma
- **Moving Toward Mechanism**
  - Collagen homeostasis
  - Matrix metalloproteinases and their inhibitors (*WT1* and *ADAMTS6*)
  - Elastin and chronic obstructive pulmonary disease (*EFEMP1*)

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What Do We Still Need to Learn about Hernia Genetics?

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What Do We Still Need to Learn about Hernia Genetics?

- **Where are the Regulatory Elements in Hernia Risk Loci?**
- **Which Variants Disrupt the Function of These Elements?**

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Genes Are Like Lightbulbs

Genes

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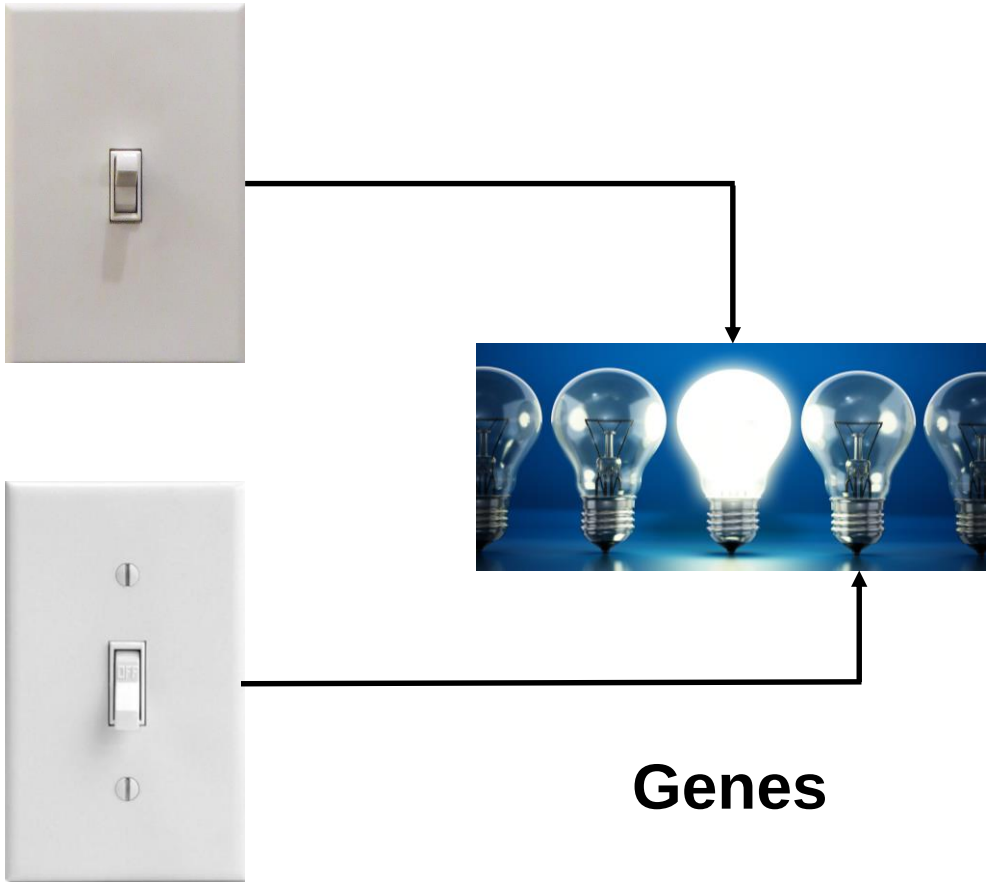
Genes Are Like Lightbulbs



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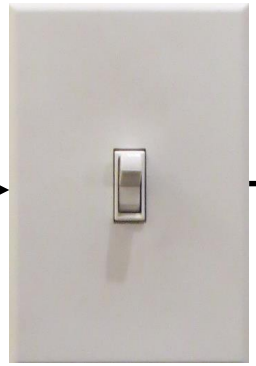

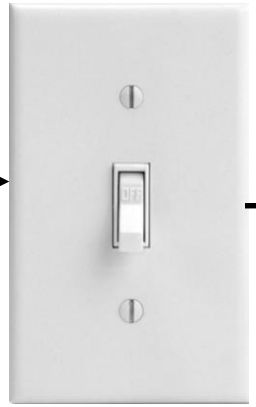

Enhancer

Promoters

Genes

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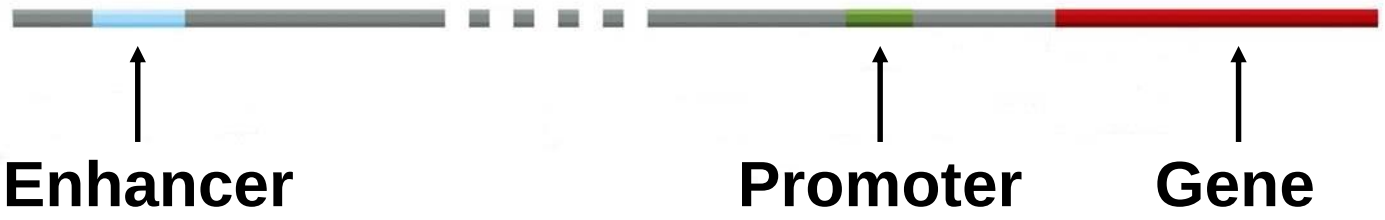
Proteins Bind Regulatory Elements



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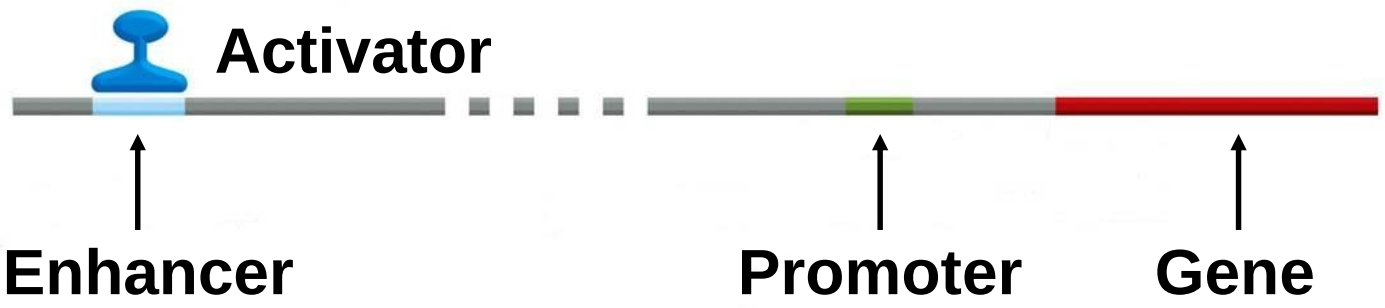

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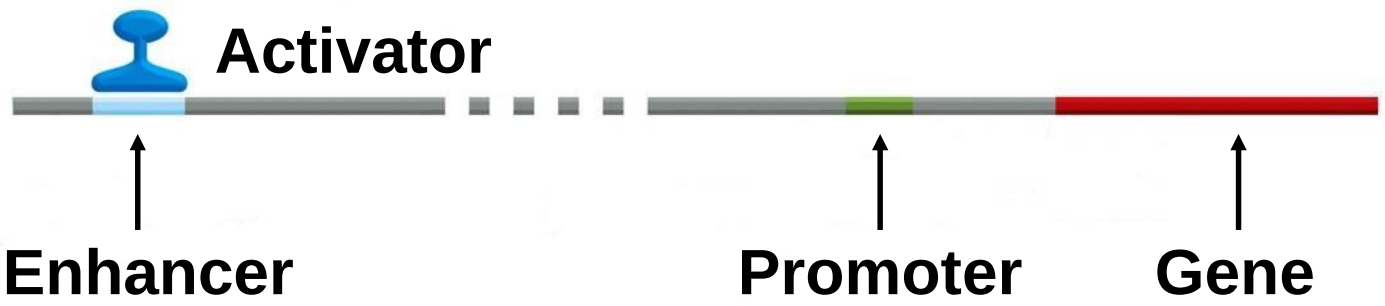
Proteins Bind Regulatory Elements



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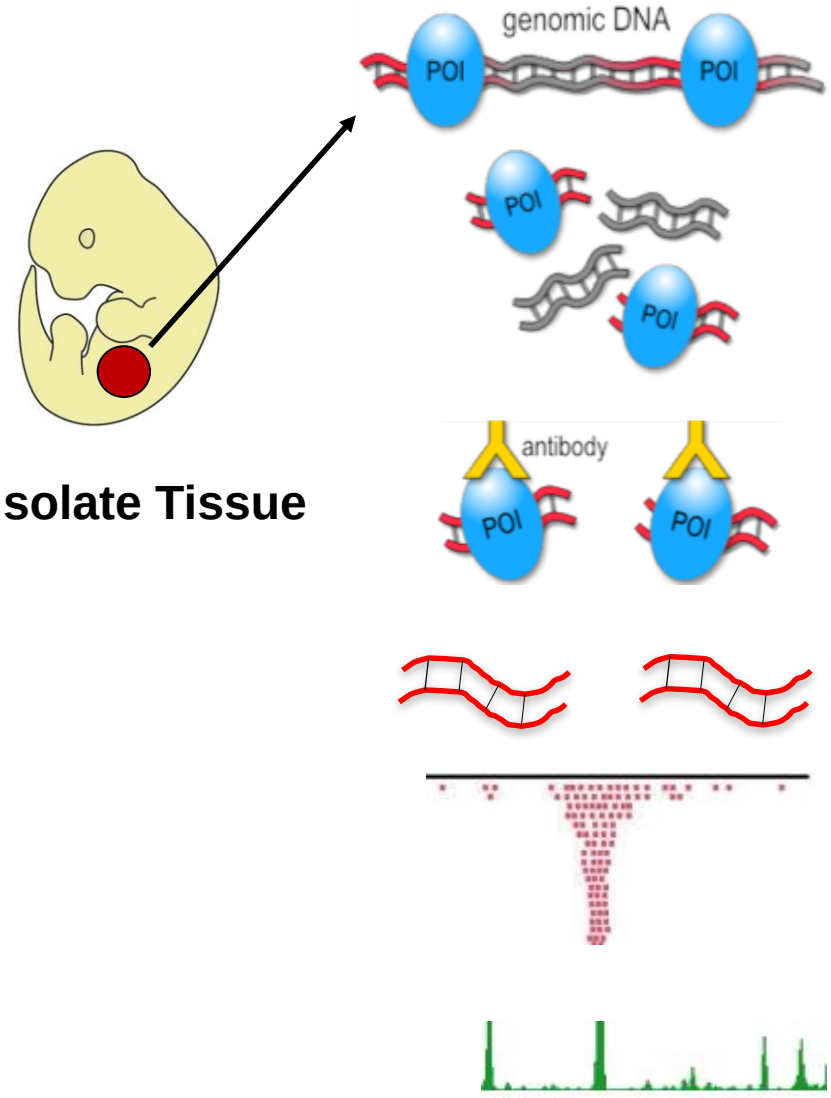
ChIP-seq Can Identify Regulatory Elements



Isolate Tissue

Cross-link DNA and Proteins

Shear DNA

Immunoprecipitate

Reverse Cross-link

Sequence and Map Reads

Determine Location

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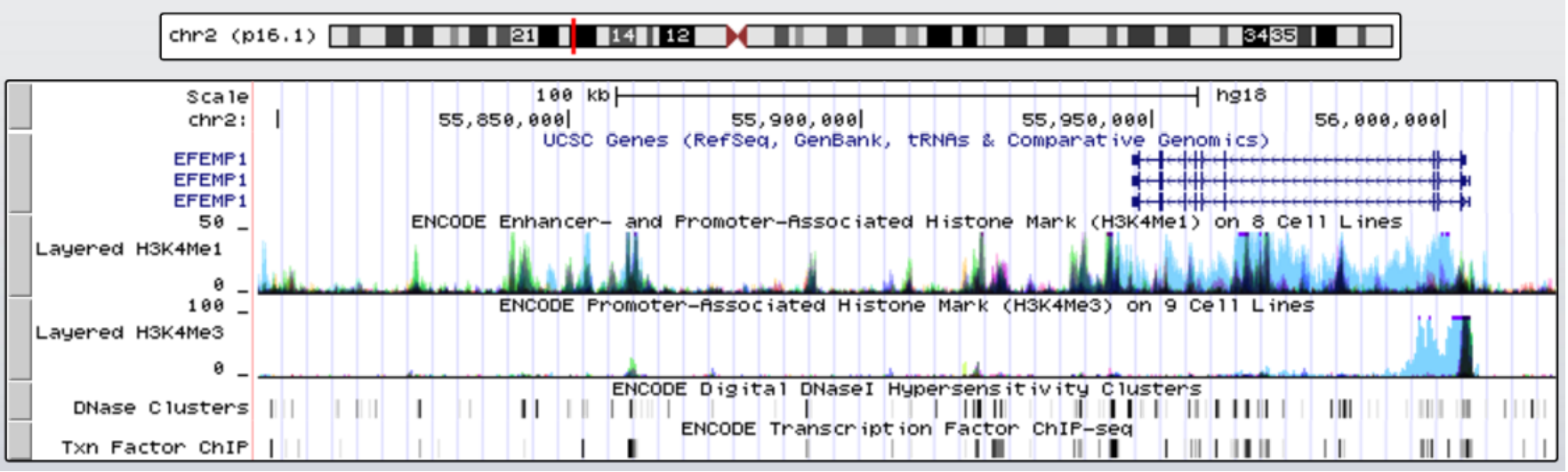
ChIP-seq for the *EFEMP1* Region from ENCODE



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What Do We Still Need to Learn about Hernia Genetics?

- **Where are the Regulatory Elements in Hernia Risk Loci?**
- **Which Variants Disrupt the Function of These Elements?**

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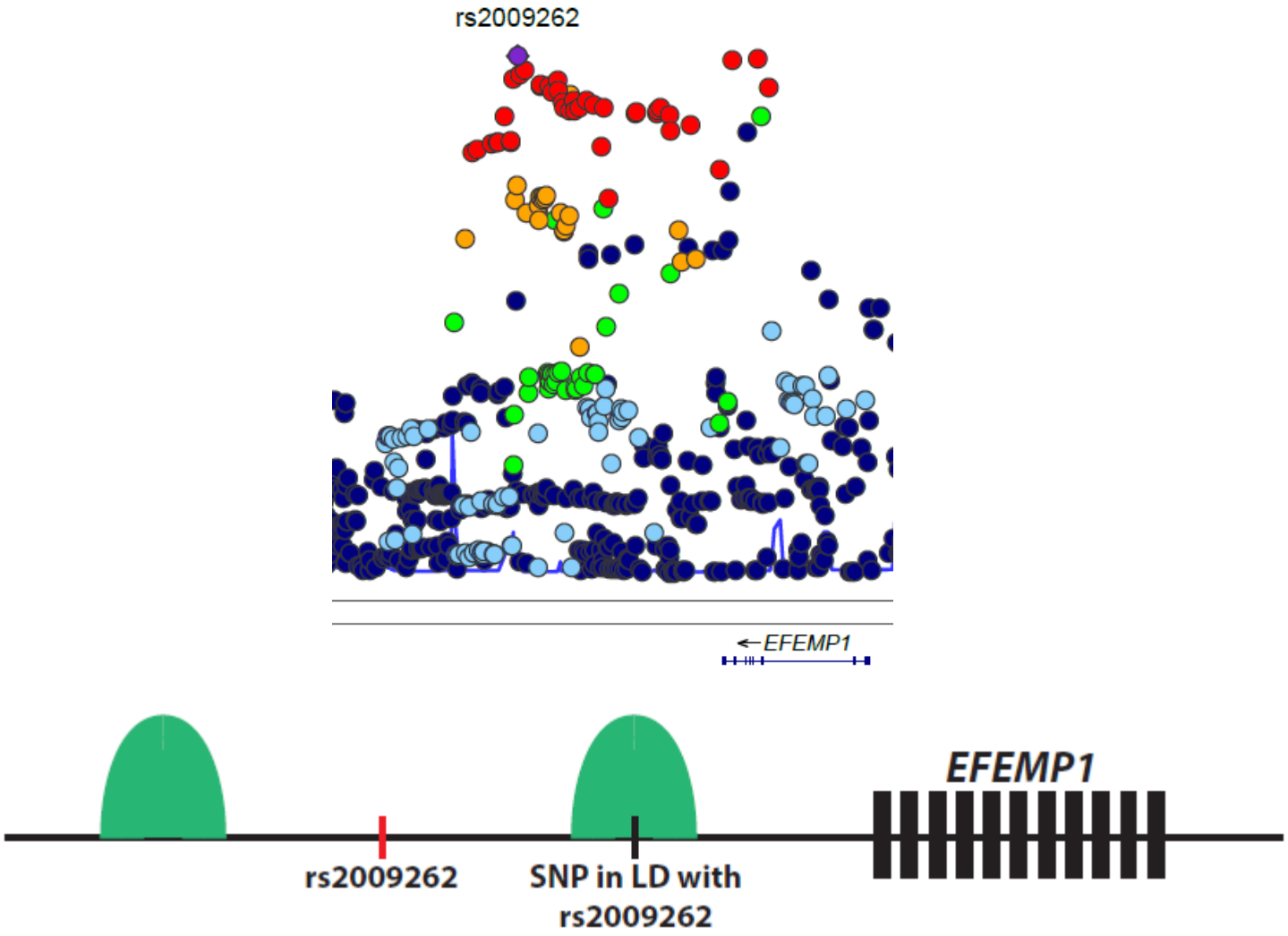
Identify SNPs in Regulatory Elements



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SNPs in the *EFEMP1* Locus Might Affect Binding

SNP	Chromosome	Position	RegulomeDB Score
rs11888023	2	56,006,984	2b
rs6739641	2	56,020,961	2b
rs3791679	2	56,096,891	2b
rs1367228	2	56,112,439	3a
rs78857879	2	56,135,098	3a
rs3762515	2	56,150,863	3a
rs6704991	2	56,179,758	3a
rs11893573	2	56,007,033	4
rs1346782	2	56,037,093	4
rs11680015	2	56,090,238	4
rs4672080	2	56,188,552	4
rs1432561	2	56,188,647	4
rs6747490	2	56,193,417	4
rs13431149	2	56,193,664	4
rs1432563	2	56,193,908	4
…and 132 other SNPs			

Jorgenson *et al*. Nature Communications 2015

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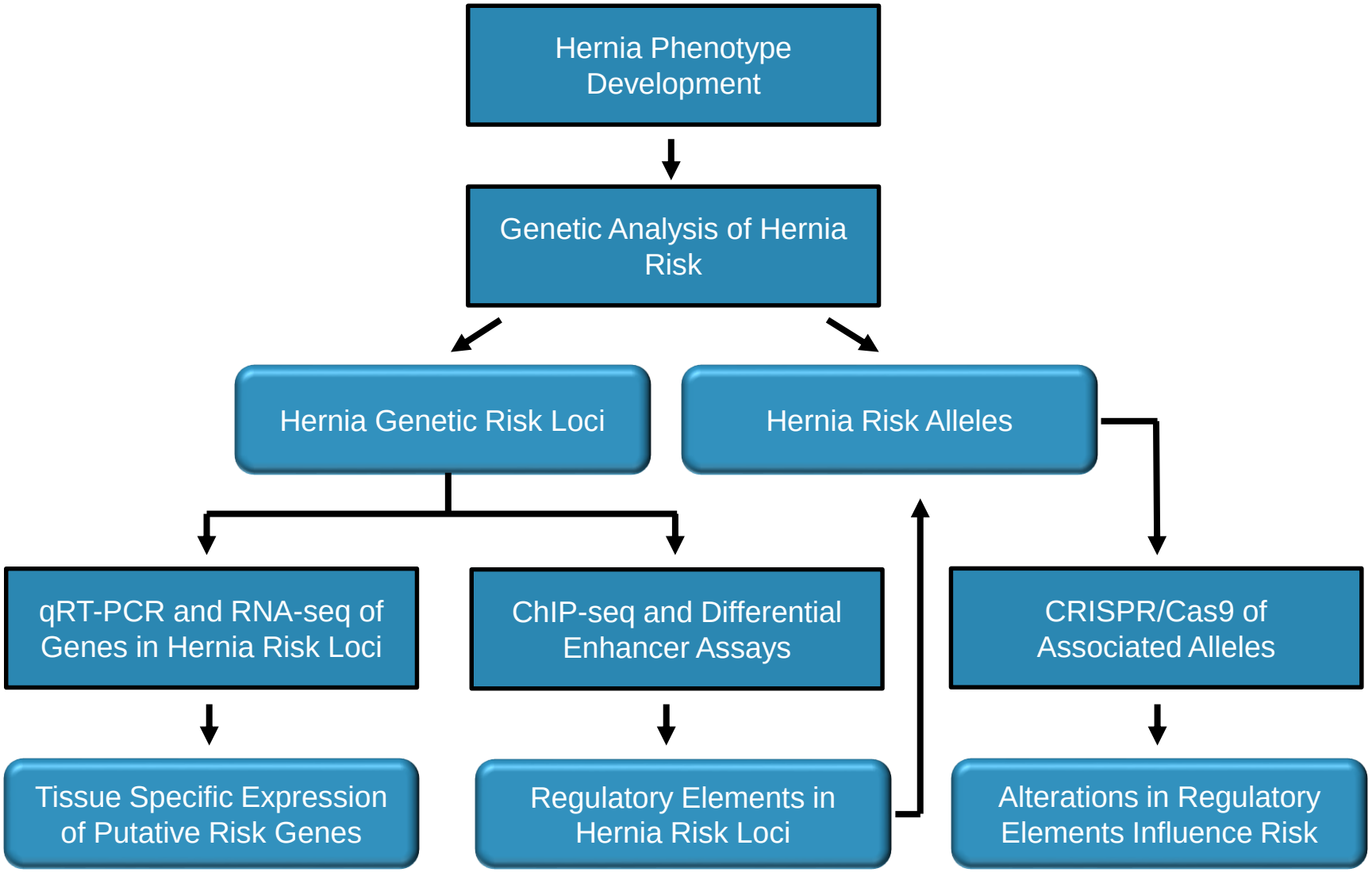
Identifying Causal Variants



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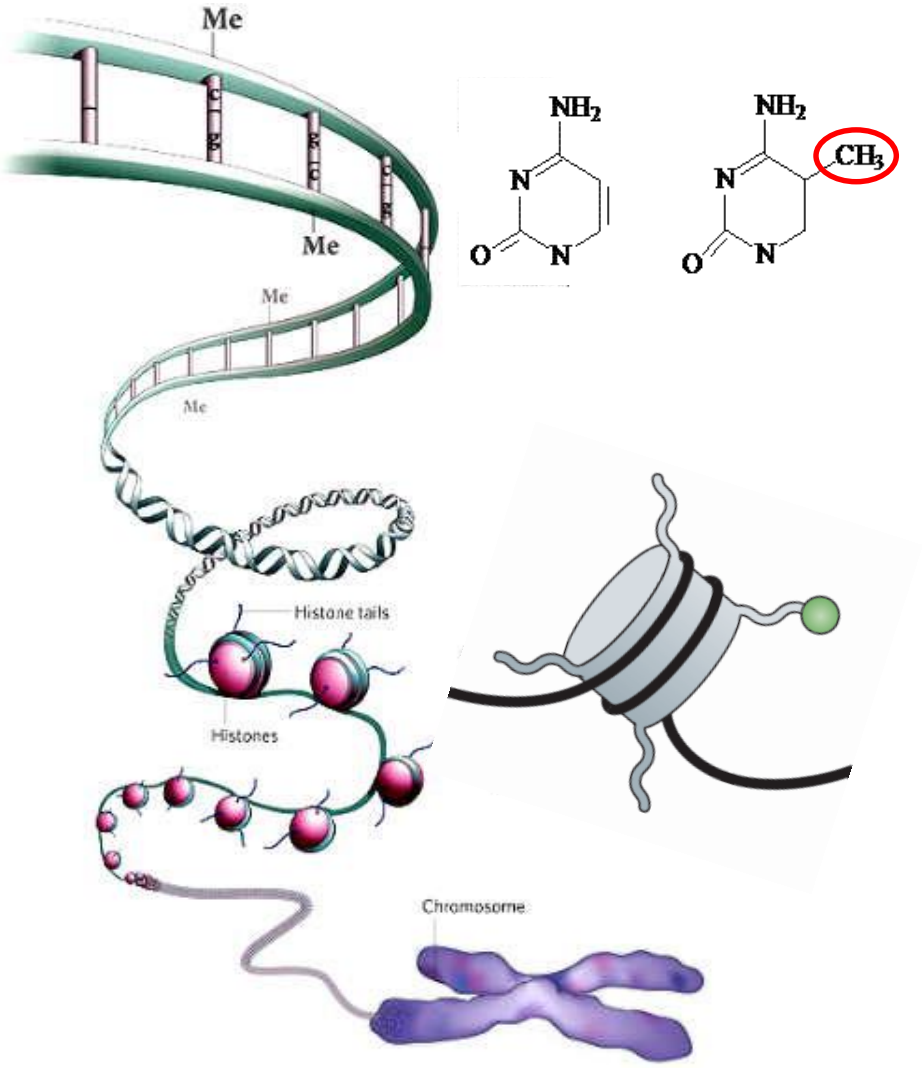
Outline

- **Epigenetics: DNA Methylation and Histone Modification**
- Parent-of-origin Effects
- Mitochondrial DNA
- *De Novo* Variation

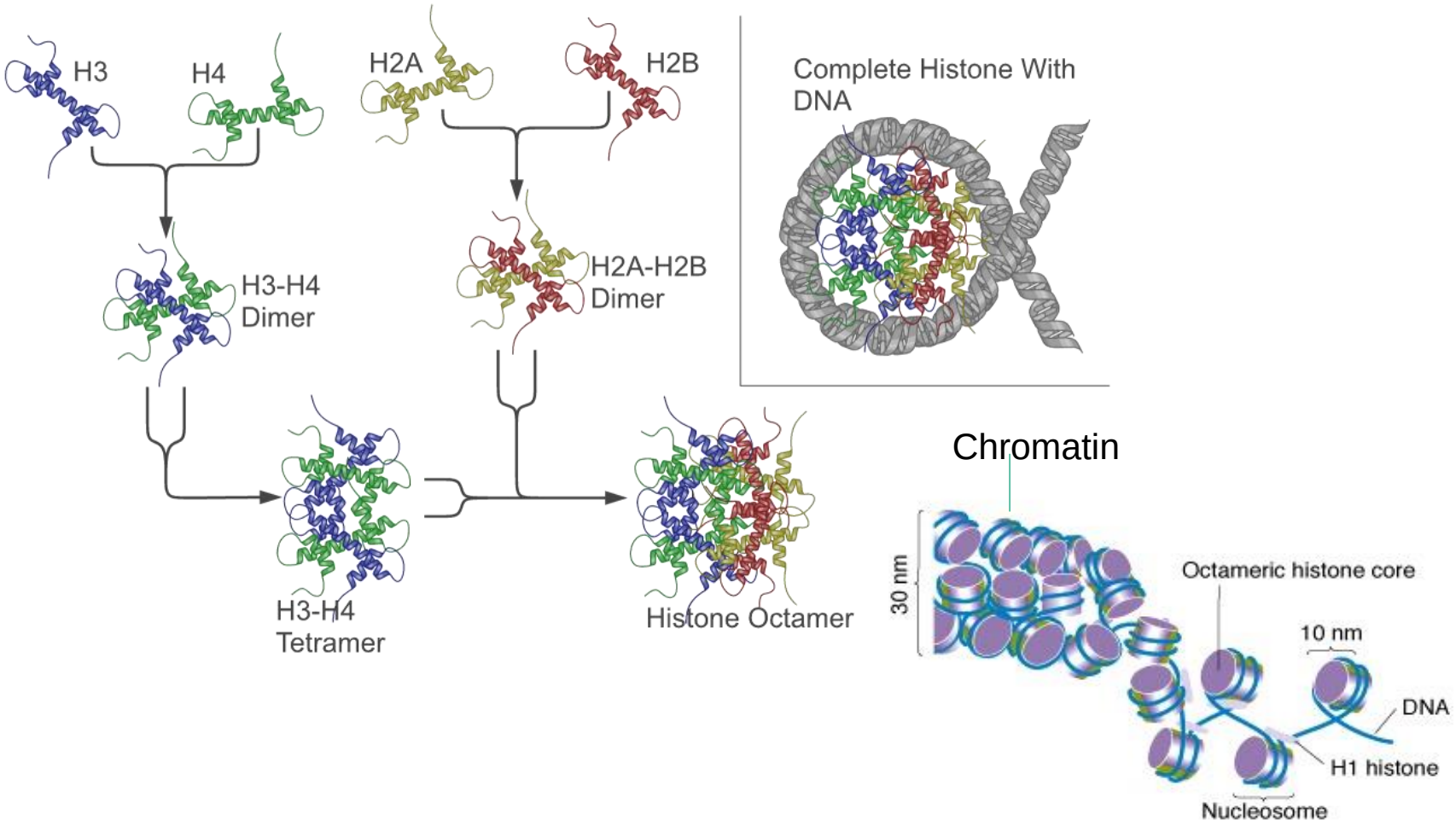
The Two Main Components of the Epigenetic Code



DNA methylation

Histone modification

Chromatin: Histones Provide Structure to DNA



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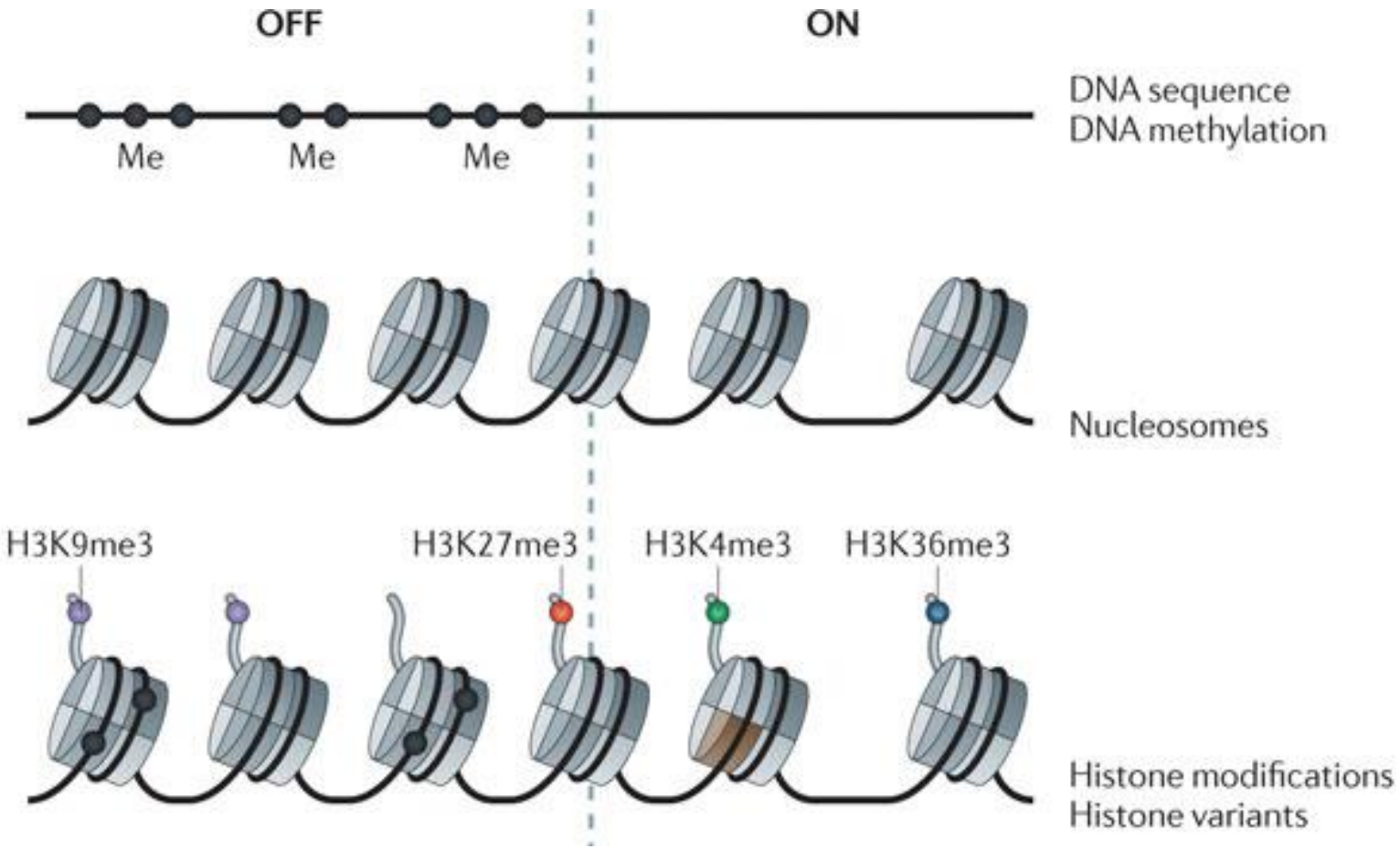
Chromatin Structure is Determined by DNA Methylation and Histone Modifications



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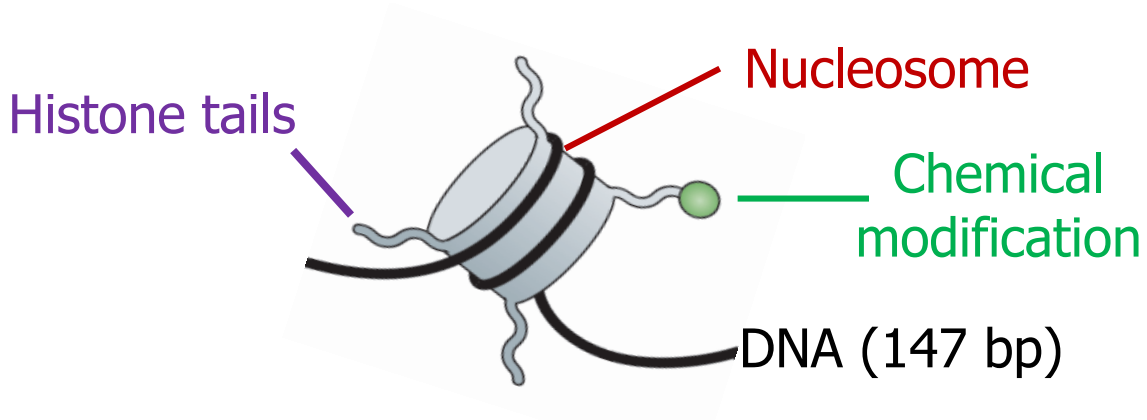
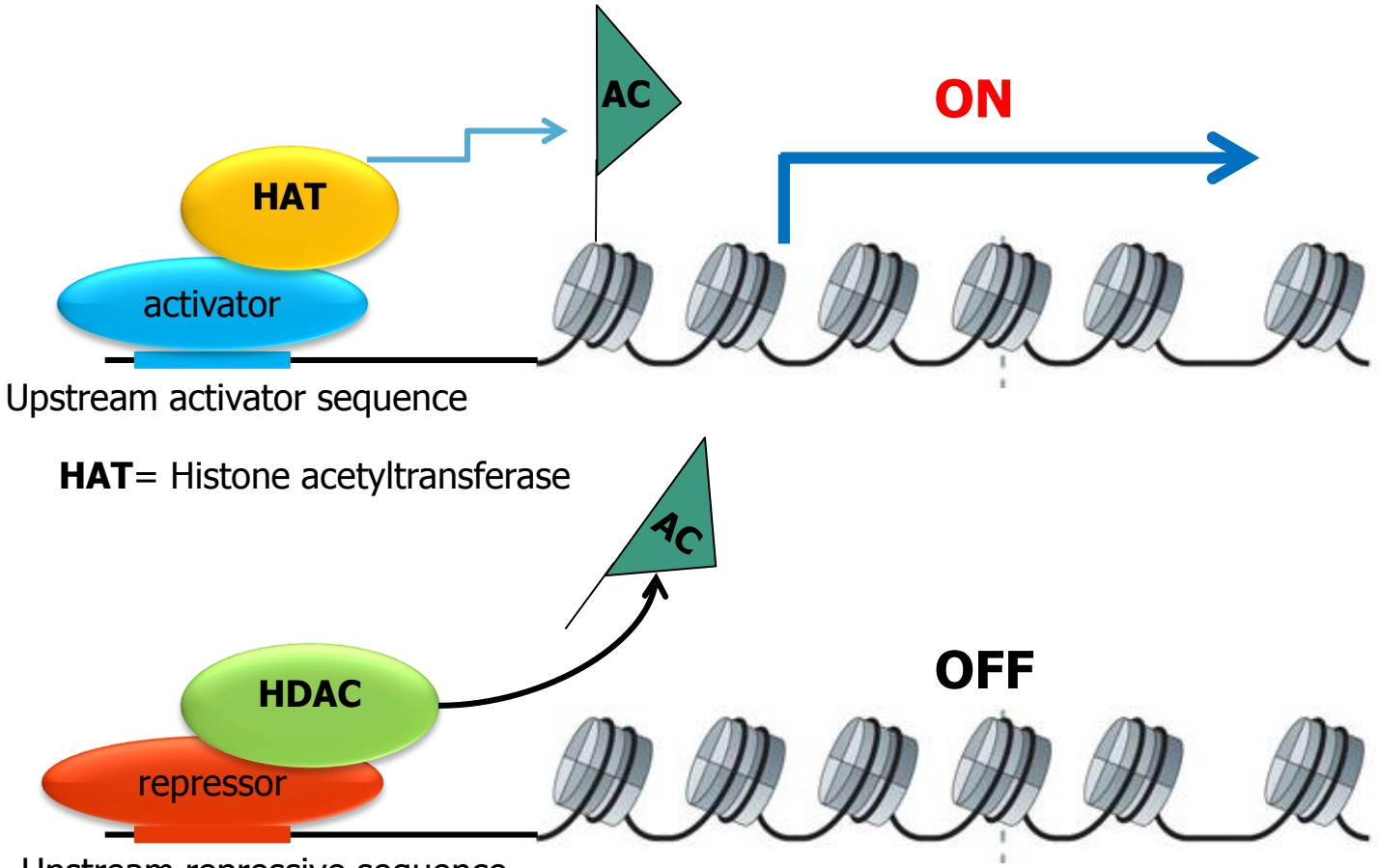
Histone Modifications

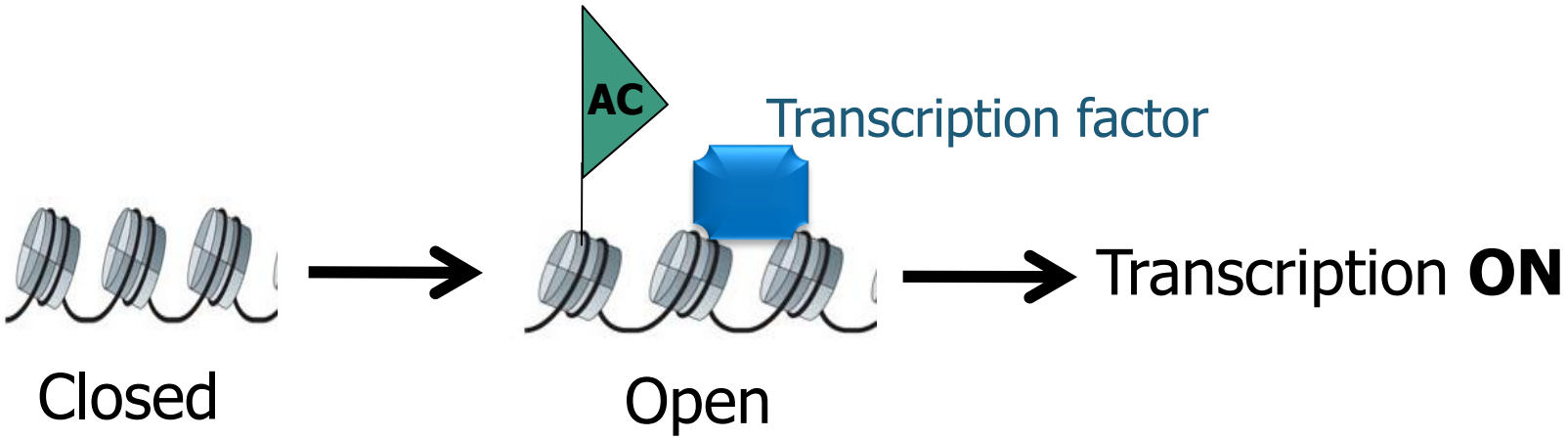


Modification	Role in transcription	Histone-modified sites
Acetylation	**Activation**	H3 (K9,K14,K18,K56)
		H4 (K5,K8,K12,K16)
		H2B (K6,K7,K16,K17)
Phosphorylation	**Activation**	H3 (S10)
Methylation	**Activation**	H3 (K4,K36,K79)
	Repression	H3 (K9,K27)
		H4 (K20)

Histone Acetylation and Deacetylation



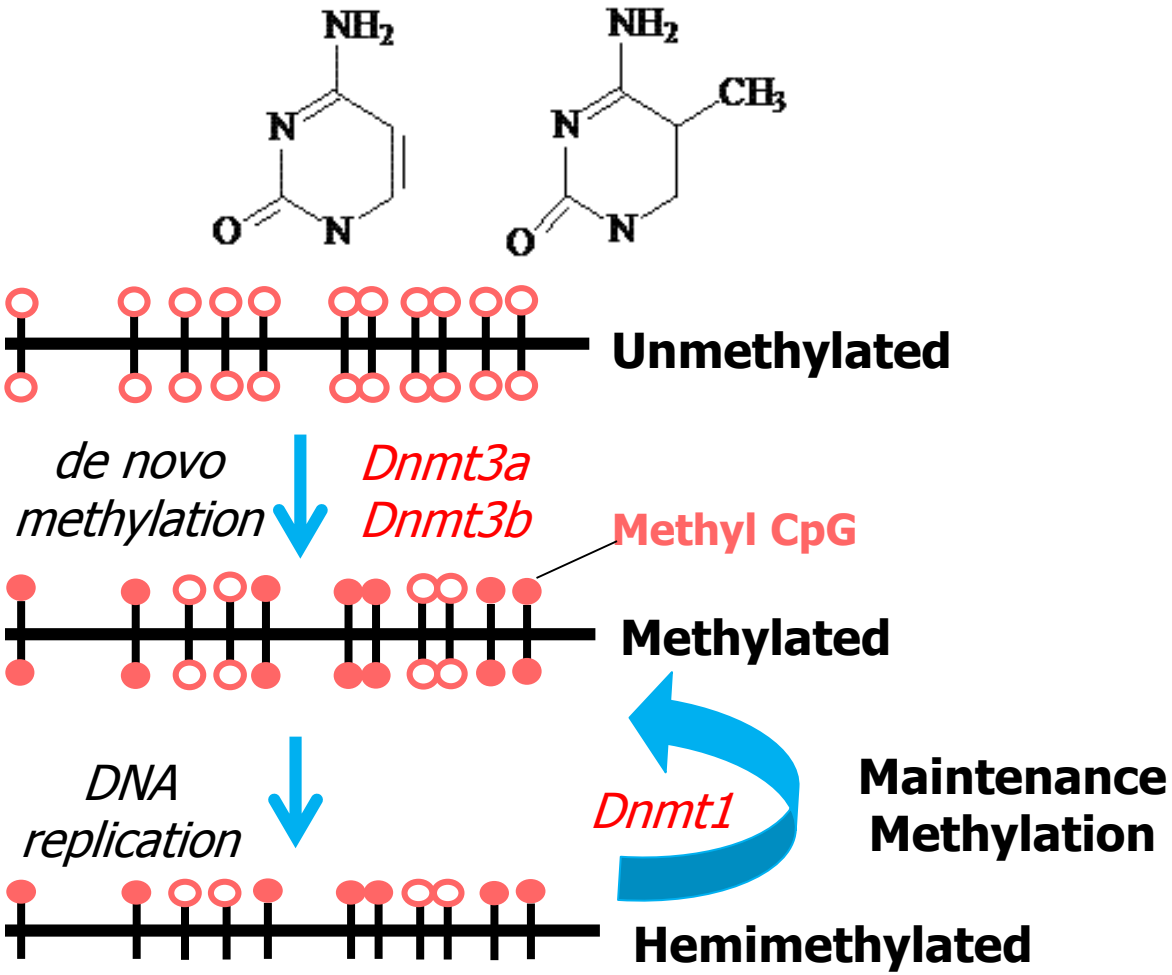
Acetylation Reduces Histone Binding to DNA



Histone Methylation

- Occurs at either lysines or arginines
- Activates or represses gene transcription depending on the position within the histone tail
- Multiple possible methylation states on each residue (-mono, -di or –tri methylated).

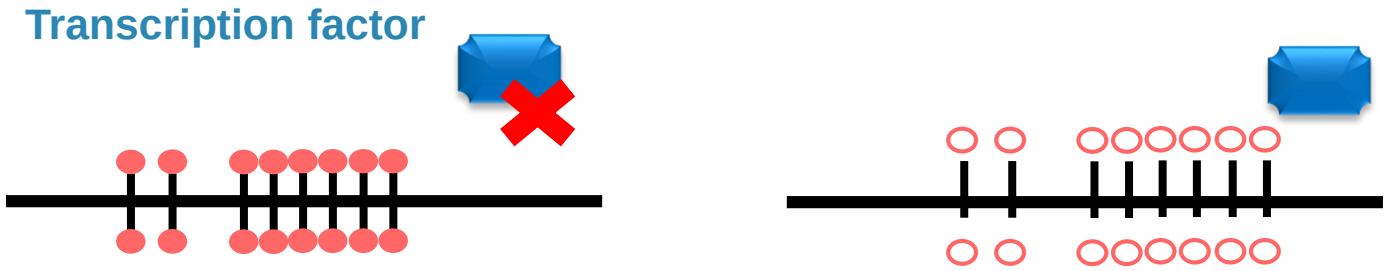
DNA Methylation



DNA Methylation Affects Gene Expression in Two Ways

Model 1

The presence of the methyl groups interferes with the binding of transcription factors that activate transcription from a specific gene.

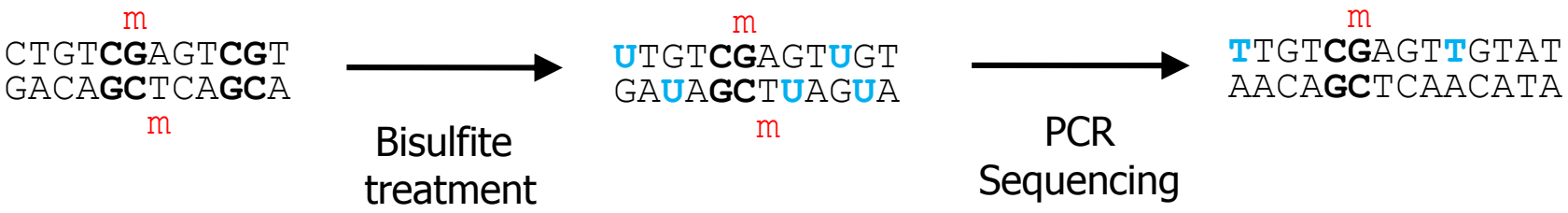


Model 2

Repressing proteins are attracted by methylated DNA.

Using Bisulfite Sequencing to Study DNA Methylation

Treatment with sodium bisufite converts all cytosines (C) in uracils (U) unless they are methylated (C^m).



DNA Methylation and Heart Disease: Incidence

	Ischemic Heart Disease			
	crude		multivariable[a]	
HR	(95%CI)	HR	(95%CI)	HR
LINE-1 methylation				
86.3-77.5 (%5mC)[b]	1.0		1.0	
77.4-68.1 (%5mC)	2.7	(1.3 to 5.6)	4.0	(1.8 to 8.9)
Age (continuous, 10 yrs)			1.3	(0.6 to 2.7)
BMI (continuous, 5 kg/ m^2)			1.0	(0.6 to 1.7)
Smoking (ever)			1.4	(0.5 to 4.1)
Pack-years (continuous, 35 pack-yrs)			1.3	(0.6 to 2.6)
Alcohol drinking (two drinks or more/day)			0.4	(0.1 to 1.4)

Baccarelli *et al*. Epidemiology 2010

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DNA Methylation and Heart Disease: Mortality

	Ischemic Heart Disease			
	crude		multivariable[a]	
	HR	(95%CI)	HR	(95%CI)
LINE-1 methylation				
86.3-77.5 (%5mC)[b]	1.0		1.0	
77.4-68.1 (%5mC)	3.4	(1.5 to 7.9)	3.3	(1.3 to 8.4)
Age (continuous, 10 yrs)			2.6	(1.3 to 5.2)
BMI (continuous, 5 kg/m^2)			1.2	(0.7 to 1.9)
Smoking (ever)			1.7	(0.6 to 4.5)
Pack-years (continuous, 35 pack-yrs)			1.1	(0.8 to 1.7)
Alcohol drinking (two drinks or more/day)			0.6	(0.2 to 2.3)

Baccarelli *et al*. Epidemiology 2010

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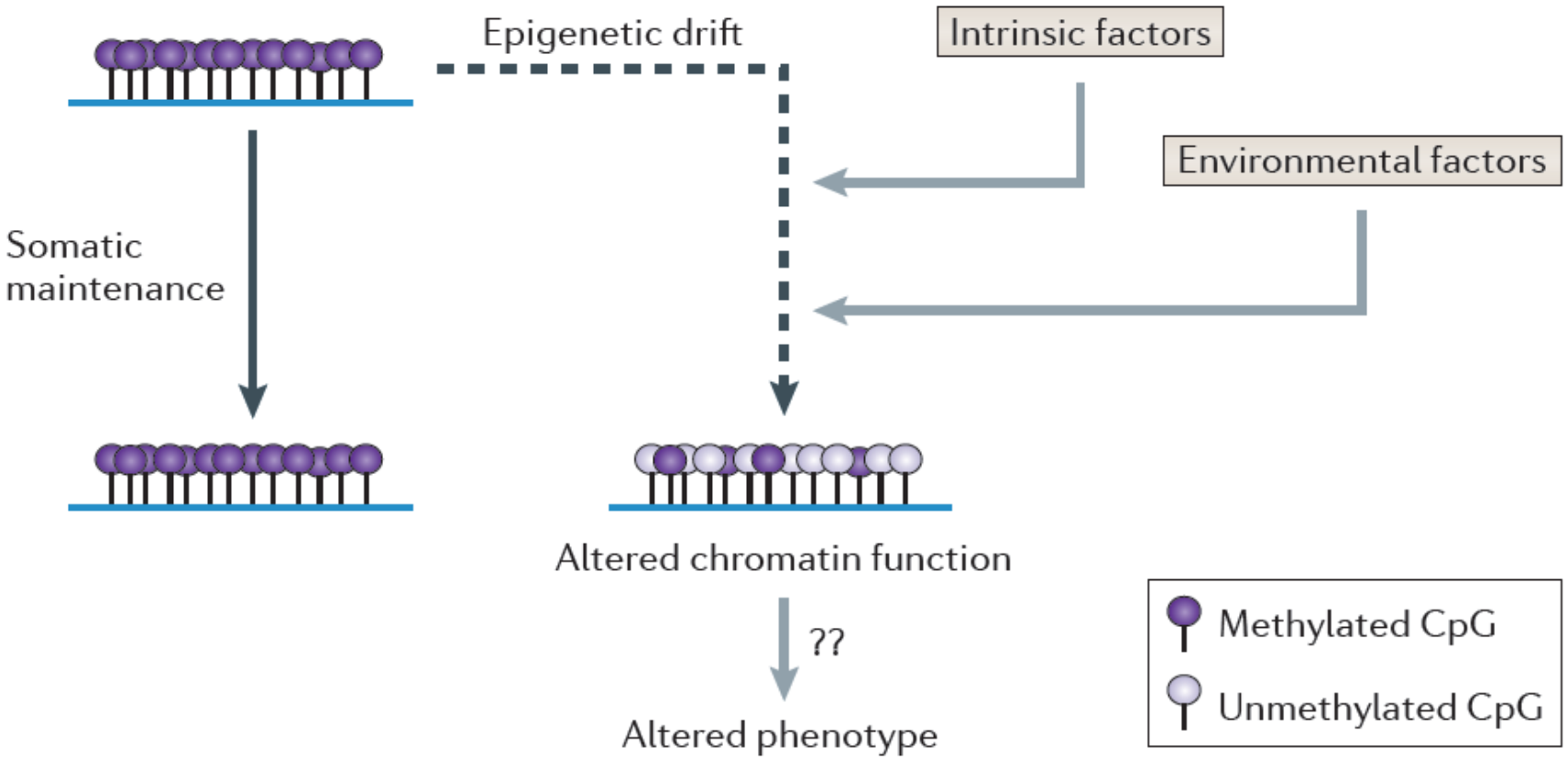
The Environment Can Affect Methylation



Feil and Fraga, 2012 Nature Reviews Genetics

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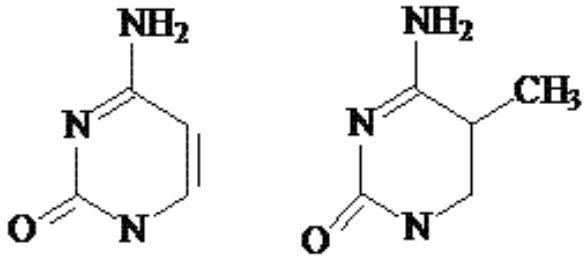
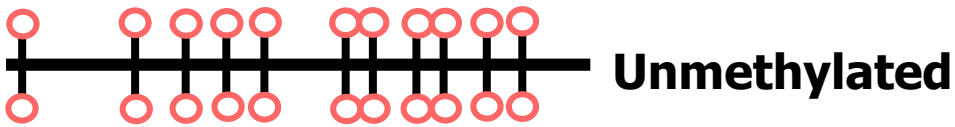
Environment and Epigenetics

Royal Jelly

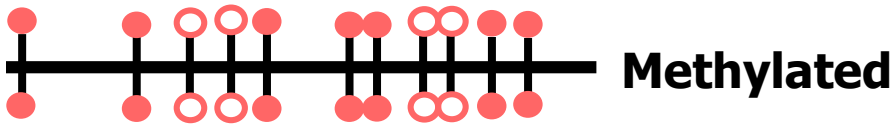
Drone Queen Worker





Unmethylated

DNMT3 silencing



Methylated

- Kucharski, R., Maleszka, J., Foret, S. & Maleszka, R.. Science 2008
- Lyko, F. *et al*. PLoS Biology 2010

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Development and Epigenetics

The Dutch famine of 1944 took place in the German-occupied part of the Netherlands near the end of World War II. About 4.5 million were affected.

Famine exposure in the periconceptional period led to an increased risk of metabolic and psychiatric disorders.

Development and Epigenetics

Table 3. Timing of famine exposure during gestation, *IGF2* DMR methylation, and birth weight

	Periconceptional exposure	Late gestational exposure	All controls
n	60	62	122
Males, %	46.7	45.2	45.9
Mean age, years	58.1 (SD, 0.35)	58.8 (SD, 0.4)	57.1 (SD, 5.5)
Birth weight, g	3612 (SD, 648)	3126 (SD, 408)	—
IGF2 DMR methylation			
Average	0.488 (SD, 0.047)	0.514 (SD, 0.045)	0.517 (SD, 0.047)
$P_{\text{vs all controls}}$	1.5×10^{-5}	.69	
$P_{\text{interaction}}$			4.7×10^{-3}

P values were obtained using a linear mixed model and adjusted for age.

Heijmans *et al*., PNAS 2008

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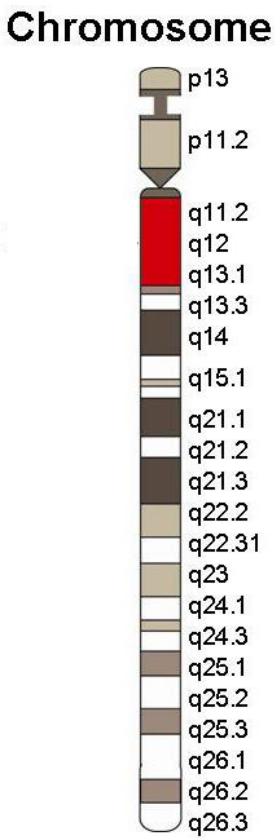
Outline

- Epigenetics: DNA Methylation and Histone Modification
- **Parent-of-origin Effects**
- Mitochondrial DNA
- *De Novo* Variation

Parent-of-Origin Effects

- Imprinting
  - Genes expressed only from one parent
  - DNA Methylation
  - <1% of genes
- In utero effects
  - Mother's genotype can affect environment

Diseases of Imprinting: Prader-Willi and Angelman



PWS/AS region

Paternal loss = **Prader-Willi** (gene *SNRPN*, Necdin gene, clusters of snoRNAs)

Maternal loss = **Angelman** (gene *UBE3A*)

Paternal allele silenced in hippocampus and cerebellum

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Parent-of-Origin Effects in Type 2 Diabetes

Disease, SNP[Alleles][a]		Standard case-control test		Tests of association with parental origins						
Position B36	(M)[b]			Paternal Allele[d]		Maternal Allele[d]		2-df test[e]	P. vs M. (case only)	
N (Case Sample Size)	Con. Frq.	OR	P †	OR	P †	OR	P †	P †	n12 : n21[f]	P †
C11 1,653,425	(34706)									
Discovery (1468)	0.412	1.11	0.017	1.41	4.3×10^{-9}	0.87	0.020	3.5×10^{-9}	437 :276	7.0×10^{-9}
Replication (783)		1.02	0.71	1.23	0.0055	0.84	0.023	0.0018	222 : 157	8.0×10^{-4}
Combined (2251)		1.08	0.034	1.35	4.7×10^{-10}	0.86	0.0020	5.7×10^{-11}	659 : 433	4.1×10^{-11}

Kong *et al*. Nature 2009

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Testicular Germ Cell Tumors and Maternal Genotype

	Gene and polymorphism					
	CYP1A2 −163C>A	*CYP1B1* Arg^{48}Gly	*CYP1B1* Leu^{432}Val	*CYP1B1* Asn^{453}Ser	*CYP3A4* −392A>G	*CYP3A5* 6986A>G
Maternal (*n*)	153	150	153	153	158	151
Reference	1.0	1.0	1.0	1.0	1.0	1.0
Heterozygote RR	0.4 (0.2-1.0)	1.4 (0.8-2.3)	0.6 (0.3-1.1)	0.9 (0.5-1.7)	0.9 (0.4-2.1)	0.8 (0.4-1.5)
Homozygote RR	0.6 (0.3-1.5)	2.6 (1.1-6.3)	0.8 (0.4-1.5)	0.4 (0.1-2.1)	0.9 (0.1-14)	0.4 (0.0-4.2)
*P**	0.010	0.005	0.156	0.897	0.962	1.000
Offspring						
Reference	1.0	1.0	1.0	1.0	1.0	1.0
Heterozygote RR	0.7 (0.3-1.4)	0.9 (0.5-1.4)	1.2 (0.7-2.0)	0.8 (0.5-1.3)	5.2† (1.5-18)	1.8† (0.9-3.6)
Homozygote RR	0.5 (0.2-1.2)	1.0 (0.4-2.4)	1.3 (0.6-2.6)	0.2 (0.0-1.4)	Undefined†	Undefined†
*P**	0.079	0.677	1.000	0.273	0.013	0.256

Starr *et al*. CEBP 2005

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Outline

- Epigenetics: DNA Methylation and Histone Modification
- Parent-of-origin Effects
- **Mitochondrial DNA**
- *De Novo* Variation

Joe's Class Demo

				taste receptors		taste phenotype	ear wax	earwax phenotype
ID #	[Pico]	[Nano]	260/280	rs10246939	rs1726866		rs17822931	
11	5.198	14.82	2.5	CC	GG	N	CC	wet
16	8.368	21.78	2.01	CT	AG	mild	CC	dry
19	7.809	22.2	2.06	CC	GG	Y	CC	dry
21	6.496	18	2.25	CT	AG	Y	TT	dry
24	13.29	29.75	2.04	CT	AG	Y	CC	dry
25	7.969	20.43	2.07	CT	AG	medium	CC	wet
26_#1	2.808	10.69	2.83	CT	AG	Y	CC	wet
26_#2	14.49	33.37	1.9	CC	GG	Y	TT	wet
27	90.22	125.8	1.89	CC	GG	Y	TT	dry
40	3.483	11	2.17	CC	GG	Y	CT	wet
42	5.533	16.43	2.15	CC	GG	Y	CT	dry
43	4.607	13.37	1.86	CT	AG	Y	CT	dry
44	11.61	24.5	2.07	TT	AA	N	TT	dry
45	4.919	14.46	2.08	TT	AA	N	CT	dry

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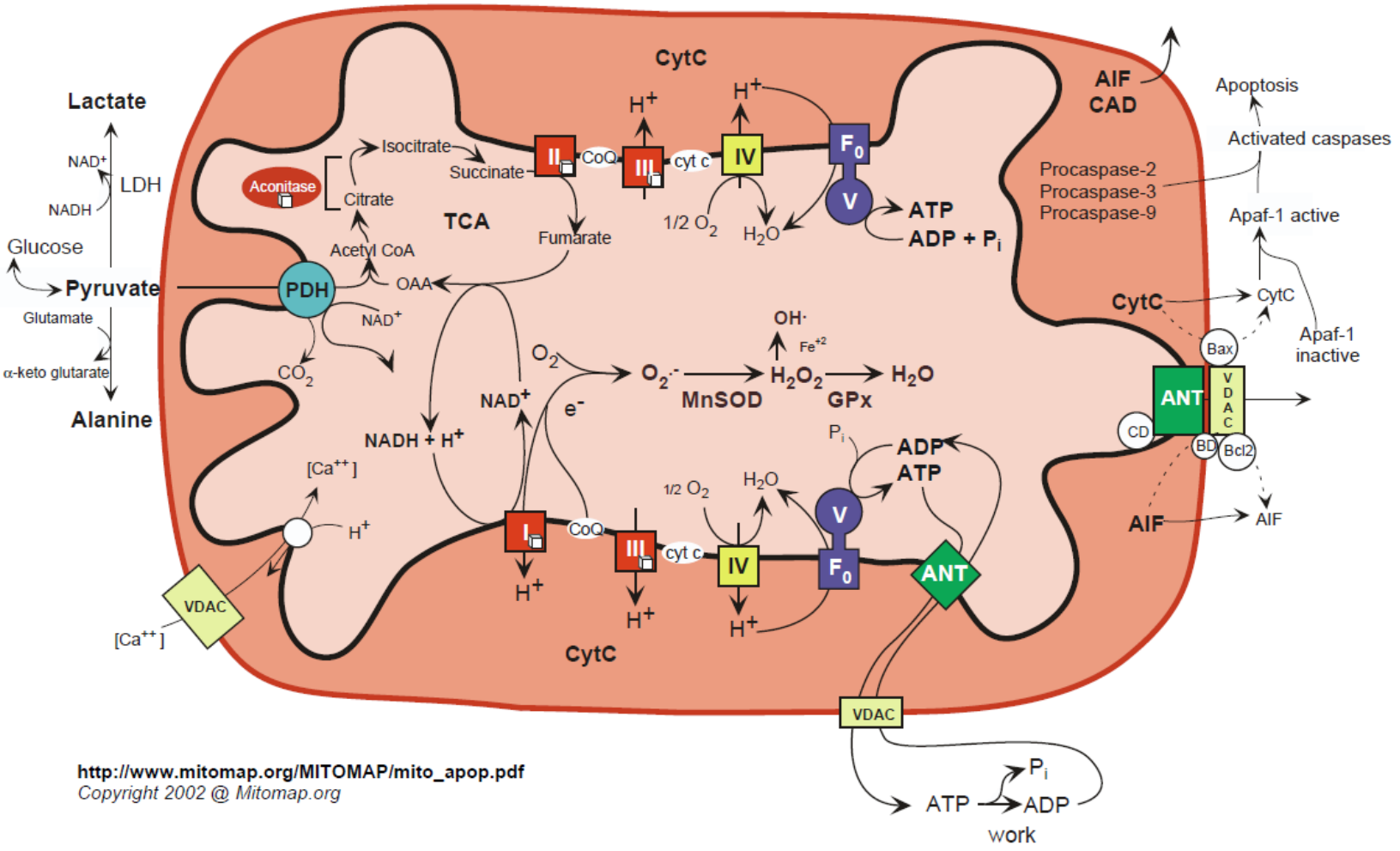
PTC Taste Perception

ID #	rs10246939	rs1726866	Taster Status
19	CC	GG	Y
26_#2	CC	GG	Y
27	CC	GG	Y
40	CC	GG	Y
42	CC	GG	Y
11	CC	GG	N
21	CT	AG	Y
24	CT	AG	Y
26_#1	CT	AG	Y
43	CT	AG	Y
25	CT	AG	medium
16	CT	AG	mild
44	TT	AA	N
45	TT	AA	N

Earwax Type

ID #	rs17822931	Earwax Type
11	CC	wet
25	CC	wet
26_#1	CC	wet
16	CC	dry
19	CC	dry
24	CC	dry
40	CT	wet
42	CT	dry
43	CT	dry
45	CT	dry
26_#2	TT	wet
21	TT	dry
27	TT	dry
44	TT	dry

Mitochondrial Energetics



http://www.mitomap.org/MITOMAP/mito_apop.pdf

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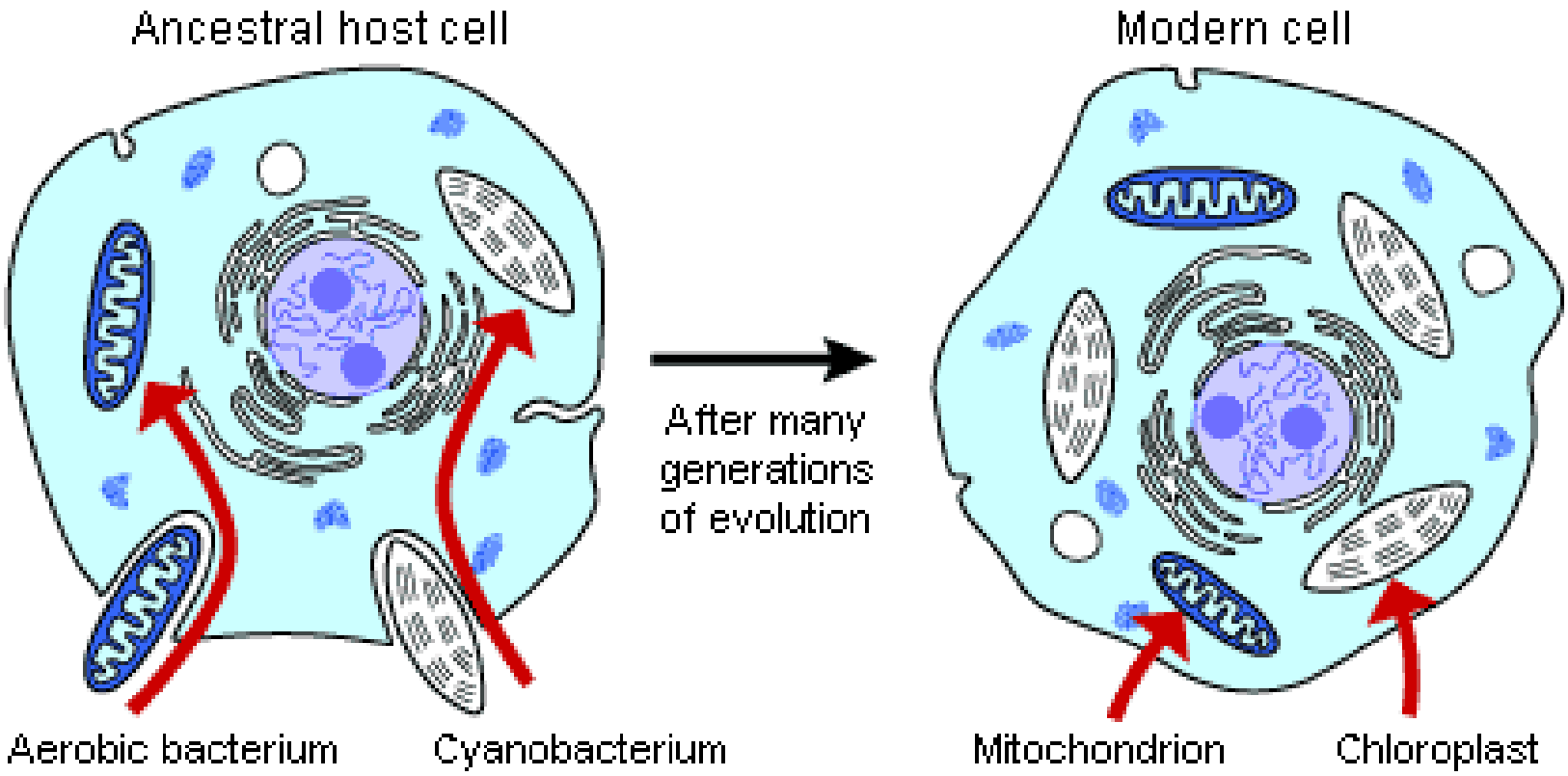
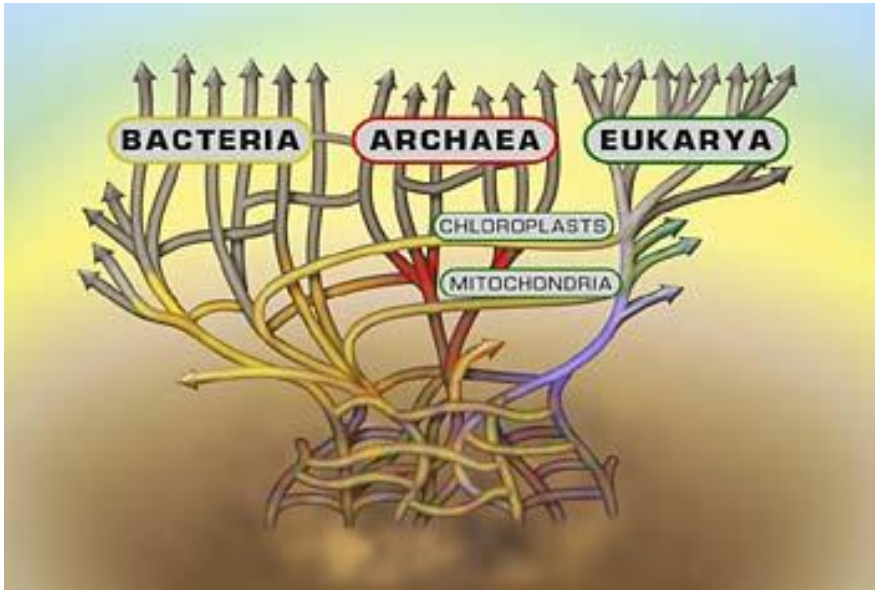
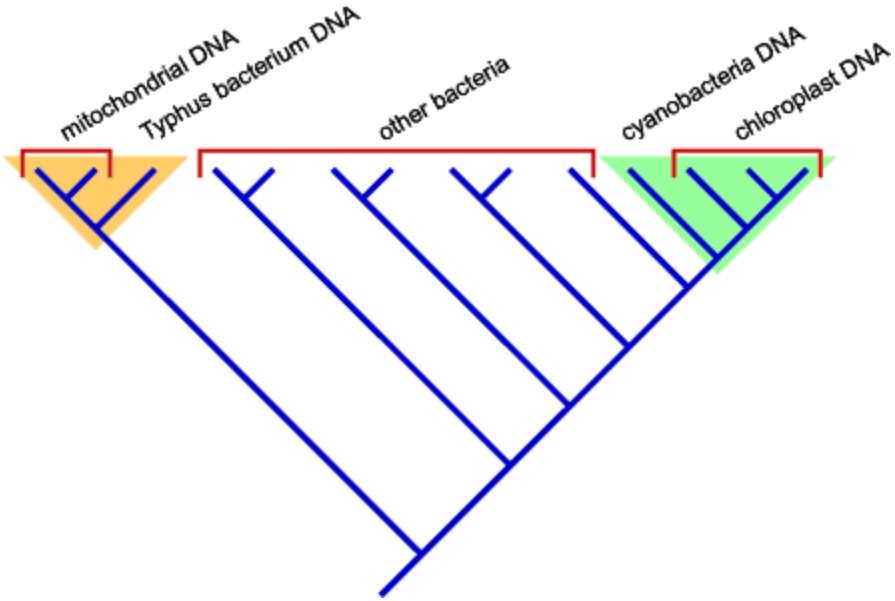
Origin of Mitochondria

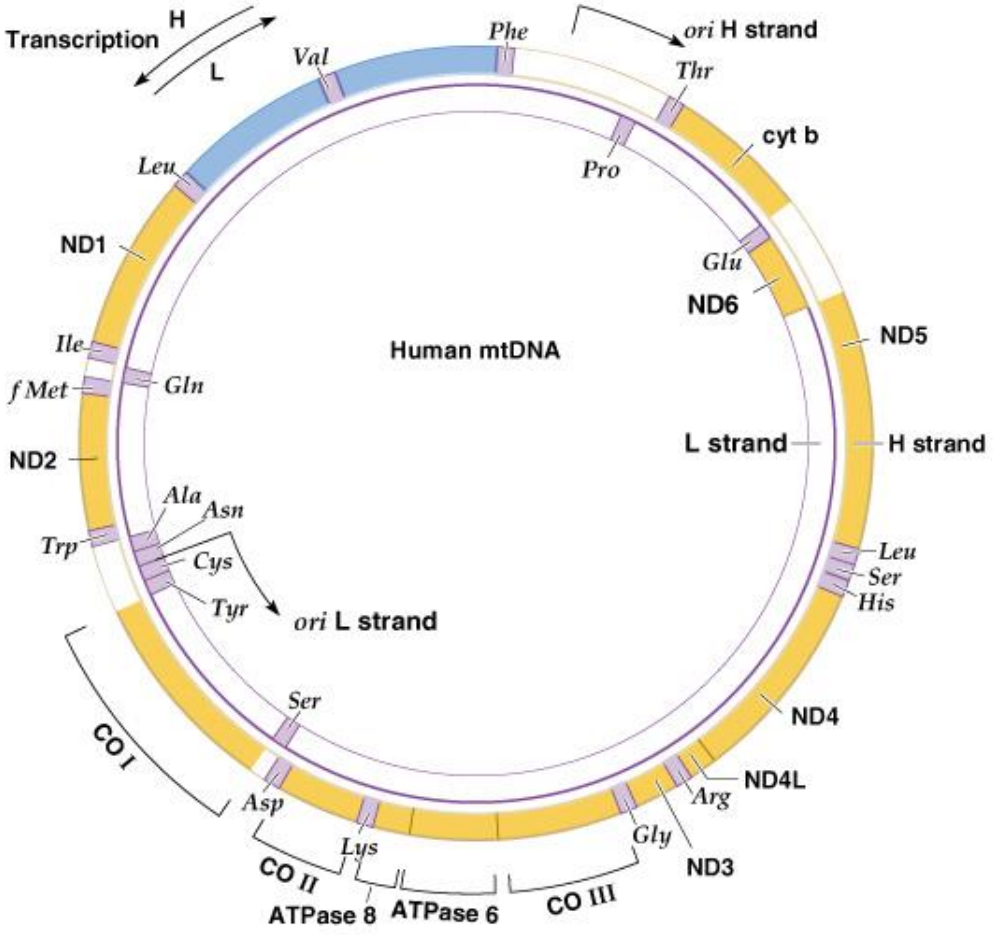


Origin of Mitochondria





Human mtDNA (16,569 bp)



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Mitochondrial Genes

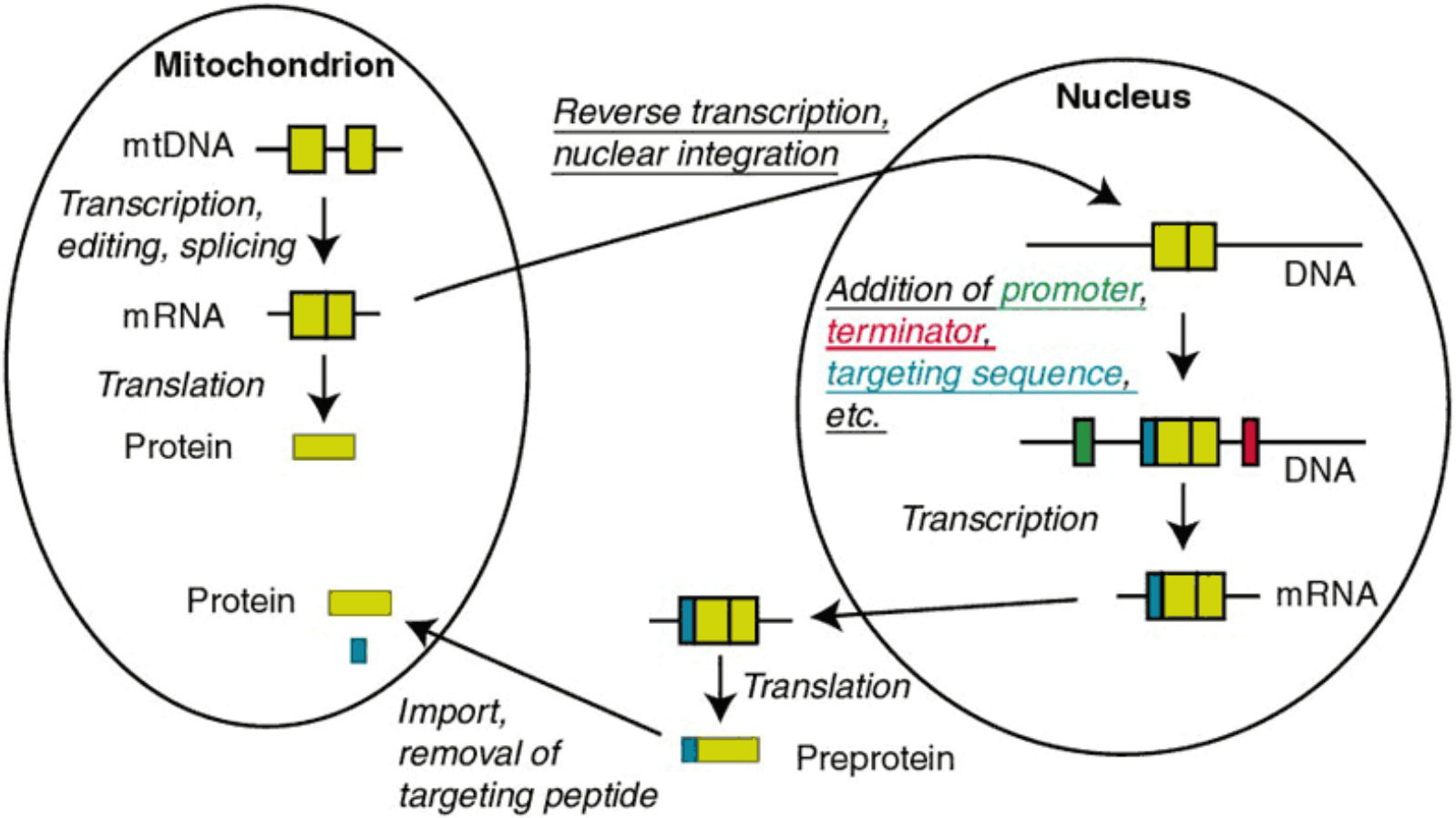
tRNAs

rRNAs

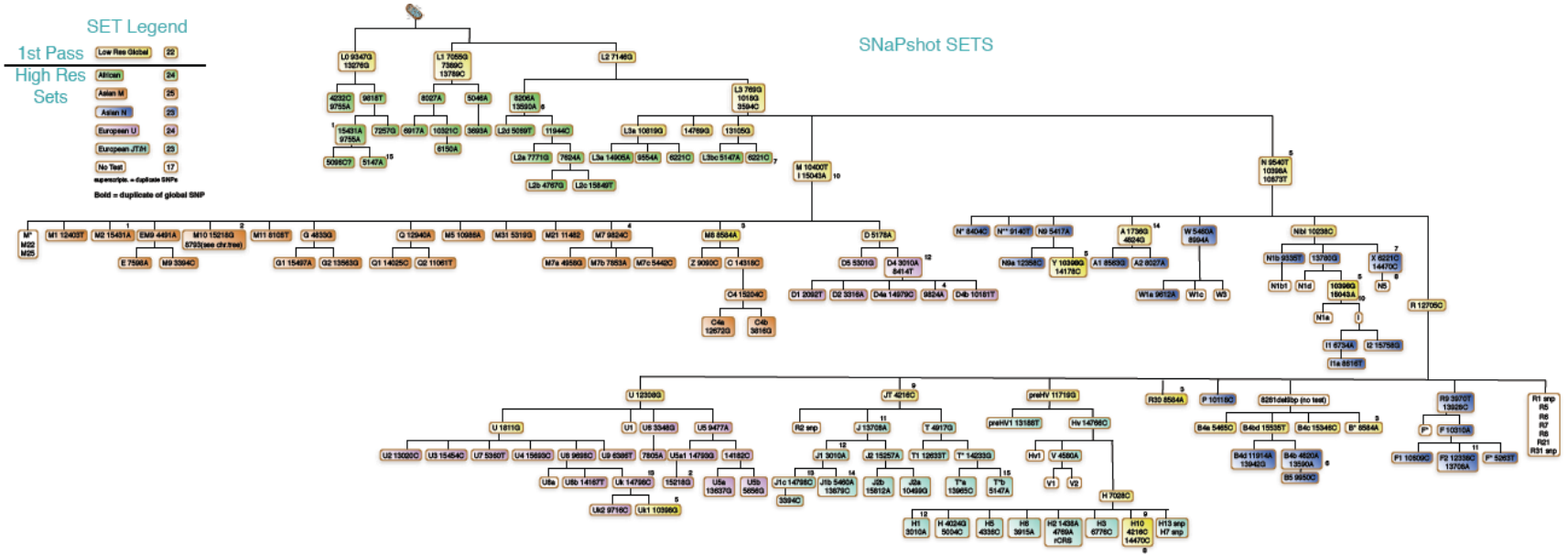
Protein subunits:

- cytochrome oxidase
- NADH dehydrogenase
- ATPase

mtDNA Products Act Inside and Outside the Mitochondrion



Human mtDNA Lineages



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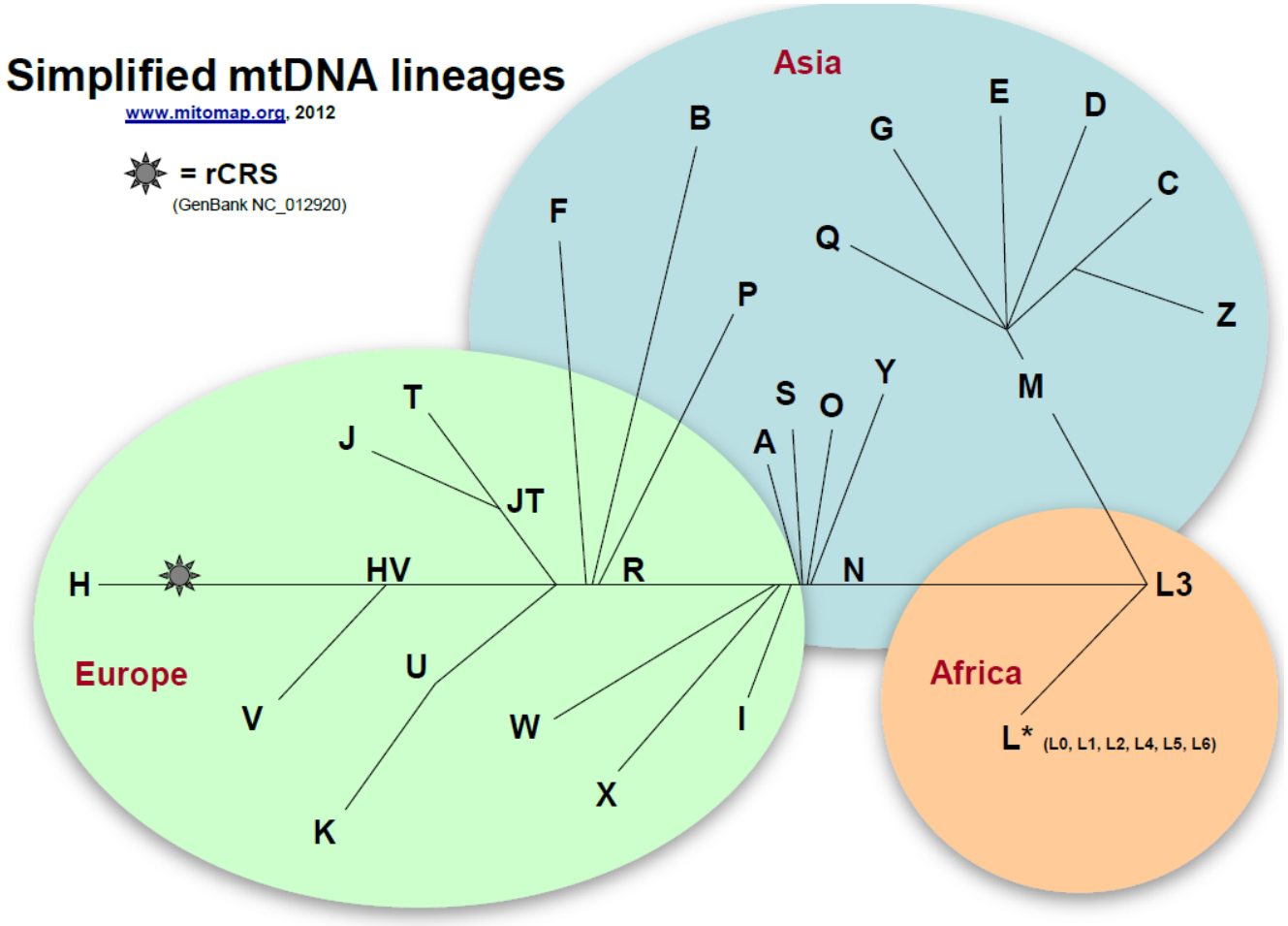
mtDNA Lineages (Simplified)



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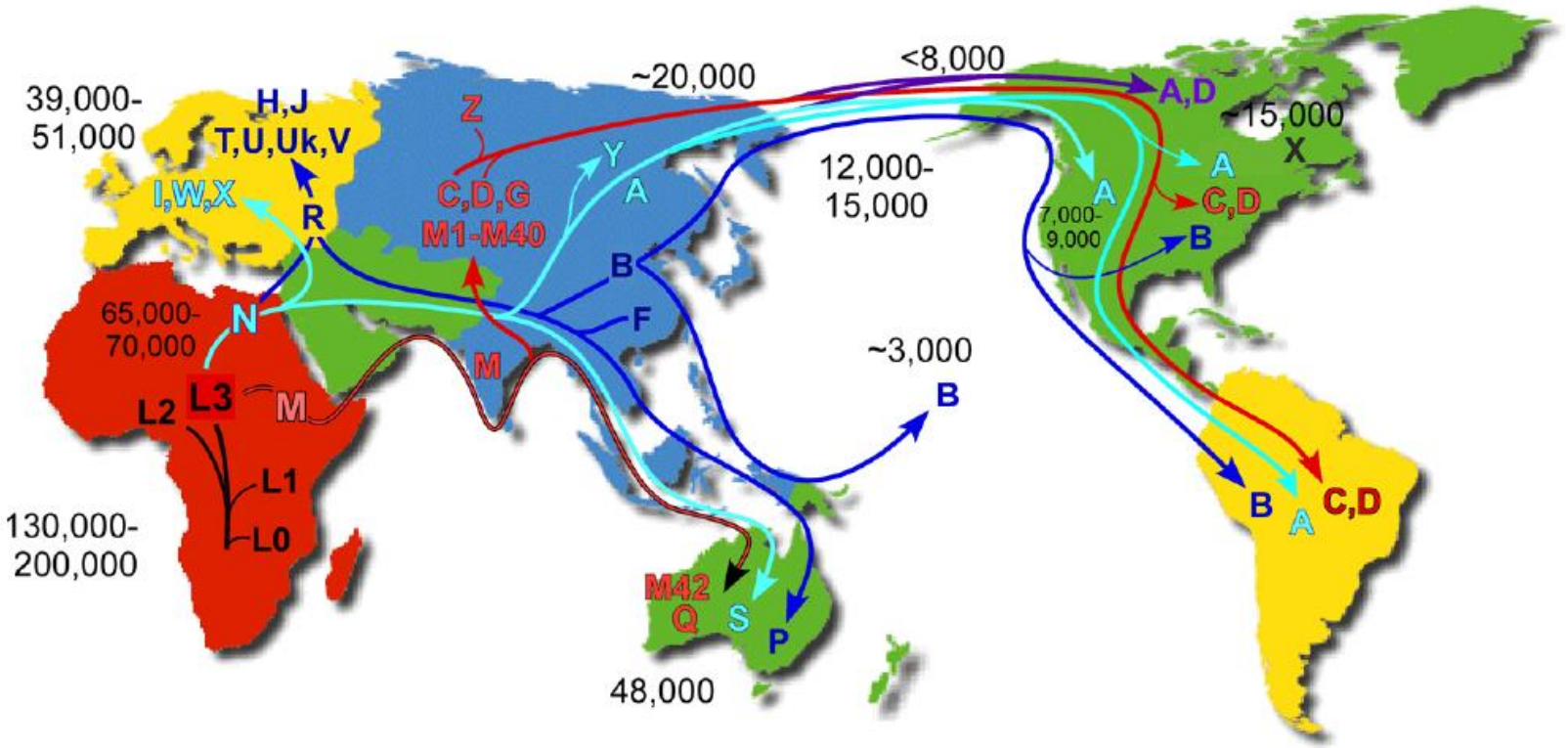
Human mtDNA Migrations



Mutation rate = 2.2 – 2.9% / MYR
Time estimates are YBP

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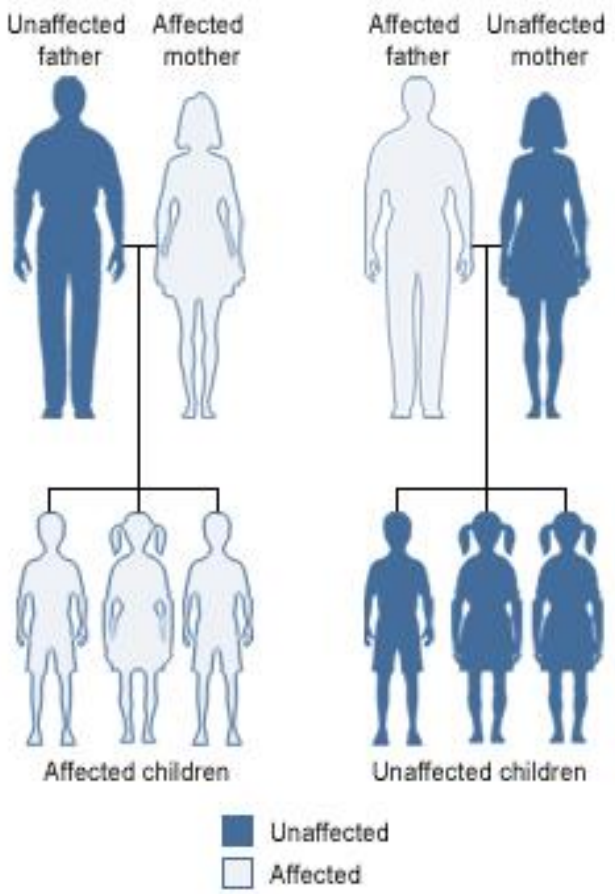
Mitochondrial Inheritance



U.S. National Library of Medicine

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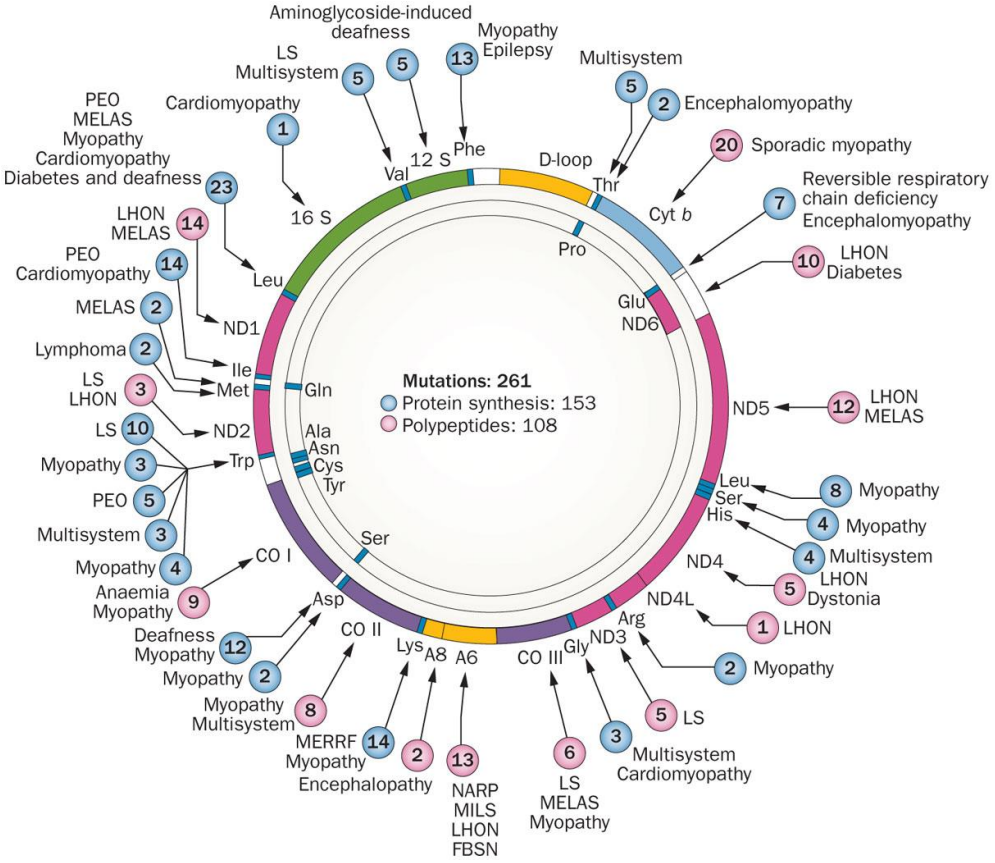
Morbidity Map of mtDNA



DiMauro, S. *et al.* Nat. Rev. Neurol. 2013

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Normal Vision Compared to Vision with Glaucoma

Normal Vision

Glaucoma

 |

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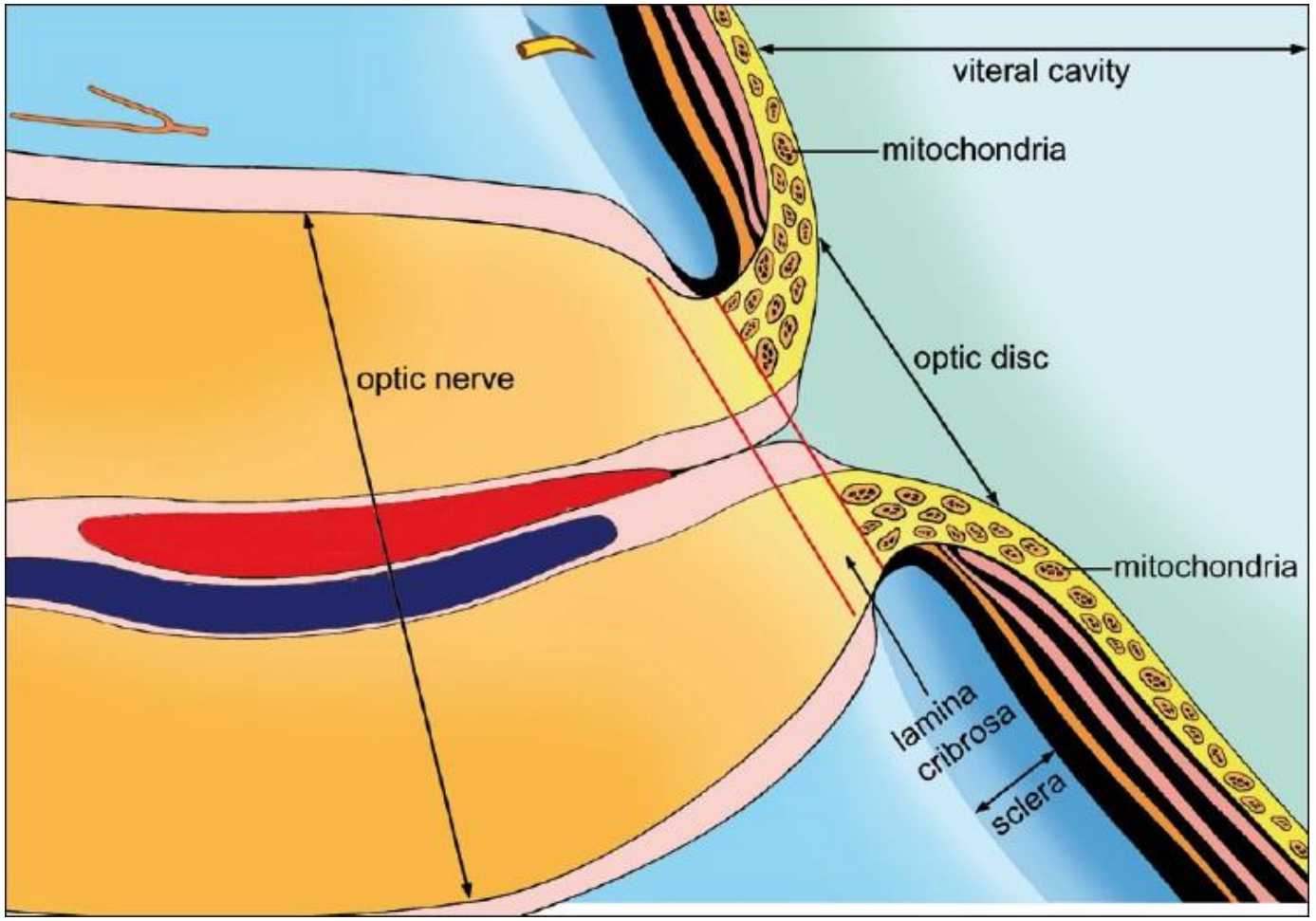
High Concentration of Mitochondria at Optic Disc



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Primary Open Angle Glaucoma Case and Control Definitions

- **Cases**
  - 35 or older
  - KPNC members from 2008-2014
  - One or more diagnoses of primary open angle glaucoma (POAG)
    - ICD-9 Codes: 365.01, 365.05, 365.1, 365.10, 365.11, and 365.15
  - No diagnosis of any other type of glaucoma
- **Controls**
  - 35 or older
  - KPNC members from 2008-2014
  - No glaucoma diagnosis of any kind
  - No documented IOP measures above 22 mmHg in either eye
  - No documented Cup-to-Disc ratio difference of more than 0.2 between eyes

Primary Open Angle Glaucoma in GERA

	Cases		Controls	
	n=4,580		n=75,766	
Age (years)	n	%	n	%
35-44	30	0.66	6,283	8.29
45-54	164	3.58	13,288	17.54
55-64	743	16.22	23,075	30.46
65-74	1,450	31.66	20,022	26.23
75-84	1,676	36.59	10,837	14.30
85+	517	11.29	2,261	2.98
Mean (SD)	72.89	(9.94)	62.45	(12.13)
Sex				
Women	2,324	50.74	43,537	57.48
Men	2,256	49.26	32,219	42.52
Race				
Non-Hispanic white	3,607	78.76	62,393	82.35
Latino	371	8.10	6,154	8.12
East Asian	313	6.83	5,167	6.82
African American	289	6.31	2,052	2.71

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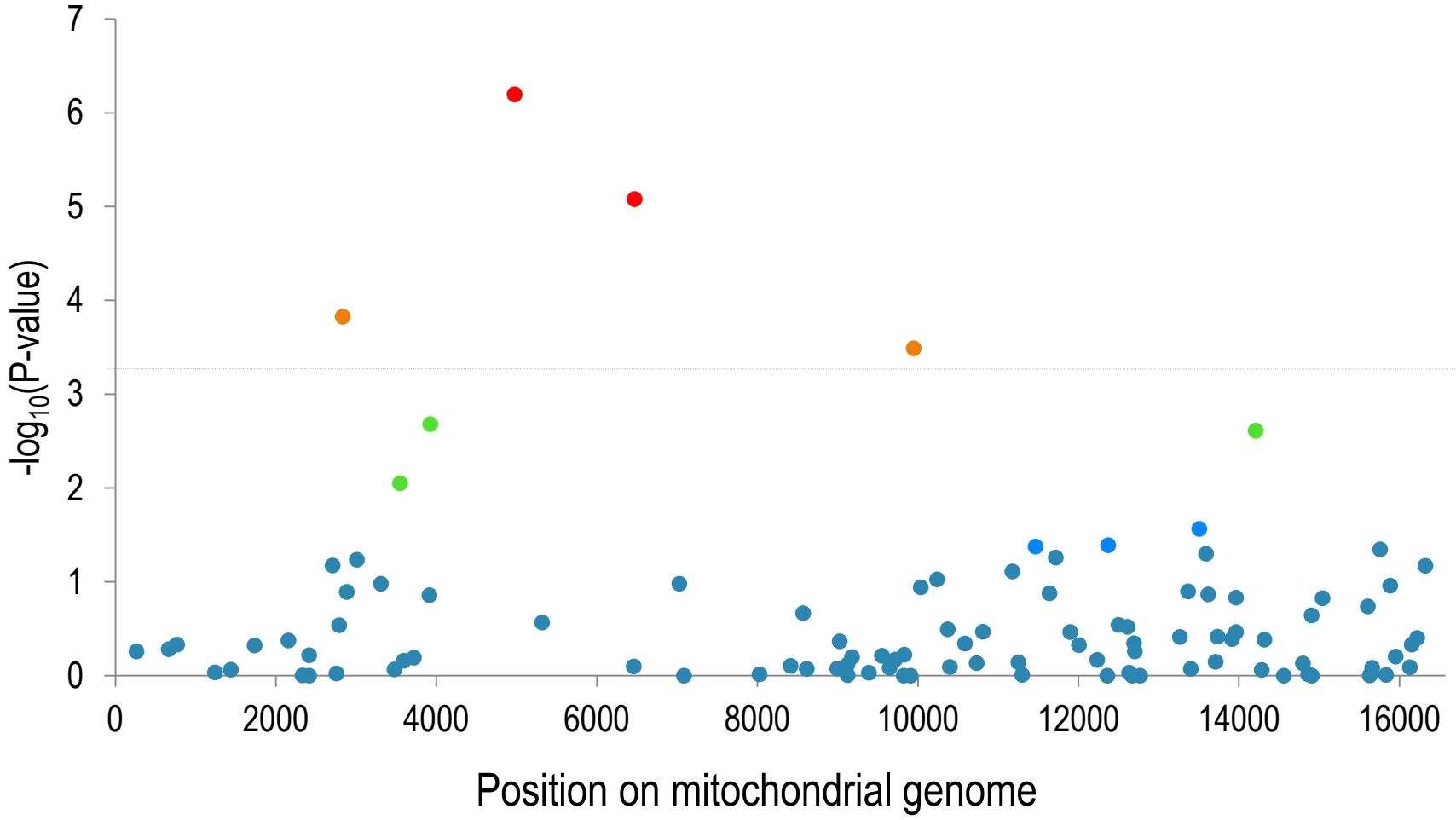
Association Results in GERA Non-Hispanic Whites



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mtSNPs Associated with POAG in Non-Hispanics Whites

SNP	Position in mtDNA (bp)	Minor Allele Frequency		OR	P-value	*Haplogroups*
		Control	Case			
rs28357981	T4977C	0.0014	0.0046	4.04	$6.39*10^{-7}$	**B2**, L4, N10
rs1029294	C6473T	0.0020	0.0055	3.05	$8.32*10^{-6}$	**B2**, M1
rs3928312	A2833G	0.0001	0.0012	12.36	$1.50*10^{-4}$	R5, M38, M67
rs3134801	T9950C	0.0026	0.0055	2.45	$3.26*10^{-4}$	**B2**, B5, HV4, L0, L3, M11, W1

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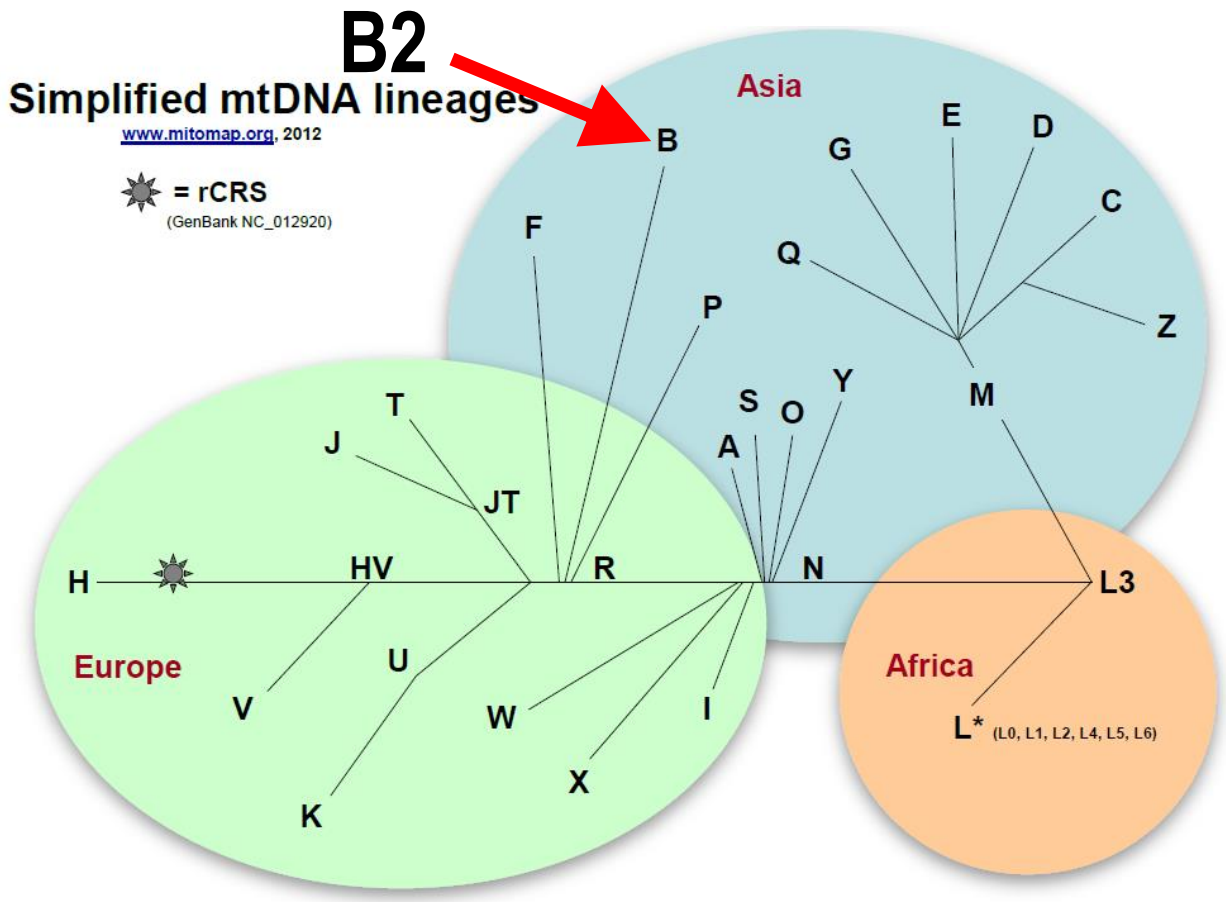
Mitochondrial Lineages Cluster by Continent



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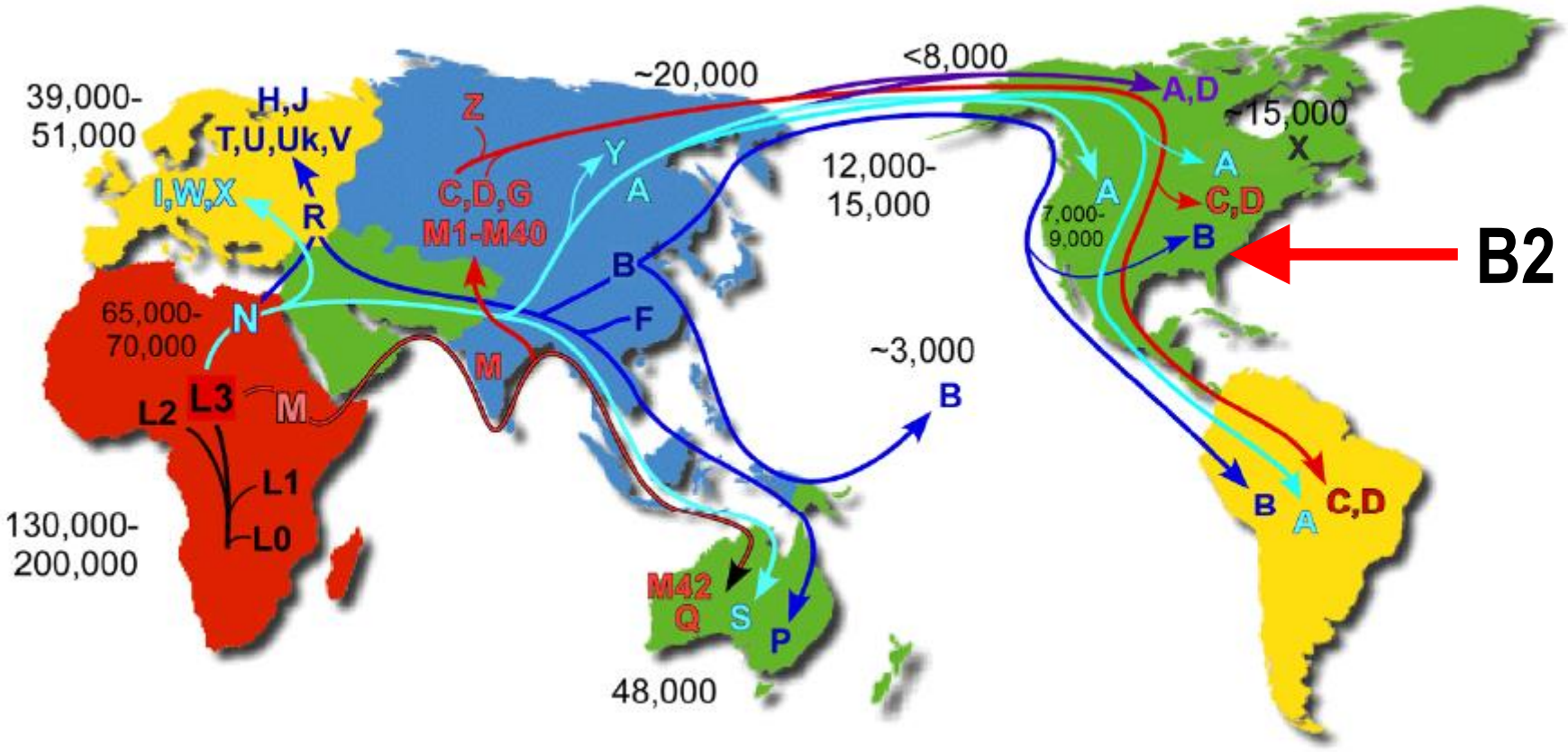
Mitochondrial Migrations



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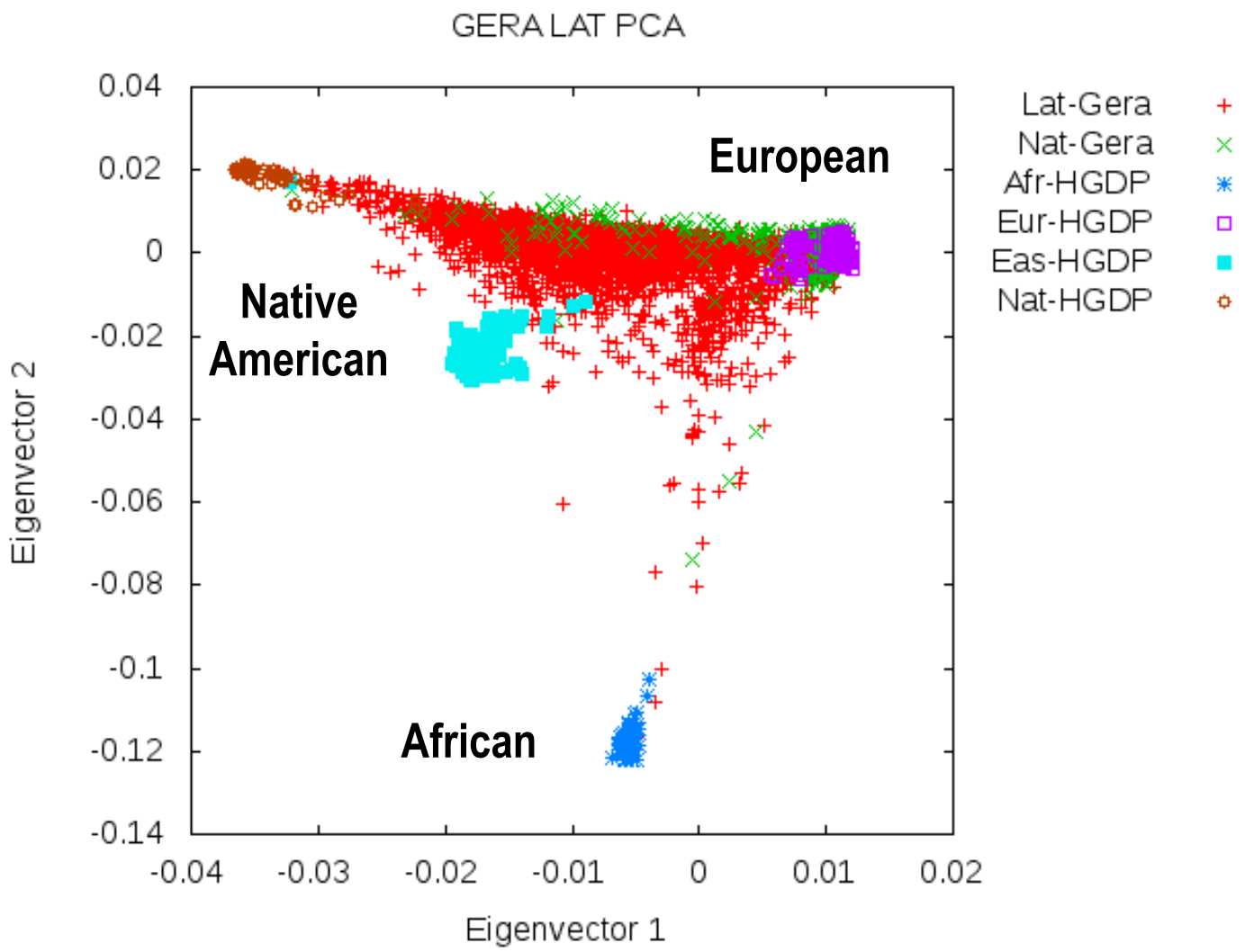
Ancestry of Latinos in GERA



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The Effect of Ancestry on B2 and mtSNP Associations

Haplogroup/SNP	Unadjusted		Adjusted	
	OR	P-value	OR	P-value
non-Hispanic white				
B2	2.95	$3.88*10^{-5}$	3.34	$1.54*10^{-5}$
rs28358586	1.71	$1.93*10^{-2}$	2.08	$2.40*10^{-3}$
rs28357981	3.59	$8.37*10^{-7}$	4.35	$1.05*10^{-7}$
rs1029294	2.96	$4.49*10^{-6}$	3.15	$5.04*10^{-6}$
rs3134801	2.20	$7.04*10^{-4}$	2.50	$2.00*10^{-4}$
Latino				
B2	1.34	0.031	1.19	0.220
rs28358586	1.33	0.038	1.20	0.206
rs28357981	1.38	0.016	1.24	0.133
rs1029294	1.34	0.031	1.19	0.229
rs3134801	1.32	0.039	1.19	0.235

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Outline

- Epigenetics: DNA Methylation and Histone Modification
- Parent-of-origin Effects
- Mitochondrial DNA
- ***De Novo* Variation**

Your *De Novo* Mutations

- 74 new SNPs
- 2 new Indels
- Rarely a CNV

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De Novo Mutation Methods

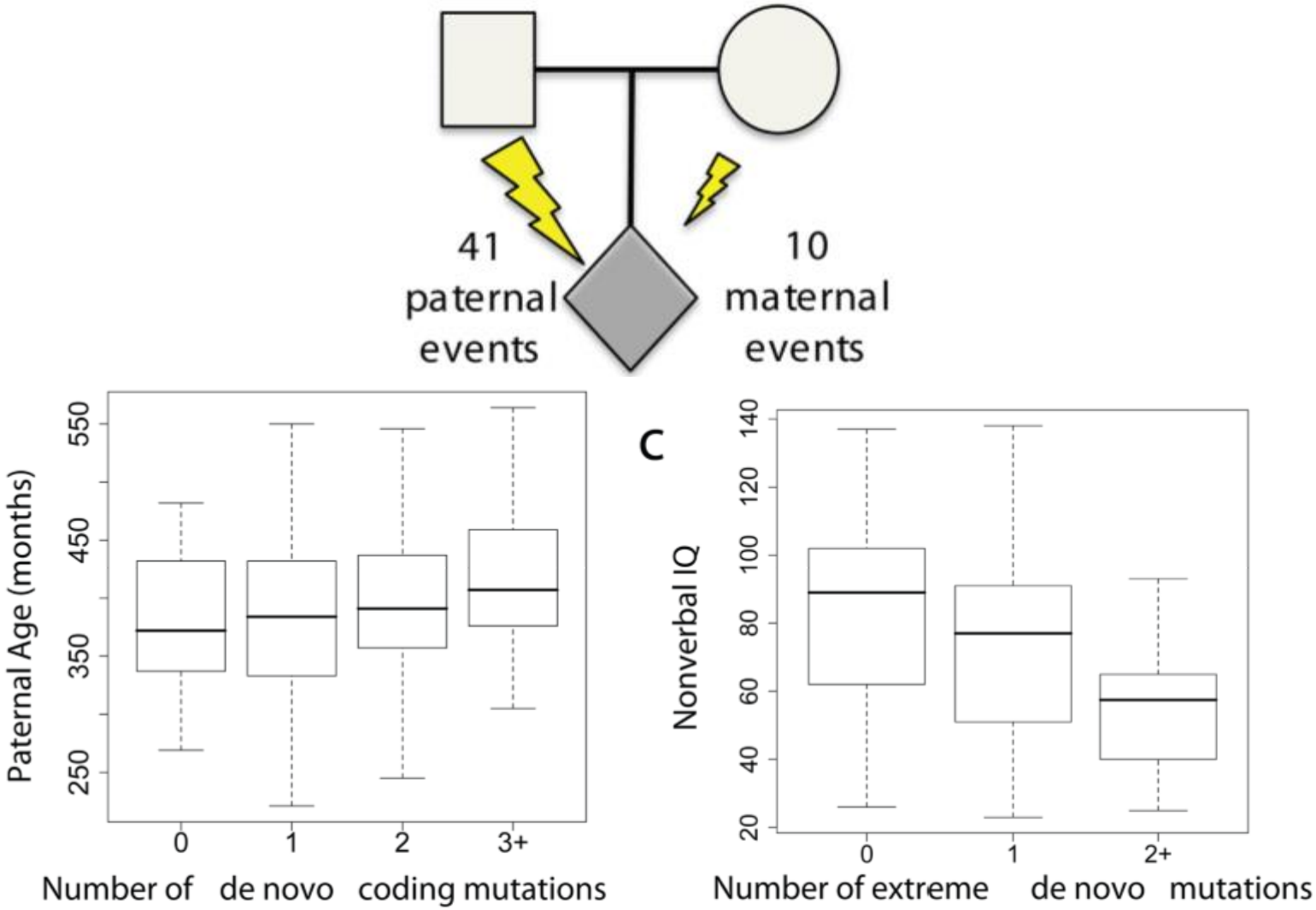
- Family Design
  - Trios
- Exome Sequencing
  - Coding Variants
- Array CGH
  - CNVs
- Bioinformatics
  - Filter artifacts
- Sanger Sequencing
  - Confirmatory

Sporadic Autism Exome Study and *De Novo* Mutations

- 209 Families, 677 Individuals
  - Trios
  - Unaffected Siblings
- Exome Sequencing
- 248 *De Novo* Events
  - 225 SNVs (single nucleotide variants)
  - 17 Indels
  - 6 CNVs
- 181 nonsynonymous changes
  - 120 severe

O'Roak *et al*. Nature 2012

De Novo Mutations and Autism Spectrum Disorder



O'Roak *et al*. Nature 2012

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Next Week

- Next generation sequencing