Forensic Genetics and Legal Medicine 2019-2020

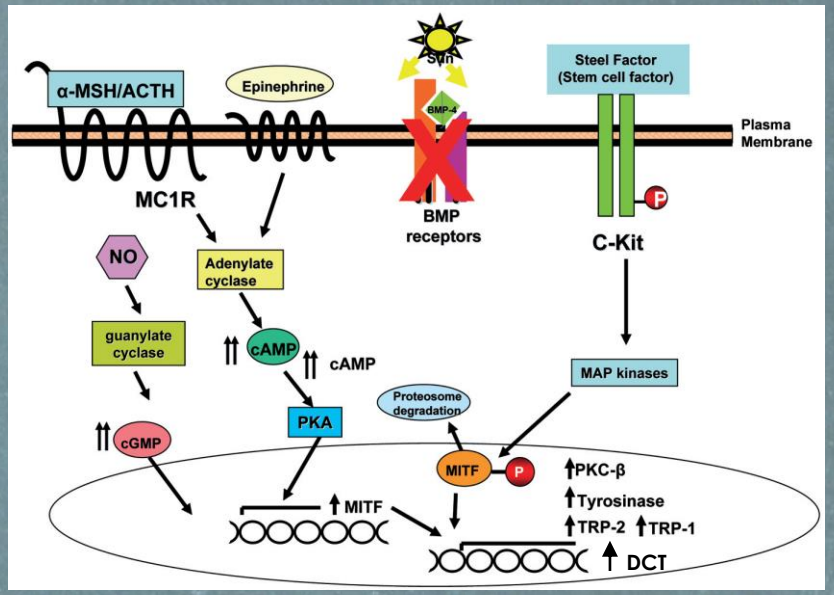
13th May 2020

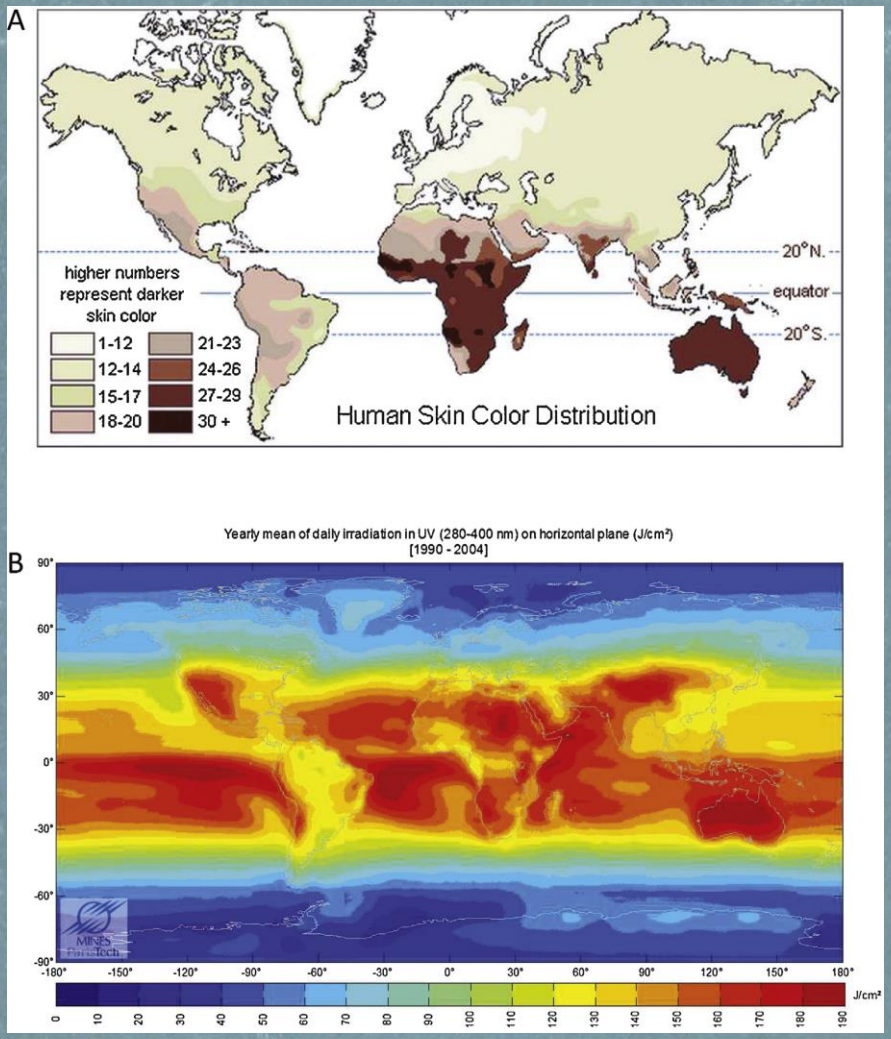
Beyond identification (EVCs and age)

Pigmentation

- ✓ human pigmentation traits are the least genetically complex of all EVCs, with a handful of genes providing most of the phenotypic information
- Phenotypic variation in pigmentation traits is determined by the amount, type, and distribution of melanin within melanocytes
- Two types of melanin (eumelanin, brown-black) and pheomelanin (red-brown) synthesized and stored in melanosomes through a chain of chemical reactions subjected to complex genetic regulation

MSH and ACTH bind melanocortin 1 receptor (MC1R) leading to: Increase in intracellular level of cAMP > activation of MAP kinases > phosphorylation (activation) of MITF > transcription of the melanogenic enzymes tyrosinase, TRP-1, TRP-2 and DCT (modified from Park et al. Cell Mol Life Sci 2009)



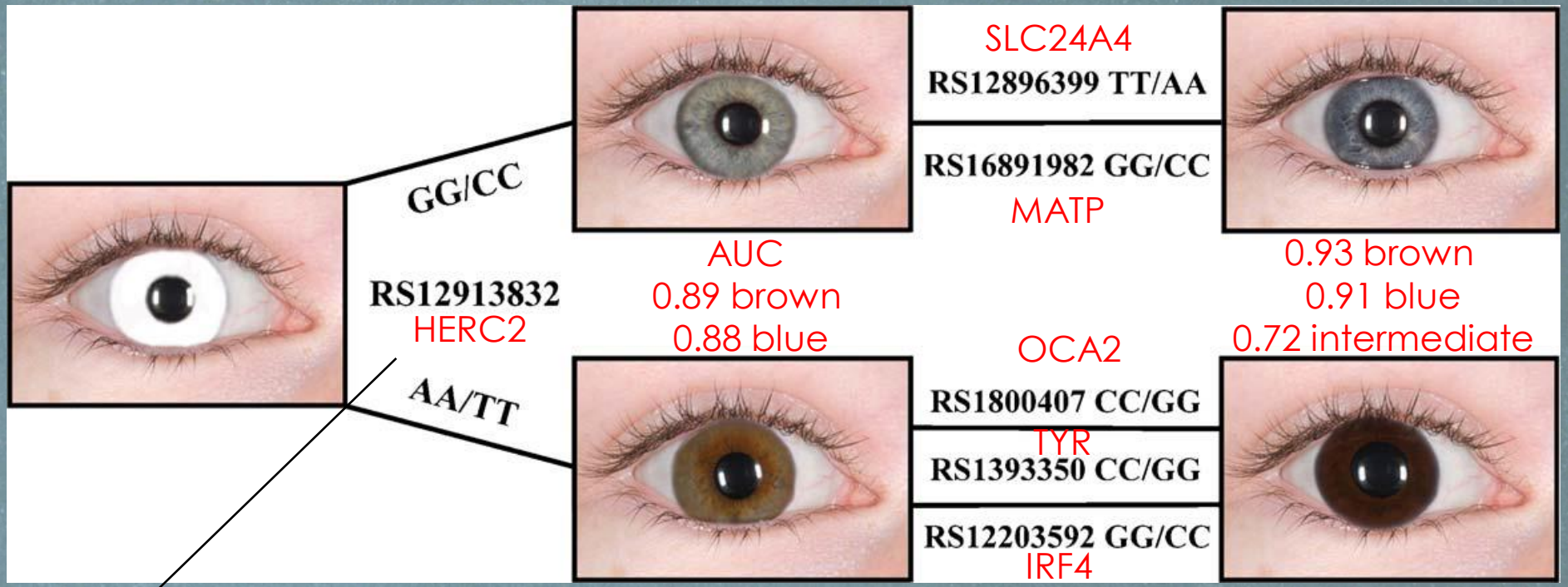


Liu et al. Seminars in Cell & Developmental Biology 2013

- ✓ strong correlation between the skin color and latitude
- ✓ adaptive trait shaped by recent evolutionary history: UV radiation stronger in equatorial regions
- ✓ UV needed for the synthesis of vitamin D in the skin (deficiency results in rickets and increases the risk of miscarriage
- ✓ Strong UV radiation degrades folate with increase risk of neonatal malformations

- ✓ absence of a plausible fitness advantage for eye and hair color variation
- ✓ It is likely that eye and hair color variation arose in Europe via genetic mutations: sexual selection of derived non-brown eye color and non-black hair color phenotypes

✓ eye color (Irisplex)



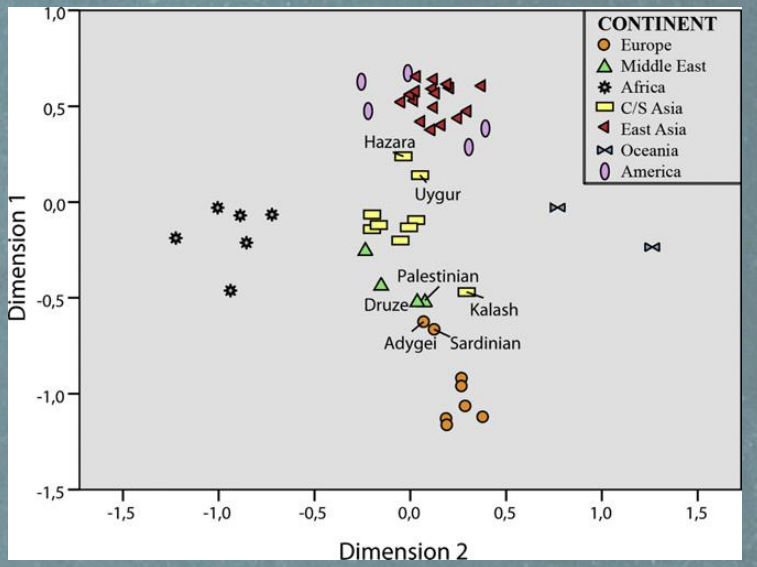
Walsh et al Forensic Sci Int Genet 2011

Located on chromosome 15 next to OCA2 gene. OCA2 (regulating pH in melanosomes and whose mutations were known to cause albinism) was believed to be the key gene for eye color. HERC2 allele A/T enhances transciption of OCA2 and melanin synthesis leading to dark pigmentation

+ Fully validated for forensic purposes (down to 30 pg DNA)
+ Though developed from a Dutch GWAS study, good performance regardless to ancestry(e.g. all East Asian, African, Oceanian and American HGDP samples predicted as brown-eyed)
+ Enhanced prediction model including non-Dutch Europeneas increase AUC up to 0.95 (brown), 0.94 (blue), 0.74 (intermediate) (Walsh et al. Forensic Sci Int Genet 2014)
- Reduced prediction accuracy for brown in admixed American populations (mostly in rs12913832 heterozygous subjects)
- Possible gender effect?

✓ hair color (HIrisplex) → 23 SNPs and 1 Indel in MC1R, **HERC2**, **OCA2**, **MATP**, KITLG, EXOC2, **TYR**, **SLC24A4**, **IRF4**, PIGU/ASIP and TYRP1 (including the 6 Irisplex SNPs) (Walsh et al Forensic Sci Int Genet 2013)

AUC
- 0.80 (blond) - 0.75(brown)
- 0.92 (red) - 0.85 (black)



+ fully validated for forensic purposes (down to 60 pg DNA)
+ good performance regardless to ancestry (e.g. all East Asian, African, Oceanian and American HGDP samples predicted as black-hair)
+ potential prediction of ancestry →
- Higher errors rates in blond/brown prediction partly due to age-dependent hair darkening (blond/brown individuals turning brown/black after childhood)

✓ Hair shape (straight vs non-straight) → 32 SNPs from 26 genes (Pospiech et al Forensic Sci Int Genet 2018)

AUC
- 0.66 (europeans)
- 0.79(non-europeans) →

AUC increase mostly influenced by highly accurate prediction of striaght hair due to a single SNP (rs3827760) in the EDAR gene, The G allele associated to thick and straight hair in East Asians is almost abstent in Europe and Africa

✓ Male pattern baldness (MPB) → 14 SNPs (Liu et al Eur J Hum Genet 2016)

↓

Many X-SNPs!

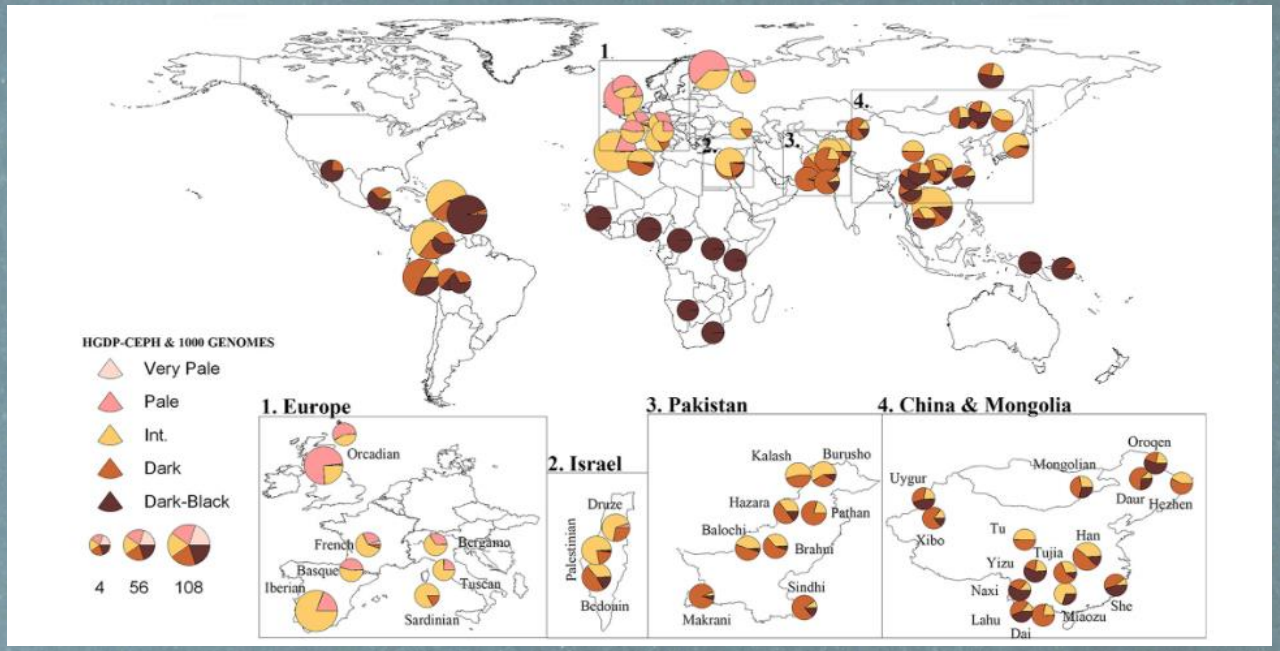
AUC
- 0.74 (early onset)

✓ Skin color (Hirisplex-S) → 17 skin SNPs from 11 genes (ANKRD11, **OCA2**, BNC2, **HERC2**, **SLC24A4**, **TYR**, SLC24A5*, **ASIP**, RALY, **MC1R**, DEF8) in a separate multiplex to complement Hirisplex markers (Chaitanya et al Forensic Sci Int Genet 2018)

AUC (based on the 17 skin markers plus 19 Hirisplex markers)
- 0.75 (very pale)
- 0.73(pale)
- 0.75 (intermediate)
- 0.84 (dark)
- 0.98 (dark/black)

*light skin associated variant allele at rs1426654 almost fixed in Europeans, whereas the ancestral allele predominates in Africa and East Asia

+ down to 60 pg
+ skin color prediction as expected in HGDP and 1000 genomes samples →



Erasmus MC (Erasmus Universitair Medisch Centrum Rotterdam) [NL] | https://hirisplex.erasmusmc.nl

HIrisPlex-S Eye, Hair and Skin Colour DNA Phenotyping Webtool

12px

Welcome to the Department of Genetic Identification of Erasmus MC

With the advancement of DNA phenotyping as a tool in Forensic and Anthropological usage, we now provide an easy to use interactive website to predict eye, hair and skin colour from DNA using the IrisPlex, HIrisPlex and HIrisPlex-S systems.

This work is in collaboration with the Walsh laboratory of Indiana-University-Purdue-University-Indianapolis (IUPUI), USA.

Please see the manual for instructions. We hope you enjoy using this tool and find it a useful addition to your analyses. If you have any issues regarding this website, please contact us using the contact form.

The HIrisPlex-S System

	Gene	SNP	Allele	No. of Alleles
1	*MC1R*	rs312262906	A	0 1 2 NA
2	*MC1R*	rs11547464	A	0 1 2 NA
3	*MC1R*	rs885479	T	0 1 2 NA
4	*MC1R*	rs1805008	T	0 1 2 NA
5	*MC1R*	rs1805005	T	0 1 2 NA
6	*MC1R*	rs1805006	A	0 1 2 NA
7	*MC1R*	rs1805007	T	0 1 2 NA
8	*TUBB3*	rs1805009	C	0 1 2 NA
9	*MC1R*	rs201326893	A	0 1 2 NA
10	*MC1R*	rs2228479	A	0 1 2 NA
11	*MC1R*	rs1110400	C	0 1 2 NA
12	*SLC45A2*	rs28777	C	0 1 2 NA
13	*SLC45A2*	rs16891982	C	0 1 2 NA
14	*KITLG*	rs12821256	G	0 1 2 NA
15	*LOC105374875*	rs4959270	A	0 1 2 NA
16	*IRF4*	rs12203592	T	0 1 2 NA
17	*TYR*	rs1042602	T	0 1 2 NA
18	*OCA2*	rs1800407	A	0 1 2 NA
19	*SLC24A4*	rs2402130	G	0 1 2 NA
20	*HERC2*	rs12913832	T	0 1 2 NA
21	*PIGU*	rs2378249	C	0 1 2 NA
22	*LOC105370627*	rs12896399	T	0 1 2 NA
23	*TYR*	rs1393350	T	0 1 2 NA
24	*TYRP1*	rs683	G	0 1 2 NA
25	*ANKRD11*	rs3114908	T	0 1 2 NA
26	*OCA2*	rs1800414	C	0 1 2 NA
27	*BNC2*	rs10756819	G	0 1 2 NA
28	*HERC2*	rs2238289	C	0 1 2 NA
29	*SLC24A4*	rs17128291	C	0 1 2 NA
30	*HERC2*	rs6497292	C	0 1 2 NA
31	*HERC2*	rs1129038	G	0 1 2 NA
32	*HERC2*	rs1667394	C	0 1 2 NA
33	*TYR*	rs1126809	A	0 1 2 NA
34	*OCA2*	rs1470608	A	0 1 2 NA
35	*SLC24A5*	rs1426654	G	0 1 2 NA
36	*ASIP*	rs6119471	C	0 1 2 NA
37	*OCA2*	rs1545397	T	0 1 2 NA
38	*RALY*	rs6059655	T	0 1 2 NA
39	*OCA2*	rs12441727	A	0 1 2 NA
40	*MC1R*	rs3212355	A	0 1 2 NA
41	*DEF8*	rs8051733	C	0 1 2 NA

Display Predicted Phenotype | Download Predicted Phenotype

→ Web tools for phenotyping

Phenotypic SNPs readily combined in new MPS assays ←

Table 1. Forensic Loci Included in ForenSeq DNA Signature Prep Kit

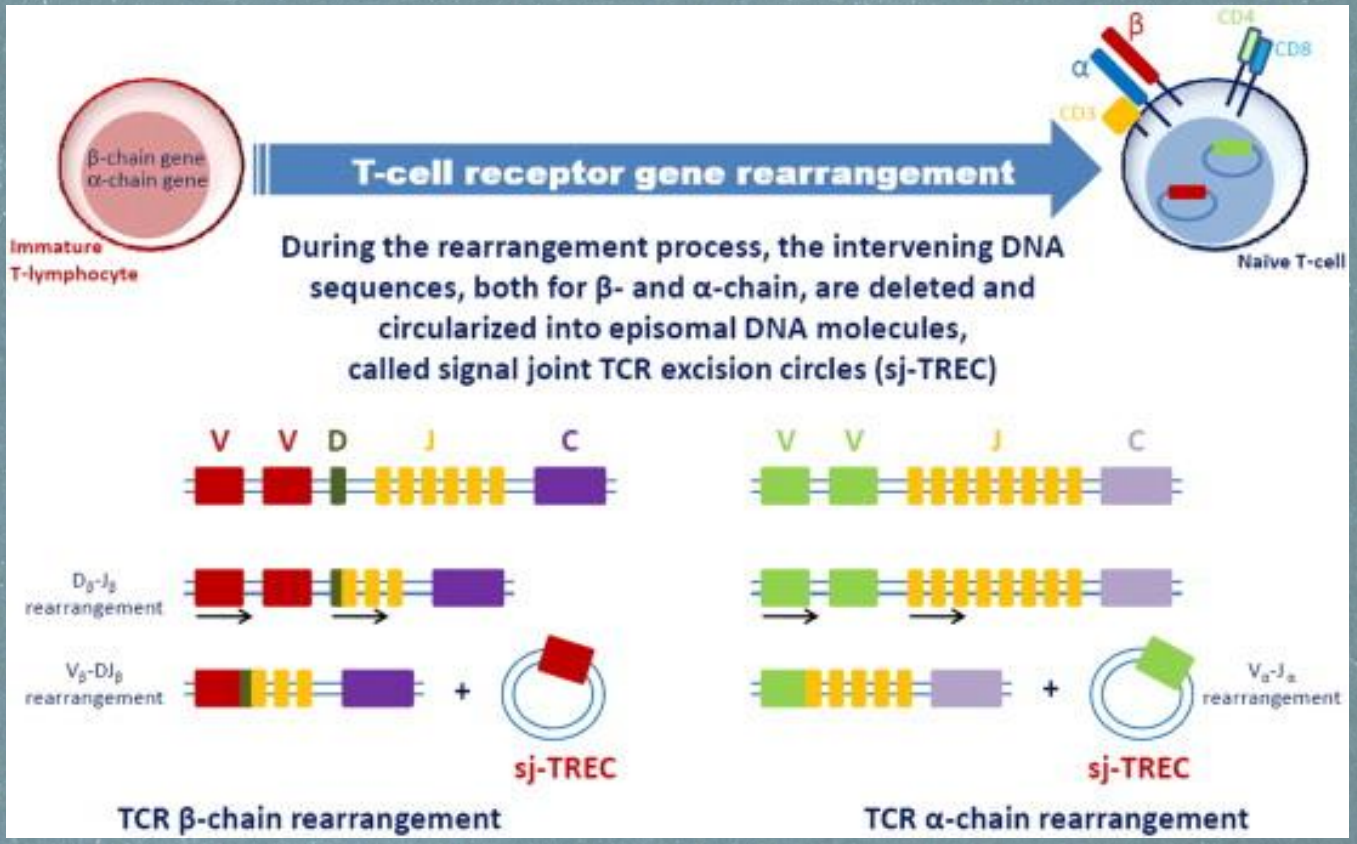
Feature	Number of Markers[a]	Amplicon Size Range (bp)
Global Autosomal STRs	27	61–467
Y-STRs	24	119–390
X-STRs	7	157–462
Identity SNPs	95	63–231
Phenotypic SNPs[b]	22	73–227
Biogeographical Ancestry SNPs[b]	56	67–200

a. SNP and STR chromosome locations can be found in the ForenSeq DNA Signature Prep Kit User Guide (support.illumina.com/downloads/forenseq-dna-signature-prep-guide-15049528.html).

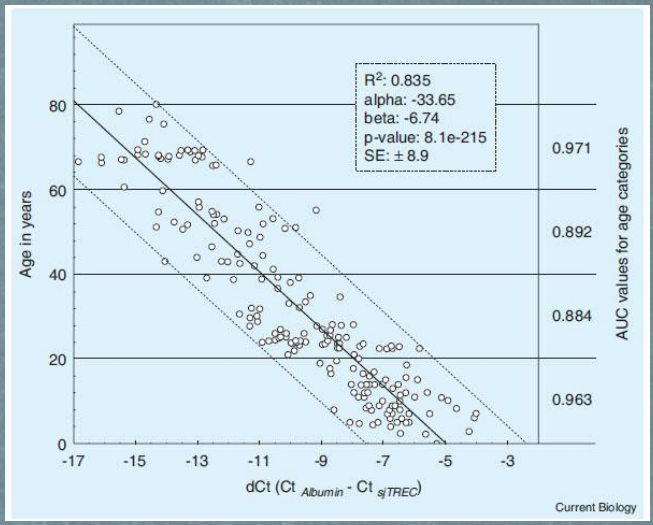
b. Two piSNPs used for hair/eye color are also used in the aiSNP marker set.

Age

- ✓ First studies describing age-dependent accumulation of point mutation (heteroplasmy) and large scale deletions in mtDNA as well as shortning of telomere sequences in chromosomes lacked accuracy



- ✓ T-cell receptor rearrangement
- concentration of sj-TREC in blood reduces over time and can be measured through real time PCR
- blood only test
- requires at least 0.5 ng of DNA



Zubakov et al Curr Biol 2010

- ✓ gene expression is known to correlate with human age

- a recent study used qPCR to measure the expression of age-correlated genes selected through genome-wide expression chips and blood-derived DNA from 52 individuals (Zubakov et al. Forensic Sci Int Genet 2016)
- results were compared to DNA rearrangement and telomere shortening and…

Table 3. Categorical age prediction based on 2 non-correlated mRNA age markers, *NRCAM* and *CFH*, identified in this study, in RNA samples from blood of 267 individuals from 22 to 84 years of age (125 males and 142 females).

Age Category, years[a]	N	AUC	MAD	p-value
22–40	67	0.871	11.889	4.21E-18
41–60	95	0.682	7.381	0.89E-3
61–84	101	0.873	9.300	3.80E-17

[a] Samples from the young age group <22 years were not available for these markers.

Table 6. Age correlation of sjTREC and telomere length markers in DNA samples from blood of 306/305 individuals from 18 to 84 years of age (166/174 males and 140/113 females).

Marker	R^2	Cross-validated R^2	R^2 SD	SEE, years	MAD, years
sjTREC	0.546	0.549	0.070	±11.935	9.757
sjTREC with sex as co-factor	0.578	0.579	0.073	±11.527	9.391
Telomere length	0.141	0.151	0.07	±15.362	12.276

MAD = mean absolute deviation

- DNA methylation markers

Table 5. Categorical age prediction based on 8 non-correlated DNA methylation age markers identified in this study, in DNA samples from blood of 216 individuals from 4 to 82 years of age (113 males, 103 females).

Age category, years	N	AUC	MAD	P-value
4–20	33	0.962	5.363	5.31E-27
21–40	68	0.949	4.531	1.72E-27
41–60	63	0.891	4.445	8.13E-20
61–82	52	0.956	5.618	4.04E-27

- ✓ significant change of global DNA methylation levels was observed to be associated with increasing age in epigenome-wide association studies

+ large public databases created in epigenetic studies through chip technology that provide a source for age-predictive tests
- most databases have only blood as tissue source (age-dependent methylation may be tissue specific)

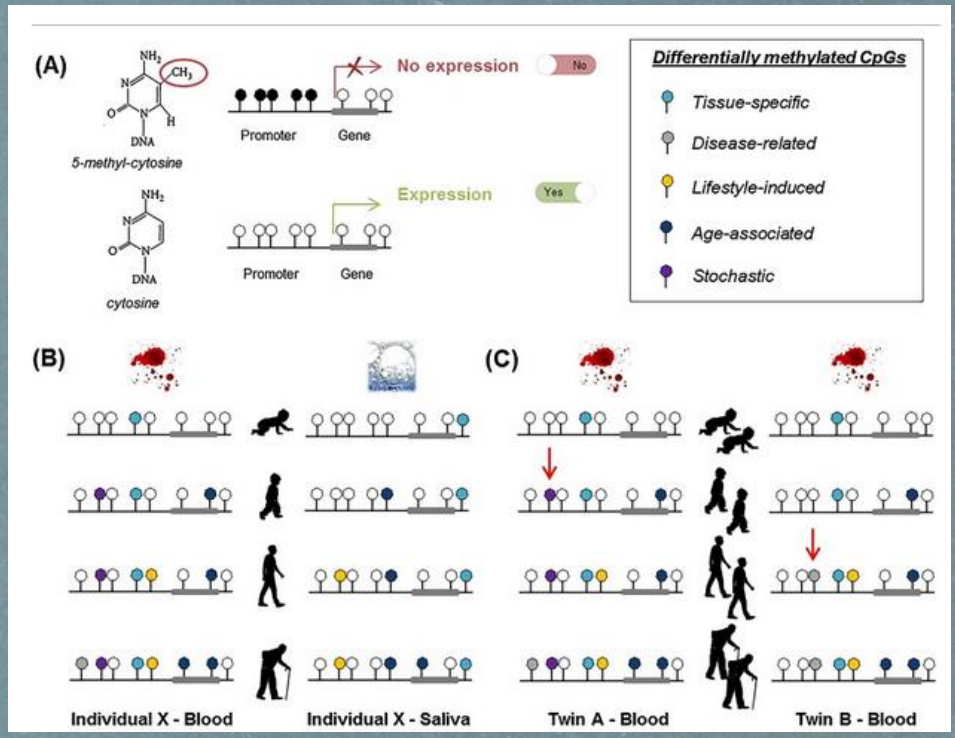
- ✓ several studies of (only partly overlapping) CpG sites generally reporting a MAD of 3-4 years

- ✓ Some reporting reproducibility of results obtained from DNA methylation markers selected on blood samples to non-blood samples e.g. saliva (Vidaki et al. Forensic Sci Int Genet 2017)

- Epigenetics refers to the 'heritable' alterations in gene expression and cellular phenotype that are triggered by molecular mechanisms other than DNA sequence changes, e.g. DNA base modifications such as cytosine methylation
- In vertebrates ~ 10% cytosines are methylated
- methylation is not random but limited to 5' C-G 3' sequences
- about 50% of our genes lay close to G and C rich regions (CpG islands)
- epigenetics regulation of gene expression works under the 'rule' - with many exceptions - that a methylated gene promoter becomes compact and non-accessible to transcription factors leading to the inactivation of the gene

The lifelong molecular responses to the 'dynamic' environment via adjusting DNA methylation levels across the genome, results in individual epigenomic variation relevant in the forensic field for:

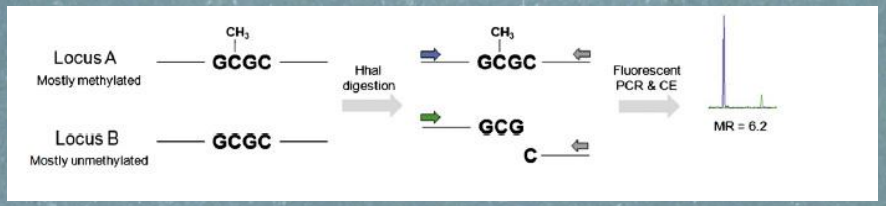
- ✓ determination of body fluids and tissues
- ✓ age estimation
- ✓ discrimination of monozygotic twins



Vidaki et al Forensic Sci Int Genet 2018

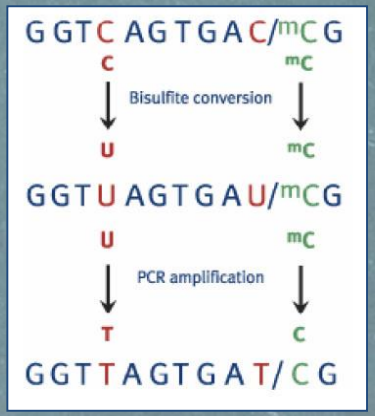
Methylation detection techniques

a) Restriction enzyme analysis
(HhaI restriction enzyme 5′-GCG^C-3′)



Methylated DNA is protected from cleavage and amplified by specific fluorescent primers

b) Bisulfite conversion



Sodium bisulfite converts unmethylated cytosine to uracil while keeping methylated cytosines unchanged

Uracil becomes thymine in PCR

Challanges

- ✓ Results affected by restriction cleavage / bisulfite conversion efficiency
- ✓ In vitro stabilty of bisulfite converted DNA still unclear
- ✓ PCR specificity (bisulfite converted DNA will mostly consist of 3 bases)
- ✓ PCR efficiency (converted and non-converted sequences may amplify at differnt rates creating an artificial bias in the final detected methylation status)
- ✓ Several techniques (end point PCR, SBE, qPCR, sequencing, pyrosequencing, NGS) used for methylation detection, making difficult to compare methylation levels and prediction models across studies