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Technology Transition Workshop| *Angela van Daal, Ph.D.*

Phenotypic SNPs to Aid in Forensic Analyses

Overview

- **Why type phenotypic SNPs?**
- **Phenotypic traits**
  - **Heritability**
- **Pigmentation**
  - **Hair, skin, eye colour**
  - **Genetics**
  - **NIJ project**
- **Height**
- **Face**
- **Applications**

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Why Type Physical Trait SNPs?

- **Current forensic DNA testing requires suspect**
- **Evidence DNA sample provides no or limited info (gender, ancestry)**
- **If can use DNA markers that describe appearance traits allows genetic prediction of appearance for tracing offender (more reliable than eye witness)**

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Privacy Concerns

- **Need only do on anonymous crime scene samples**
- **No privacy issues – sample not linked to specific person**
- **Predictive/investigative of external traits only**
- **On suspect arrest obtain sample for STR typing**

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Single Nucleotide Polymorphisms (SNPs)

- **These single base changes play a significant role in determining our differences**
- **Only need one base change for significant difference (for example, severe disease such as cystic fibrosis)**

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Physical Appearance

- **Physical traits**
- **High heritability traits**
  - **Pigmentation**
  - **Height**
  - **Facial morphology**
- **Causal SNPs**

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Genetics Versus Environment

- **Heritability measures genetic component**
- **H = 80 to 90% for pigmentation and height**
- **Face – identical twins**

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Pigmentation

- **Hair, skin, eye colour is highly heritable**
- **>120 genes in mouse affect coat colour variation**

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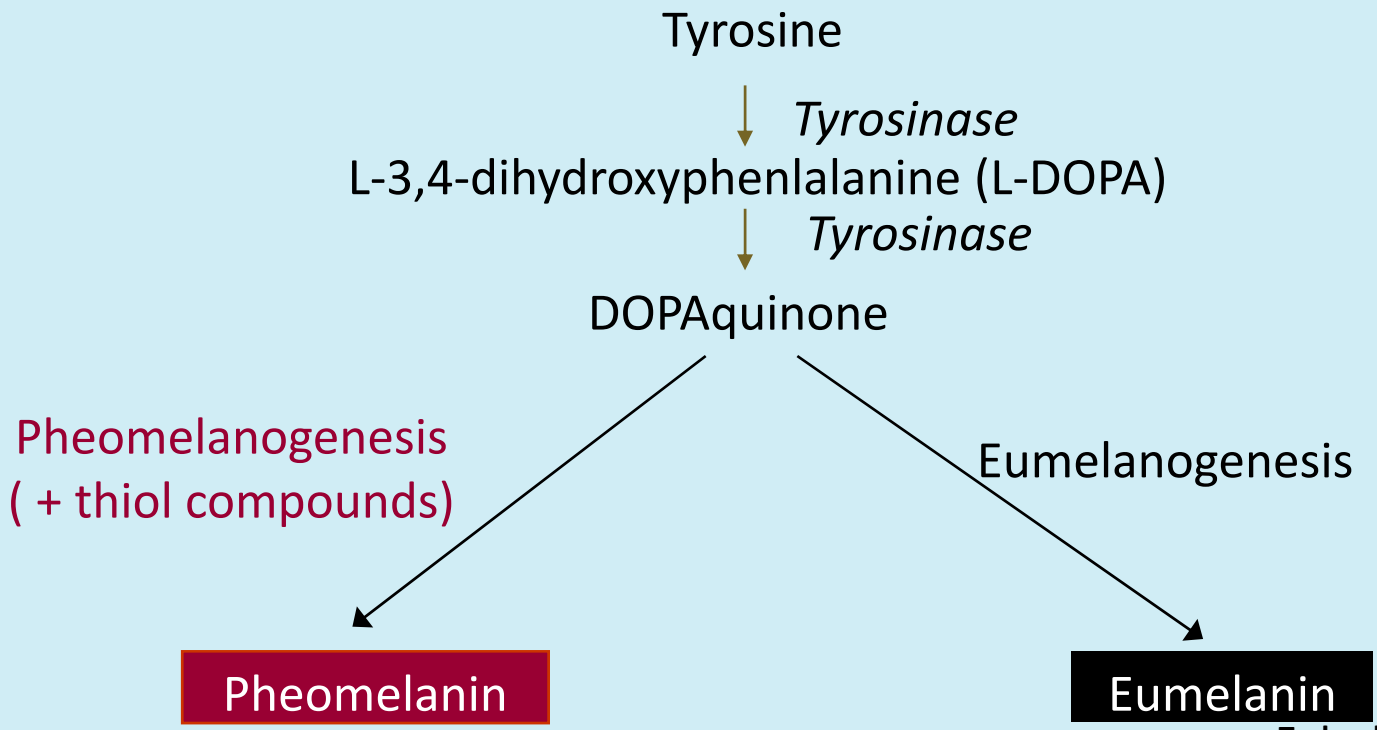
Pigmentation

- **Melanin**
  - **Compound responsible for skin, hair and eye colour**
  - **Also protects the skin from UV radiation**
  - **Exists in two forms**
    - **Eumelanin (black-brown pigments)**
    - **Pheomelanin (yellow-red pigments)**
  - **The amount and type of these two compounds are responsible for the wide variety of pigmentation phenotypes**

Melanin Synthesis

- **Melanin**
  - **Synthesized from amino acid tyrosine**



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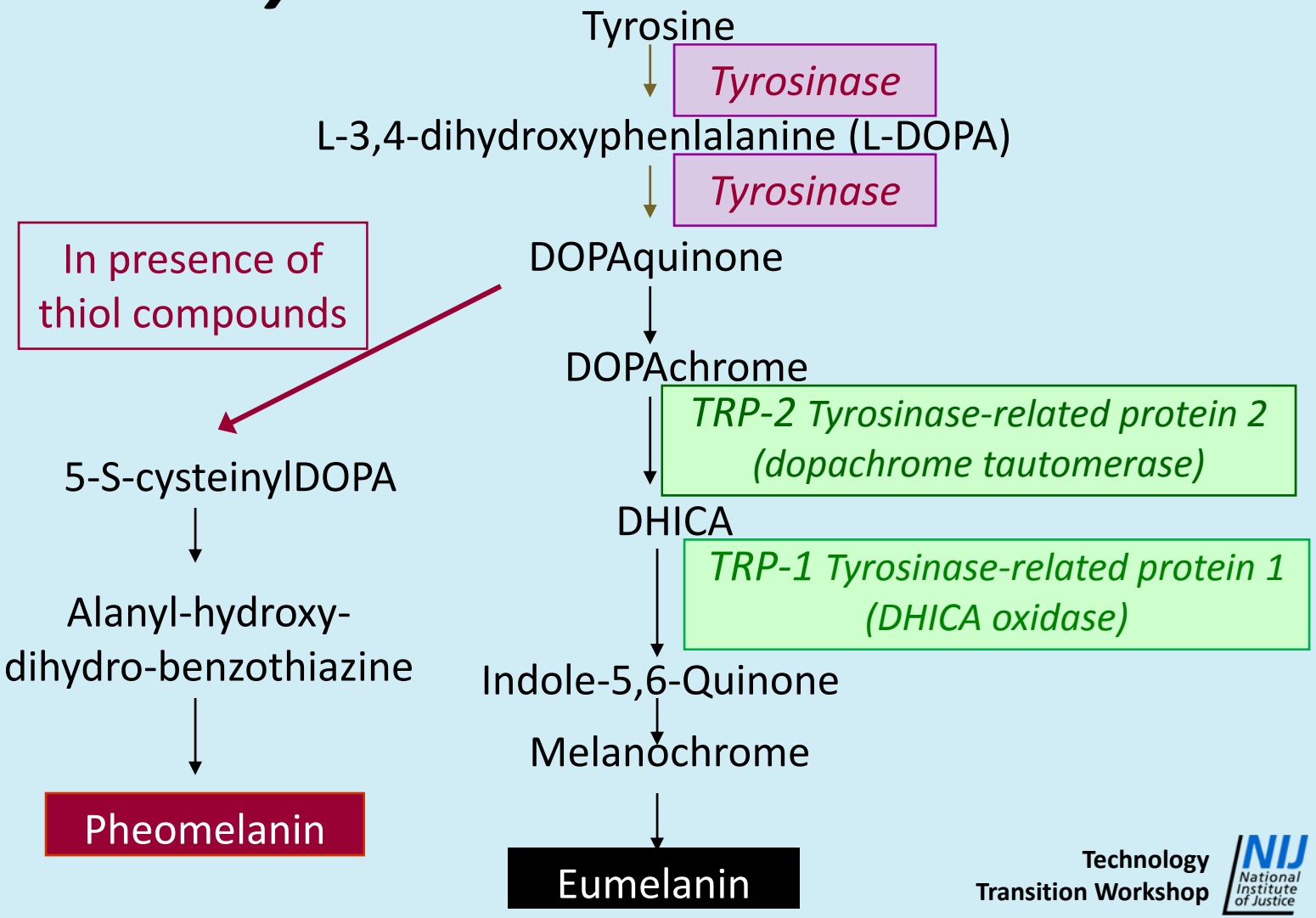
Melanin Synthesis



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Pigmentation Biology

- **Melanin is synthesized in melanosomes**
  - **Specialised organelles**
  - **Within melanocyte cells**
  - **Distribute melanin to keratinocytes**

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Pigmentation Gene Classes

- **Disease (albinism)**
- **Melanosomes**
- **Melanosome transport**
- **Melanocyte function**
- **Development**

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Pigmentation Disease

- **Oculocutaneous albinism (OCA)**
  - **Overall frequency of 1 per 20,000**
  - **Hypopigmentation of the hair, skin and eyes**
  - **High risk for developing skin cancers**
  - **Four known forms (OCA1, 2, 3 and 4)**

OCA1
Tyrosinase (TYR)

- **Large number of different mutations throughout the gene**
- **Two clusters of mutations in the copper A and B binding sites**
    - **Probably disrupt metal ion-protein interaction necessary for enzyme function**

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OCA3
Tyrosinase-related Protein 1 (TYRP-1)

- **43% amino acid homology with tyrosinase**
- **Block in the formation of eumelanin with continuing pheomelanin production**

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OCA2

P gene

- **25 exons spanning between 250 and 600 kb**
- **838-amino acid protein with 12 transmembrane domains**
- **Similar to various transporters**

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OCA4
MATP

- **Encodes a 530 amino acid protein with 12 transmembrane domains**
- **Protein ~ 58 kD predicted to span lipid bilayer 12 times**

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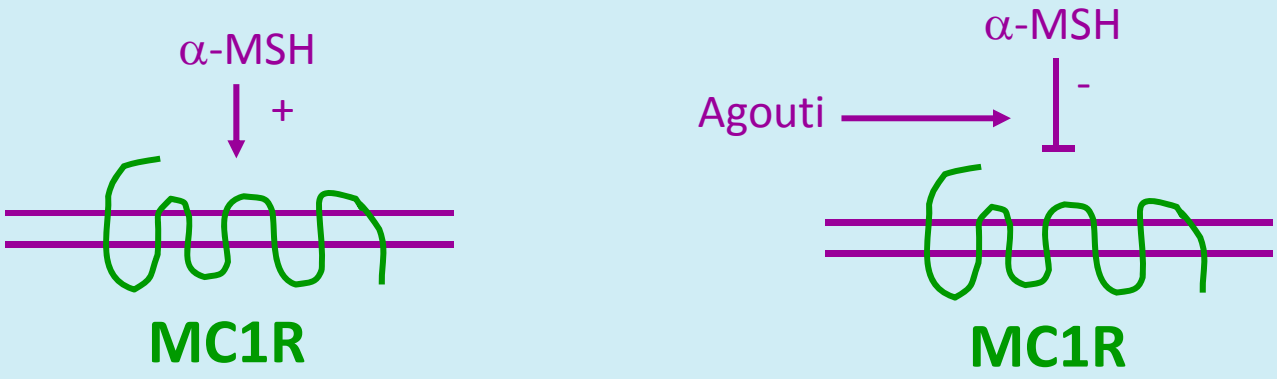
MC1R/Agouti Regulation of Pigmentation



Tyrosine
↑ cAMP
DOPA
DOPAquinone
DOPAchrome
Eumelanin

↓ cAMP
Tyrosine
DOPA
DOPAquinone
cysteinylDOPA
Phaeomelanin

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Agouti

- **Mouse**
  - **Paracrine signaling molecule that causes hair follicle melanocytes to synthesize pheomelanin**
  - **Transient agouti expression causes switching of eumelanin to pheomelanin from days 4 to 6 of the hair cycle by blocking the binding of α-MSH to MC1R**

Human MC1R SNPs

- **MC1R gene SNPs occur in:**
  - **> 80% of individuals with red hair and/or fair skin**
  - **< 20% of individuals with brown or black hair**
  - **< 4% of individuals with a good tanning response**

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Agouti/ASIP Gene

- **ASIP = antagonist of MC1R**
- **Screened ASIP gene**
  - **No coding SNPs**
  - **One 3' UTR SNP (g8818A →G)**

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Frequency in Different Phenotypes

- **Significant association with dark hair colour**

Phenotype	n[a]	Frequency 8818A allele	Frequency 8818G allele	P[b]
Hair colour				
Black	32	0.87	0.13	0.041
Brown	364	0.91	0.090	
Blonde	86	0.98	0.020	
Red	60	0.98	0.020	
Hair colour				
Dark	396	0.91	0.090	0.003
Light	146	0.98	0.020	
Eye colour				
Brown	132	0.93	0.070	0.671
Hazel	94	0.93	0.070	
Blue	206	0.92	0.080	
Green	110	0.95	0.050	
Eye colour				
Dark	226	0.93	0.070	0.544
Light	316	0.93	0.070	
Hair and eye colour				
Dark	120	0.93	0.070	0.544
Light	114	0.98	0.02	

[a]n represents the number of alleles tested.
[b]P-value as determined by Pearson chi-square test.

Voisey, Box and van Daal *Pigment Cell Res* (2001) **14** 264–267

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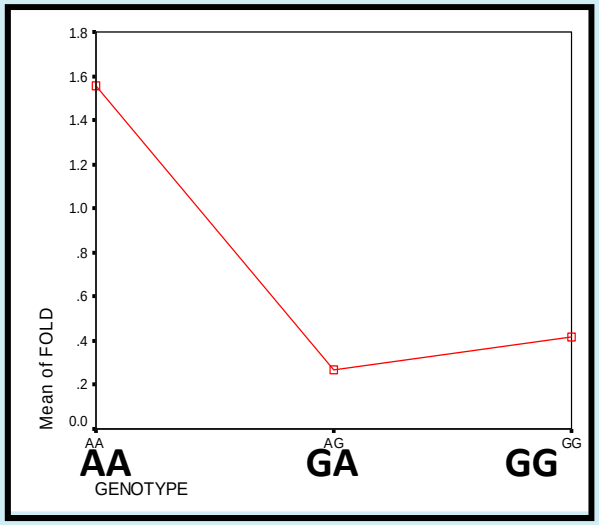
Agouti SNP Function – mRNA Stability?

- **Different expression levels in melanocytes**
  - **Genotypes AA, AG and GG**

Genotype	Cell line	Fold change[a]
AA	QF712	19 ± 2.4
	QF718	16.5 ± 2.6
	QF6	6 ± 3.4
AG	QF812	3.5 ± 1.7
	QF967	1 ± 0.26
	QF181	1 ± 0
GG	QF321	1.6 ± 0.31

[a]Values are the mean *ASIP* mRNA fold change of nine replicates for each cell line ± SD.



Agouti mRNA levels in melanocytes

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P Gene SNPs

- **P gene also associated with natural eye colour variation – brown/blue eye colour gene?**

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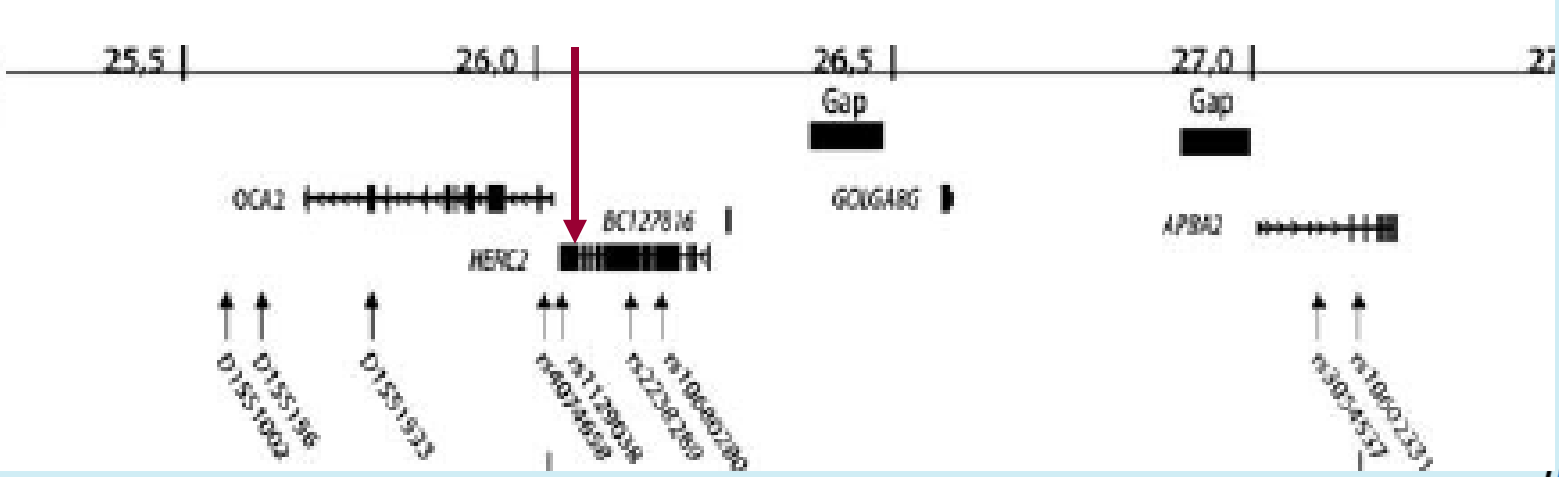
Blue Eye Gene?

Blue eye color in humans may be caused by a perfectly associated founder mutation in a regulatory element located within the *HERC2* gene inhibiting *OCA2* expression

Hans Eiberg · Jesper Troelsen · Mette Nielsen · Annemette Mikkelsen · Jonas Mengel-From · Klaus W. Kjaer · Lars Hansen

- **SNP located 21kb upstream OCA2 promoter**
- **Highly conserved sequence in intron 86 of HERC2**
- **Perfectly associated with the blue eye colour**



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MATP Gene SNPs

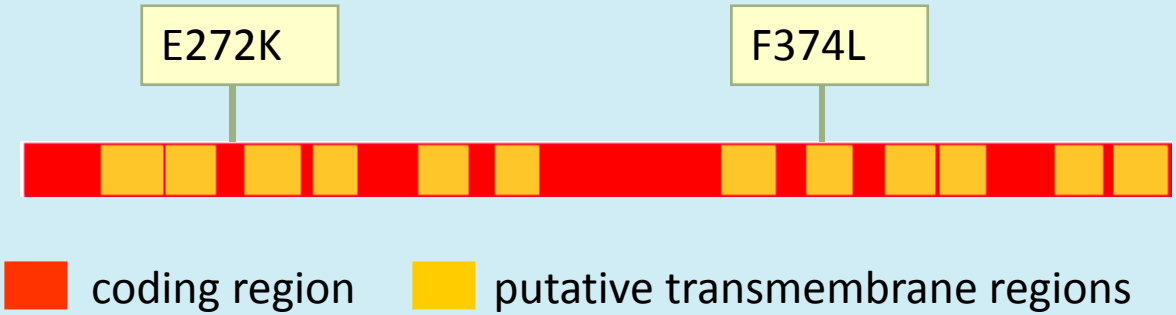

TABLE 2. *MATP* Allele Frequencies of Different Population Groups

		Allele frequency of MATP variants			
		p.Phe374Leu		p.Glu272Lys	
	n[b]	Phe	Leu	Glu	Lys
Population group[a]					
Caucasian	912	0.934	0.066	0.972	0.028
African American	140	0.414	0.586	0.750	0.250
Australian Aboriginal	102	0.275	0.725	0.971	0.029
Asian	62	0.113	0.887	0.661	0.339

[a]p value < 0.0001 for p.Phe374Leu and p.Glu272Lys.
[b]n represents number of alleles tested.

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MATP SNPs: Caucasian Phenotypes

TABLE 3. Summary of Allele Frequencies for *MATP* Variants in Caucasian Population

		Allele frequency of *MATP* variants					
		p.Phe374Leu			p.Glu272Lys		
Phenotype	n[a]	p[b]	Phe[c]	Leu[c]	p[b]	Glu[c]	Lys[c]
Hair color:		<0.0001			0.002		
Blonde	84		0.988 (166)	0.012 (2)		1 (168)	0 (0)
Red	44		0.989 (87)	0.011 (1)		0.989 (87)	0.011 (1)
Brown	302		0.925 (559)	0.075 (45)		0.967 (584)	0.033 (20)
Black	26		0.769 (40)	0.231 (12)		0.904 (47)	0.096 (5)
Skin color:		<0.0001			0.001		
Fair	199		0.977 (389)	0.023 (9)		0.984 (392)	0.016 (6)
Average	213		0.944 (402)	0.056 (24)		0.974 (415)	0.026 (11)
Olive	44		0.693 (61)	0.307 (27)		0.898 (79)	0.102 (9)
Eye color:		<0.0005			0.002		
Blue	206		0.968 (399)	0.032 (13)		0.990 (408)	0.010 (4)
Green	69		0.935 (129)	0.065 (9)		0.978 (135)	0.022 (3)
Hazel	68		0.897 (122)	0.103 (14)		0.963 (131)	0.037 (5)
Brown	113		0.893 (202)	0.107 (24)		0.938 (212)	0.062 (14)

[a]n represents number of individuals.
[b]p value as determined by chi-squared test.
[c]Numbers in parentheses represents number of alleles in each pigmentation category.

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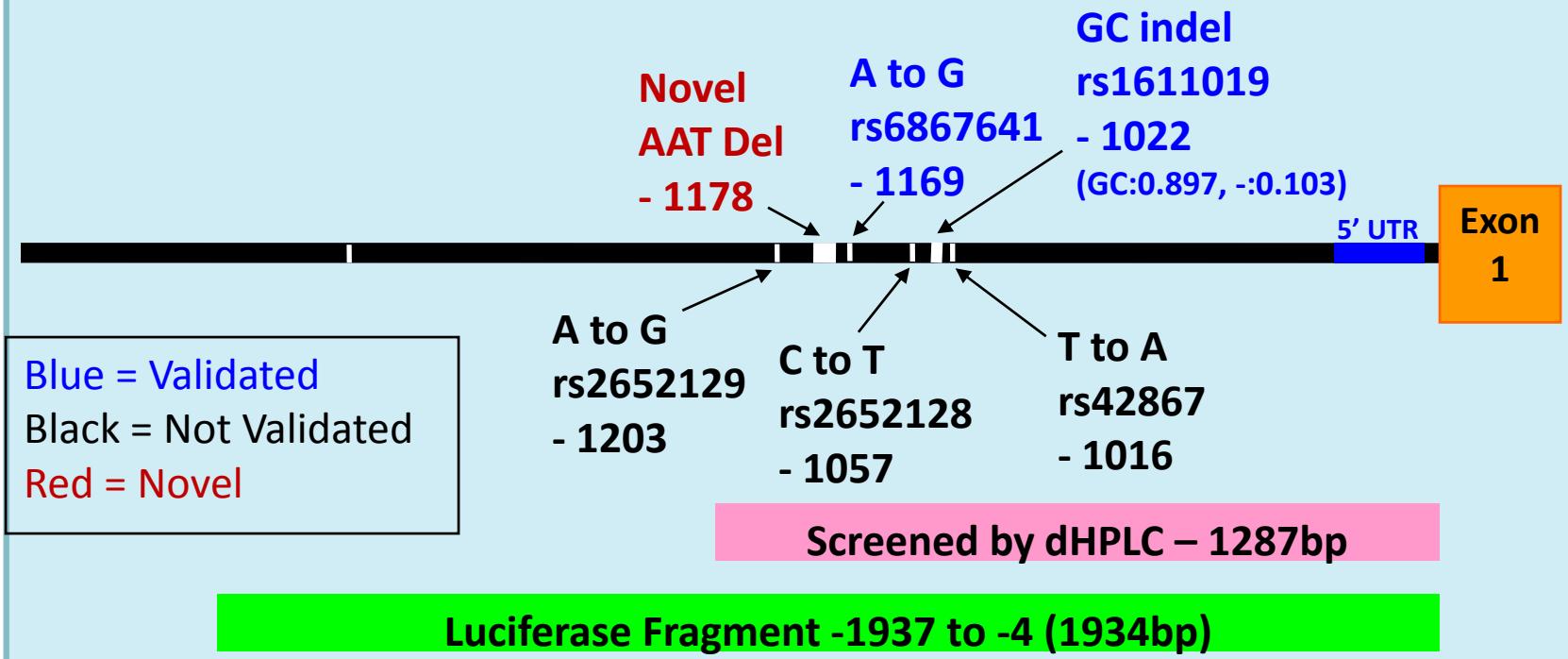
MATP Promoter

- **Three polymorphisms: 1721G, + dup, and 1169A**



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MATP Promoter Polymorphisms

- **1721G, + dup, and 1169A significantly associated with olive skin colour**
- **In LD with each other**
- **Not in LD with F374L and E272K**

		Allele frequency of *MATP* promoter variants					
		c.−1176_−1174dupAAT[a]			c.−1169G>A[a]		
Phenotype	n	P*	−Dup[b]	+Dup[b]	P*	G[b]	A[b]
Hair color		0.186			0.494		
Blonde	98		0.566 (111)	0.434 (85)		0.622 (122)	0.378 (74)
Red	53		0.679 (72)	0.321 (34)		0.698 (74)	0.302 (32)
Brown	353		0.588 (415)	0.412 (291)		0.640 (452)	0.360 (254)
Black	25		0.660 (33)	0.340 (17)		0.700 (35)	0.300 (15)
Skin color		0.0003			0.003		
Fair	235		0.645 (303)	0.355 (167)		0.689 (323)	0.319 (147)
Average	245		0.584 (286)	0.416 (204)		0.633 (310)	0.367 (180)
Olive	49		0.429 (42)	0.571 (56)		0.510 (50)	0.490 (48)
Eye color		0.311			0.113		
Blue	237		0.627 (297)	0.373 (177)		0.684 (324)	0.316 (150)
Green	82		0.555 (91)	0.445 (73)		0.598 (98)	0.402 (66)
Hazel	87		0.575 (100)	0.425 (74)		0.609 (106)	0.391 (68)
Brown	123		0.581 (143)	0.419 (103)		0.630 (155)	0.370 (91)

[a]Polymorphism numbering is based on using the cDNA GenBank accession No. NM_016180.3 as a reference.
[b]Numbers in parentheses represents number of alleles.
*P value as determined by chi-squared test.
n, number of individuals.

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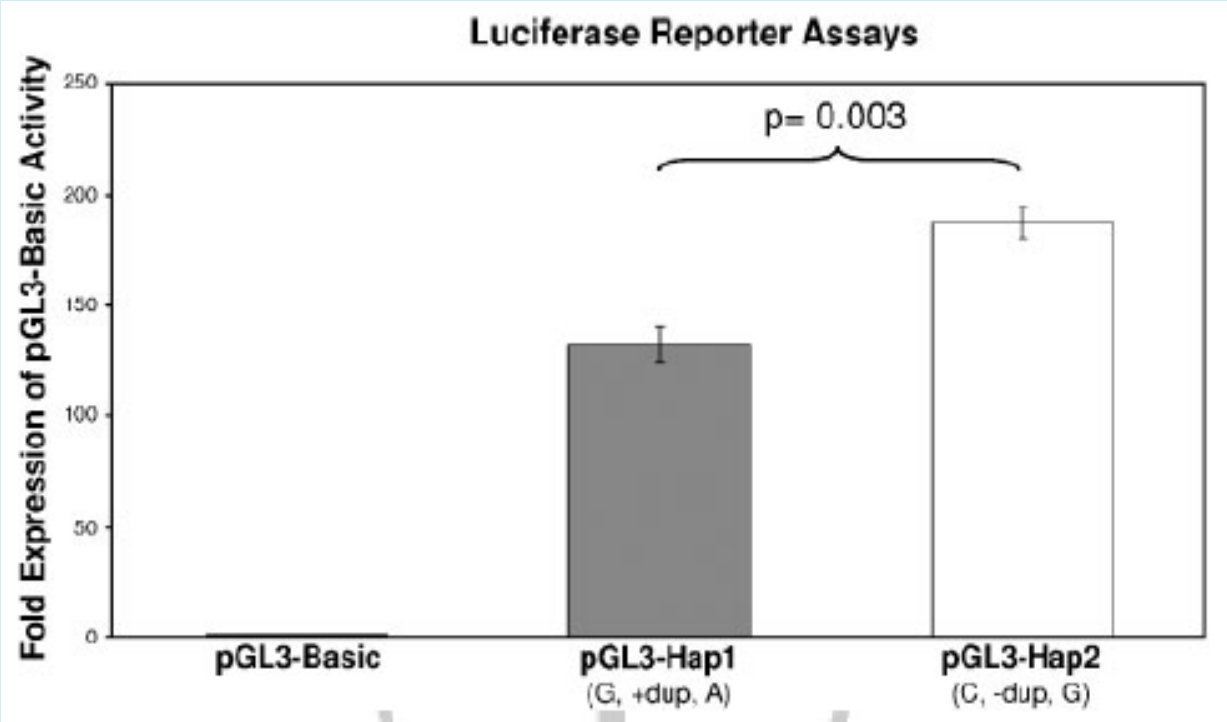
MATP Promoter Polymorphisms

- **Functional analyses in a melanoma cell line**
- **Haplotype 1721G, + dup, 1169A → ↓MATP transcription**



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NIJ Pigmentation SNP Panel Project

- **Project participants**
  - **Bond University (Professor Angela van Daal / Dr. Wenji Liu)**
  - **UNT (Professor Art Eisenberg / Professor John Planz)**
- **Literature review of SNP associations**
- **≥ 53 SNPs in 14 genes**
  - **MC1R, OCA2, HERC2, SLC24A5, SLC24A4, SLC45A2, TYR, TYR1, ASIP, TPCN2, KITLG, IRF4, EXOC2 and SILV**
- **Functionally characterized as responsible for pigmentation differences among populations**
- **Account for LD among these SNPs**
  - **≥ 40 SNPs selected**
  - **SNP panel to predict eye, hair and skin colour from DNA**

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Human Height

- **Heritability ~80%**
- **Adult height related to long bone length**
- **Longitudinal bone growth is due to bone elongation at the epiphyseal growth plate**
- **Endochondral ossification is the process by which long bones form and grow**
- **Mediated by numerous hormones, transcription factors and growth factors**

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Skull Development

- **Development of specific facial types is governed by the shape of the head/skull**
- **Premature fusion of skull sutures results in mild to severe facial abnormalities (craniosynostosis)**
- **Mutations in several genes cause craniosynostosis**
  - **FGFR1 and TWIST**

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Applications

- **Forensic DNA Identikit**
  - **Predict perpetrator appearance**
- **Facial reconstruction**
  - **Provide pigmentation and soft tissue information**

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Acknowledgements

- **Bond University**
  - **Dr. Wenji Liu**
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  - **Professor Bruce Budowle**
  - **Professor Art Eisenberg**
  - **Associate Professor John Planz**
- **Funding**
  - **NIJ (http://www.ojp.usdoj.gov/nij/about/welcome.htm)**
  - **TSWG (http://www.tswg.gov/about.html)**

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Questions?

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