Molecular Epidemiology of BRAF and NRAS Mutations in Melanomas

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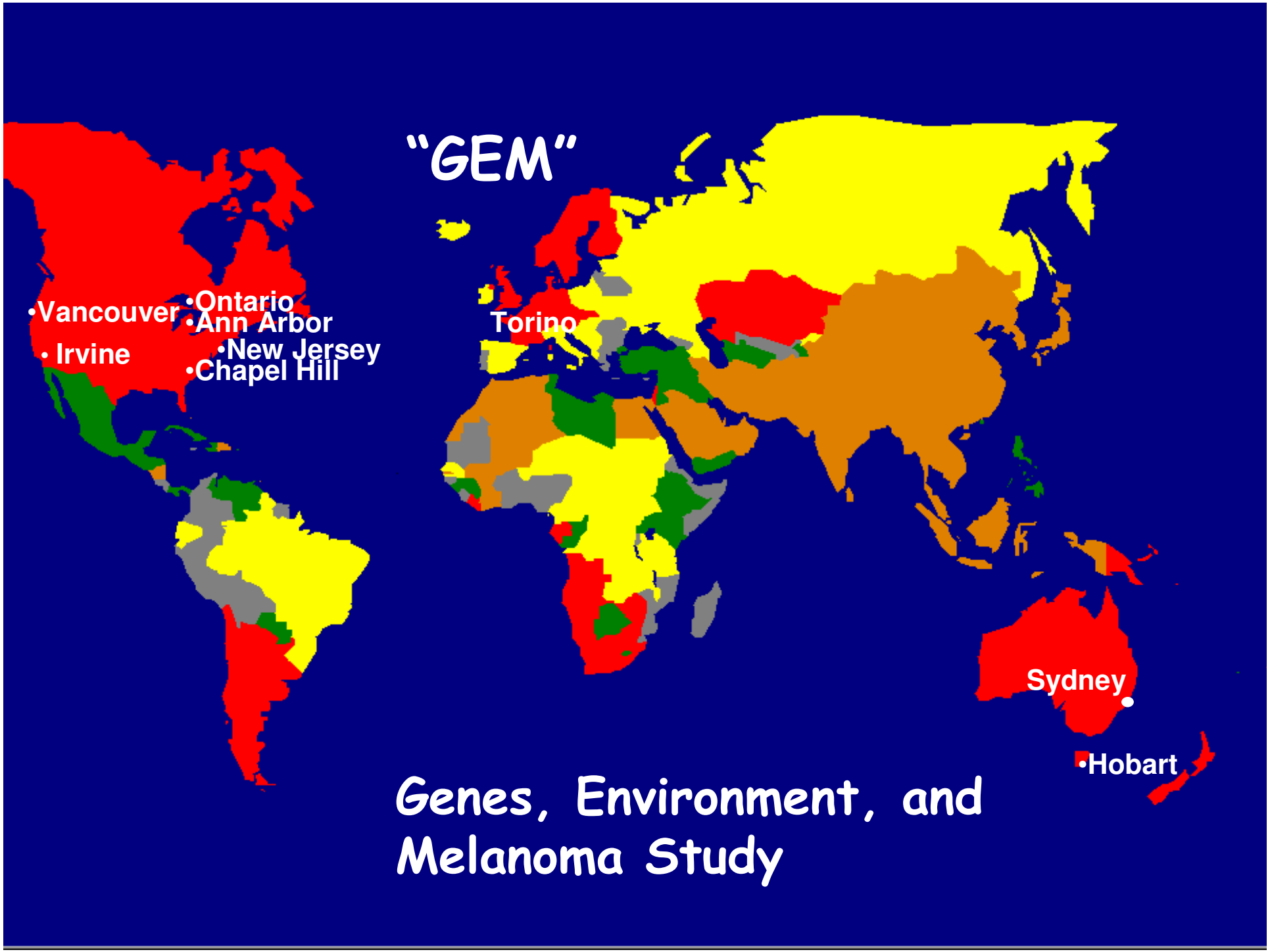
Questions

Do childhood and adult sun exposure increase melanoma risk?

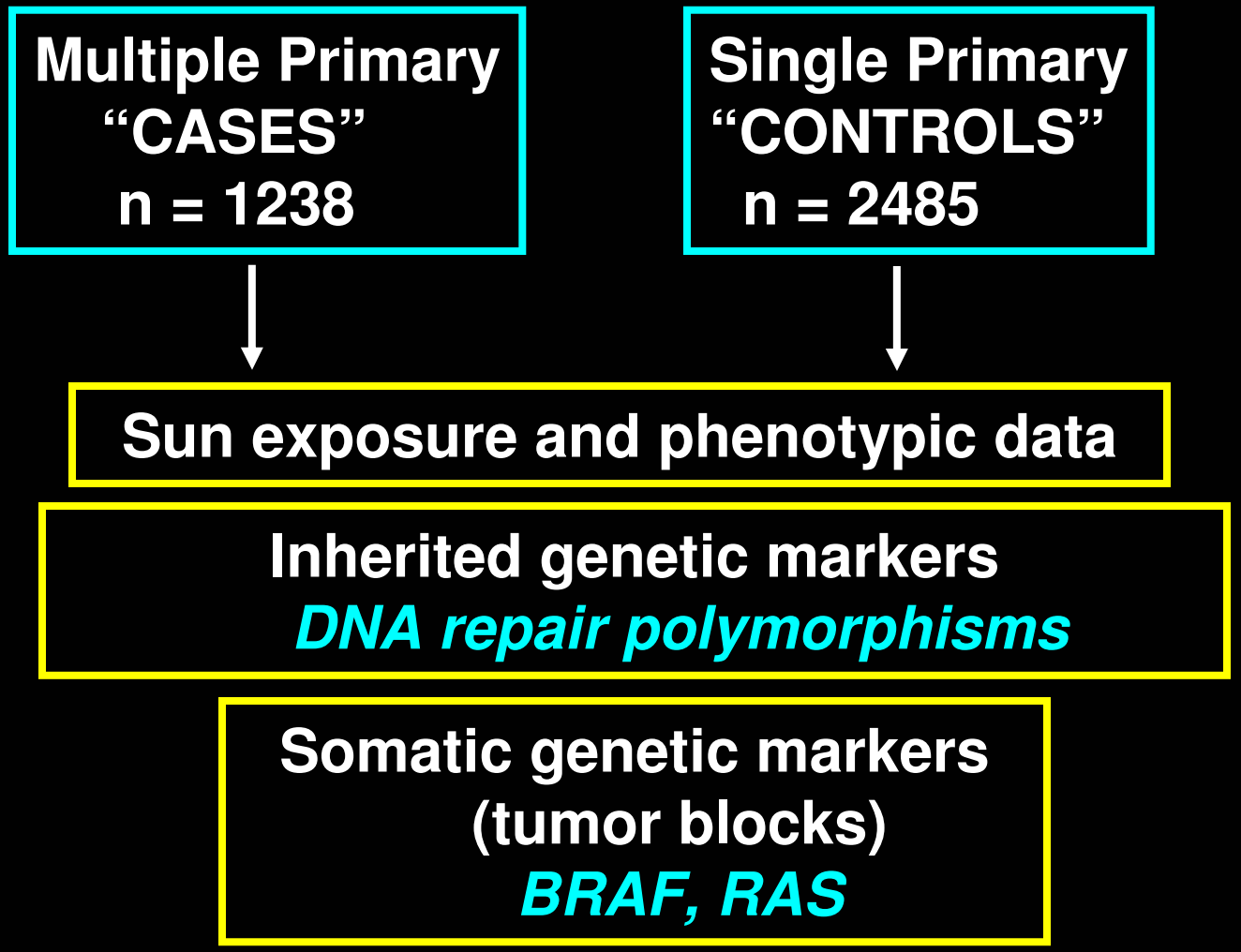
Do common NER polymorphisms increase melanoma risk?

Are melanoma pathways (denoted by mutational status) differentially associated with sun exposure and moles?

Is there a mechanism by which BRAF mutations could arise related to sun exposure?


"GEM"
•Vancouver
•Ontario
•Ann Arbor
• Irvine
•New Jersey
•Chapel Hill
Torino
Sydney
•Hobart
Genes, Environment, and Melanoma Study

GEM Study Design



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Interpretation of Results

Risk of second or higher order melanoma among persons with a first diagnosis of melanoma

approximates

Risk of first primary melanoma among persons who were previously unaffected

Begg. Int J Epidemiol, 2006

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Sunlight Exposure in GEM

Ecologic level	Residence history linked to latitude, zenith angle, ozone column, surface elevation, cloud cover
Individual level	Recreational, occupational, vacations, sunburns

Strong Melanoma Risk Factors

Ecologic level	Childhood sun exposure by residence OR ~ 2
Individual level	Lifetime beach activities & holidays OR ~ 1.5

Kricker et al. Cancer Causes Control, 2006

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DNA repair genes

Nucleotide excision repair gene polymorphisms

XPD, HR23B, XPG, XPC, XPF, ERCC6

DNA repair genes

XPD 312 OR = 1.5 (1.2-1.9), P = 0.004

XPD 751 OR = 1.4 (1.1-1.7), P = 0.004

Strongest for diagnosis before age 30.

Number of *XPD 312 + 751* haplotypes:

trend P = 0.002

Millikan et al. Carcinogenesis 2006

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DNA repair genes

Increased risk with increasing number of variant alleles for all NER genes combined:

trend P = 0.02

GEM Results

Somatic genetic markers

(tumor blocks)

BRAF, RAS

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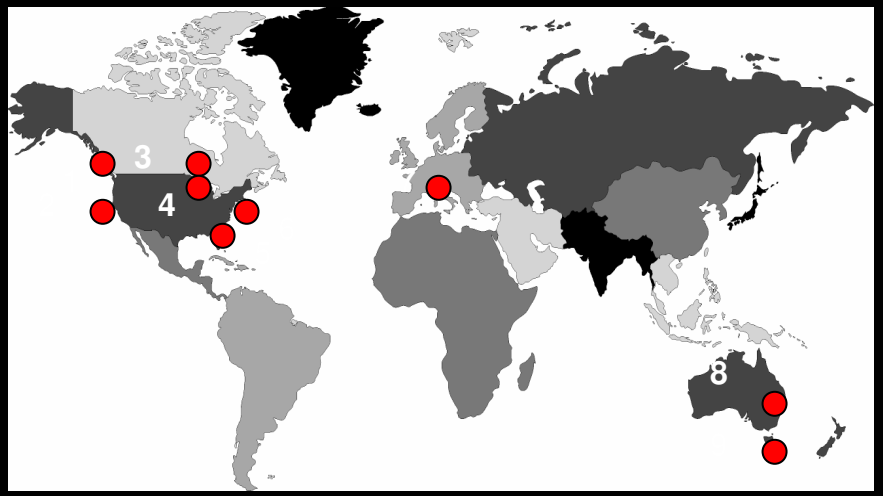
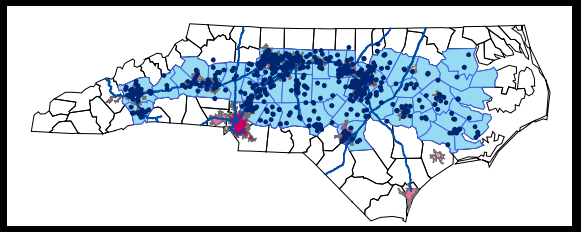
Nested GEM Study



214 cases in North Carolina, 2000



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NC Cases (N=214)

Mean 51.8 years; 55% male

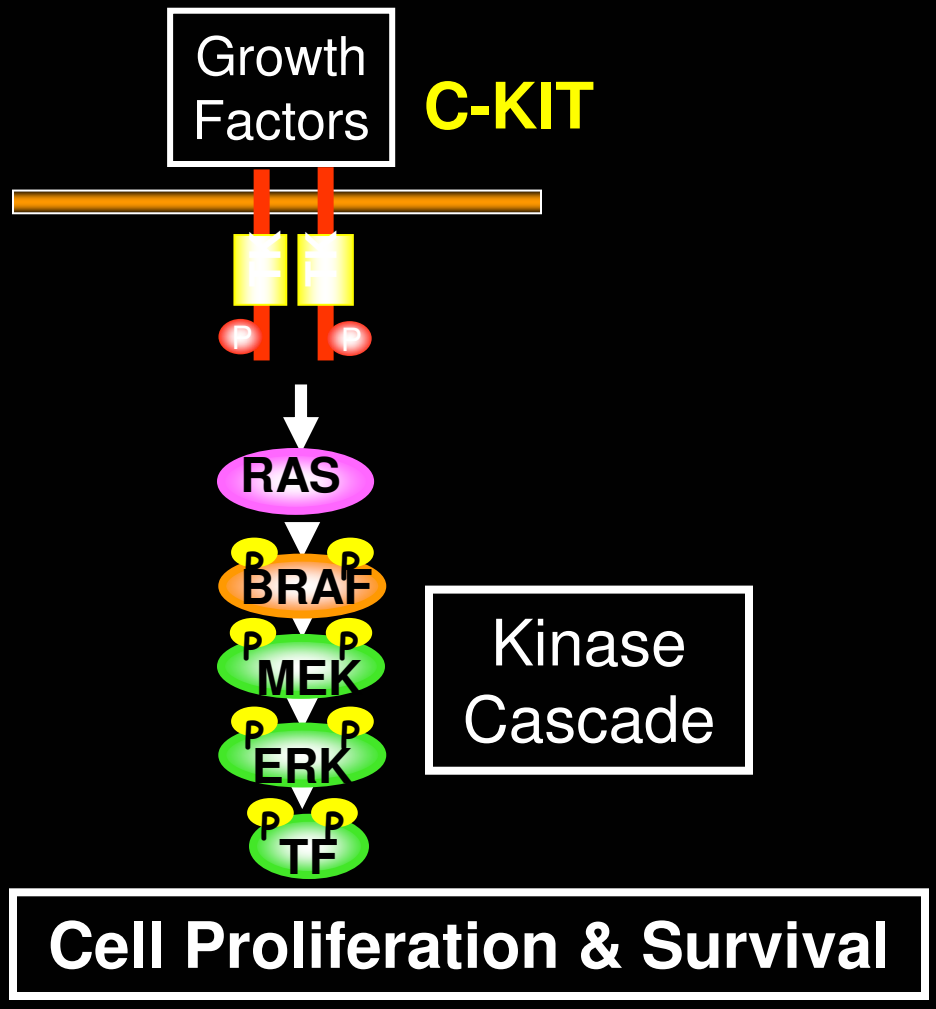
55.5% thin (< 0.75 mm)

79.7% SSM, 4.5% NM, 10.4% LMM, 5.5% other

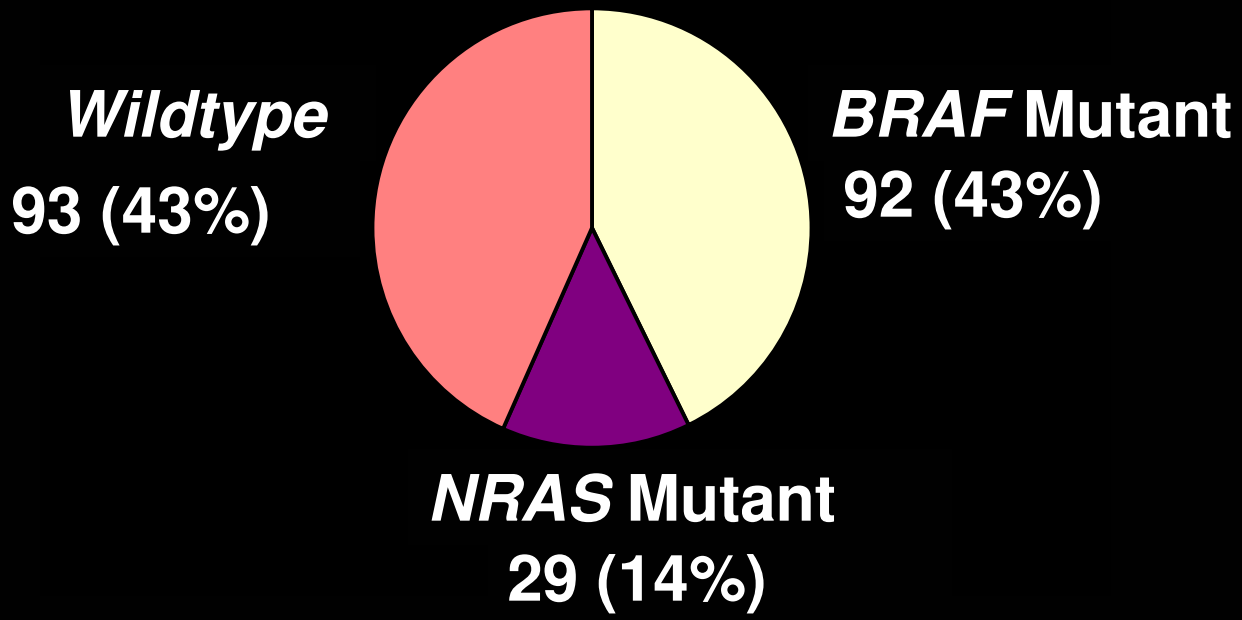
Mutually Exclusive

BRAF+	BRAF-	BRAF-
NRAS-	NRAS+	NRAS-
		(wildtype)

MAPK Kinase Pathway Activation



Prevalence



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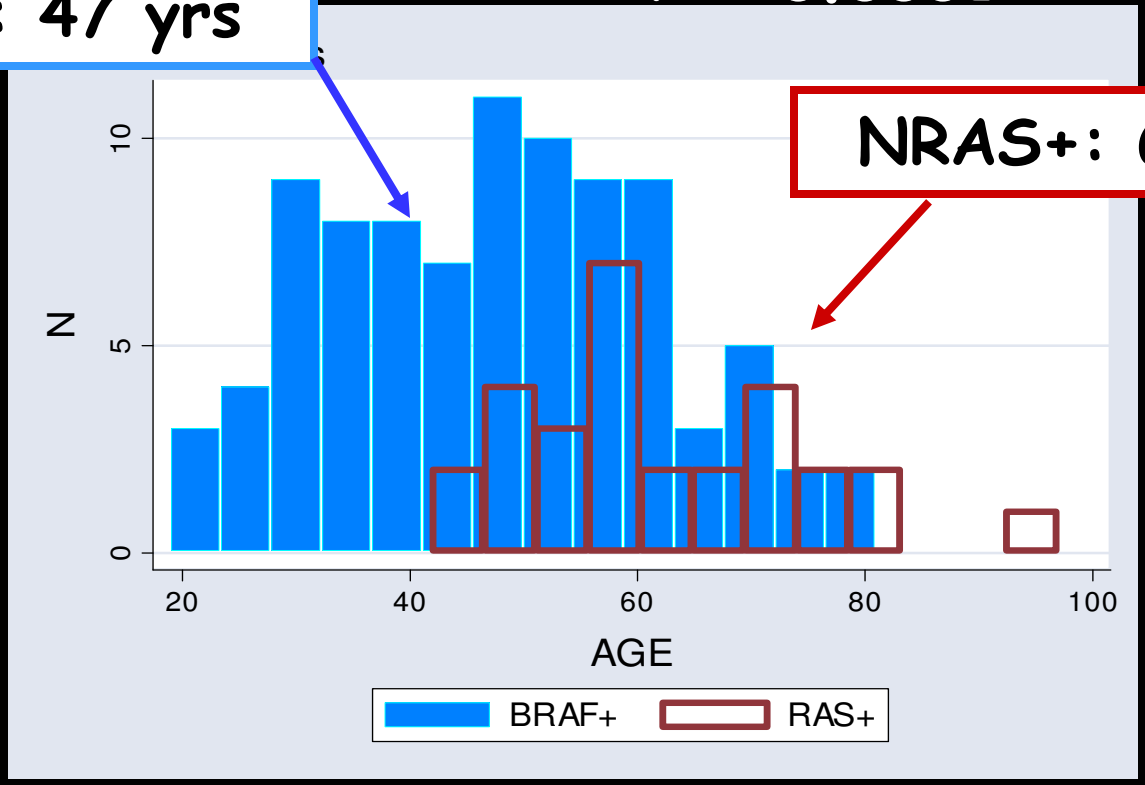
NRAS+ Decade older than BRAF+

BRAF+: 47 yrs

P < 0.0001



Thomas et al., CEBP 2007

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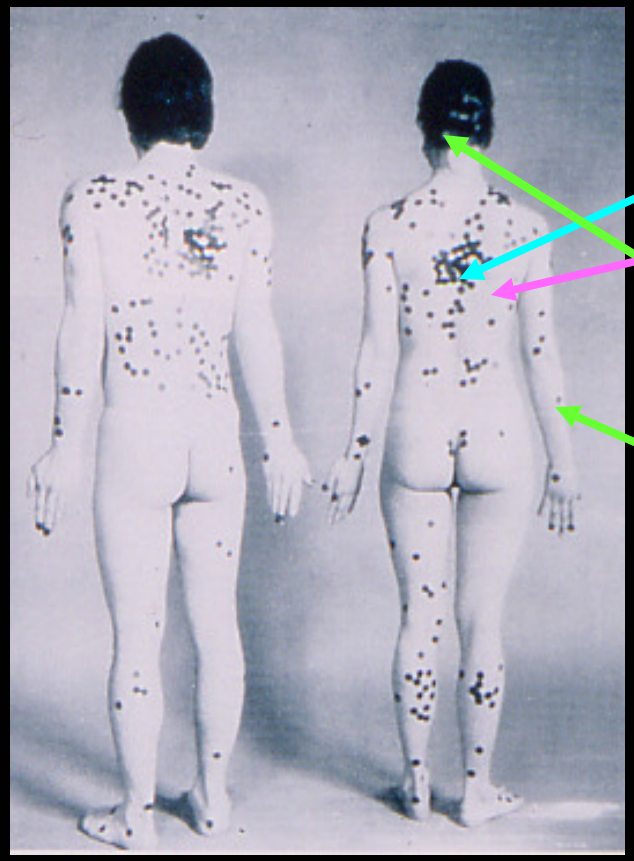
Anatomic Site



BRAF+

NRAS+

BRAF-

NRAS-

(wildtype)

Thomas et al. Cancer Epidemiol Biomarkers Prev 2007

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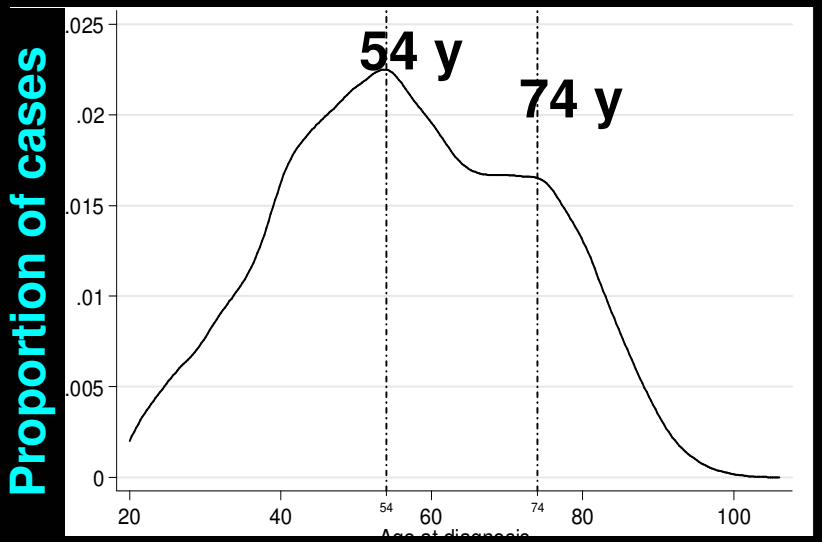
Similar Evidence in SEER



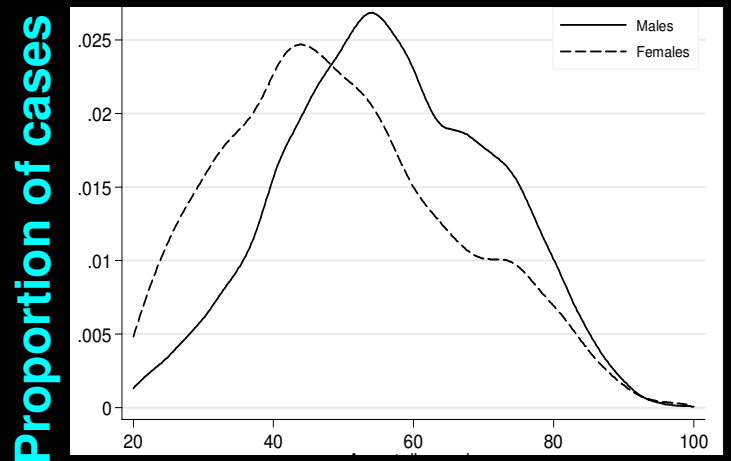
Age-density distribution

2000-2004

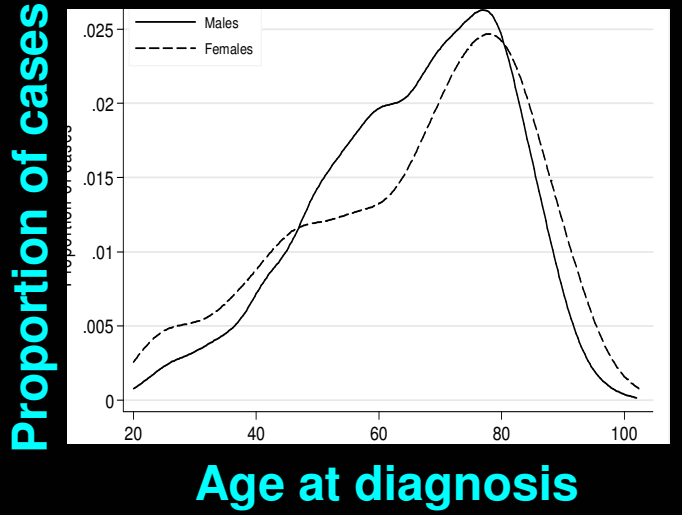
(n=48,673)

Lachiewicz et al. JID 2007

Trunk



Face, ears



North Carolina GEM Cases
Tumor characteristics

BRAF +	• SSM, NM • Low solar elastosis
NRAS +	• SSM, NM
BRAF – RAS –	• LMM • High solar elastosis

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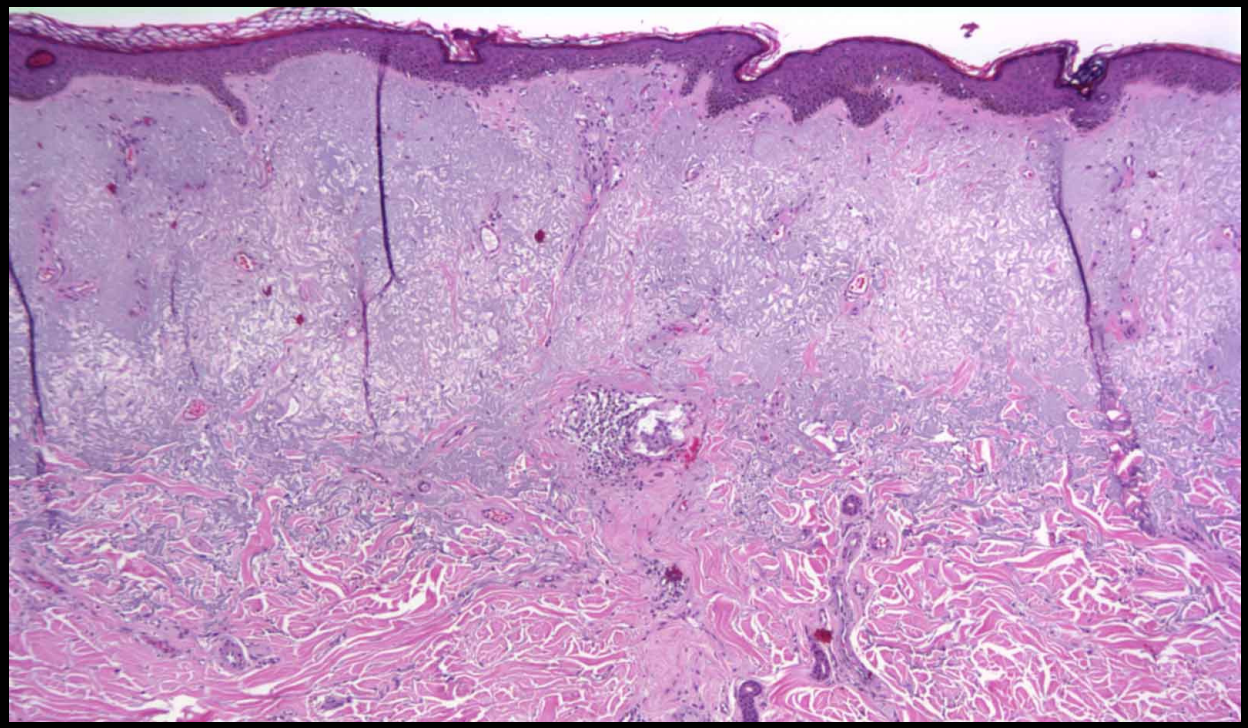
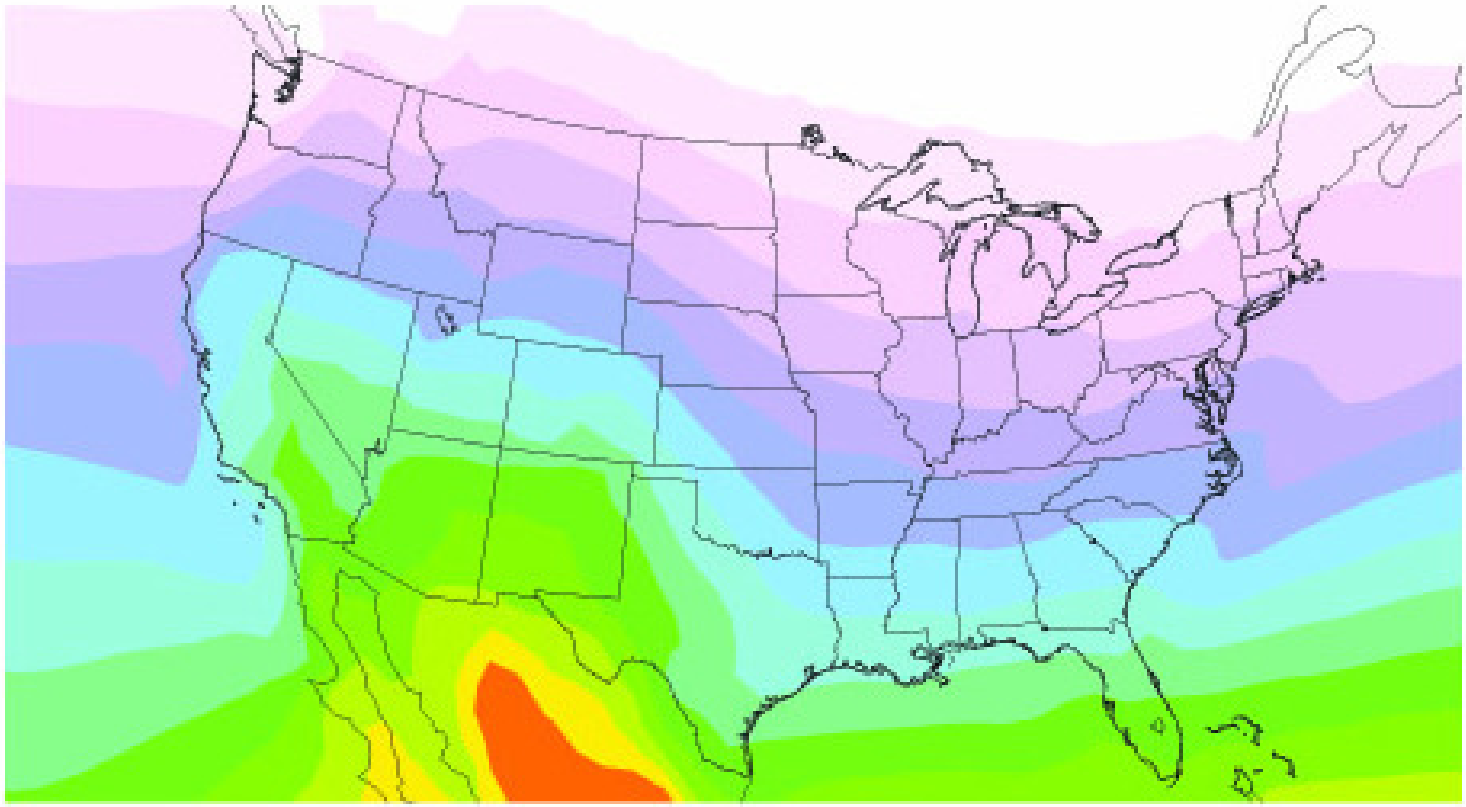
Histologic Evidence of Solar Elastosis

Homogenization of the superficial dermis



Erythemal UV Irradiance



Erythemal UV (250-400) KJ/m^2/yr 1979-2000 avg.

600 800 1000 1200 1400 1600 1800

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Ambient Erythemal UV Exposure

Ambient Annual UV	*BRAF*+ vs WT Age-adj OR (95% CI)	*NRAS*+ vs WT Age-adj OR (95% CI)
Lifetime		
Low UV	1.0	1.0
High UV	2.0 (1.0-4.0)	1.1 (0.4-2.7)
Early life		
Low UV	1.0	1.0
High UV	2.6 (1.2-5.3)	0.9 (0.4-2.2)

Thomas et al. Cancer Epidemiol Biomarkers Prev 2007

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Age of Ambient Erythemal UV Exposure

High UV irradiance	*BRAF*+ vs Wt Age-adj OR (95% CI)	*NRAS*+ vs WT Age-adj OR (95% CI)
Birth year	2.0 (1.0-4.1)	0.9 (0.4-2.2)
Age 10	1.9 (1.0-3.9)	0.8 (0.3-1.9)
Age 20	2.7 (1.3-5.7)	0.8 (0.3-1.9)
Age 30	1.0 (0.5-1.9)	0.7 (0.3-1.8)
Age 40	1.4 (0.6-3.3)	1.3 (0.5-3.4)
Age 50	1.2 (0.4-3.8)	2.5 (0.7-8.5)
Age 60	1.1 (0.2-7.0)	2.0 (0.4-9.8)

BRAF and *NRAS* Mutations in Moles

- About 70% of moles have *BRAF* mutations
- Some moles have *NRAS* mutations
- Great majority of moles do not progress to melanoma

Pollock Nat Genet 2002; Kumar JID 2003; Yazdi JID 2003

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Associations with Moles

Characteristic	*BRAF*+ vs WT Age-adj OR (95% CI)	*NRAS*+ vs WT Age-adj OR (95% CI)
Back mole counts		
0-4	1.0	1.0
5-14	2.4 (1.1-5.5)	1.2 (0.4-3.7)
> 14	3.2 (1.4-7.0)	1.7 (0.6-4.8)
P_{trend}	0.006	0.34
Mole density diagrams		
None	1.0	1.0
Low	2.3 (1.0-5.2)	2.7 (0.8-8.6)
Medium to high	3.8 (1.4-10.4)	3.3 (0.7-14.9)
P_{trend}	0.009	0.10

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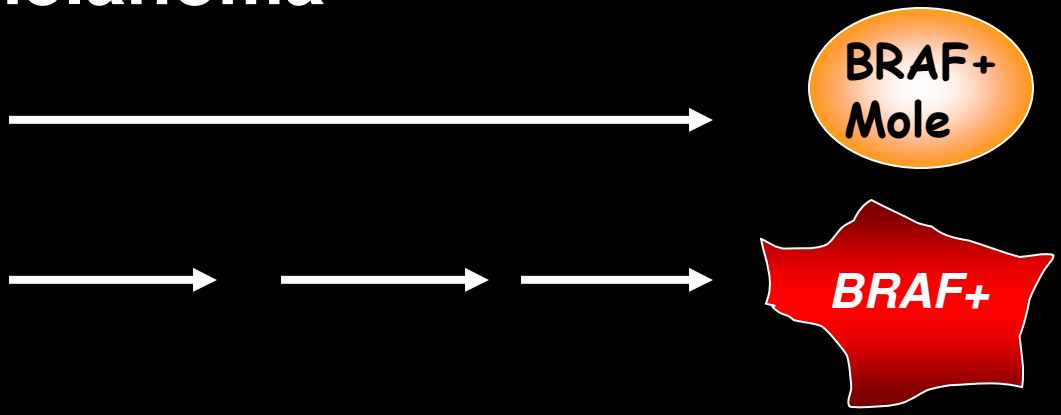
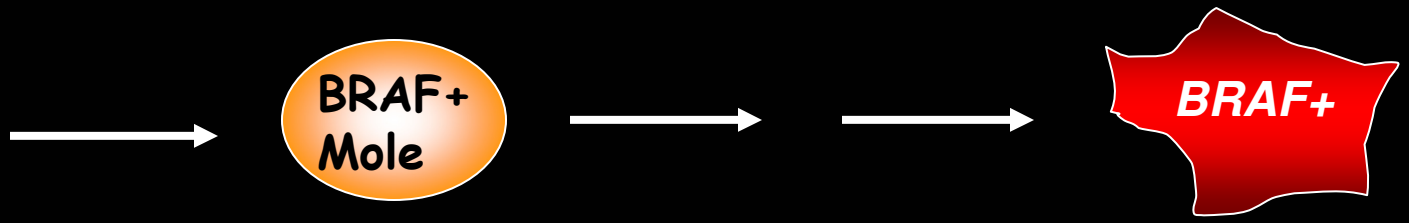
Multivariate Model

Characteristic	*BRAF*+ vs Wt Age-adj OR (95% CI)	*NRAS*+ vs Wt Age-adj OR (95% CI)
Age at diagnosis (per 10 yrs)	0.8 (0.7-1.0)	1.4 (1.1-1.9)
Back mole counts		
0-4	1.0	1.0
5-14	2.8 (1.2-6.4)	1.1 (0.4-3.3)
> 14	3.4 (1.5-7.8)	1.9 (0.6-5.5)
P_{trend}	0.004	0.27
Early life UV		
Low UV	1.0	1.0
High UV	2.6 (1.2-5.6)	0.9 (0.4-2.2)

Are Moles Causal Intermediates for Some *BRAF*+ Melanomas?

- **Mole-prone individuals are more likely to have *BRAF*+ melanoma**





- **Presence of a contiguous mole was associated with *BRAF*+ melanomas**

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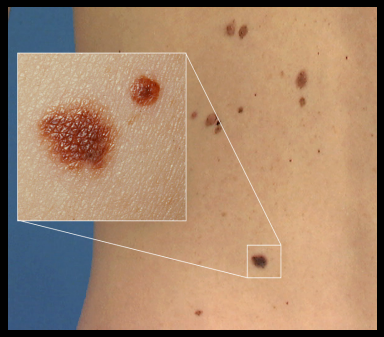
Melanoma Models



Mole Prone

Early life

BRAF+

Later life?

NRAS+

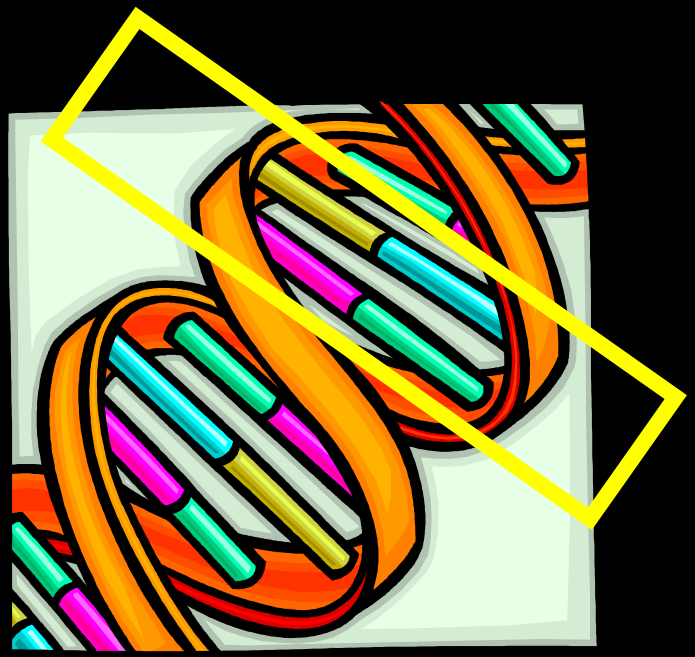
Mole Resistant

Habitual Sun?

NRAS- BRAF-

↑ AGE

Tandem BRAF Mutations



10% of melanomas; rare in other BRAF-mutant tumors

Tissue-specific UV exposure?

Proposed mechanism:

- Nearby potential pyrimidine dimer sites
- Specialized DNA polymerases

J Invest Dermatol 122:1245-50 (2004) and 126:1693-6 (2006)

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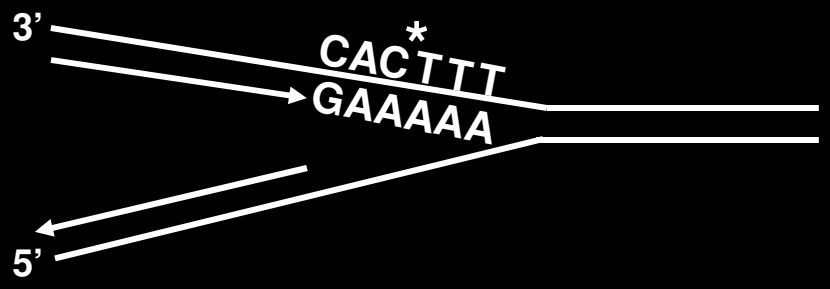
BRAF Mutations in Melanomas

Wild-type:

```
          * * *
3' CGATGACACTTTAGA
5' GCTACAGTGAAATCT
    *      * *
```

* Di-pyrimidines, potential sites for photoproduct formation



Mutagenic bypass of UVB-induced DNA lesions?

```
3' CGATGACTCTTTAGA
5' GCTACAGAGAAATCT
```

t1799a Mutant

```
3' CGATGACTTTTTAGA
5' GCTACAGAAAAATCT
```

tg1799aa Tandem Mutant

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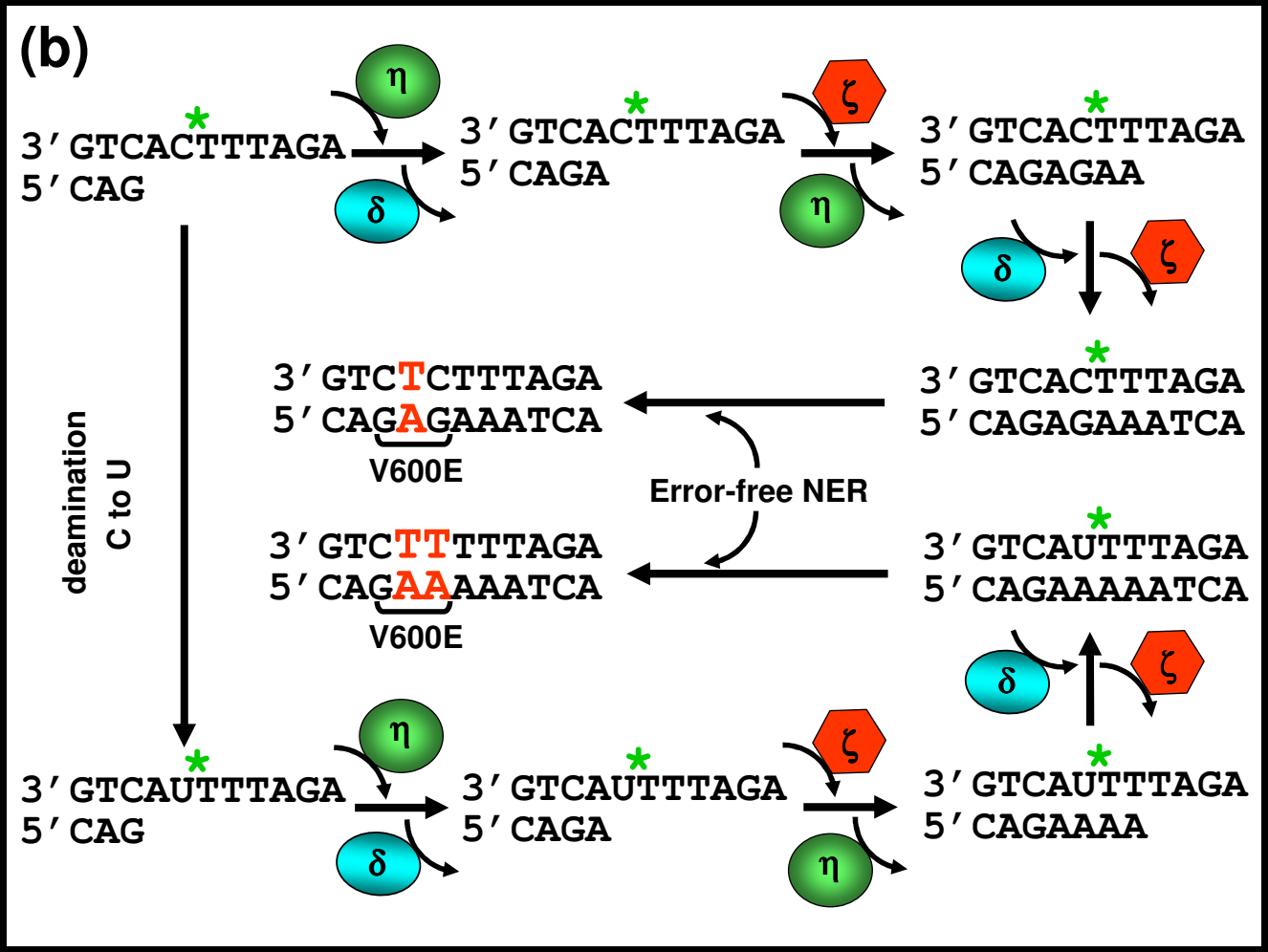
Inaccurate Polymerization?



Thomas NE, Berwick M, Cordeiro-Stone M, JID 2006

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Answers

Childhood & adult sun exposure increase melanoma risk

Common NER polymorphisms increase melanoma risk

> OR with high waterside sun exposure

Melanomas pathways are differentially associated with sun exposure, modified by nevus propensity

BRAF mutations could arise from a mechanism involving nearby potential pyrimidine dimer sites, specialized DNA polymerases, and powerful selection

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Future Plans

7 GEM sites participating in somatic tumor BRAF NRAS analysis

~1000 cases for analysis of risk and outcome

Relationship of XPD polymorphisms with NRAS and BRAF somatic mutations is being examined

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Collaborators

UNC-GEM Melanoma Group

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NCAR - Atmospheric Chemistry Division

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