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# Possible genetic controls of differing susceptibility to skin sensitisation

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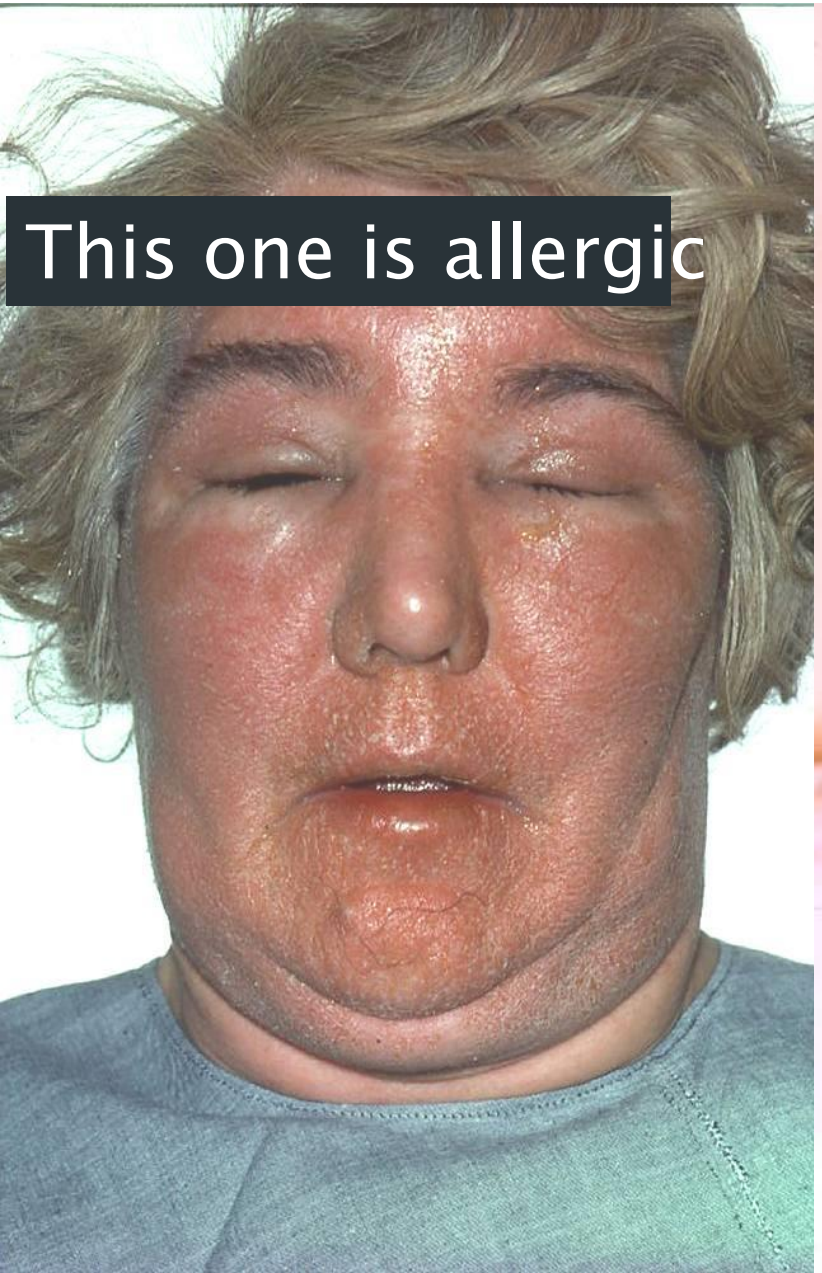
**University of Southampton**

# What's the difference between these women??

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# Is there evidence for genetic controls of susceptibility to contact sensitisation?

- What can we learn from drug allergy?
- Consider whether there is any evidence of different susceptibilities
- Is susceptibility specific for particular chemicals or general – susceptibility to becoming allergised?

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# Drug Allergy – very strong genetic evidence

| Reaction Type | Drug | HLA Association |
|---|---|---|
| Hypersensitivity Syndrome (DRESS/DIHS) | Abacavir<br>Allopurinol<br>Carbamazepine | B*57:01<br>B*58:01<br>A*31:01 |
| Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis | Allopurinol<br>Carbamazepine<br>Phenytoin<br>Sulfamethoxazole | B*58:01<br>A*31:01<br>B*15:02<br>B38 |
| Drug exanthem | Carbamazepine | A*31:01 |

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# Conclusion

- **For certain drugs there is very clear and strong evidence of genetic determination of susceptibility**

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# What is the evidence that genetic controls exist for contact allergy?

- Animal studies
- Family incidence of susceptibility

# Animal studies

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- **Chase (1941)**
  - **Reactivity to DNCB or poison ivy could breed true – both strong and weak reactivity**

Chase (1941) J Exp Med, 73: 711 – 726

- **Various strains of mice shown to have genetic linkage controlling reactivity to various metals, DNFB and other. Relevant linkage almost all with H2 and Ia regions (Homologues of human HLA Class I and II).**
- **Most mice cannot be sensitised to Ni because of a lack of key histidine residues in the TLR4 receptor – Ni binds histidines, hence in humans, TLR4 is activated**

# Animal studies 2

- **Simple but very clear data from Polak (1968)**
  - Guinea pigs of strain II can be made allergic to dichromate and Beryllium but NOT Hg
  - Guinea pigs of strain XIII are the reverse

Polak, Barnes & Turk (1968) Immunology, 14: 707-711

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# Family incidence of susceptibility

- Anecdotal history – someone else in my family is also allergic to ….
- Forsbeck (1971): 404 relatives of 94 people with ACD were patch tested; +ve reactions in 30% of relatives of ACD cf 18% in relatives of controls
- Experimental data: Walker used 2 sensitisers – DNCB (v potent) and NDMA (moderate).
  - 99 families, 301 individuals
  - Sensitised parents and children with both chemicals
  - With DNCB – only weak evidence of familial susceptibility
  - With NDMA if both parents were sensitised then 91% of children were
  - If only 1 parent was sensitised then 53% of children were

Walker, Smith & Maibach (1967) Int Arch Allergy Appl Immunol 32: 453-62

# What's the problem with the last slide?

- Forsbeck lumped ALL allergies together while Walker looked at basically 1 allergen
- So we must bear in mind at least 2 possibilities:
  - 1) Genetic control of general susceptibility to sensitisation by anything – non-specific susceptibility
  - 2) Specific susceptibility to sensitisation by the allergen(s) of interest

# Family incidences of susceptibility – Twin studies

- **Conflicting data**
  - Forsbeck et al (1968): Insignificant concordance in monozygotic; no difference in sensitisation between MZ and DZ twins
  - Menne & Holm (1983): Ni sensitisation more in monozygotic twins cf dizygotic twins
  - Bryld et al (2004): Very small concordance rate in MZT; Nickel sensitisation dependent on environmental factors (suspenders, piercing etc)

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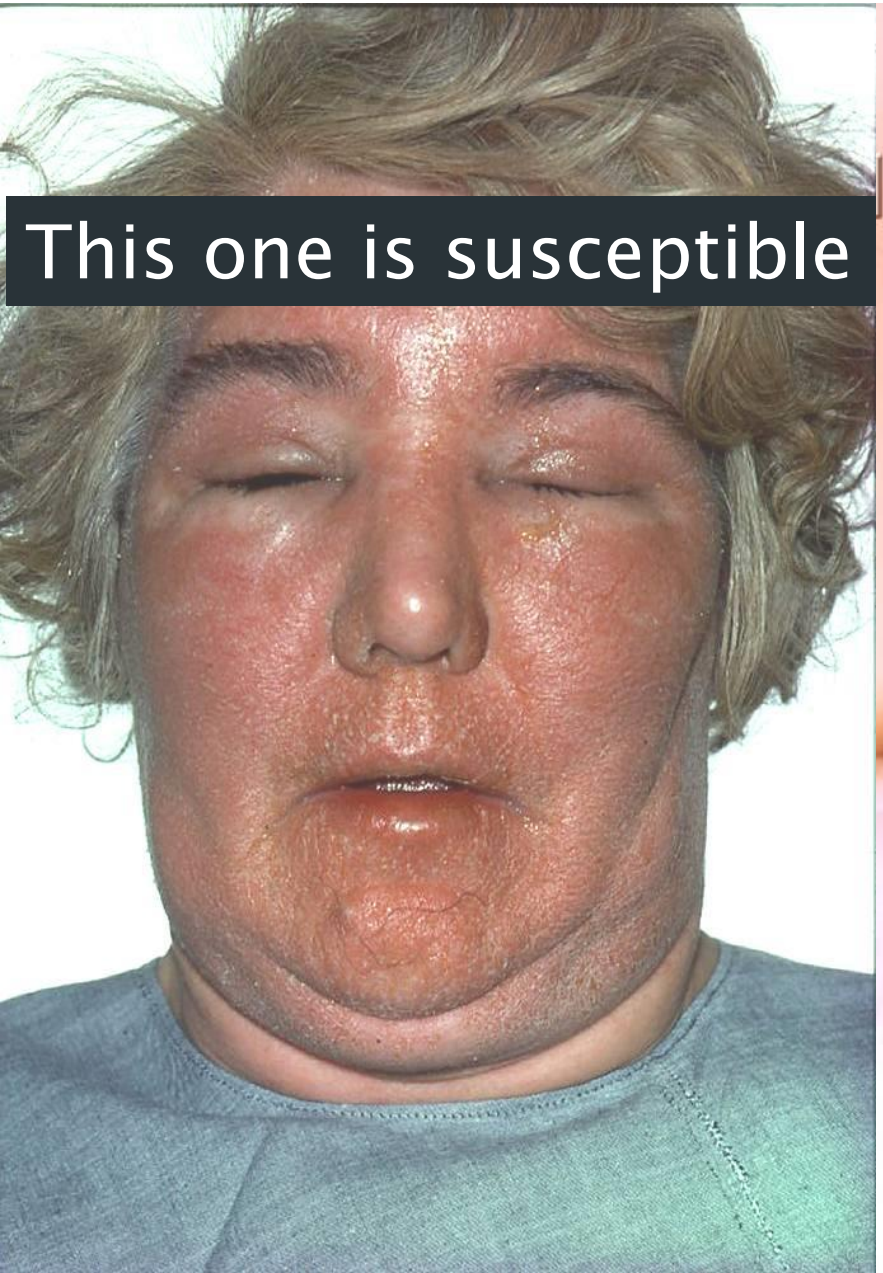
# What evidence for a phenotype of increased susceptibility?

# What's the difference between these women??




# Human dose response studies with DNCB

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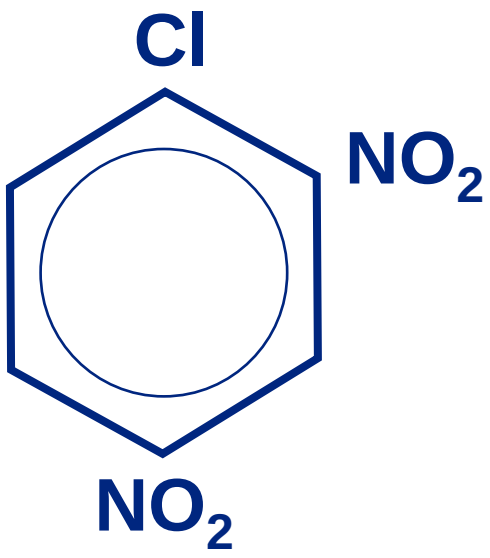
1. **Sensitise normal volunteers with increasing doses of**

   **Dinitrochlorobenzene (DNCB)**




5 groups of normal

Each received a
Different sensitising dose:
62.5, 125, 250, 500, 1000μg
on a 3cm circle on forearm

4 weeks later challenge with
4 small doses

# Proportions sensitised by increasing doses of DNCB

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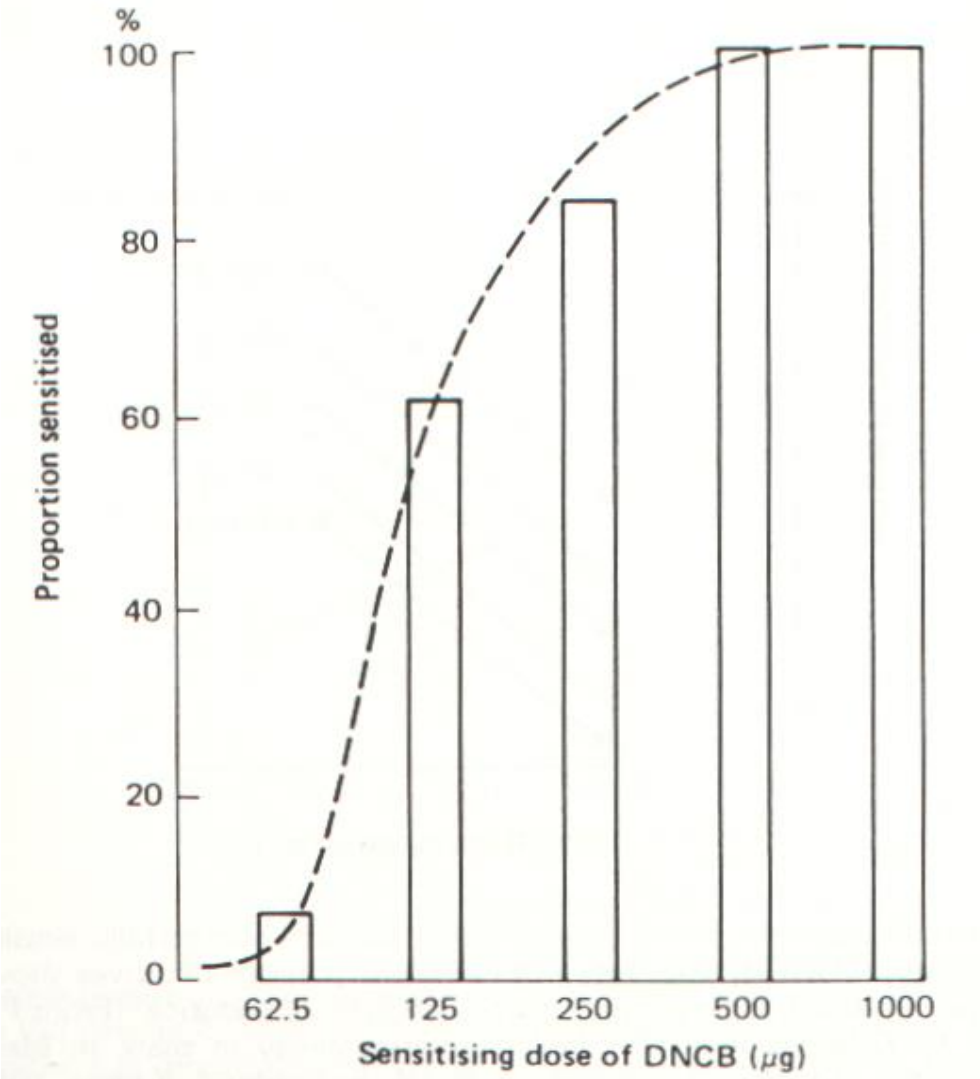

# Increase of sensitivity with increasing sensitising dose

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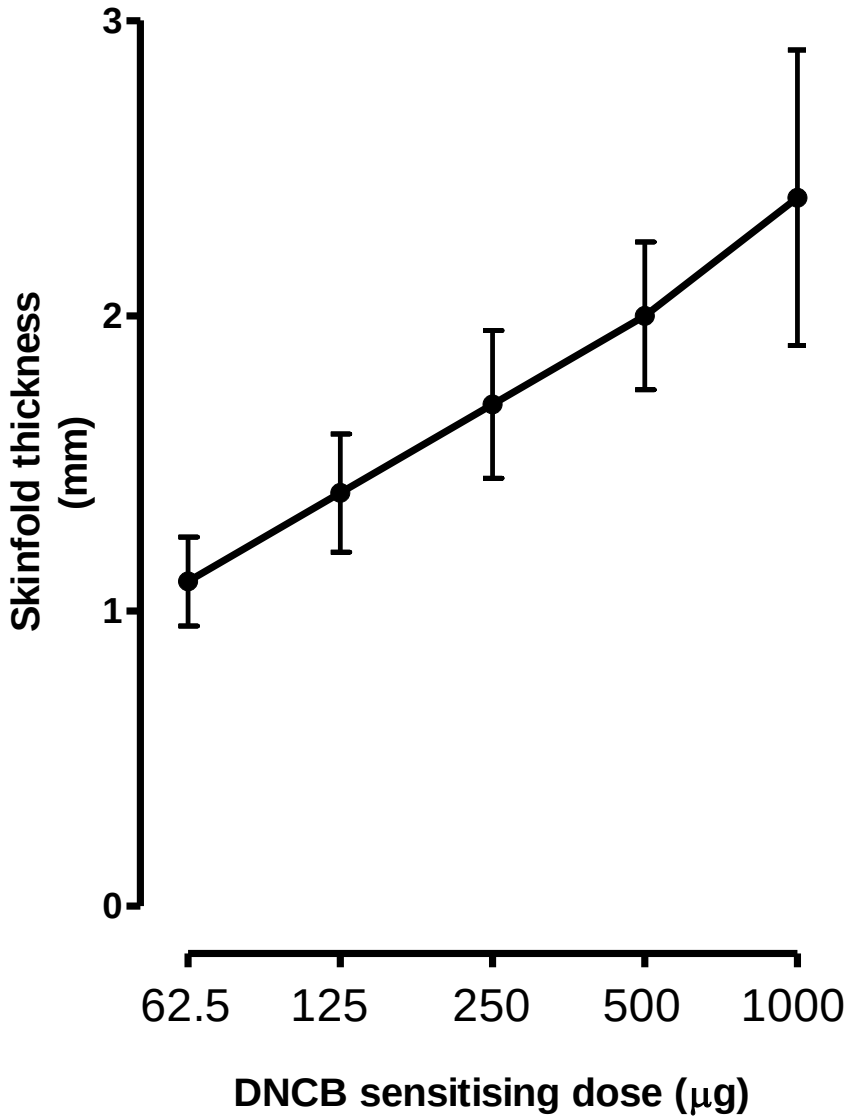

# Proportions sensitised by increasing doses of DNCB

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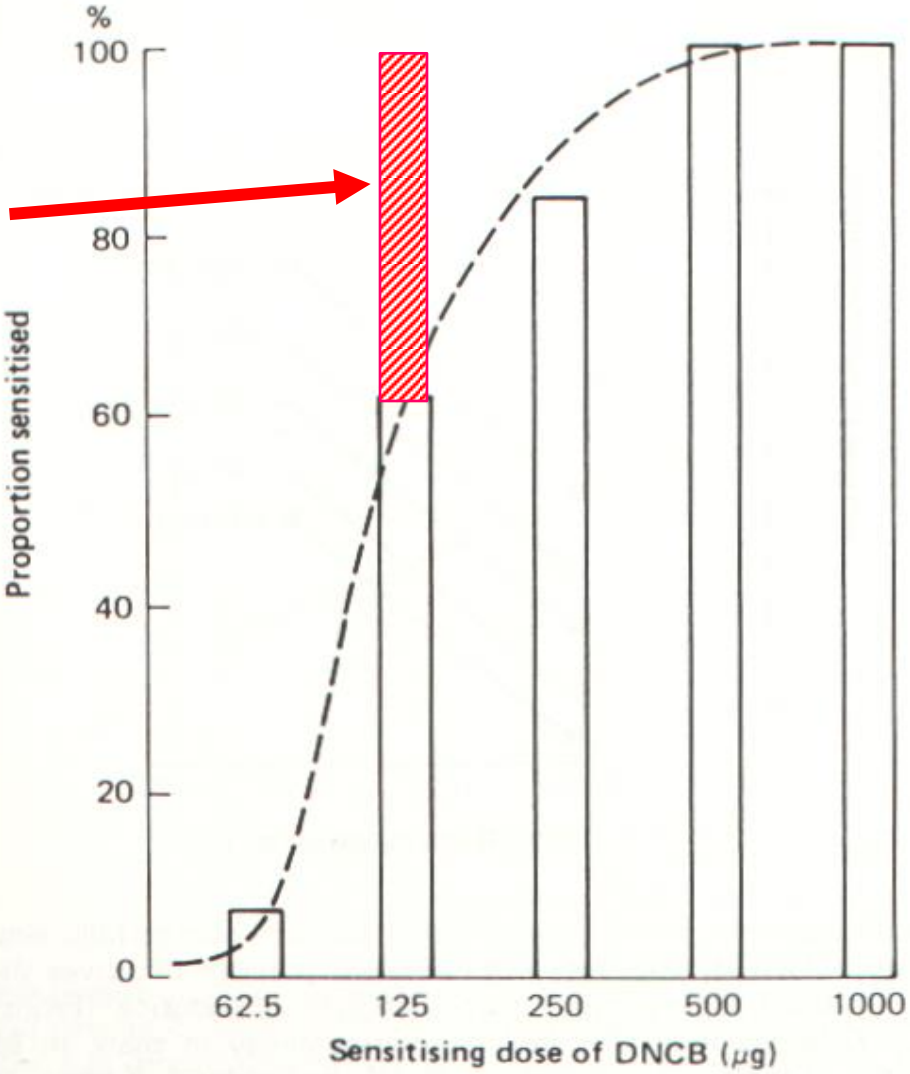
What about the 40% who Did not sensitise?



# Can we demonstrate "increased susceptibility"?

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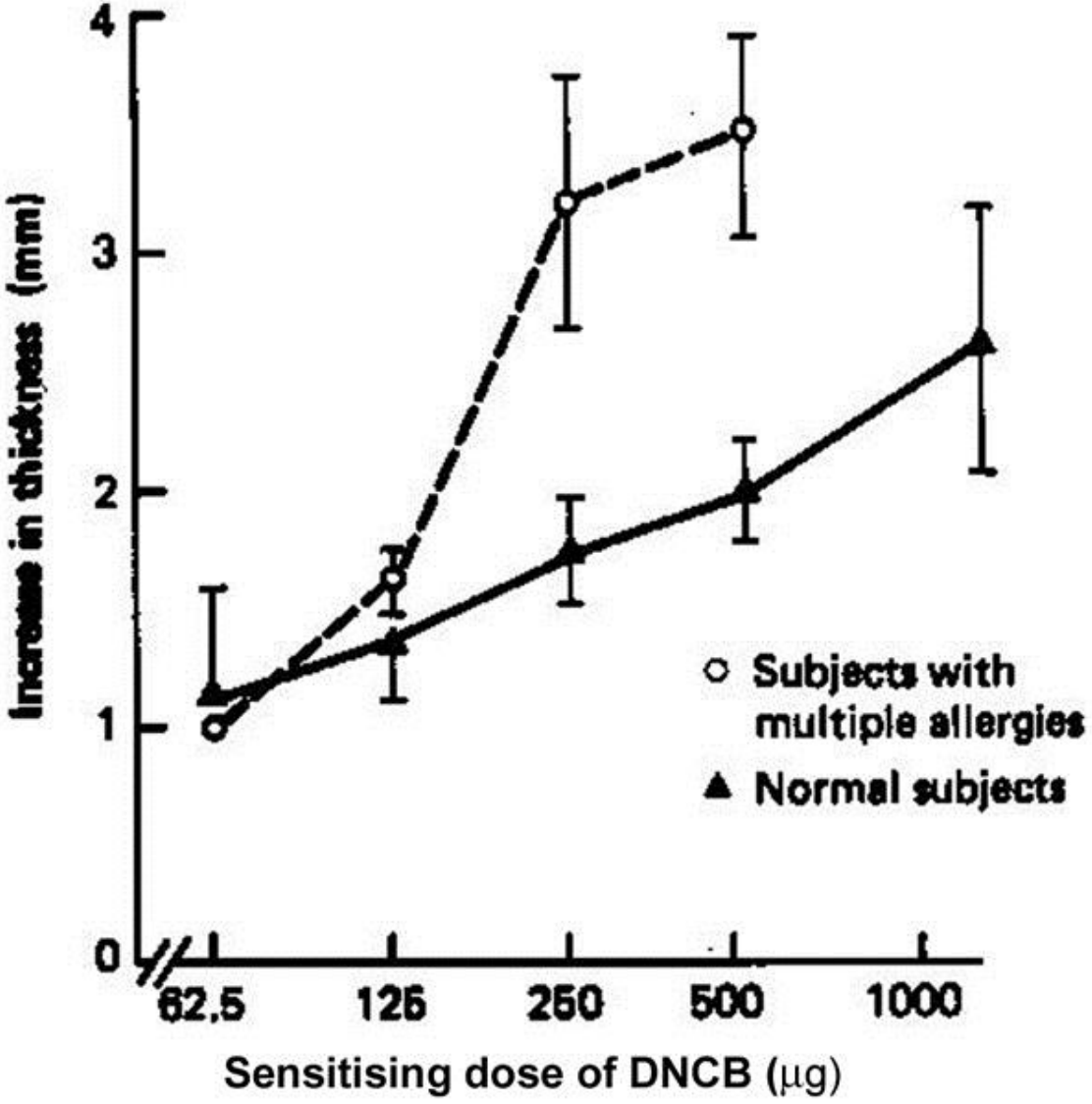
- Two studies have done so in formal experimental tests
  - Moss, Friedmann et al 1985
  - Bangsgaard et al 2010
- Test a group of individuals with "polysensitivities" – at least 3 distinct contact allergies and a group allergic only to 1 allergen or nickel
- Moss: Repeat the DNCB sensitisation protocols using 4 different sensitising doses
- Bangsgaard: Used a single SD of DPCP

## AUGMENTATION OF RESPONSIVENESS WITH INCREASING SENSITISING DOSE OF DNCB



# Comparison of Moss & Bangsgaard

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- **MOSS**
- Sensitise with 4 doses (focus on 35 *μg/cm²* )
- Proportion sensitised:

100%, 100% and 80% controls (N.S.)

- Stronger responses to challenge in polysensitized (P<0.001)
- Elicitation thresholds would be lower – we did not look formally

- **Bangsgaard**
- Sensitise with DPCP *(26μg/cm² = 60 μg/cm² of DNCB)*
- Proportion sensitised:

57% cf 59% cf 65% controls! (N.S.)

- Stronger responses to challenge in polysensitized (n.s.)
- Lower elicitation threshold in polysensitised

# Bangsgaard – response to DPCP challenge

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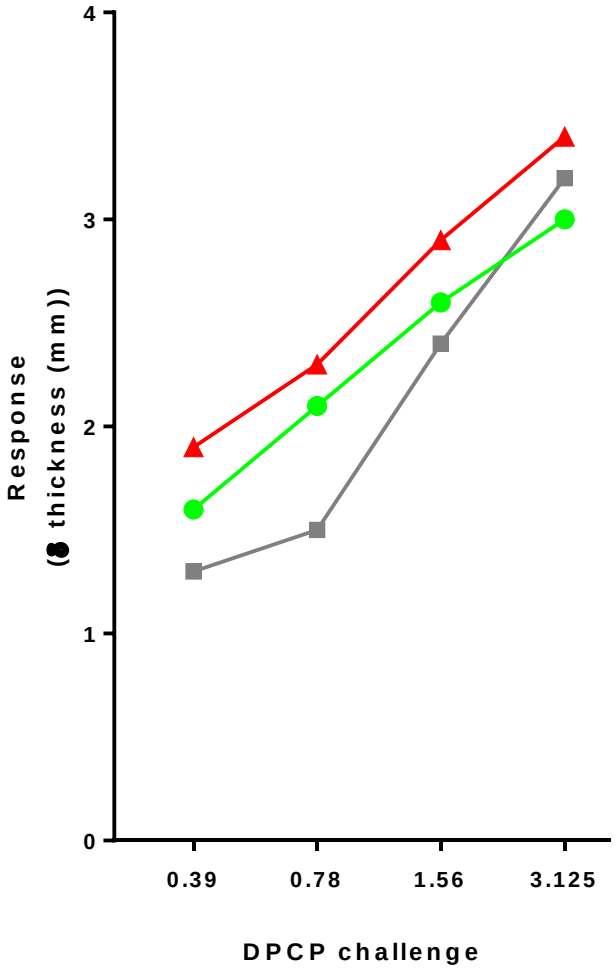
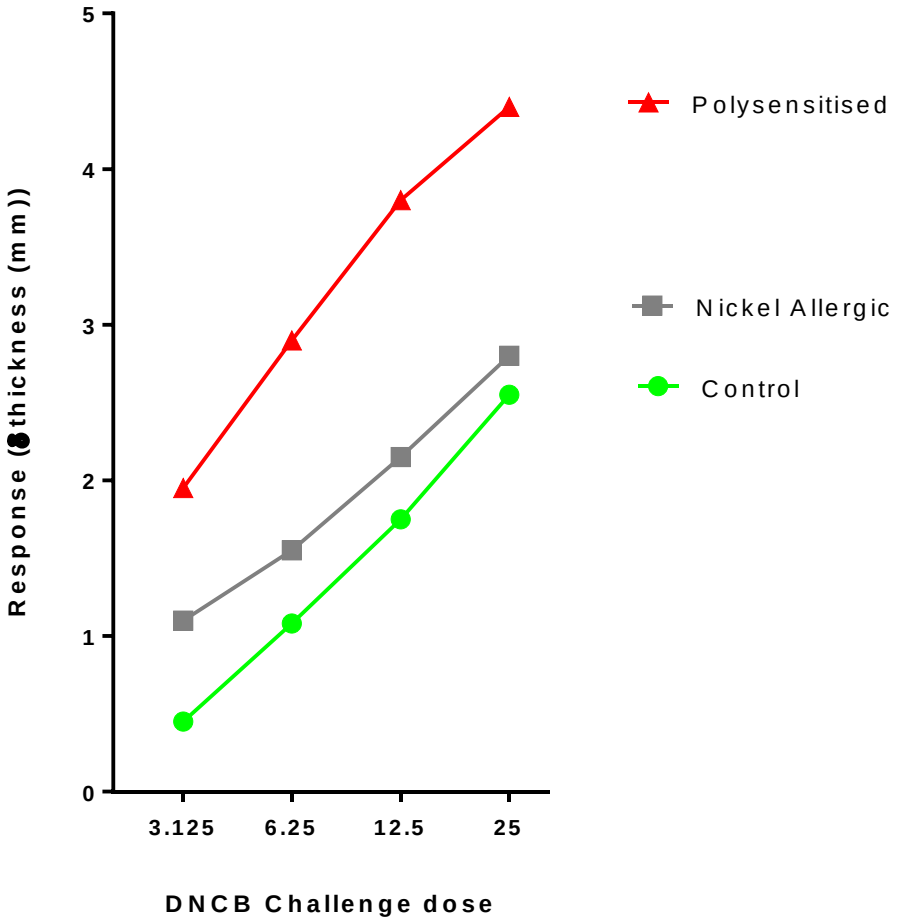
**Responses to challenge with DPCP (sensitised with 26μg/cm²)**




**Responses to challenge with DNCB (sensitised with 35μg/cm²)**



Bangsgaard

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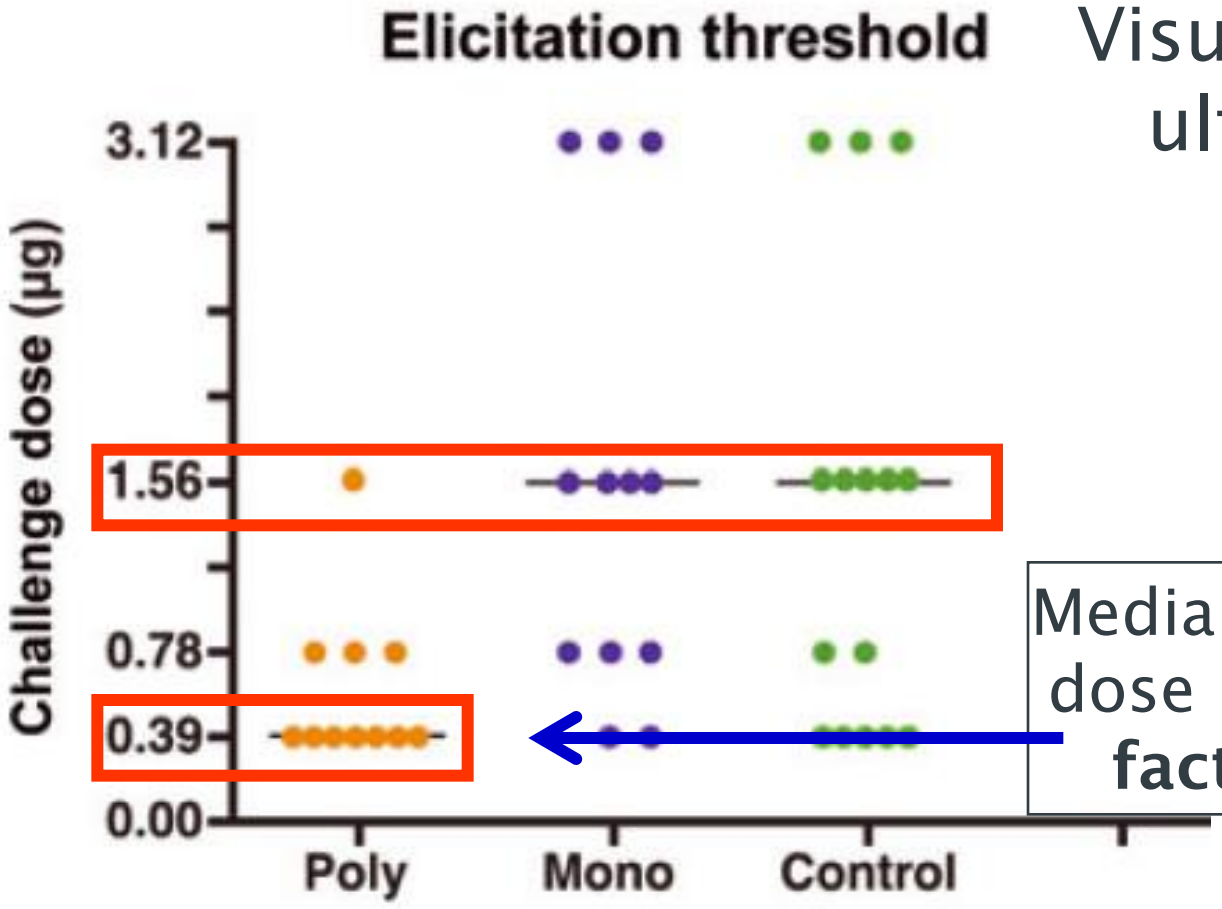


Visual scoring ultrasound

Median challenge dose lower by a **factor of 4 !**

*Bangsgaard N, Carlsen BC, JohansenJD, Menné T, Skov L.* ***2010***

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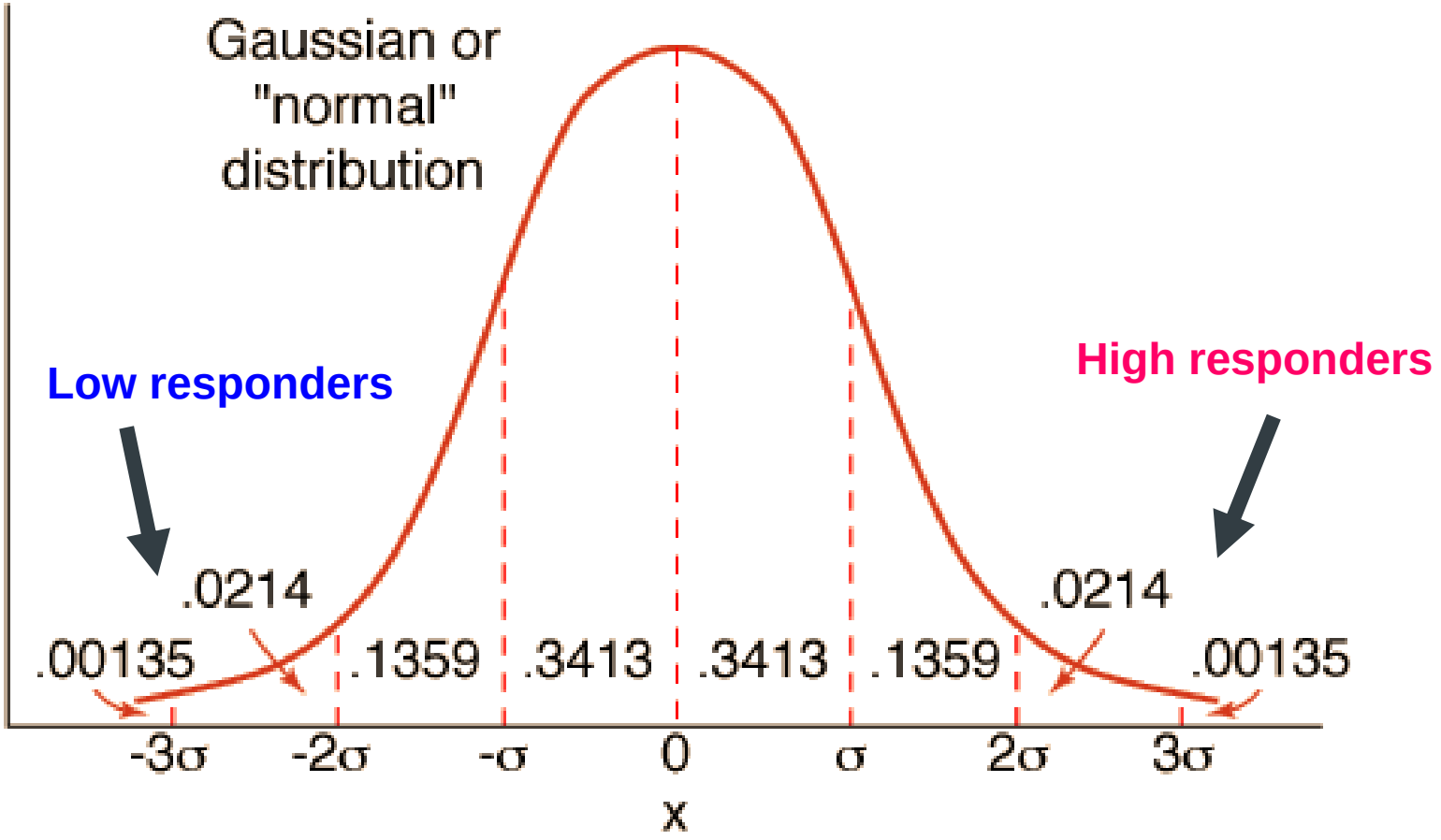
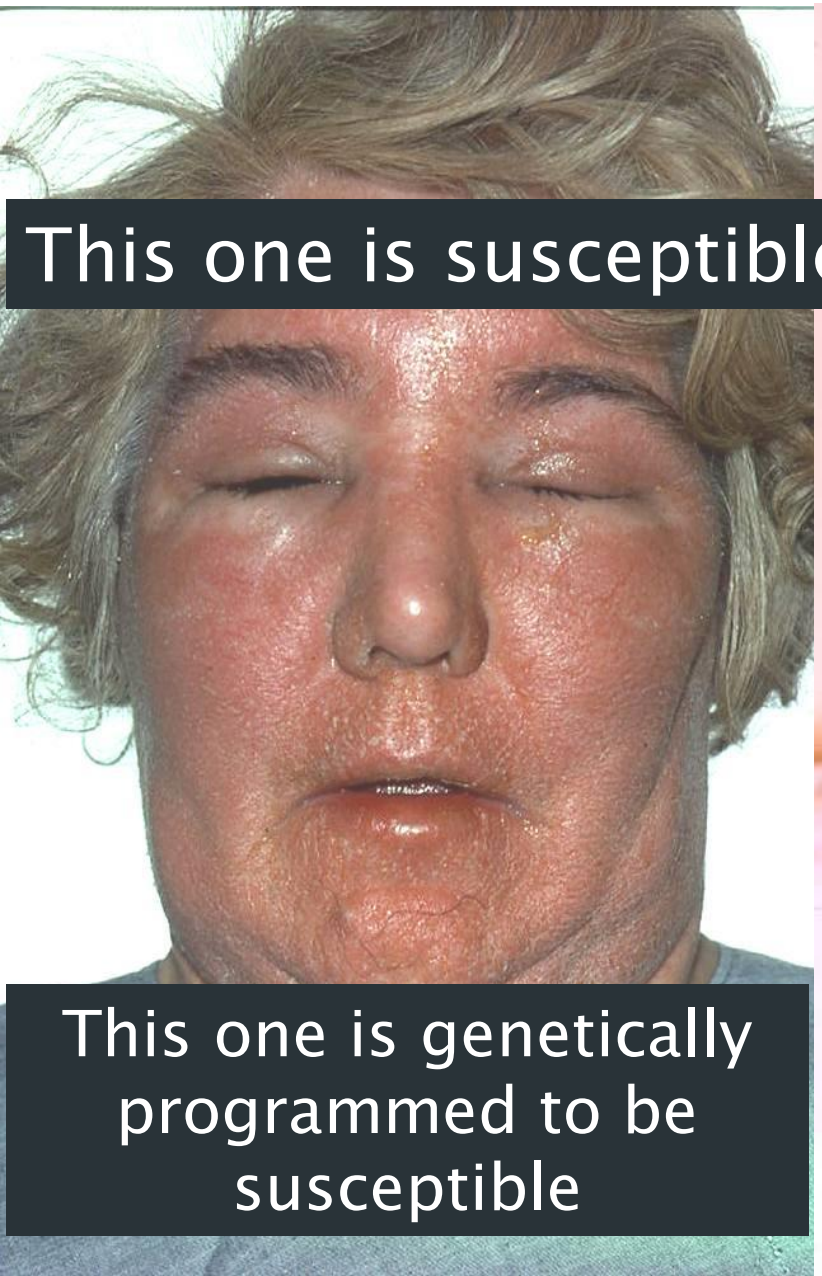
# Who becomes allergic to contact sensitisers??



# What's the difference between these women??



This one is susceptible

This one is genetically programmed to be susceptible

This one is resistant

This one is genetically programmed to be resistant

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# What processes/pathways could be relevant

- Immune response determining
- Drug metabolism
- Epidermal barrier defences

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# What processes/pathways could be relevant

- **Immune response determining:**
  - HLA
  - TCR structure
  - TLR and other PAMPs
  - Cytokine polymorphisms:
    - ✓TNF, IL-6

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# What processes/pathways could be relevant 2

- **Drug metabolism**
  - Phase 1: Cytochrome P450's
  - Phase 2: N-acetyl transferases, GSTs,
  - Anti-oxidant pathways
    - ✓Individual enzymes e.g. MnSOD
    - ✓Broader oxidative stress sensors – Nrf2/keap1
    - ✓Nalp3 inflammasome

# Drug Metabolism

Phase 1

Oxidation
Reduction
Hydrolysis

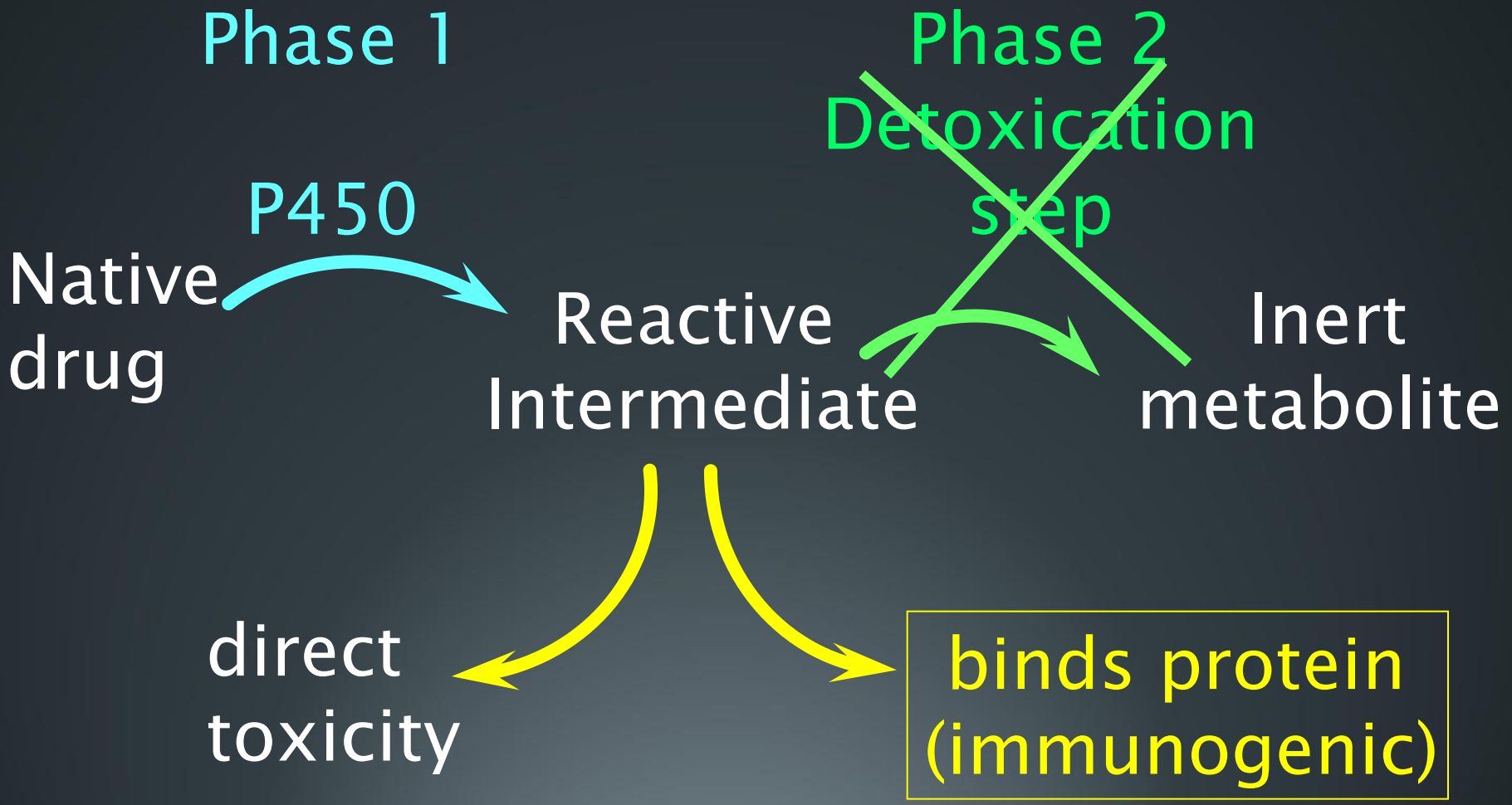
Intermediate metabolite

Phase 2

Glutathione conjugn
Glucuronidation
Sulphation
Methylation
Acetylation
(Sulphas, Dapsone)

# Drug Metabolism





# Xenobiotic metabolic pathways Acetylation

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- **NAT1 & NAT2 acetylate hydroxylamine metabolites – important for PPD.**
  - **Traditionally, slow acetylators are more susceptible to drug allergy and cancer**
  - **Schnuch (2000)**

| Acetylator Status | ACD | Control |
|---|---|---|
| **Fast** NAT2*4 or NAT2*12A | 45% | 30% |
| **Slow** NAT2*5b NAT2*6c | 15% | 31% |

  - **Paradoxical – generation of haptens/sensitisers**

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# Xenobiotic metabolism – glutathione transferase

- GSTs – 7 main classes, GSTM1 and GSTT1 are most important
- Genetic variations e.g. null alleles of GSTM1 or GSTT1 increase risk of some cancers
- GSTs involved in inactivation of organic mercury – deficiency more common in people with Thiomersal allergy
- GSTs involved in inactivation of chromate – GSTT1 (but not GSTM1) polymorphisms increase in Taiwanese cement workers allergic to chromate: 18% cf 3%
- GST important in de-toxication and export of DNCB

# Anti-oxidant defence/stress

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- **Wide range of anti-oxidant enzymes and scavengers**
  - Superoxide dismutase, catalase, heme oxygenase, quinone reductase, Glutathione, Thioredoxin, PUFAs etc
- **Oxidative stress sensors**
  - Many transcription factors – AP1, NFκB
  - Keap1
  - Nalp3 inflammasome
- **Xenobiotics activate innate immune pathways**

# Mechanisms of chemical-induced innate immunity in allergic contact dermatitis

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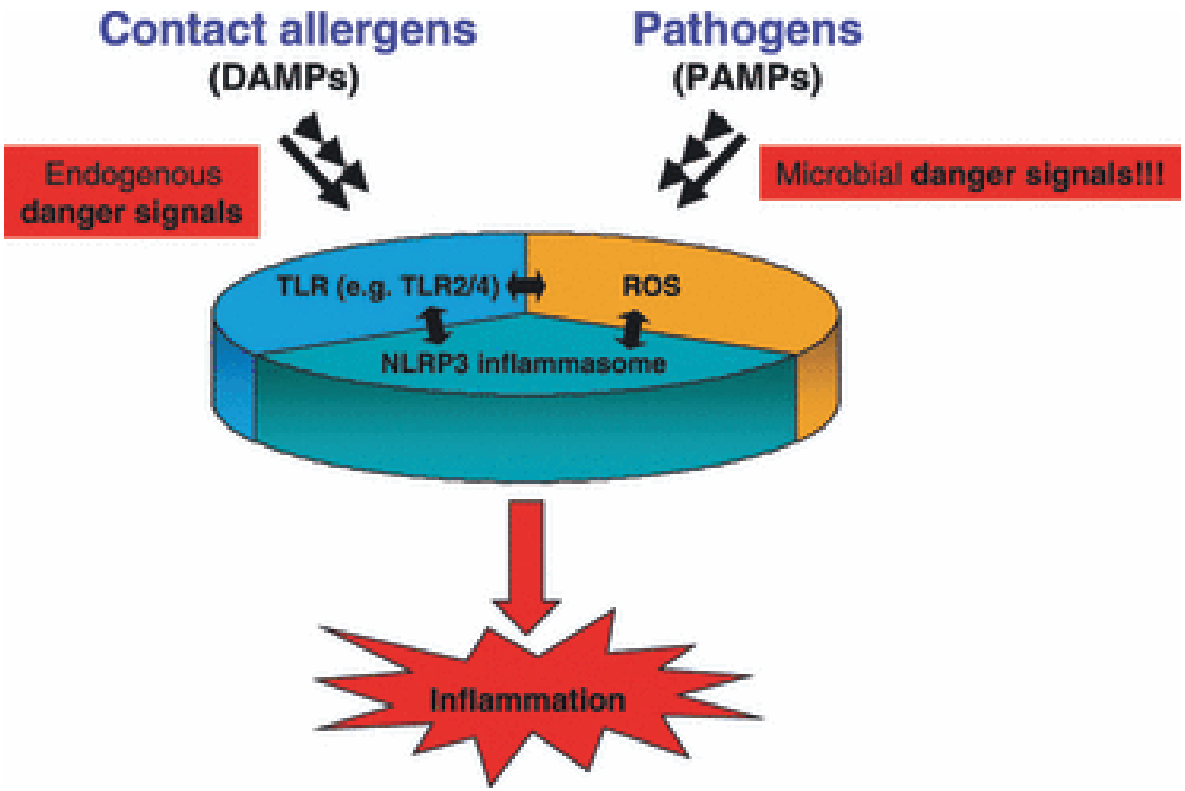


**Allergy**
Volume 66, Issue 9, pages 1152-1163, 21 MAY 2011 DOI: 10.1111/j.1398-9995.2011.02652.x
http://onlinelibrary.wiley.com/doi/10.1111/j.1398-9995.2011.02652.x/full#f2

# Mechanisms of chemical-induced innate immunity
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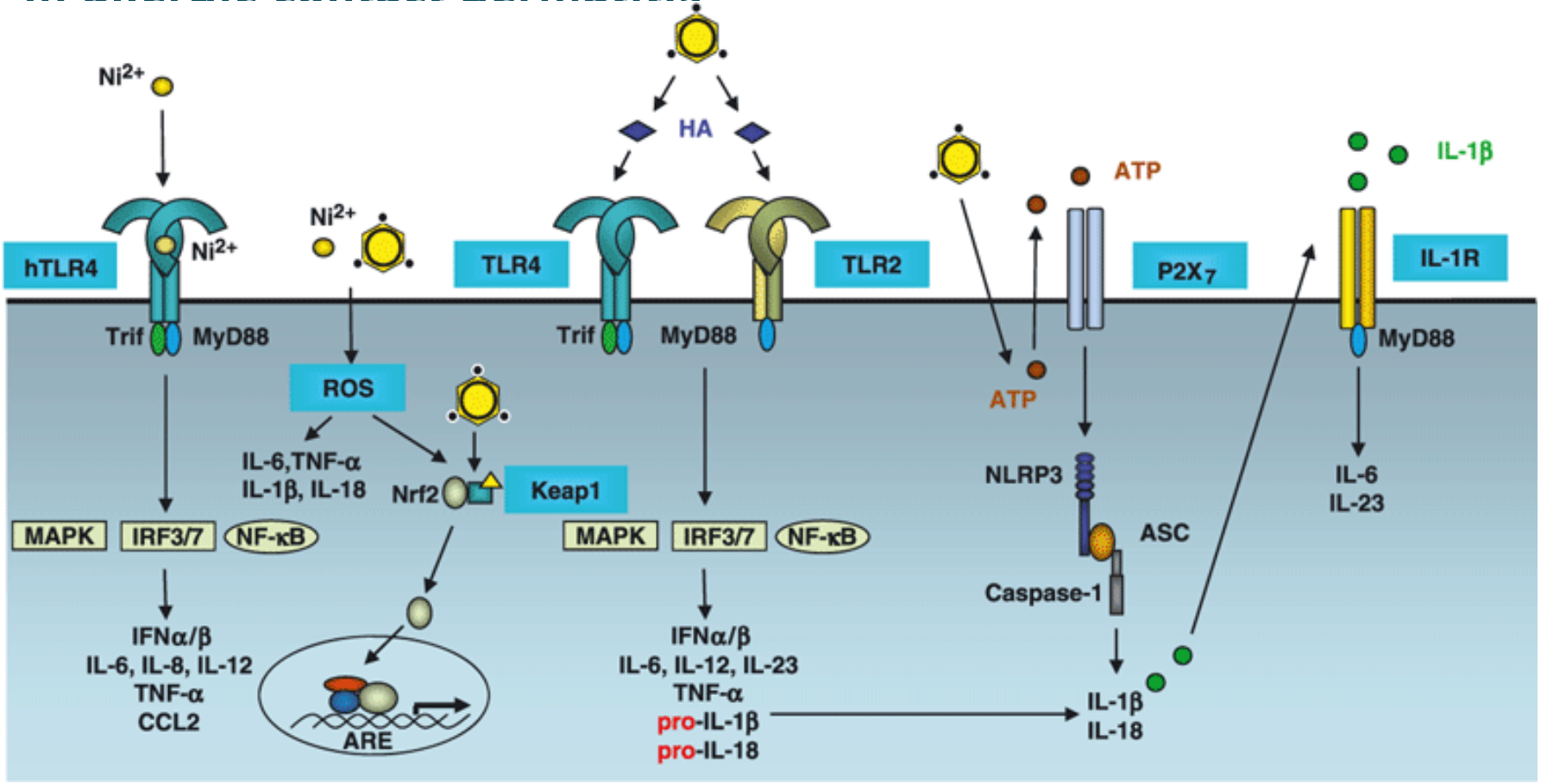


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# Anti-oxidant defence/stress

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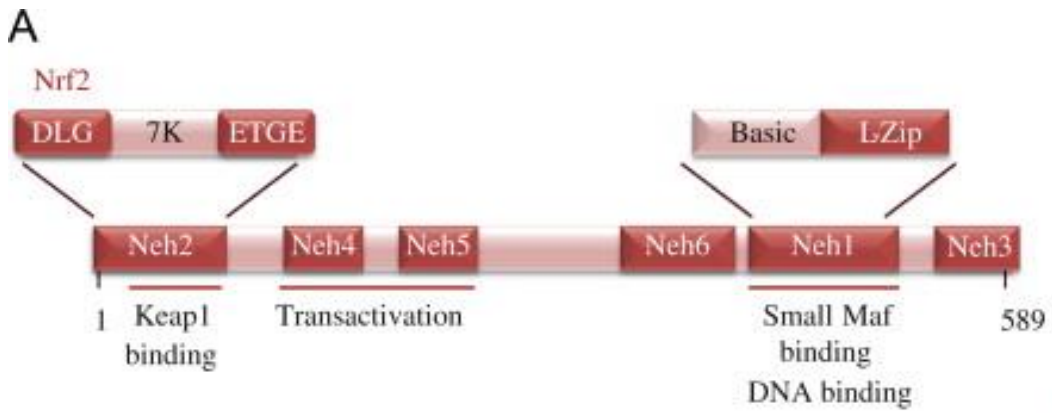
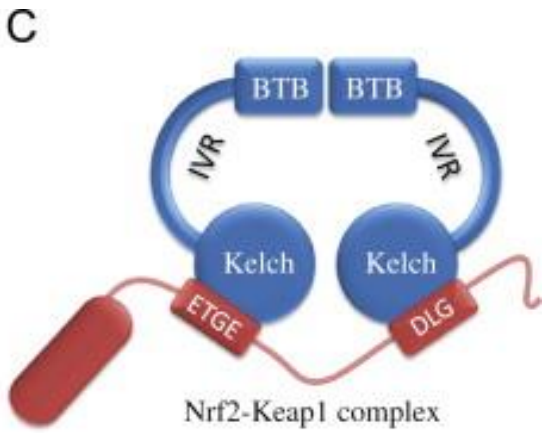
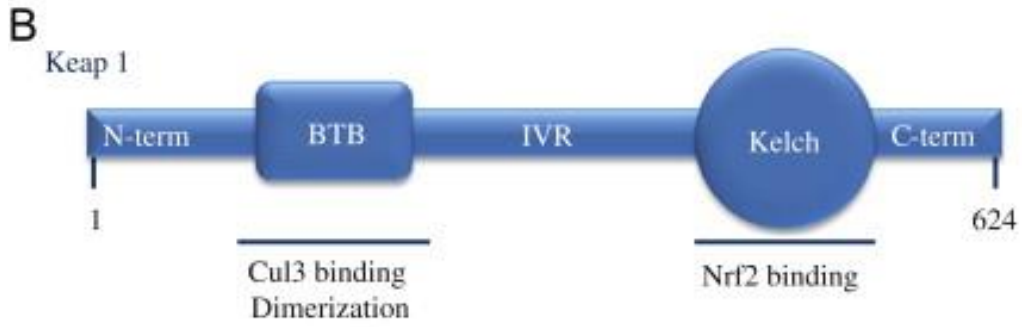
- Keap1 is a transcription factor activating genes containing ARE (Anti-oxidant response element) sequence.
- Nrf2 is an inhibitor that binds to Keap1 and prevents it localising in nucleus
- Pro-oxidant stressors modify key Cys residues allowing dissociation
  - **From Kansanen et al 2013 Redox Biology, 1; 45-49**



# Anti-oxidant defence/stress

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- Natsch et al (2008) examined 102 chemicals of known skin sensitizing potential for their capacity to activate AREs via the Keap1/Nrf2 pathway.
- Specific read-out: ARE-dependent activation of Quinone reductase and a construct containing 8 ARE repeats to activate luciferase
- For QR induction assay – extreme, strong and moderate sensitisers were good activators but weak and very weak ones did not activate.
- For ARE-Luc induction there was much better correlation

| Sensitisation class | No. of chemicals | Luc induction | No induction |
|---|---|---|---|
| Extreme | 5 | 5 | 0 |
| Strong | 10 | 9 | 1 |
| Moderate | 35 | 31 | 4 |
| Weak | 20 | 12 | 8 |
| V weak/none | 30 | 4 | 26 |

# Anti-oxidant defence/stress

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- Mutations in Keap1 found in lung cancer cells.
- Many mutations in either Nrf2 or Keap1 identified in a wide range of cancers
- Will mutations of Nrf2 or Keap1 be identified in people with ACD to a specific sensitiser or a generally increased susceptibility to sensitization?

# Genes involved in epidermal defence

- **Genes for structural proteins**
  - Filaggrin – integrity affects water permeability but probably not lipid permeability
- **Genes involved in biochemical defences**
  - Reactive thiol-rich barrier of stratum corneum
  - Intra-epidermal xenobiotic metabolising and anti-oxidant systems

# Biochemical barrier in stratum corneum

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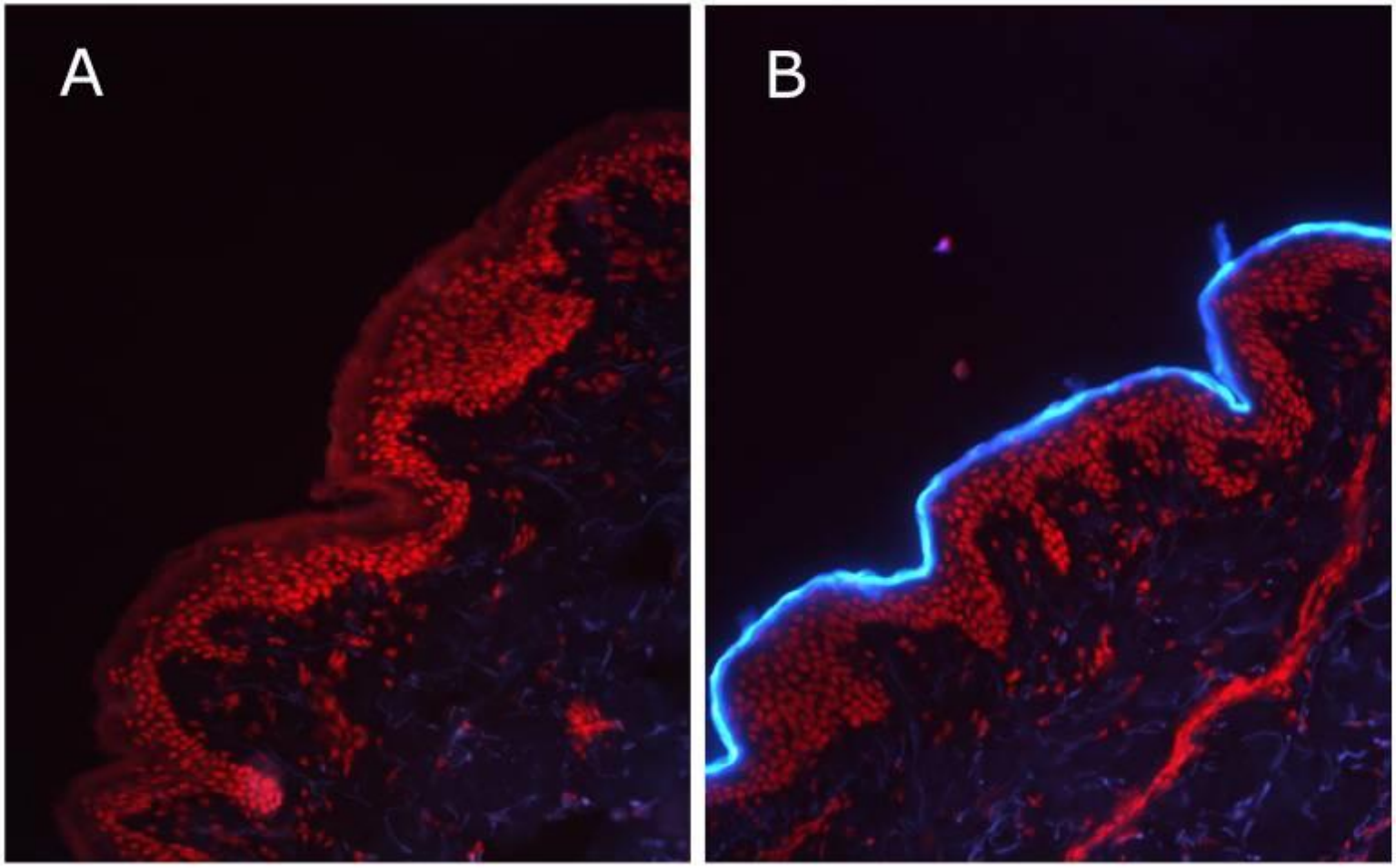


Control

Reactive Thiols stained blue by surface application of MBB

# General Considerations

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- Must bear in mind difference between general susceptibility to sensitisation (high/low responder) and genetics of potential to be sensitised by any specific chemical
- For specific chemicals, single gene products may be critical – HLA molecules, TCR, specific proteins that get haptenated etc etc – as in drug allergy.
- Susceptibility may not be "congenital" but may be acquired – following viral infection (Measles, Herpes, HIV and ??others)
  - HIV predisposes to drug allergy via i) depletion of glutathione stores ii) loss of regulatory T cells
- For general susceptibility it is more likely that broad areas/processes involved in responses are modified. Potential candidates include:
  - transcription factors which control many genes
  - Epigenetic changes to DNA methylation and acetylation – acquired genetics
  - microRNAs which can control expression of multiple proteins