Research Directions in Genetically-Mediated Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis:

Basic science of pathogenesis, functional genomics and mechanisms

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Mechanism of SJS/TEN

- Drug antigens
- Environmental factors
- Host factors:

  Genetics: HLA and non-HLA associations

  Non-genetic factors

  Immune mechanism:

    drug-peptides/proteins-HLA-TCR interaction

    immune molecules and cytotoxic proteins

  Cell death mechanism

  Therapeutic targets

Historical milestones of SJS/TEN research

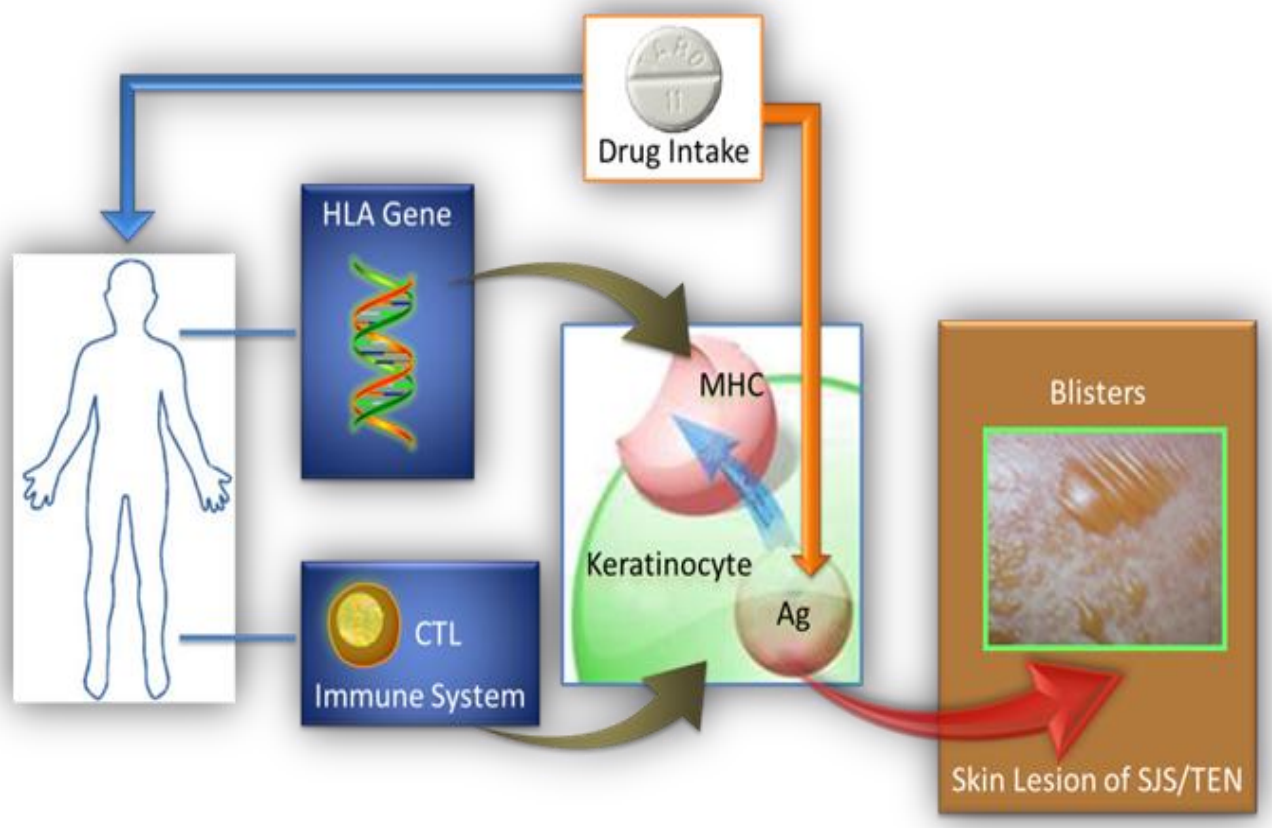
Table 3 Key dates and publications related to epidermal necrolysis.

Date	Author(s)	Concept	Refs
1866	von Hebra	Erythema exsudativum multiforme	12
1916, 1917	Rendu, Fiessinger and Rendu	Multiorificial ectodermosis	13,14
1922	Stevens and Johnson	Stevens–Johnson syndrome	15
1939	Debré et al	Bullous erythroderma with epidermolysis	11
1956	Lyell	Toxic epidermal necrolysis	1
1968	Bergoend H et al	High risk of long-acting sulfonamides	18
1968, 1970	Billingham and Streilein, Streilein and Billingham	TEN and a graft-versus-host model in hamsters	37,38
1970	Mellish and Glasgow	Staphylococcal scalded skin syndrome	3
1972	Peck et al	Human graft-versus-host reaction as a cause of TEN	39
1972	Kauppinen	Drug rechallenge often negative in SJS and TEN	9
1985	Ruiz-Maldonado	ADEN types 1, 2, and 3	22
1985	Revuz et al	First international meeting on TEN, Créteil, France	—
1985	Lyell A (Créteil meeting)	The Jackpot hypothesis	—
1986	Roujeau et al	Mild links between HLA and TEN	58
1987	SCAR study group	Initiation of multinational case-control study on EN	—
1990	Roujeau and Revuz	Acute skin failure	64
1993	Correia et al	Studying T cells in blister fluid of TEN	43
1993	Bastuji-Garin et al	Consensus definition of EEMM, SJS, and TEN	23
1995–2002	Pichler et al	Drug-specific T cell clones, p-i concept	44–47
1995	Roujeau et al	Results from first case-control study of drug risks in EN	32
1998	*Viard I, et al.*	Inhibition of Toxic Epidermal Necrolysis by Blockade of CD95 by IVIG	
2002	Nassif et al	Drug-specific cytotoxic cells in blister fluid of TEN	48
2004	Chung et al	Carbamazepine (CBZ)-related TEN and HLA-B*15:02	29
2005	Hung et al	Allopurinol-related TEN and HLA-B*58:01	59
2008	Mockenhaupt et al	EuroSCAR case-control study of SJS/TEN	33
2008	Chung et al	Granulysin as the key cytokine in necrolysis	50
2011	Ko et al	Restricted TCR needed for CBZ-related SJS/TEN	52
2011	Genin et al	GWAS on a large European series of SJS/TEN cases	60
2012	Wei et al	Direct noncovalent link between CBZ and HLA-B1502	53
2012	Chen et al	Eradication of CBZ-induced TEN from Taiwan	63
2013	Sekula et al	SJS/TEN even more severe than suspected	28

ADEN = acute disseminated epidermal necrosis; EN = epidermal necrolysis; GWAS = genome-wide association studies; HLA = human leukocyte antigen; p-i concept = pharmoco-immune concept; RCT = randomized controlled trial; SCAR study = Severe Cutaneous Adverse Reactions study; SJS = Stevens–Johnson syndrome; TCR = T-cell receptor; TEN = toxic epidermal necrolysis.

Adapt from Jean-Claude Roujeau, Dermatologica Sinica, 2013

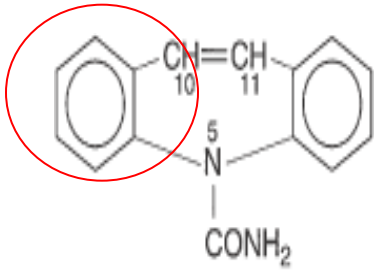
Pathogenesis of SJS/TEN



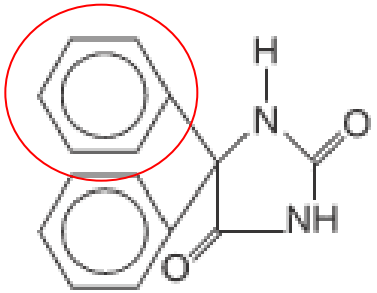
$$\text{Frequency / Severity of Drug Hypersensitivity} = f_1\left(\text{Chemistry of drug}\right) + f_2\left(\text{Biology of individual}\right)$$

Werner J. Pichler,et al. J Allergy Clin Immunol 2011
Chung WH et al, J Dermatol Sci, 2012

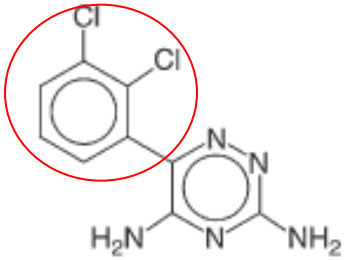
Chemical structures of aromatic antiepileptic drugs frequently associated with SJS/TEN



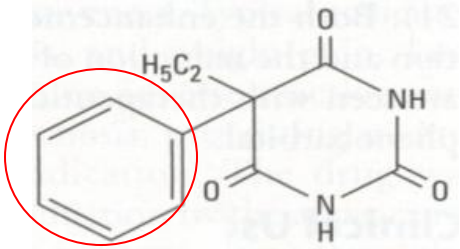
CARBAMAZEPINE



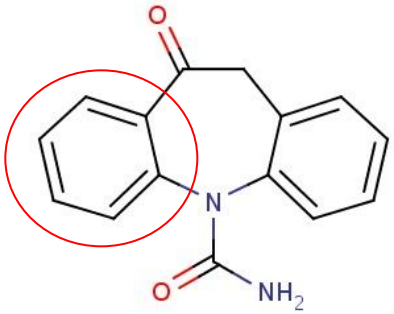
PHENYTOIN



LAMOTRIGINE

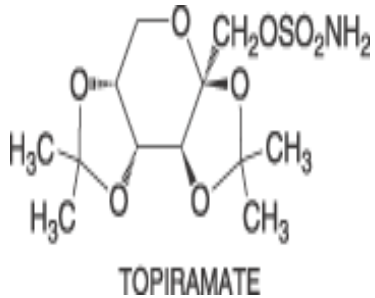


Phenobarbital

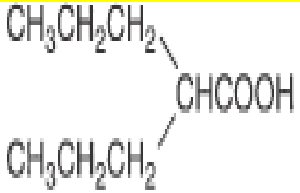


Oxcarbazepine

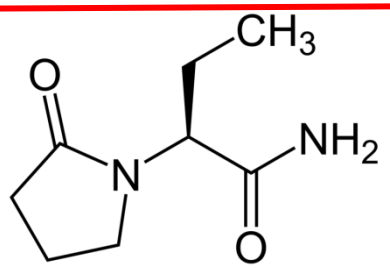
Non-aromatic AED



TOPIRAMATE



VALPROIC ACID



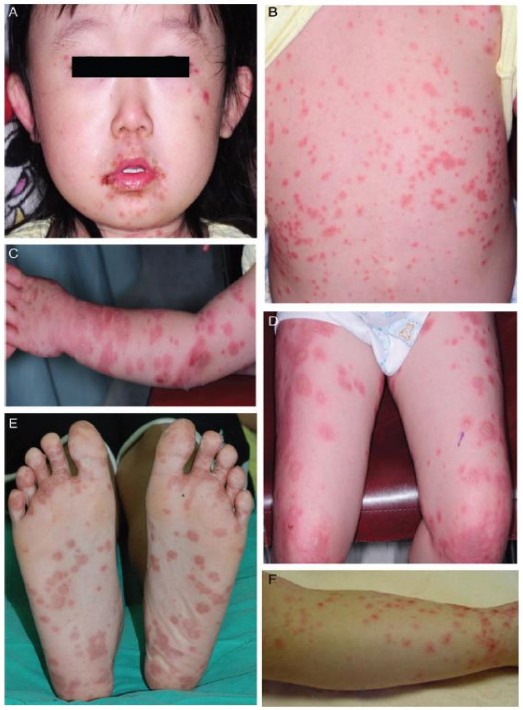
Levetiracetam

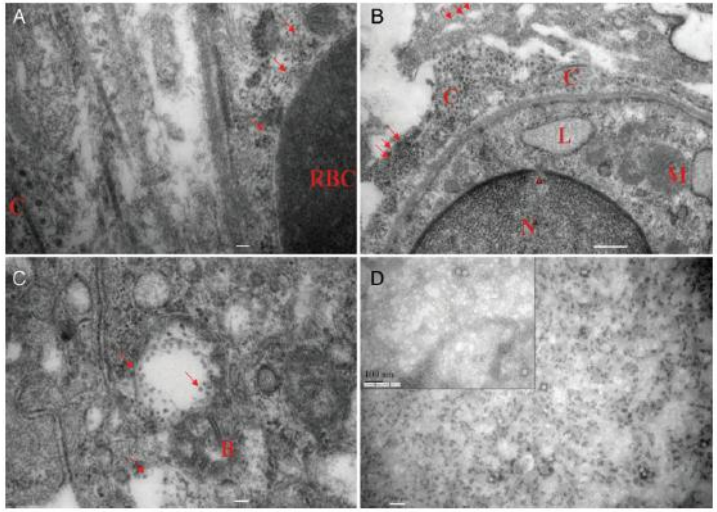
Pan et al., Current Immunology Reviews, 2014

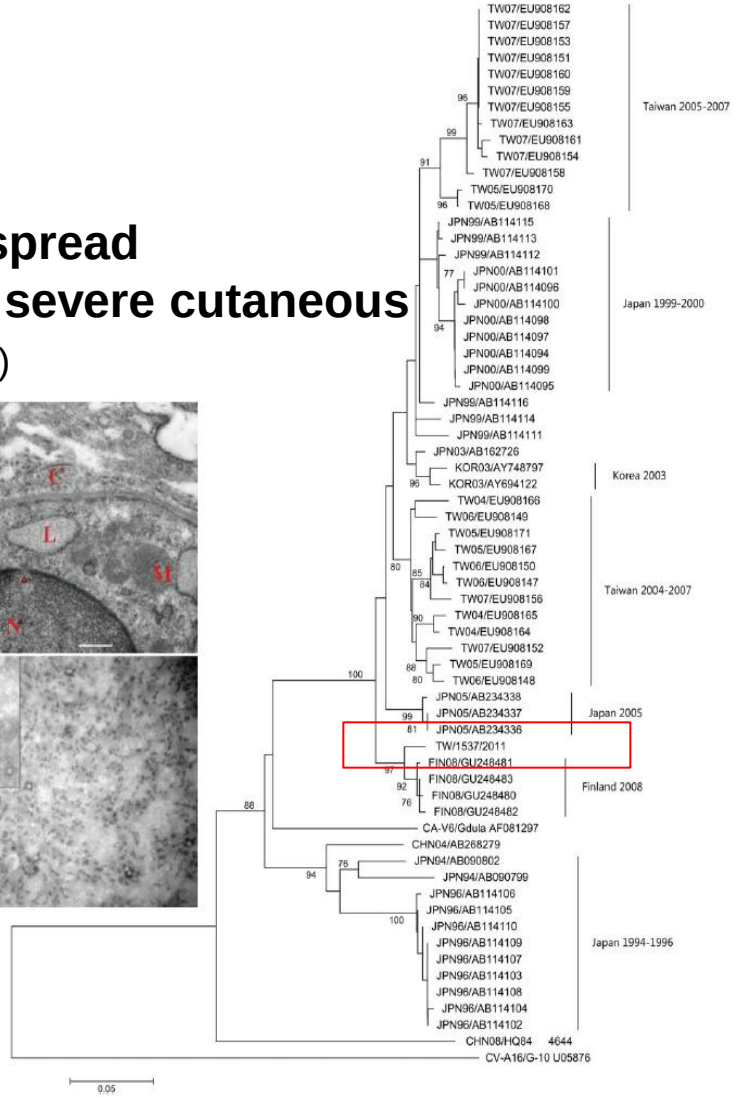
Environmental factors

Mycoplasma
HSV
~20% Unknown ?

Coxsackievirus A6 new variant induced widespread mucocutaneous bullous reactions mimicking severe cutaneous adverse reactions (Chung WH et al ., J Infect Dis. 2013)







Host factors- genetics

Association of serious drug hypersensitivity and HLA alleles

Culprit Drug	Drug Hypersensitivity	HLA Association	OR	Population	References
Aromatic anticonvulsants-induced SCAR					
Carbamazepine	SJS/TEN	HLA-B*15:02	2504	Han Chinese in Taiwan	[30]
	MPE	HLA-A*31:01	17.5	Han Chinese in Taiwan	[31]
	cADR	HLA-A*31:01	9.5	Japanese	[48]
	SJS/TEN	HLA-A*31:01	25.93	Northern European	[49]
	MPE	HLA-A*31:01	8.33	Northern European	[49]
	DRESS/SCAR	HLA-A*31:01	8.8	Korean	[50]
Oxcarbazepine	SJS/TEN	HLA-B*15:02	80.7	Han Chinese	[82]
Phenytoin	SJS	HLA-B*15:02	18.5	Thai	[95]
	SJS/TEN	HLA-B*15:02	5.1	Han Chinese	[82]
Lamotrigine	SJS/TEN	HLA-B*38:01	4.7	European	[55]
	SJS	HLA-B*15:02	5.1	Han Chinese	[82]
Antibiotics-induced drug hypersensitivity					
Abacavir	MPE/DRESS	HLA-B*57:01	117	Australian, Caucasians	[28, 29]
	MPE/DRESS	HLA-B*57:01	960	Australian	[96]
	Hypersensitivity	HLA-B*57:01	>900	White and Black	[97]
Aminopenicillin	DHS	HLA-A2	7	Italian	[98]
	DHS	HLA-DRW52	9	Italian	[99]
Nevirapine	DRESS	HLA-DRB1*01:01	18	Australian	[57]
	DHS	HLA-Cw8-B14	15	Sardinians	[70]
	SJS/TEN	HLA-C*04:01	5.17	Malawian	[71]
	DRESS	HLA-B*35:05	49	Thai	[72]
Sulfamethoxazole	SJS/TEN	HLA-B*38:02	76	European	[55]
Dapsone	DRESS	HLA-B*13:01	20.53	Han Chinese	[73]
Other drugs-induced SCAR					
Allopurinol	SCAR	HLA-B*58:01	580.3	Han Chinese	[9]
	SJS/TEN	HLA-B*58:01	41	Japanese	[100]
	SJS/TEN	HLA-B*58:01	80	European	[55]
	SJS/TEN	HLA-B*58:01	348.3	Thai	[78]
	SCAR	HLA-B*58:01	97.8	Korean	[101]
Methazolamide	SJS/TEN	HLA-B*59:01	249.8	Korean	[102]
Oxicam	SJS/TEN	HLA-B*73:01	152	European	[55]

Abbreviations: cADR, cutaneous adverse drug reactions; MPE, maculopapular eruption; DHS, delayed-type hypersensitivity reaction; DRESS, drug rash with eosinophilia and systemic syndrome; SCAR, severe cutaneous adverse reactions; SJS, Stevens-Johnson syndrome; TEN, toxic epidermal necrolysis.

Phillips EJ, Chung WH ; J Allergy Clin Immunol. 2011
Ren-You Pan et al., Current Immunology Reviews, 2014

Nature **428**, 486 (01 April 2004); doi:10.1038/428486a

Medical genetics: A marker for Stevens – Johnson syndrome

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Table 1 **Frequency of *HLA* alleles in patients with Stevens–Johnson syndrome**

HLA allele	*CBZ–SJS*	*CBZ-tolerant*	*Normal*
*B*1502*	44 (100%)	3 (3%)*	8 (8.6%)†
*Cw*0801*	41 (93.2%)	17 (16.8%)	13 (14%)
*A*1101*	36 (81.8%)	51 (50.5%)	53 (57%)
*DRB1*1202*	33 (75%)	12 (11.9%)	18 (19.4%)
*B*1502, Cw*0801*	41 (93.2%)	3 (3%)	7 (7.5%)
*B*1502, A*1101*	36 (81.8%)	2 (2%)	6 (6.5%)
*B*1502, DRB1*1202*	33 (75%)	1(1%)	5 (5.4%)
*B*1502, Cw*0801, A*1101, DRB1*1202*	29(66%)	0 (0%)	3 (3.2%)

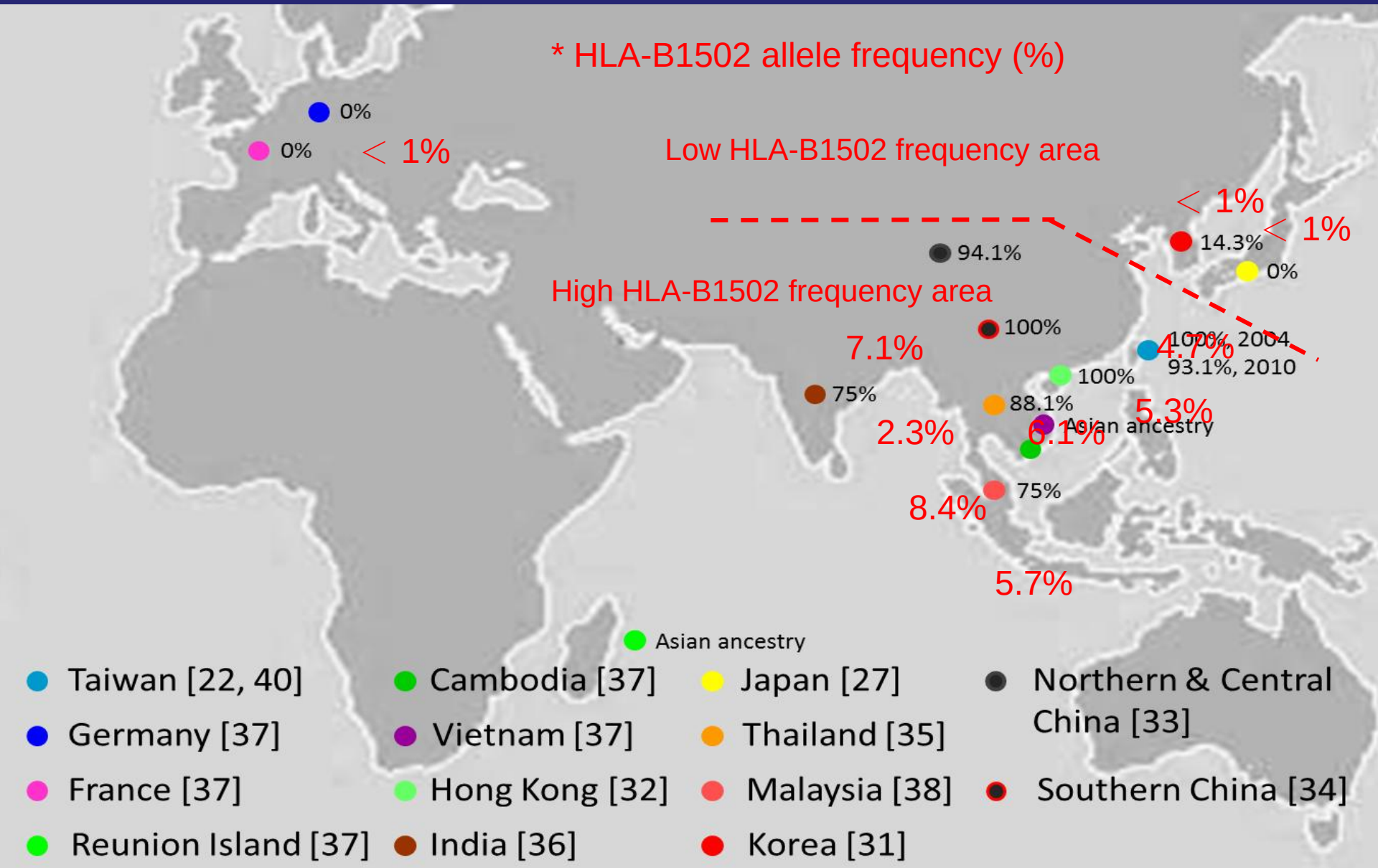
Frequencies (by number and percentage) of individual or combined loci of the *B*1502* ancestral haplotype are shown in patients with carbamazepine-induced Stevens–Johnson syndrome (CBZ–SJS; $n = 44$), and in carbamazepine-tolerant ($n = 101$) and normal subjects ($n = 93$). For methods, see supplementary information.

*Odds ratio (CBZ–SJS/CBZ-tolerant): 2,504 (95% CI, 126–49,522); corrected *P* value $P_c = 3.13 \times 10^{-27}$.

†Odds ratio (CBZ–SJS/normal): 895 (95% CI, 50–15,869); $P_c = 1.38 \times 10^{-21}$.

Association of HLA-B*1502 with CBZ-SJS/TEN in different populations

WH Chung , Journal of Dermatological Science (2012)



Phenotype & Genotype : HLA-B*1502 in CBZ-cADR (n=194)

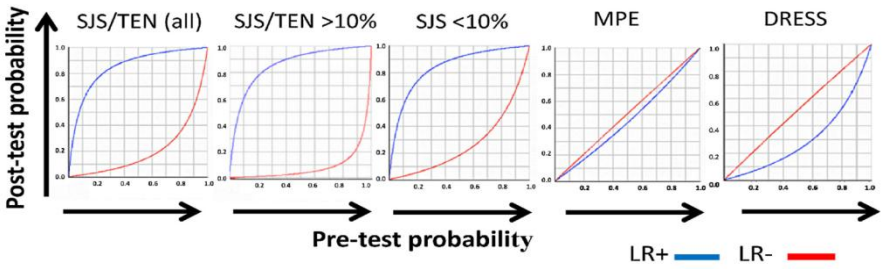
Follow-up study in Taiwan

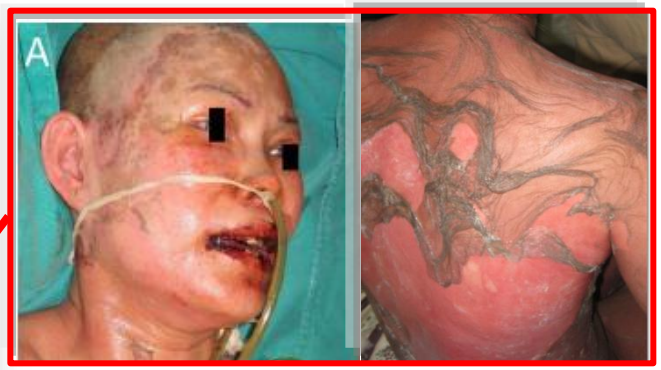
Phenotypes (% BSA detachment)	No. with HLA-B*1502 (%)	Total No.	Odds Ratio	(95% CI), p-value
SJS/TEN (all)	99 (88%)	112	97.6	(42.0 - 226.8), $p=1.6\times10^{-44}$
SJS/TEN (>5%)	25 (100%)	25	627.5	(35.8 - 10987.1), $p=3.5\times10^{-22}$
TEN (≥30%)	6 (100%)	6	160.0	(8.5 - 3021.5), $p=6.31\times10^{-7}$
SJS/TEN overlap (10-29%)	10 (100%)	10	258.4	(14.2 - 4696.3), $p=1.4\times10^{-10}$
SJS (6-9%)	9 (100%)	9	233.8	(12.8 - 4277.3), $p=1.1\times10^{-9}$
SJS/TEN (≤5%)	74 (85%)	87	73.0	(31.2 – 170.9), $p=9.2\times10^{-36}$
SJS (2-5%)	50 (87.7%)	57	91.6	(33.6 - 249.1), $p=1.2\times10^{-29}$
SJS (≤1%)	24 (80%)	30	51.3	(17.3 - 151.7), $p=2.8\times10^{-16}$
Other presentation*	2 (25%)	8	4.3	(0.8 - 23.7), p=0.13
MPE	4 (7.8%)	51	1.1	(0.3 – 3.6), p=1.00
DRESS	0 (0%)	23	0.3	(0.01 - 4.6), p=0.36
Tolerant control	11 (7.2%)	152		

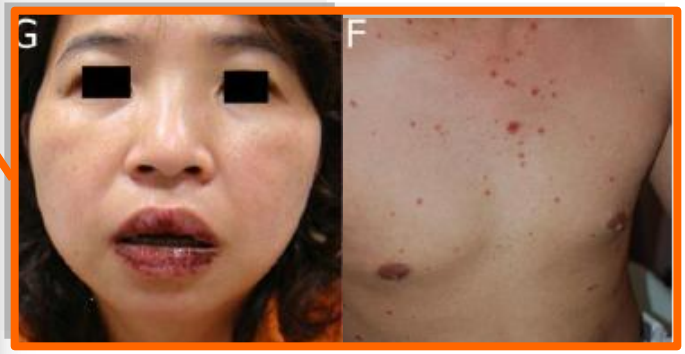
0 1 10 100 1,000 10,000

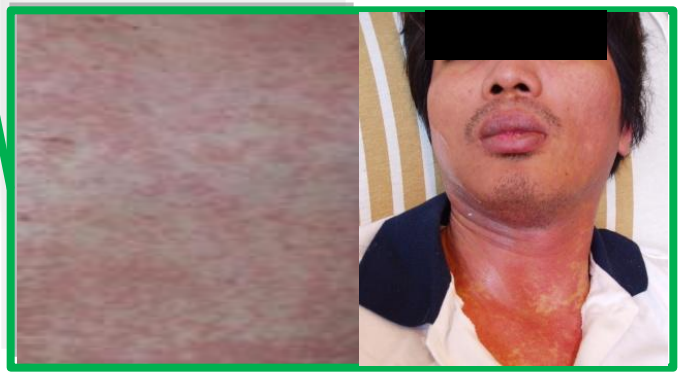
<------ HLA-B*15:02 less likely

------> HLA-B*15:02 more likely









Y.-H. Hsiao et al. Journal of Dermatological Science, 2014

Phenotype and ethnicity specific HLA association

Populations	phenotype / allele	CBZ-SJS/TEN	CBZ-DRESS
Han Chinese (*Hung SI, Pharmacogene Genomics. 2006 and updated*)	**HLA-A*3101**	**1/60 (NS)**	**6/19**; P=9x10^{-7} OR= 16.15
	HLA-B*1502	59/60 ; P= 4x10^{-43} OR=1357	0/19 (NS)
Japanese *(Ozeki T, Hum Mol Genet. 2011)*	**HLA-A*3101**	**5/6; P= 2.35x10^{-4} OR= 33.9**	**21/36**; P= 2.06x10^{-4} OR= 9.5
	HLA-B*1502	0 (NS)	0 (NS)
European *(McCormack M, NEJM. 2011)*	**HLA-A*3101**	**5/12; P= 8x10^{-5} OR= 25.93**	**10/27**; P= 0.03 OR= 12.41
	HLA-B*1502	0 (NS)	0 (NS)

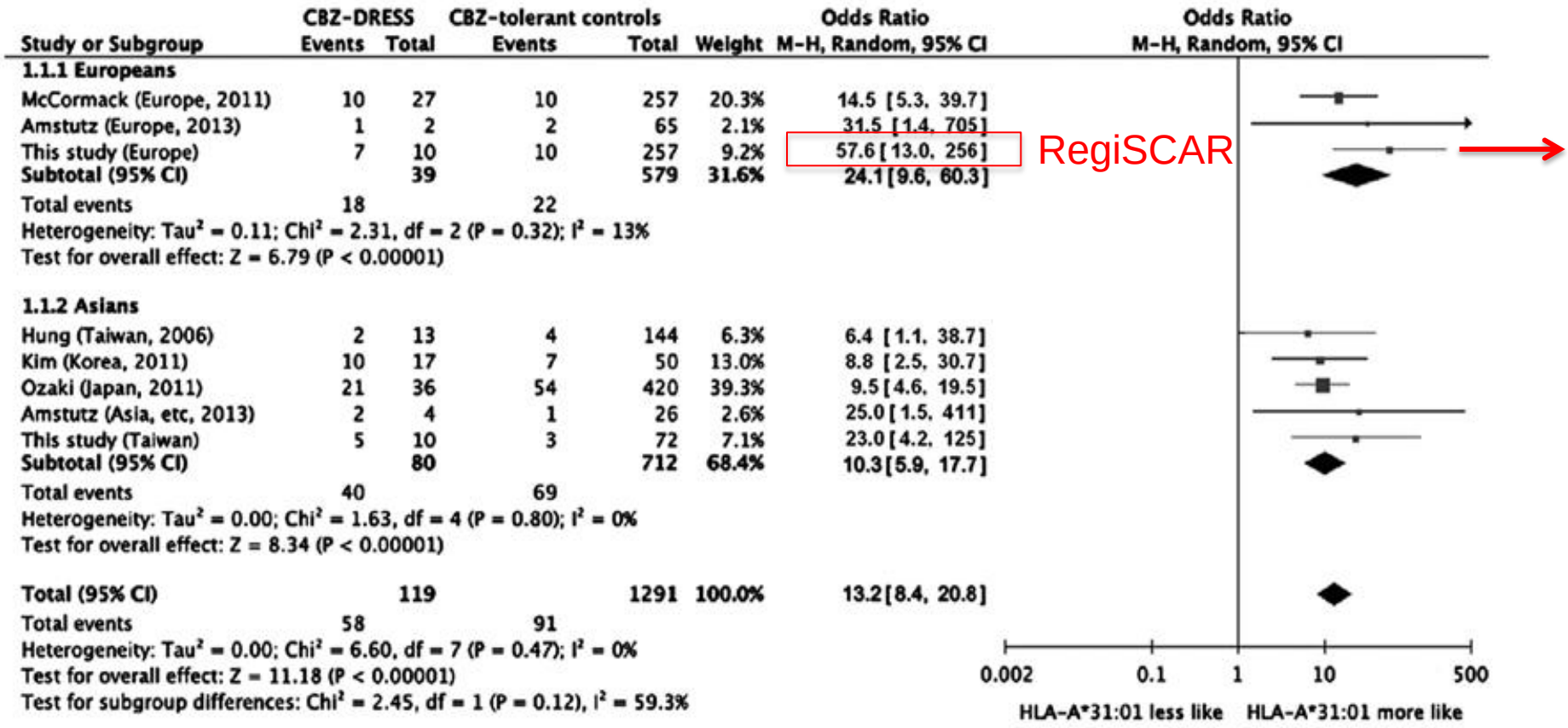
* NS: no significance

ORIGINAL ARTICLE

*HLA-A*31:01* and different types of carbamazepine-induced severe cutaneous adverse reactions: an international study and meta-analysis

E Genin[1,12], D-P Chen[2,3,12], S-I Hung[4,12], P Sekula[5], M Schumacher[5], P-Y Chang[2,3], S-H Tsai[2,3], T-L Wu[2,3], T Bellón[6], R Tamouza[7,8], C Fortier[7,8], A Toubert[7,8], D Charron[7,8], A Hovnanian[8], P Wolkenstein[8], W-H Chung[9], M Mockenhaupt[10] and J-C Roujeau[11]

HLA-A3101 is strongly associated with CBZ-DRESS in all populations



HLA-A*31:01 shows weaker association with CBZ-SJS/TEN of Europeans from RegiSCAR study: only (+) in 3/20

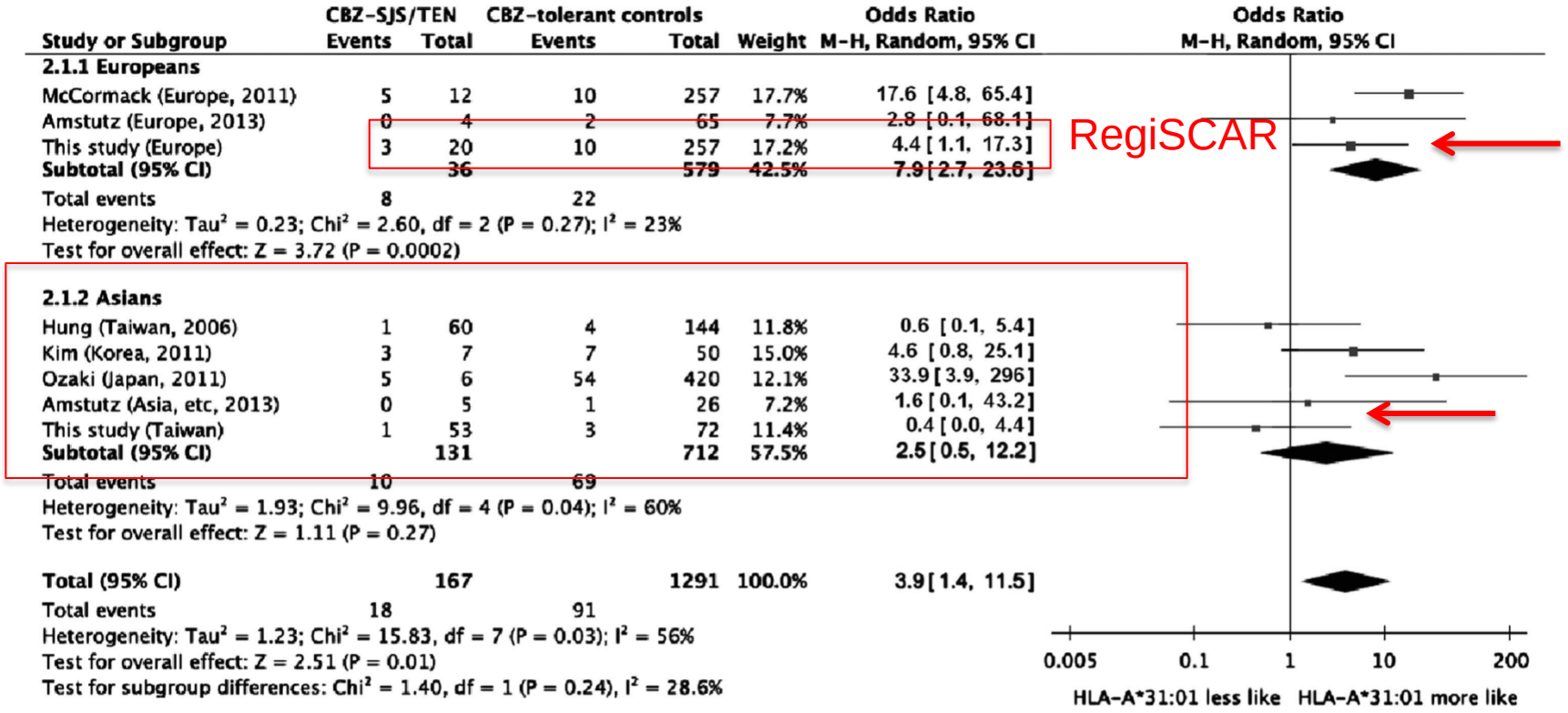


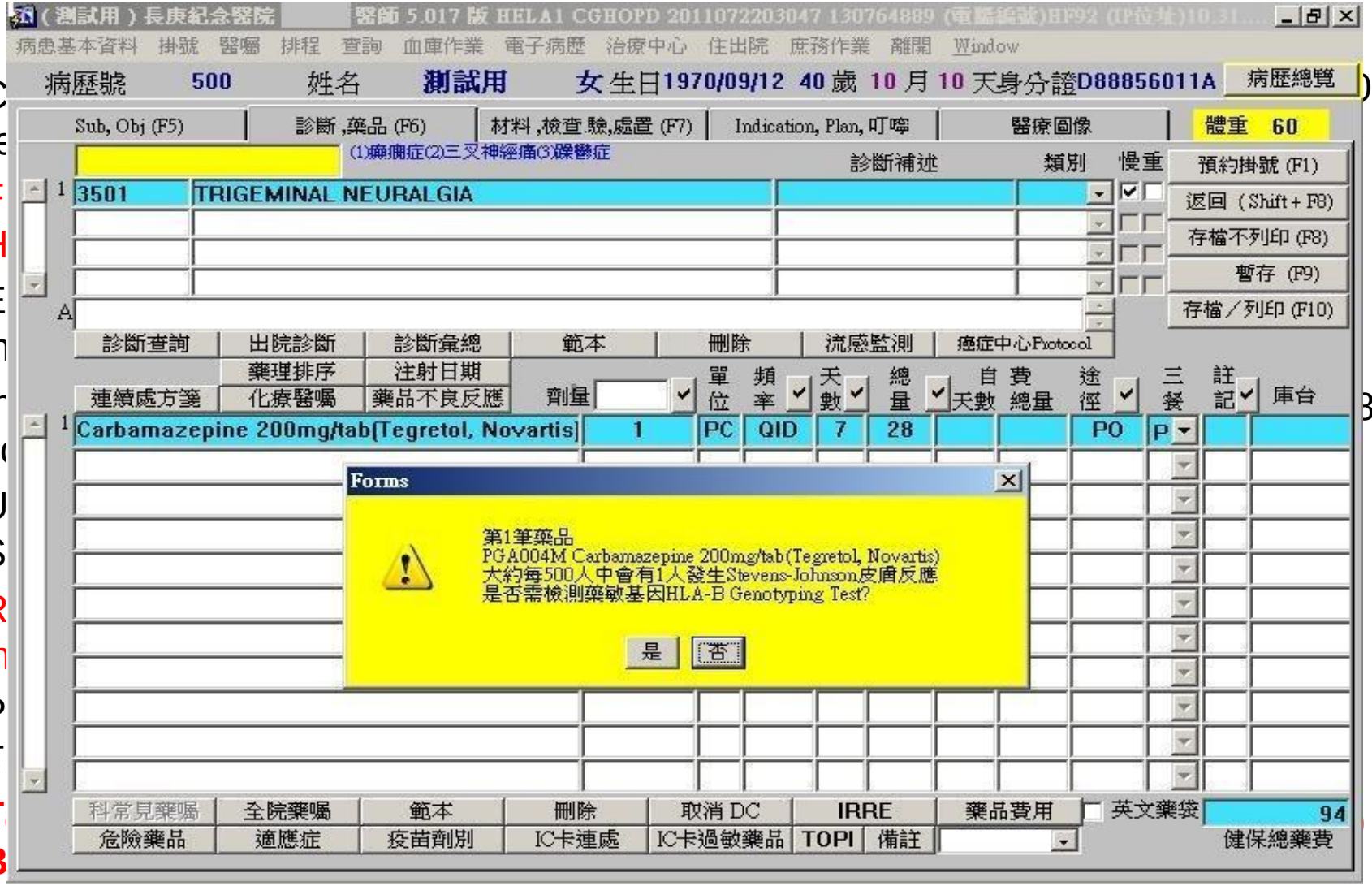
Figure 2. Analysis of the association between *HLA-A*31:01* and carbamazepine (CBZ)-induced Stevens–Johnson syndrome/toxic epidermal necrolysis (SJS/TEN) in multiple populations against tolerant controls. Events represent the carriers of *HLA-A*31:01*. Studying weighting (indicated by the squares) refers to the proportion of subjects who were recruited from each study. Tau^2 and I^2 represent the measures of heterogeneity. Diamonds represent pooled odds ratios (ORs), and horizontal lines indicate 95% confidence intervals (CIs). The data published in the literatures and in the present study was used for meta-analysis.[15,23,24,35,36] d.f., degrees of freedom; M–H, Mantel–Haenszel method.

Carbamazepine Translational Roadmap

- C
- F
- E
- In
- U
- R
- P
- T
- **T**

B

CBZ in Taiwan now)



HLA-B*5801 allele as a genetic marker for severe cutaneous adverse reactions caused by allopurinol

Shuen-Iu Hung[a,b], Wen-Hung Chung[a,b,c,d], Lieh-Bang Liou[e], Chen-Chung Chu[f], Marie Lin[f], Hsien-Ping Huang[a], Yen-Ling Lin[a], Joung-Liang Lan[g], Li-Cheng Yang[c], Hong-Shang Hong[c], Ming-Jing Chen[c], Ping-Chin Lai[h], Mai-Szu Wu[h], Chia-Yu Chu[i], Kuo-Hsien Wang[j], Chien-Hsiun Chen[a], Cathy S. J. Fann[a], Jer-Yuarn Wu[a,k], and Yuan-Tsong Chen[a,l,m]

SCAR (51)= SJS/TEN(21) ; DRESS (30)

Table 3. Frequencies of individual or combined loci of HLA-B*5801 extended haplotype in patients with allopurinol-induced SCAR, allopurinol tolerant control, and general population control

Genotype	Allopurinol-SCAR (n = 51)	Tolerant control (n = 135)	Odds ratio	Pc value*	General population control (n = 93)	Odds ratio	Pc value*
B*5801	51 (100)	20 (15)	580.3	4.7×10^{-24}	19 (20)	393.5	8.1×10^{-18}
Cw*0302	48 (94)	19 (14)	97.7	1.4×10^{-19}	19 (20)	62.3	2.5×10^{-13}
A*3303	34 (67)	24 (18)	9.3	2.2×10^{-4}	20 (22)	7.3	4.7×10^{-2}
DRB1*0301	33 (65)	17 (13)	12.7	2.8×10^{-6}	14 (15)	10.3	8.5×10^{-4}
B*5801, Cw*0302	48 (94)	19 (14)	97.7	1.4×10^{-19}	19 (20)	62.3	2.6×10^{-13}
B*5801, Cw*0302, A*3303	34 (67)	17 (13)	13.9	5.4×10^{-7}	16 (17)	9.6	1.7×10^{-3}
B*5801, Cw*0302, DRB1*0301	30 (59)	11 (8)	16.1	7.4×10^{-7}	10 (11)	11.9	7.8×10^{-4}
B*5801, Cw*0302, A*3303, DRB1*0301	21 (41)	9 (7)	9.8	0.039	9 (10)	6.5	>0.05

Numbers in parentheses indicate percentage.

*The Pc values were adjusted by using Bonferroni's correction for multiple comparisons to account for the observed alleles.

PNAS 102(11): 4134 – 4139 , 2005.

Validate the association between HLA-B*5801 and Allopurinol-SCAR in different populations

Table 1. HLA-B*5801 in Allopurinol-induced Severe Cutaneous Adverse reactions (SCAR).

Study number	1	2 (European study)		3	4
Study population	Han Chinese[a]	Caucasian[b]	Non-European ancestry (two Asians)	Japanese[c]	Thai[d]
Case	51/51 (100%)	15/27 (55%)	4/4 (100%)	7/13 (54%)	27/27 (100%)
Control	20/135 (15%)	28/1822 (1.5%)		6/493 (1.2%)	7/54 (13%)
Odds ratio	580.3	80		94.7	348.3
(95% C.I.)	(34.4 - 9780.9)	(34 - 187)		(24.4-367.3)	(19.2 – 6336.9)
P value	4.7×10^{-24} *	$<10^{-6}$ *		1.71×10^{-9}	1.61×10^{-13}
Reference	Hung, et al. PNAS, 2005.	Lonjou, et al. Pharmacogenetics and Genomics, 2008.		Kaniwa, et al. Pharmacogenomics , 2008. Dainichi, et al. Dermatology, 2007.	Wichittra, et al., Pharmacogenetics and Genomics, 2009.

[a] Case: Allopurinol-SCAR; Control: Tolerant control.

[b] Case: Allopurinol-SJS/TEN; Control: A mixed European population.

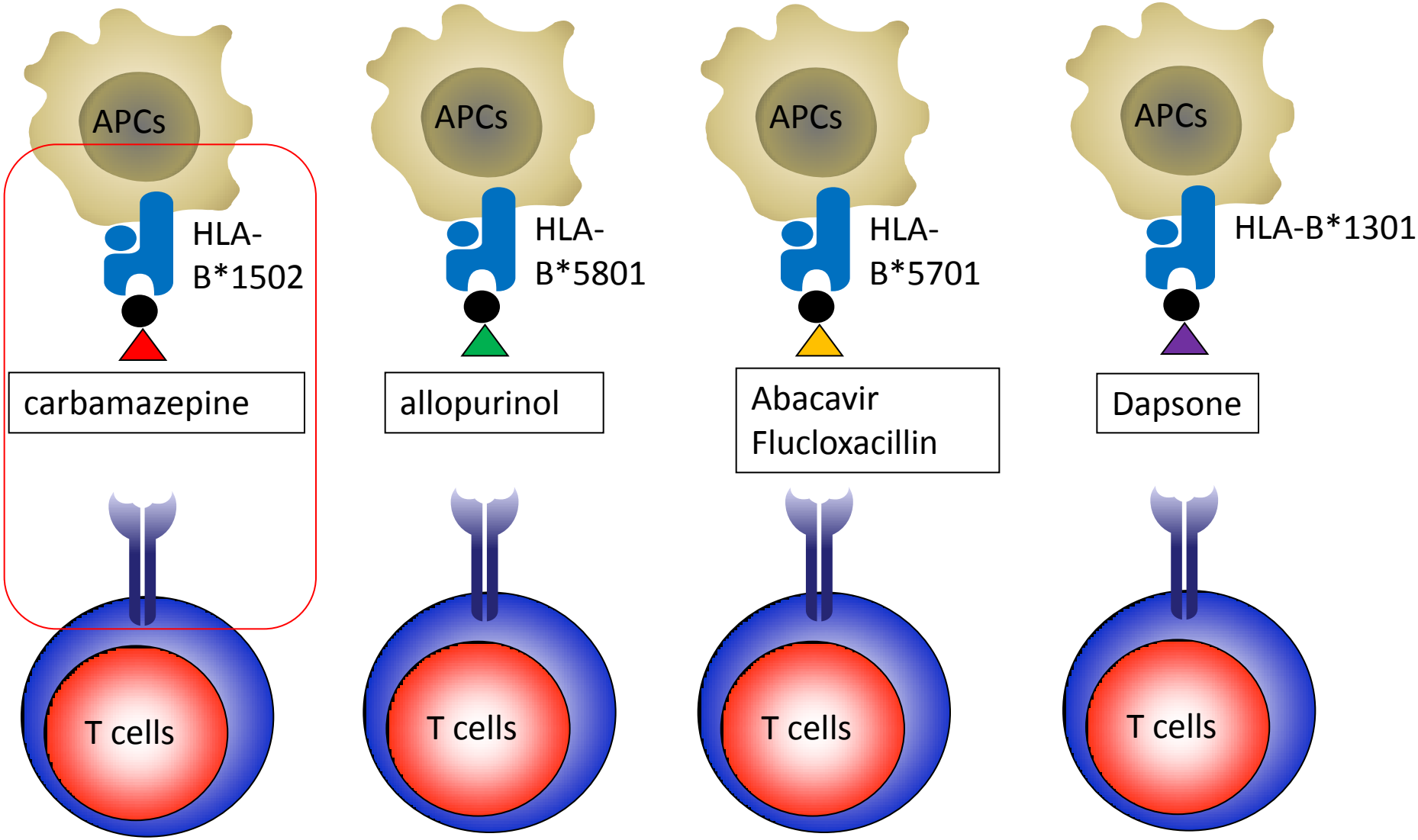
[c] Case: Allopurinol-SJS/TEN; Control: Japanese population.

[d] Case: Allopurinol-SJS/TEN; Control: Tolerant control.

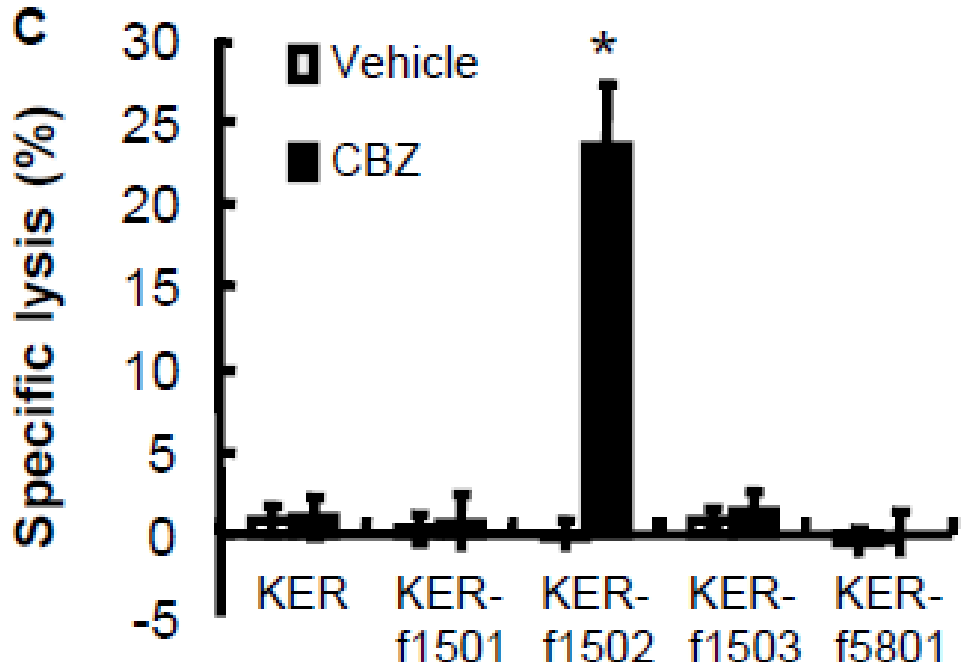
* Adjusted using Bonferroni's correction for multiple comparisons to account for observed alleles.

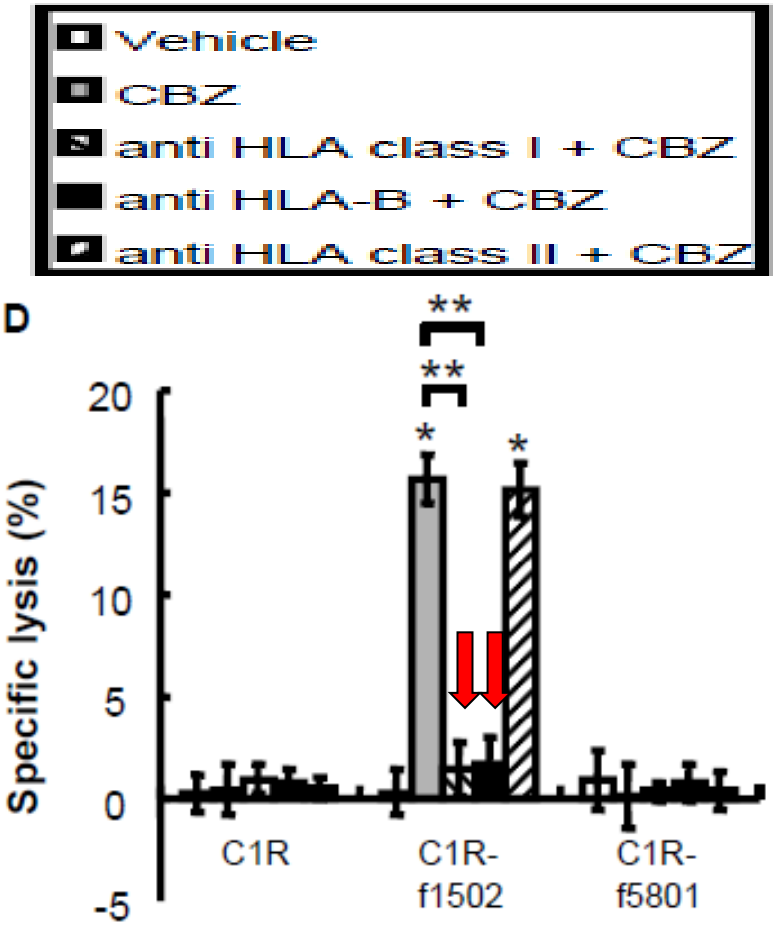
Role of HLA in drug hypersensitivity: HLA-restricted

Hypothesis: HLA proteins have different affinity to the drug/peptide complex.



HLA-B*15:02-dependent cytotoxicity of CTL in carbamazepine (CBZ)-induced SJS/TEN





- Stable transfection of different HLA-B cDNAs into C1R B cells, or keratinocytes.
- CBZ-SJS CTLs only kill target cells expressing B*15:02.

- Abolish the cytotoxicity of CTL by anti- HLA class I/HLA-B antibodies

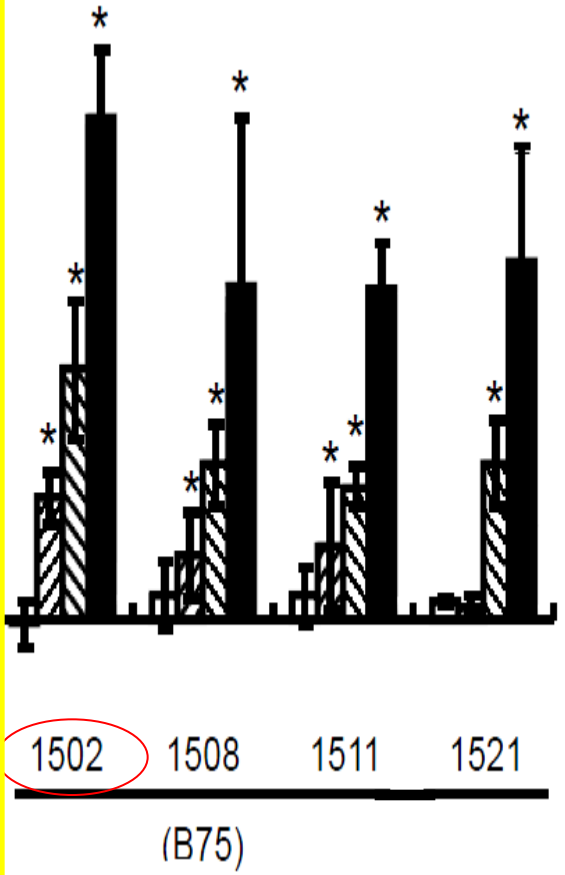
Wei CY, et al. JACI, 2012

The same HLA serotype members (B75) could present CBZ to T cells.

Other HLA-B*15 subtypes associated with CBZ-SJS/TEN in different ethnic populations

Allele (HLA-B*)	Serotype	Population	Allele frequency	Patients	Ref
1501	B62	Japanese	7.5%*	1/7 (14.2%)	Kaniwa et al., 2008
1502	B75	Han Chinese	8%#	59/60 (98.3%)	Hung et al., 2006
		Han Chinese	19.8%*	4/4 (100%)	Man et al., 2007
		Indian	1%*	6/8 (75%)	Mehta et al., 2010
		Thai	8.2%*	37/42 (88.1%)	Tassaneeyakul et al., 2010
		Asians in Europe	6.9%*	4/4 (100%)	Lonjou et al., 2006
1508	B75	Indian	1%*	1/8 (12.5%)	Mehta et al., 2010
1511	B75	Thai	0.1%*	1/42 (2.38%)	Tassaneeyakul et al., 2010
		Japanese	0.8%*	1/7 (14.2%)	Kaniwa et al., 2008
		Han Chinese	0.9%*	2/101 (1.9%)	Our unpublished data
1518	B71	Japanese	0.9%#	1/5 (20%)	Ikeda et al., 2010
1521	B75	Thai	0.7%*	2/42 (4.76%)	Tassaneeyakul et al., 2010
1558	B62	Han Chinese	0.9%*	1/60 (1.6%)	Hung et al., 2006

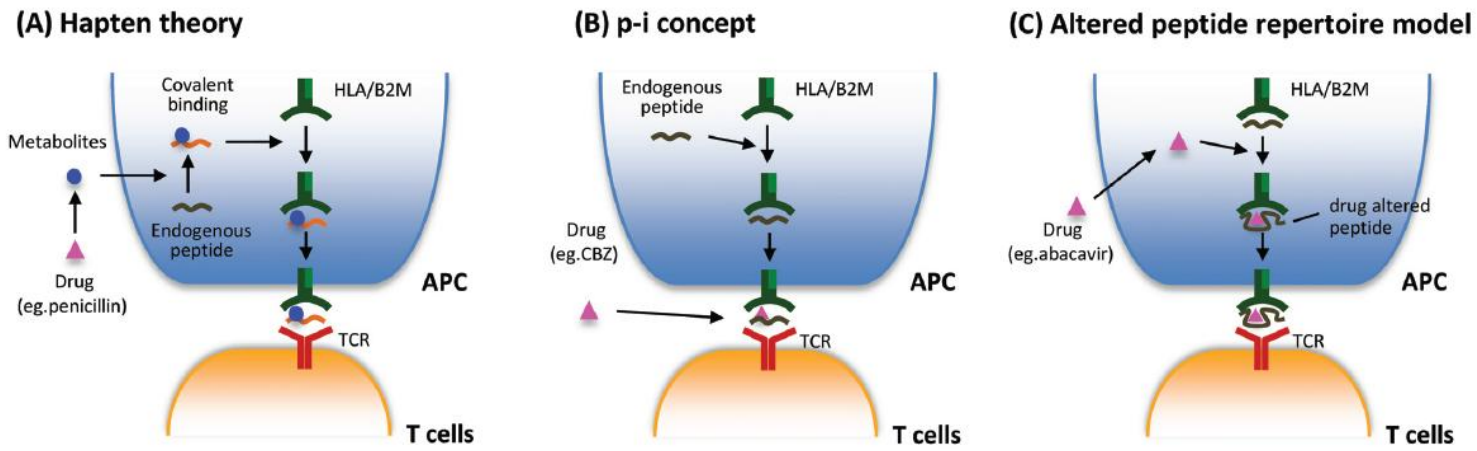
*Allele frequency from database: http://www.allelefrequencies.net/
Allele frequency adapted from reference paper.



- Stable transfection of different HLA-B cDNAs into keratinocytes.
- HLA-B75 members could elicit the cytotoxicity of CBZ-specific CTLs.

Wei CY, et al. JACI, 2012

How HLA and TCR recognize drugs in drug hypersensitivity?



	Hapten concept	p-i concept	altered self-peptide repertoire
Peptide-drug interaction	Covalent binding	Non-covalent	Non-covalent
Drug activity	Reactive (e.g penicillin)	inert (e.g.carbamazepine)	Inert (e.g. abacavir)
Ag processing	Processing, Non-processing	Non-processing	Processing
MHC restriction	MHC-restricted	MHC-restricted, non-restricted	MHC-restricted
TCR types	oligoclonal	Oligoclonal,polyclonal	polyclonal

Ren-You Pan et al., Current Immunology Reviews, 2014; Pichler WJ , Allergology International 2006 Jan;55; Illing P et al, Nature 2012

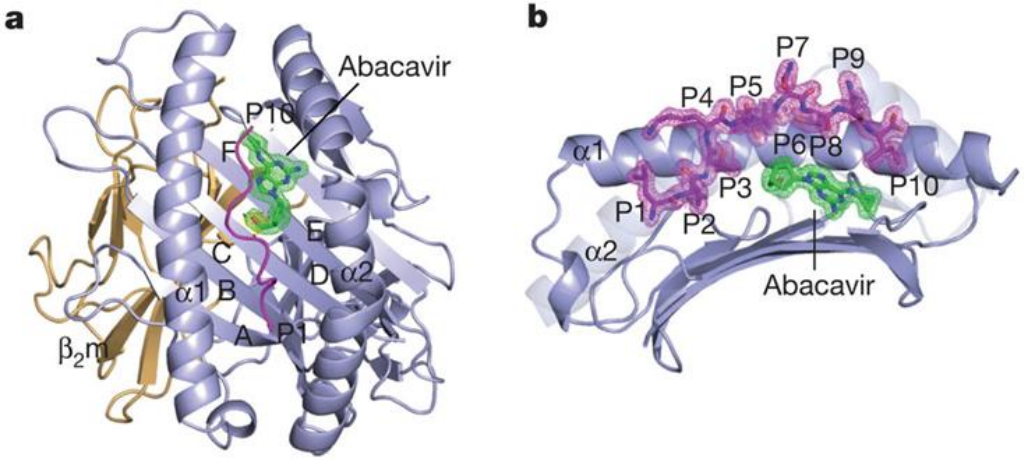
LETTER

Nature 2012, May

doi:10.1038/nature11147

Immune self-reactivity triggered by drug-modified HLA-peptide repertoire

Patricia T. Illing[1,2], Julian P. Vivian[3], Nadine L. Dudek[2], Lyudmila Kostenko[1], Zhenjun Chen[1], Mandvi Bharadwaj[1], John J. Miles[4,5], Lars Kjer-Nielsen[1], Stephanie Gras[3], Nicholas A. Williamson[2], Scott R. Burrows[4], Anthony W. Purcell[2]*, Jamie Rossjohn[3,5]* & James McCluskey[1,6]*



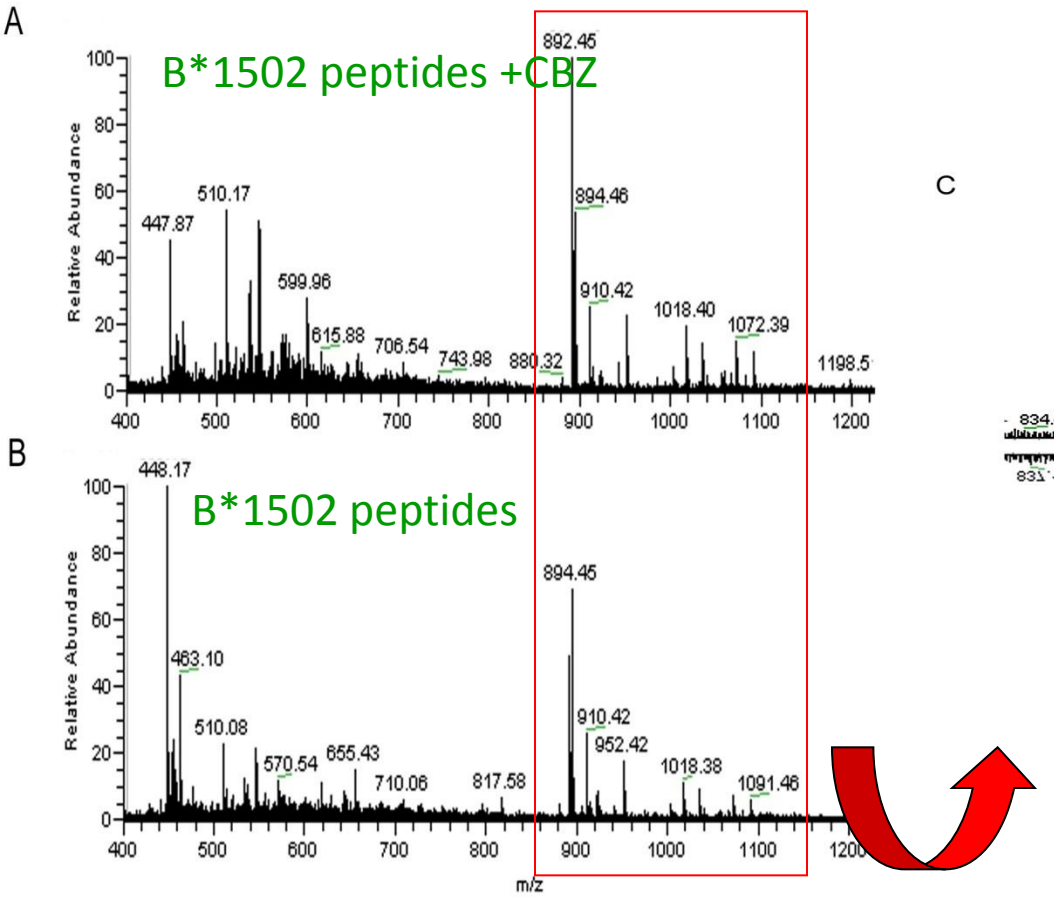
Abacavir (ABC) binds to the F-pocket of HLA-B*57:01;

the ABC filled F-pocket leads to selection of peptides with valin at position 9 (instead of tryptophan / phenylalanin)

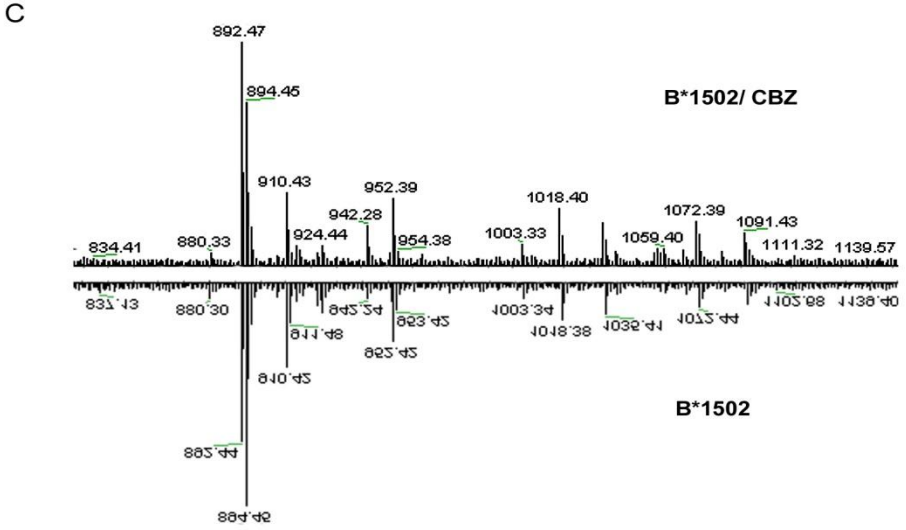
(ca 20% of peptides are altered, 80% not altered; described for very high ABC concentrations (100μg/ml)) (Illing P et al, Nature 2012)

Peptide repertoire: CBZ-HLA-B*1502 model

Using LC-MS/MS to investigate the peptides bound by HLA-B*1502.



B*1502 peptides + CBZ



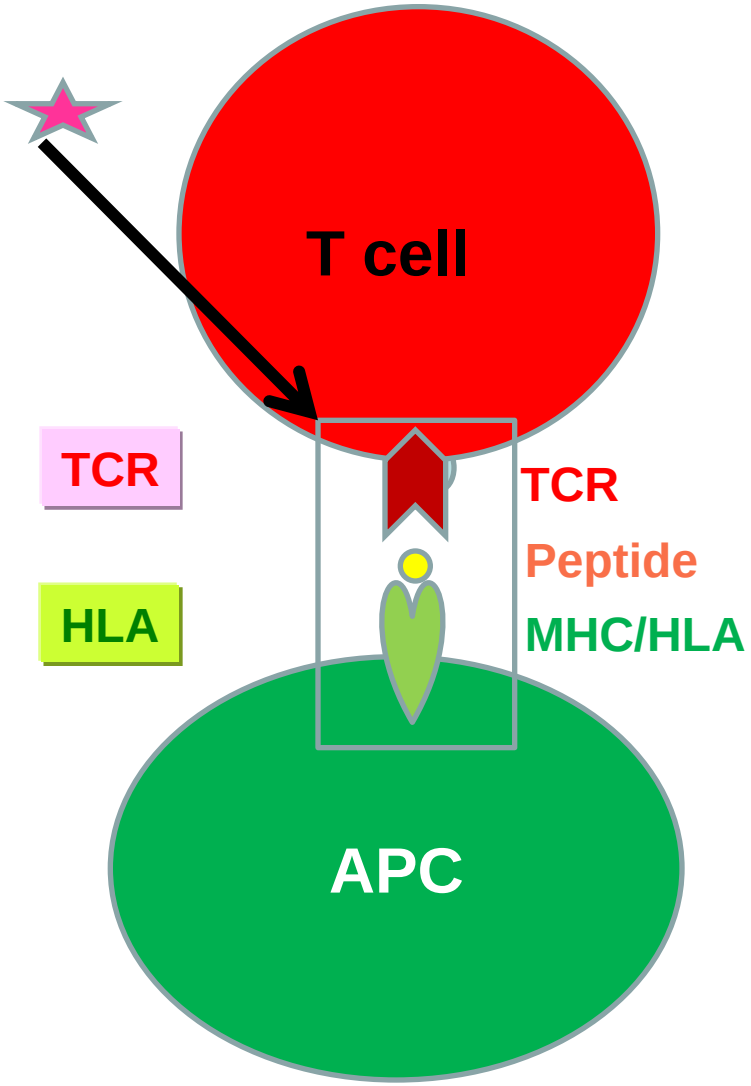
B*1502 peptides

➔No detection of CBZ covalently modified peptides.

➔No detection of altered self-peptide repertoire

Ou Yang CW, et al., JACI, 2007

TCR: shared VB-11 CDR3 clonotypes of CBZ-stimulated CD8+ T cells from patients with CBZ-SJS/TEN. (oligoclonal)

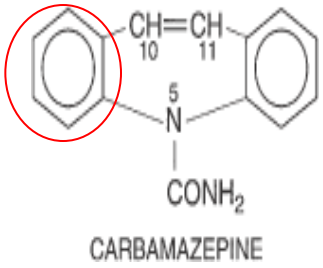


Patient	VB	TRBV	TRBD	TRBJ	CDR3	Frequency
S1	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	*CASSISGSYNEQFF*	82%
	B11	TRBV25-1*01	TRBD2*02	TRBJ2-1*01	*CASSGLAGVDNNEQFF*	18%
S2	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	*CASSISGSYNERFF*	100%
S3	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	*CASSISGSYNEQFF*	50%
	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	*CASSISGSYNEQSF*	42%
	B11	TRBV25-1*01	TRBD2*01	TRBJ2-5*01	CASSEYMGIQETQYF	8%
S4	B11	TRBV25-1*01	TRBD2*02	TRBJ2-1*01	*CASSGLAGVDNNEQFF*	39%
	B11	TRBV25-1*01	TRBD2*01	TRBJ2-7*01	CASSLGYEQYF	22%
	B11	TRBV25-1*01		TRBJ1-1*01	CASSDNTEAFF	11%
S5	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	*CASSISGSYNEQFF*	100%
S6	B11	TRBV25-1*01		TRBJ2-7*01	CASSAHEQYF	83%
	B11	TRBV25-1*01	TRBD1*01	TRBJ1-2*01	CASSEWGEVGKGYTF	17%
S7	B11	TRBV25-1*01	TRBD2*01	TRBJ2-1*01	*CASSISGSYNEQFF*	100%
S8	B11	TRBV25-1*01	TRBD1*01	TRBJ2-7*01	CASSEDRSPYEQYF	100%

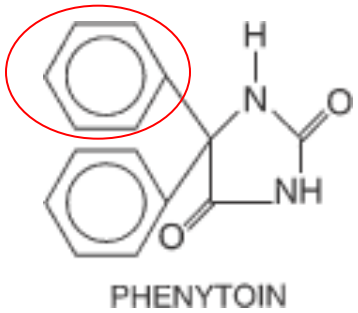
VB-11-ISGSY (5/8)
VB-11-GLAGVDN (2/8)

Ko TM et al., JACI, 2011

Is HLA-B*1502 as marker for phenytoin-induced SJS/TEN?



CARBAMAZEPINE



PHENYTOIN

Genotype	**PHT-SJS/TEN** (case: 24 vs. PHT tolerant: 103) case/tolerant(**p value/ OR**)	**PHT-MPE/HSS** (case: 38 vs. PHT tolerant: 103) case/tolerant(**p value/OR**)
HLA-A		
*1101	14/49(0.47/1.54)	28/49 (0.01/3.01)
*2402	6/32 (0.736/0.76)	8/32 (0.337/0.59)
*3101		.16)
HLA-B		
*1502	9/8 (p=4.3x 10^{-4}, OR: 7.13)	4/8 (**0.856/1.40**)
*4001	5/38(0.208/0.45)	11/38 (0.497/0.70)

Only weak association with SJS/TEN

Hung SI& Chung WH et al, Pharmacogenomics. 2010

Research

JAMA August 6, 2014 Volume 312, Number 5

Original Investigation

Genetic Variants Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions

Wen-Hung Chung, MD, PhD; Wan-Chun Chang, MS; Yun-Shien Lee, PhD; Ying-Ying Wu, MS; Chih-Hsun Yang, MD; Hsin-Chun Ho, MD; Ming-Jing Chen, MD; Jing-Yi Lin, MD; Rosaline Chung-Yee Hui, MD, PhD; Ji-Chen Ho, MD; Wei-Ming Wu, MD, PhD; Ting-Jui Chen, MD; Tony Wu, MD, PhD; Yih-Ru Wu, MD, PhD; Mo-Song Hsih, MD; Po-Hsun Tu, MD; Chen-Nen Chang, MD, PhD; Chien-Ning Hsu, PhD; Tsu-Lan Wu, PhD; Siew-Eng Choon, MD; Chao-Kai Hsu, MD, PhD; Der-Yuan Chen, MD, PhD; Chin-San Liu, MD, PhD; Ching-Yuang Lin, MD, PhD; Nahoko Kaniwa, PhD; Yoshiro Saito, PhD; Yukitoshi Takahashi, MD, PhD; Ryosuke Nakamura, PhD; Hiroaki Azukizawa, MD, PhD; Yongyong Shi, PhD; Tzu-Hao Wang, MD, PhD; Shiow-Shuh Chuang, MD, PhD; Shih-Feng Tsai, MD, PhD; Chee-Jen Chang, PhD; Yu-Sun Chang, PhD; Shuen-Iu Hung, PhD; for the Taiwan Severe Cutaneous Adverse Reaction Consortium and the Japan Pharmacogenomics Data Science Consortium

GWAS: 60 phenytoin-induced SCAR (38 SJS/TEN, 22 DRESS) v.s. 412 subjects of general population, Taiwan

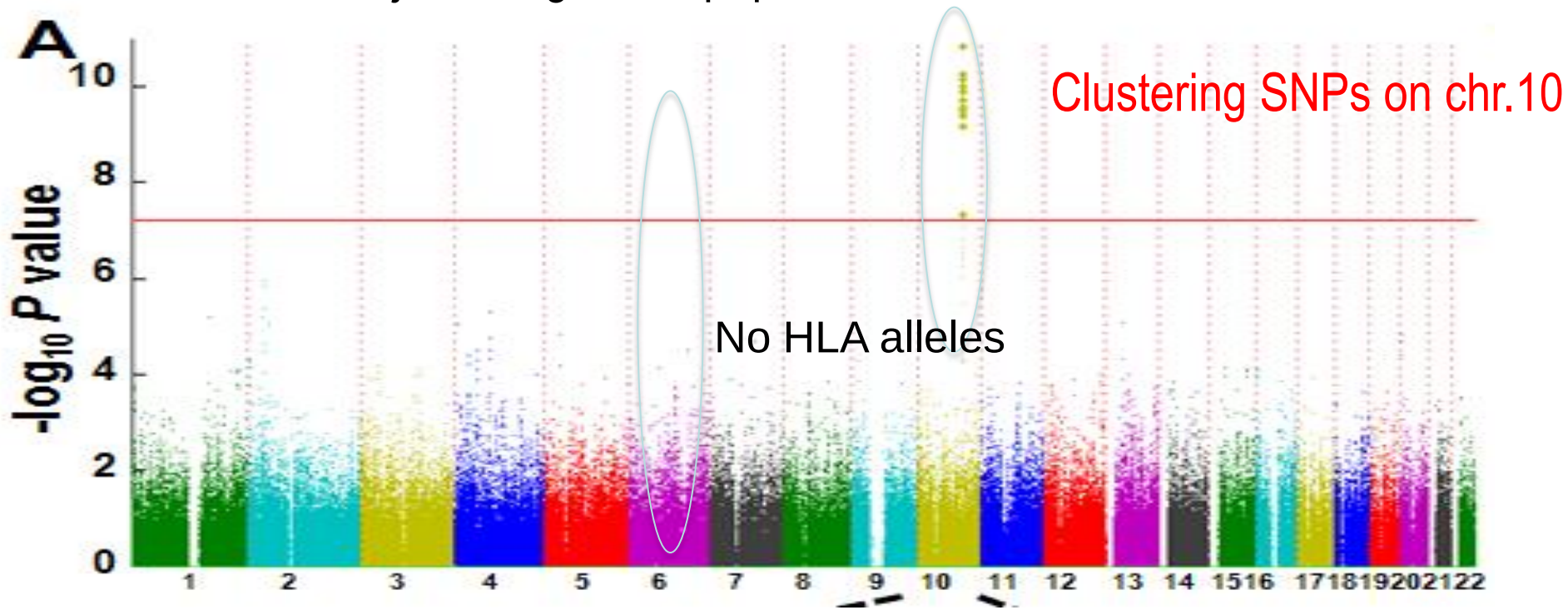
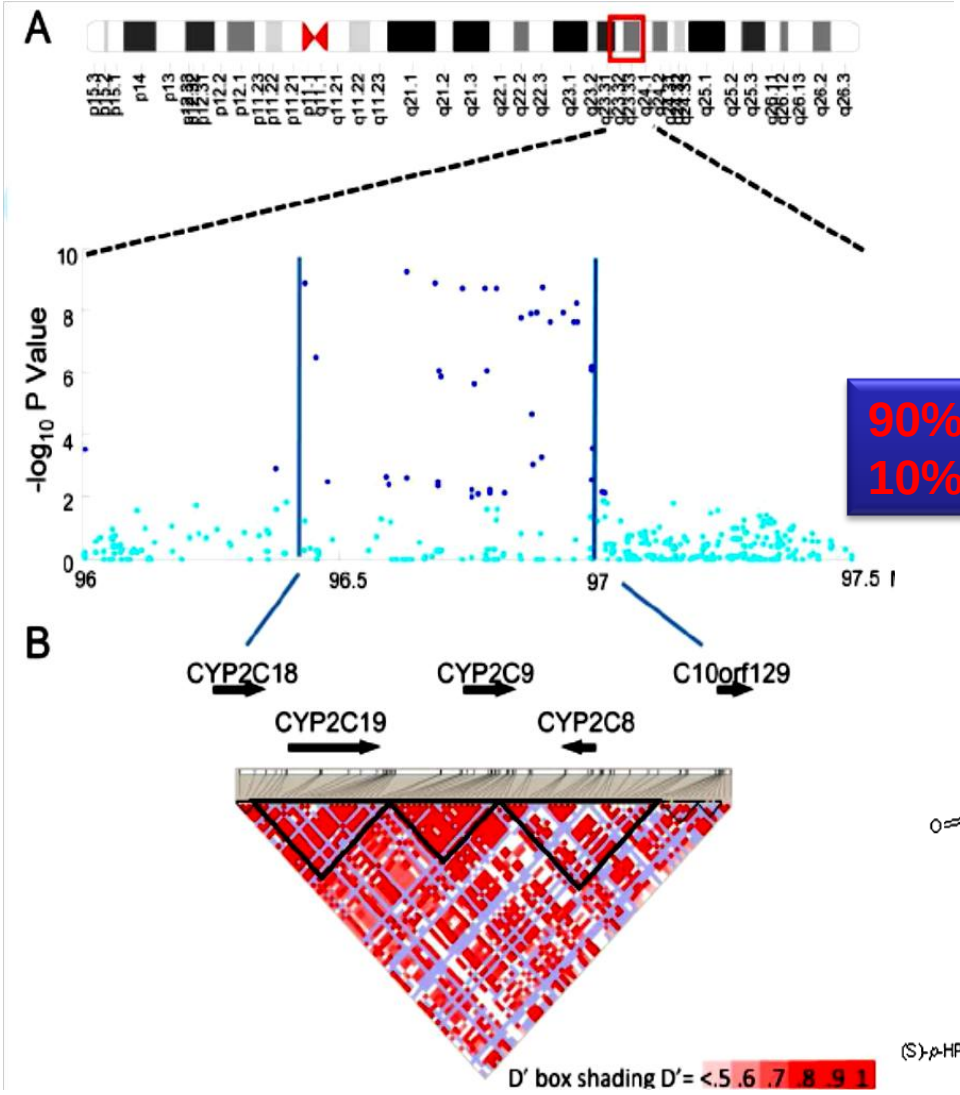
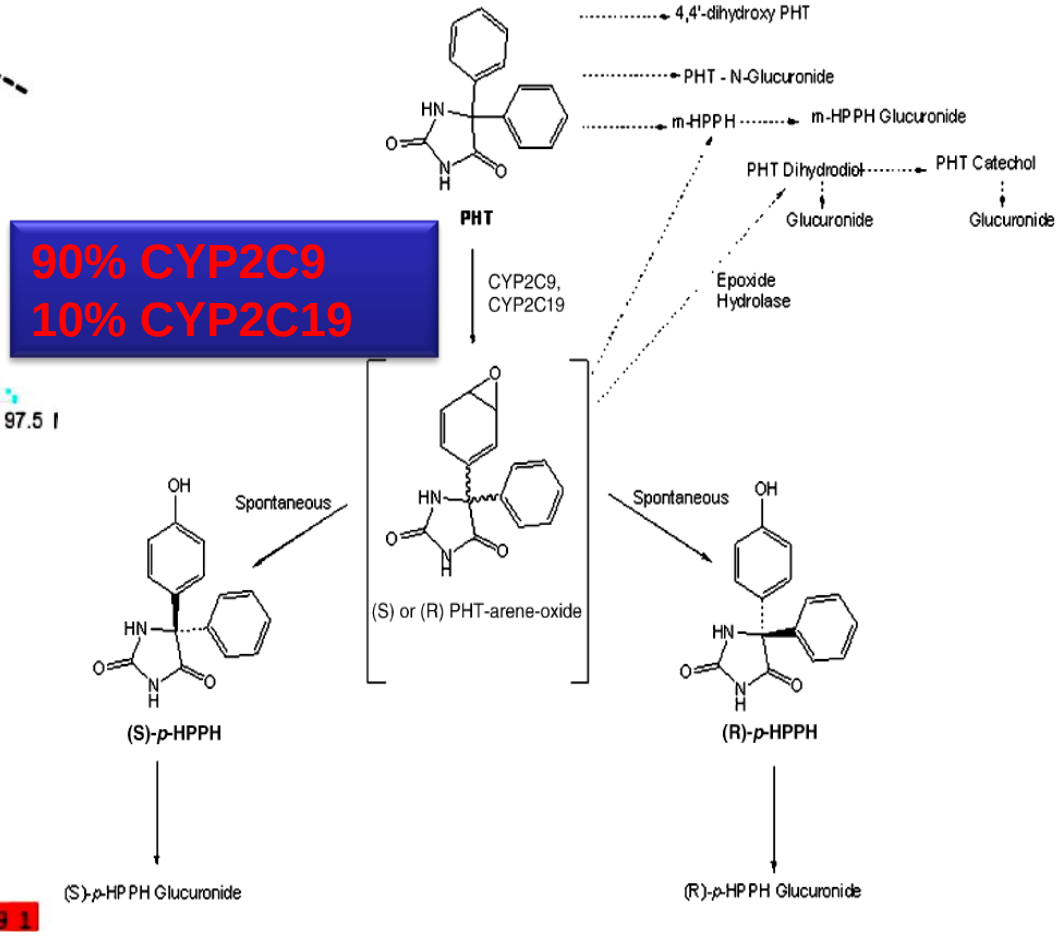


Figure 2. Linkage Disequilibrium Heat Maps for the *CYP2C* Region Associated With Phenytoin-Related Severe Cutaneous Adverse Reactions



The major metabolizing enzymes of Phenytoin



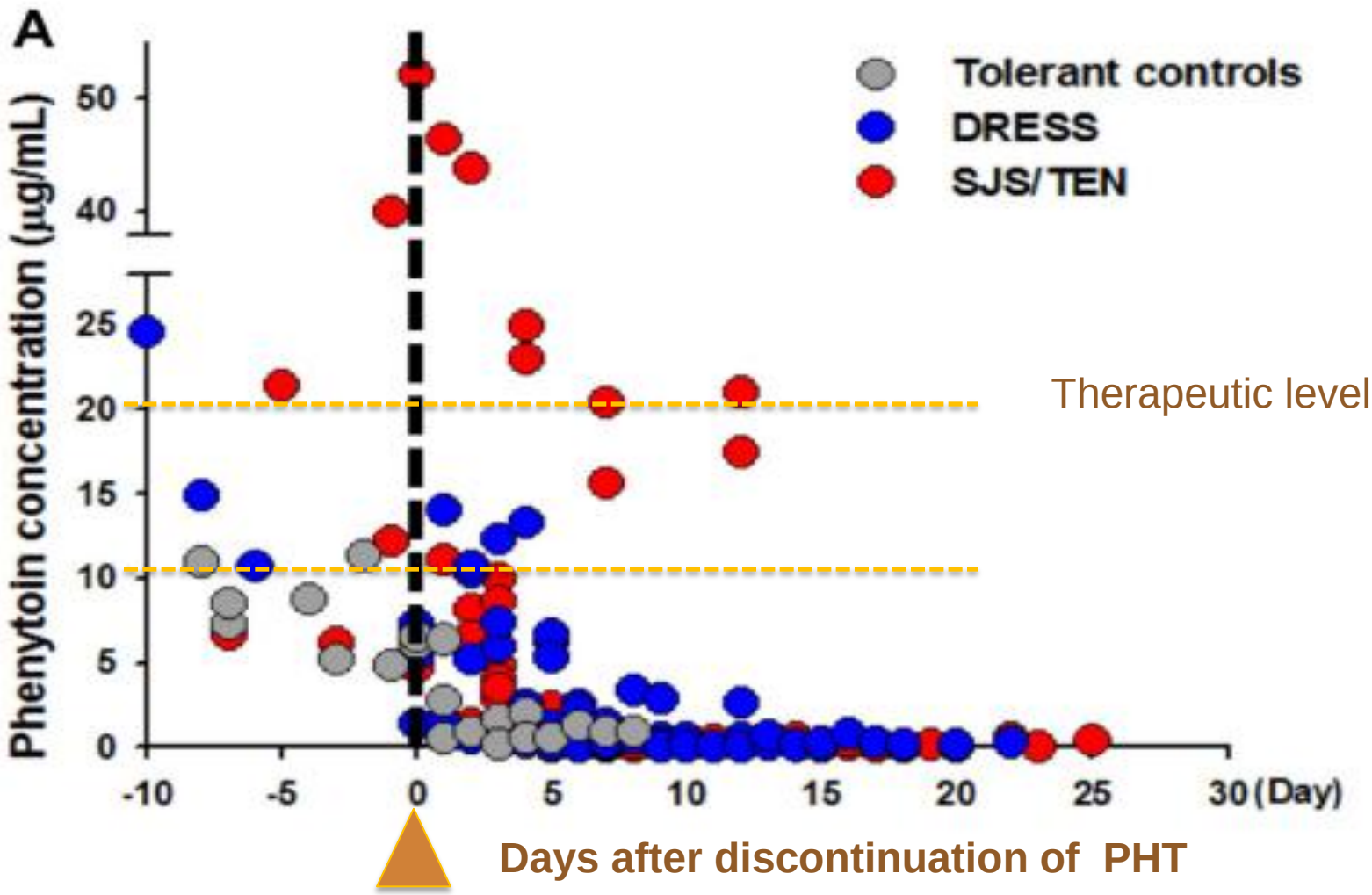
CYP2C*3 is strongly associated with PHT-SJS/TEN & DRESS, but less with PHT-maculopapular exanthem

eTable 7. Association of rs1057910 (*CYP2C9*3*) with phenytoin-induced cutaneous adverse reactions.[+]

Subgroup (Participants number, *N*)	Positive Participants *N* (%)	*P* value	ARR (95% CI)	Odds Ratio (95% CI)	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)
Tolerant controls (130)	3 (2.3%)					
SCAR (90)	33 (36.7%)	5.7×10^{-12}	-0.34 (-0.45 to -0.24)	25 (7.2-83)	36.7 (27-48)	97.7 (93-99)
SJS/TEN (48)	20 (41.7%)	1.2×10^{-10}	-0.39 (-0.54 to -0.25)	30 (8.4-109)	41.7 (28-57)	97.7 (93-99)
DRESS (42)	13 (31.0%)	7.0×10^{-7}	-0.29 (-0.43 to -0.14)	19 (5.1-71)	31.0 (18-47)	97.7 (93-99)
MPE (78)	9 (11.5%)	0.011	-0.092 (-0.17 to -0.017)	5.5 (1.5-21)	11.5 (6-21)	97.7 (93-99)
Population controls (412)	20 (4.9%)					
SCAR (90)	33 (36.7%)	1.3×10^{-14}	-0.32 (-0.42 to -0.22)	11 (6.1-21)	36.7 (27-48)	95.2 (92-97)
SJS/TEN (48)	20 (41.7%)	1.3×10^{-11}	-0.37 (-0.51 to -0.23)	14 (6.8-29)	41.7 (28-57)	95.2 (92-97)
DRESS (42)	13 (31.0%)	8.6×10^{-7}	-0.26 (-0.40 to -0.12)	8.8 (4.0-19)	31.0 (18-47)	95.2 (92-97)
MPE (78)	9 (11.5%)	0.033	-0.067 (-0.14 to 0.007)	2.6 (1.1-5.9)	11.5 (6-21)	95.2 (92-97)

[+]The reported data are based on the analysis of the dominant-inheritance model for the risk genotype. Abbreviations: ARR, absolute risk reduction; CI, confidence interval; DRESS, drug reaction with eosinophilia and systemic symptoms; MPE, maculopapular exanthema; SCAR, severe cutaneous adverse reactions; SJS/TEN, Stevens-Johnson syndrome/toxic epidermal necrolysis.

Poor metabolism of phenytoin in the PHT-SCAR patients

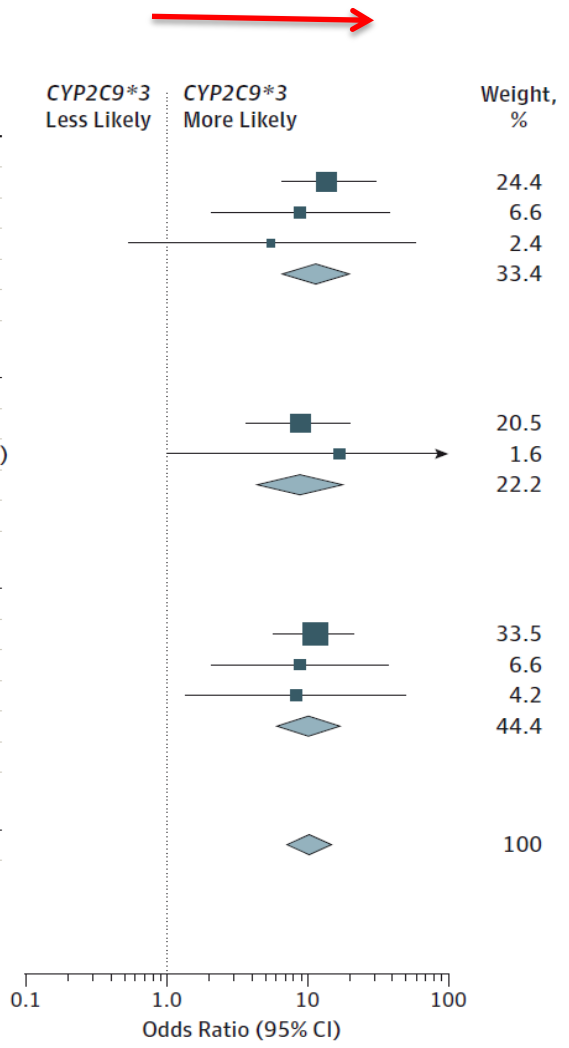


The levels of plasma phenytoin were measured by fluorescence polarization immunoassay analysis.

Same significant association of *CYP2C9*3* with PHT-SCAR was observed in patients from Taiwan, Japan, and Malaysia

Figure 3. Distribution of the *CYP2C9*3* Variant in Cases With Phenytoin-Related Severe Cutaneous Adverse Reactions and Population Controls

Subgroup	Cases of Phenytoin-Related Severe Cutaneous Adverse Reactions, No.		Population Controls, No.		Odds Ratio (95% CI)
	*CYP2C9*3* Carriers	Total Participants	*CYP2C9*3* Carriers	Total Participants	
SJS/TEN					
Taiwan	20	48	20	412	14.00 (6.75-29.02)
Japan	3	9	153	2869	8.88 (2.20-35.83)
Malaysia	1	4	21	374	5.60 (0.56-56.20)
Subtotal		61		3655	11.96 (6.42-22.28)
Total No. of *CYP2C9*3* carriers	24		194		
Heterogeneity: $\tau^2 = 0.00$; $\chi^2_2 = 0.77$; $P = .68$; $I^2 = 0\%$					
Test for overall effect: $z = 7.82$; $P < .00001$					
DRESS					
Taiwan	13	42	20	412	8.79 (3.97-19.43)
Malaysia	1	2	21	374	16.81(1.02-278.24)
Subtotal		44		786	9.22 (4.30-19.78)
Total No. of *CYP2C9*3* carriers	14		41		
Heterogeneity: $\tau^2 = 0.00$; $\chi^2_1 = 0.19$; $P = .66$; $I^2 = 0\%$					
Test for overall effect: $z = 5.70$; $P < .00001$					
All severe cutaneous adverse reactions (SJS/TEN and DRESS)					
Taiwan	33	90	20	412	11.35 (6.10-21.12)
Japan	3	9	153	2869	8.88 (2.20-35.83)
Malaysia	2	6	21	374	8.40 (1.46-48.54)
Subtotal		105		3655	10.63 (6.20-18.24)
Total No. of *CYP2C9*3* carriers	38		194		
Heterogeneity: $\tau^2 = 0.00$; $\chi^2_2 = 0.18$, $P = .91$; $I^2 = 0\%$					
Test for overall effect: $z = 8.58$; $P < .00001$					
Total	76	210	429	8096	10.71 (7.48-15.35)
Heterogeneity: $\tau^2 = 0.00$; $\chi^2_7 = 1.41$; $P = .99$; $I^2 = 0\%$					
Test for overall effect: $z = 12.92$; $P < .00001$					
Test for subgroup differences: $\chi^2_2 = 0.27$; $P = .87$; $I^2 = 0\%$					



Non-genetic factors: metabolism and drug hypersensitivity

Clinical and epidemiological research

EXTENDED REPORT

Insights into the poor prognosis of allopurinol-induced severe cutaneous adverse reactions: the impact of renal insufficiency, high plasma levels of oxypurinol and granulysin

Wen-Hung Chung,[1,2,3] Wan-Chun Chang,[4] Sophie L Stocker,[5,6] Chiun-Gung Juo,[7] Garry G Graham,[5,6] Ming-Han H Lee,[5,6] Kenneth M Williams,[5,6] Ya-Chung Tian,[3,8] Kuo-Chang Juan,[3,8] Yeong-Jian Jan Wu,[3,9] Chih-Hsun Yang,[2,3] Chee-Jen Chang,[10,11] Yu-Jr Lin,[10,11] Richard O Day,[5,6] Shuen-Iu Hung[4]

Ann Rheum Dis. 2014 Aug 12

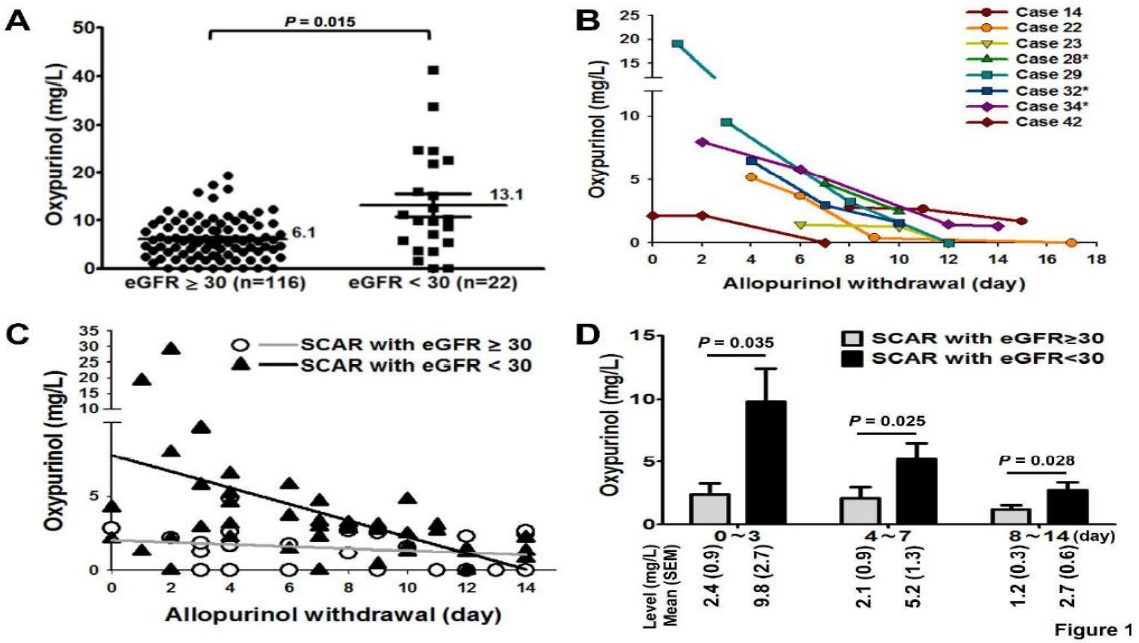


Figure 1

Correlation between the levels of plasma oxypurinol and prognosis of allopurinol-SCAR

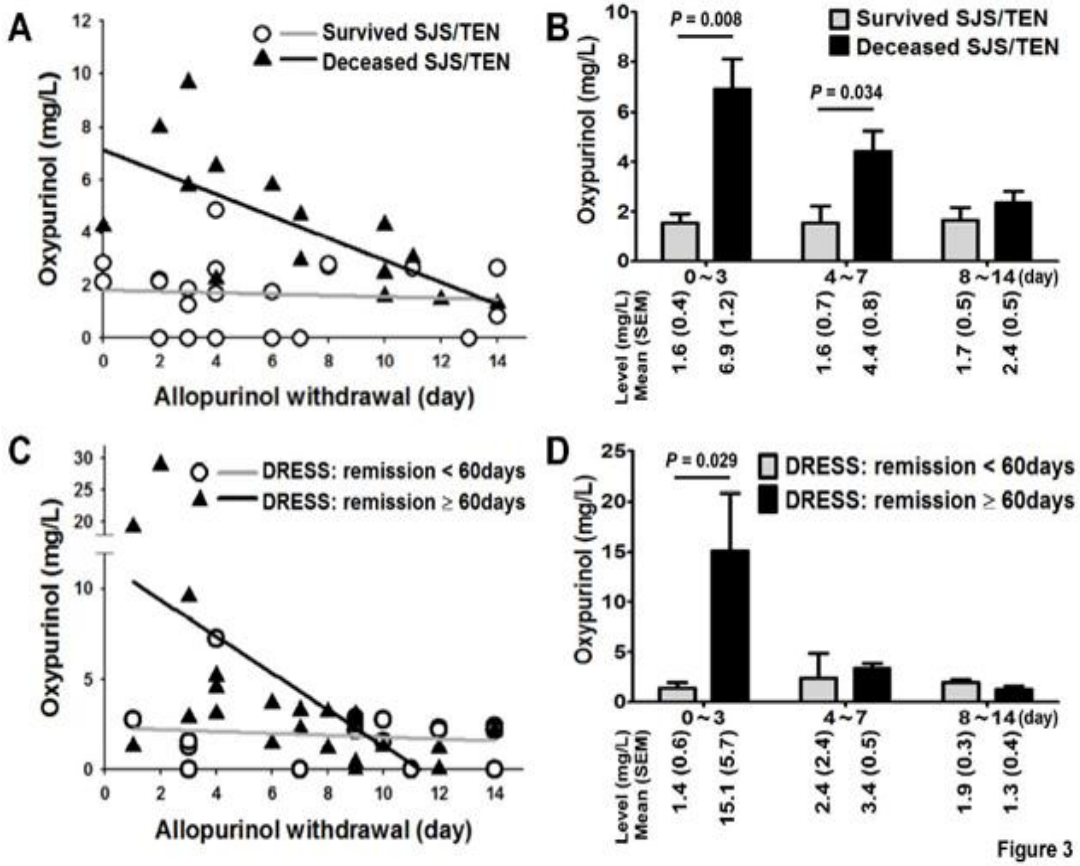
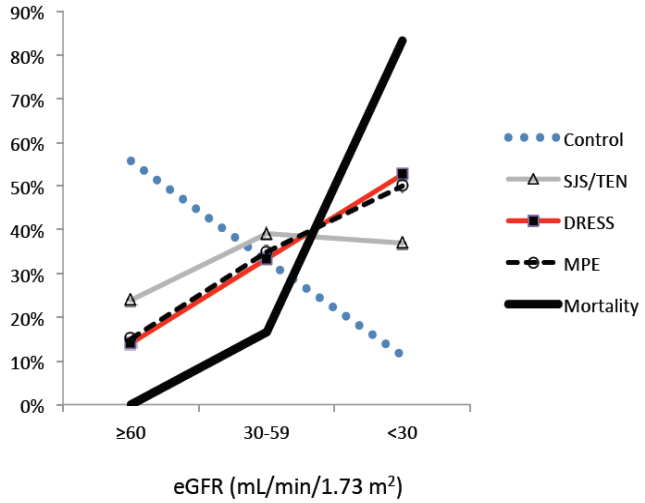


Figure 3



Chung WH et al, Ann Rheum Dis. 2014

doi: 10.1111/cea.12184

Clinical & Experimental Allergy, **43**, 1246–1255

ORIGINAL ARTICLE Clinical Mechanisms in Allergic Disease

Allopurinol hypersensitivity is primarily mediated by dose-dependent oxypurinol-specific T cell response

J. Yun[1,2], J. Mattsson[3], K. Schnyder[1], S. Fontana[4], C. R. Largiadèr[3], W. J. Pichler[1] and D. Yerly[1]

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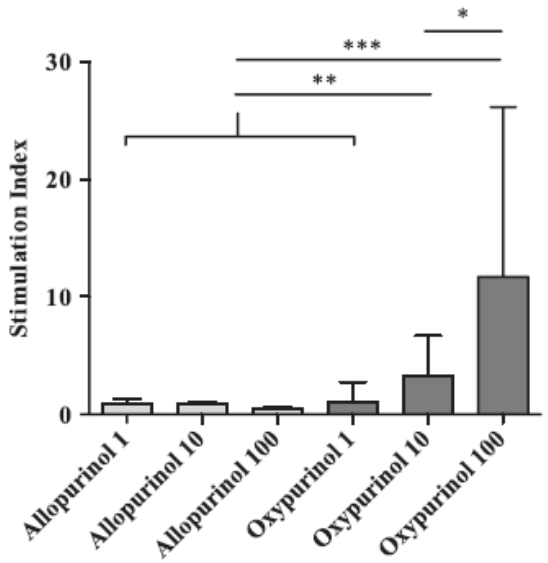


Fig. 1. Lymphocyte transformation test (LTT) results of allopurinol allergic patients with positive LTT (n = 12). Patients' peripheral blood mononuclear cells (PBMC) are tested against varying concentrations (1, 10 and 100 μg/mL) of allopurinol and oxypurinol. Bars represent median with interquartile range. *P < 0.05, **P < 0.01, ***P < 0.001.

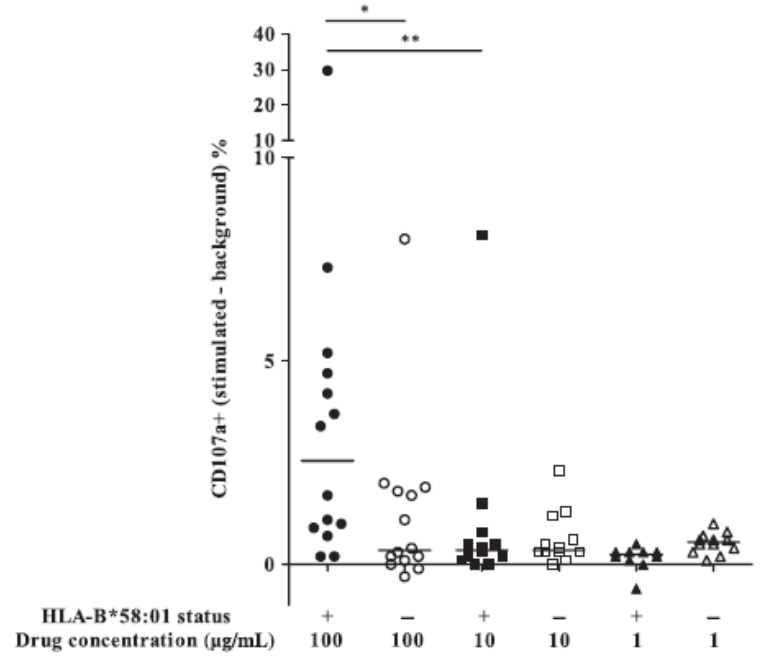


Fig. 3. The maximum observed differences in upregulation of CD107a on CD8 T cells in ALP/OXP-TCL from the background. The lines represent median values. Mann–Whitney U-test was used. *P < 0.05, **P < 0.01.

Cytotoxic mechanisms for extensive keratinocyte death in SJS/TEN

Maculopapules, SJS/TEN

Nassif A. et al. J Allergy Clin Immunol. 2004

SJS/TEN

cytotoxic T cells

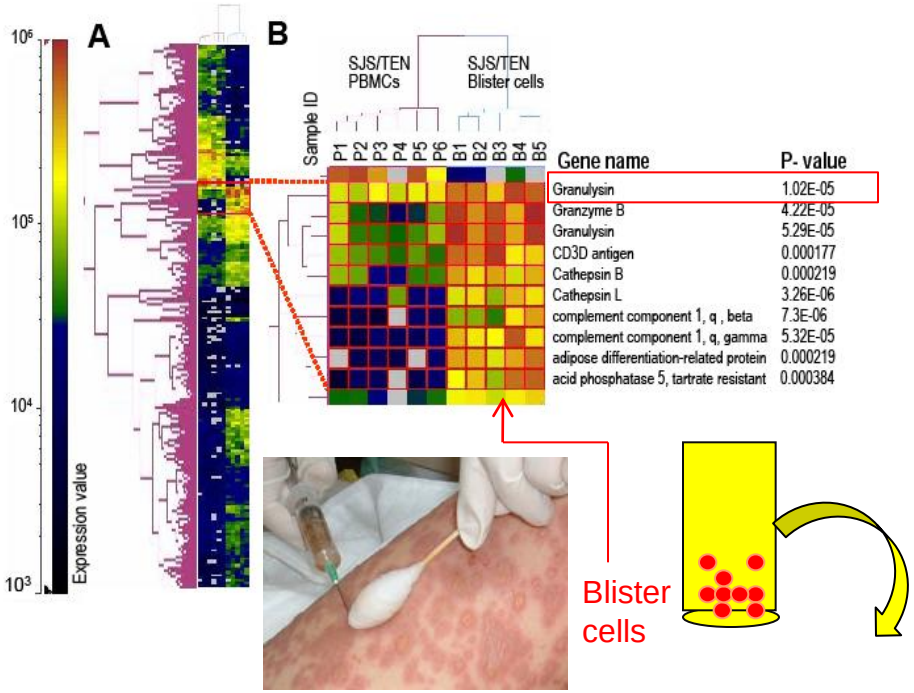
*Viard I, et al.*Science. 1998

nature medicine

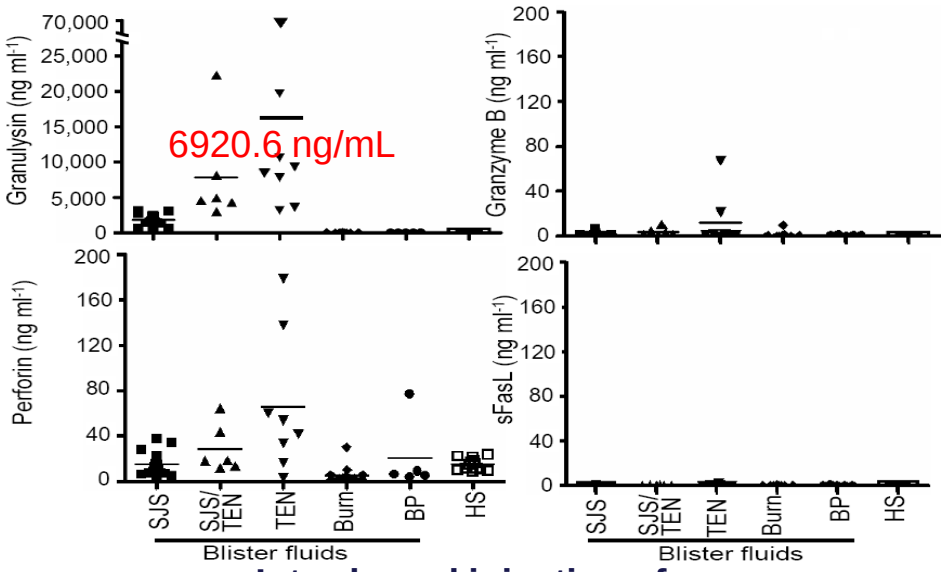
2008

Granulysin is a key mediator for disseminated keratinocyte death in Stevens-Johnson syndrome and toxic epidermal necrolysis

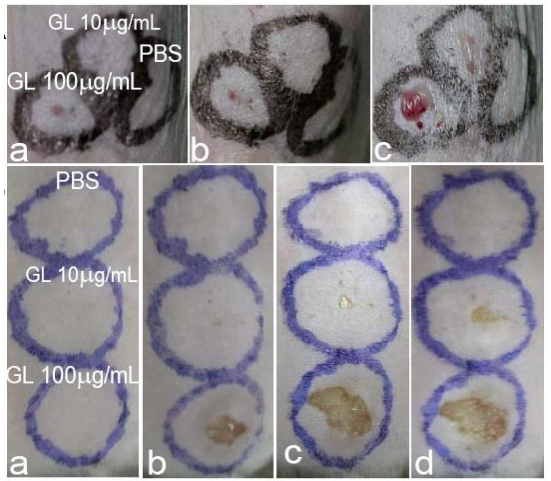
Wen-Hung Chung[1–3,9], Shuen-Iu Hung[2,4,9], Jui-Yung Yang[5], Shih-Chi Su[2], Shien-Ping Huang[2], Chun-Yu Wei[2], See-Wen Chin[4], Chien-Chun Chiou[1], Sung-Chao Chu[6], Hsin-Chun Ho[1], Chih-Hsun Yang[1], Chi-Fang Lu[7], Jer-Yuarn Wu[2], You-Di Liao[2] & Yuan-Tsong Chen[2,8]



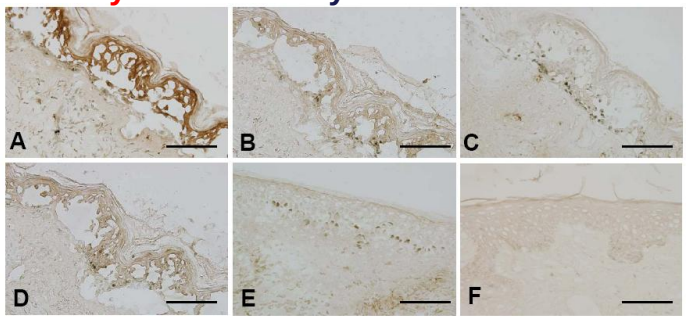
Blister fluid ELISA



Intradermal injection of granulysin in mice skin

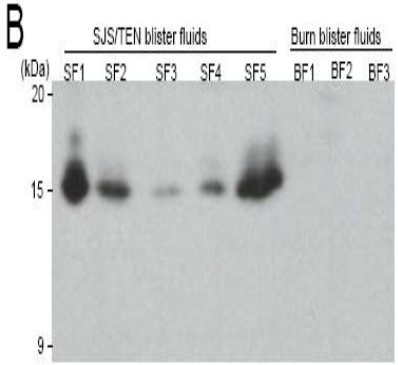


Granulysin/SJS Granzyme B/SJS Perforin/SJS

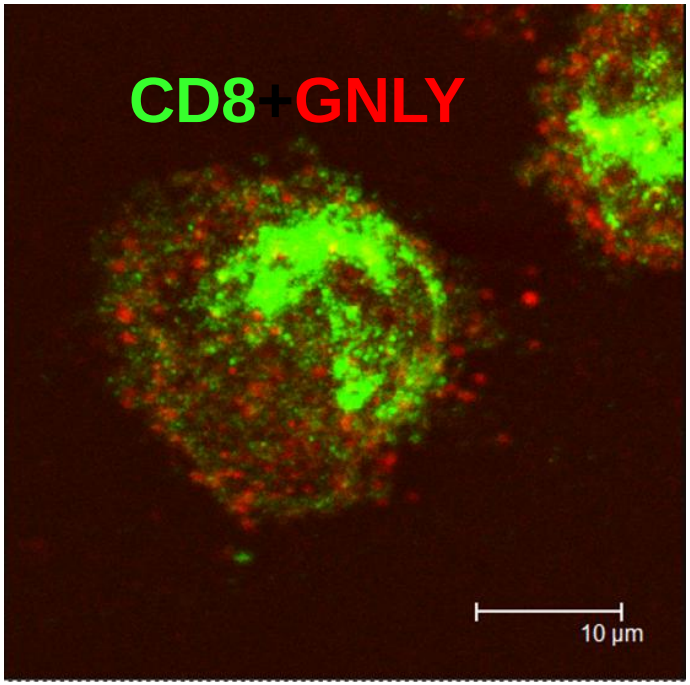


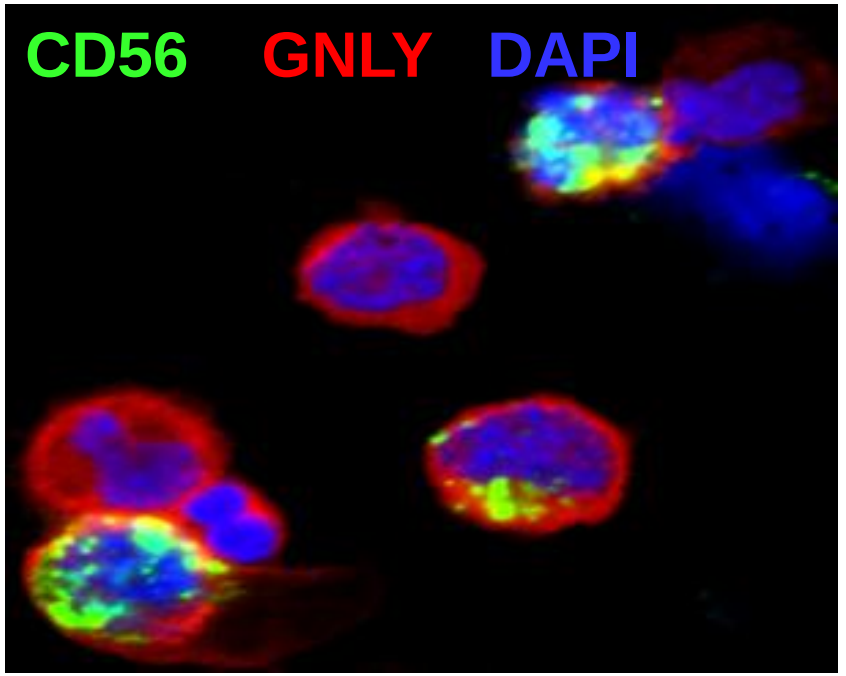
FasL/SJS Granulysin/MPE Granulysin/health

Granulysin in the blister fluids is a 15 kDa form



Expression of granulysin (GNLY) in CTLs/NK/NKT cells of SJS/TEN blister cells





Surface marker	Cell Type	Percentage (average, n=6)
$CD3^+$ $CD8^+$	**$CD8^+$ T cell**	**55.7 + 15%**
$CD8^+$ GL^+	GNLY expressing $CD8^+$ T cell	27.4+ 15.2 %
$CD3^-CD56^+$ GL^+	GNLY expressing NK cell	31.1+ 17.4 %
$CD8^+CD56^+$ GL^+	GNLY expressing NKT cell	25.8+ 17.1 %

Serum level

Granulysin as a Marker for Early Diagnosis of the Stevens–Johnson Syndrome

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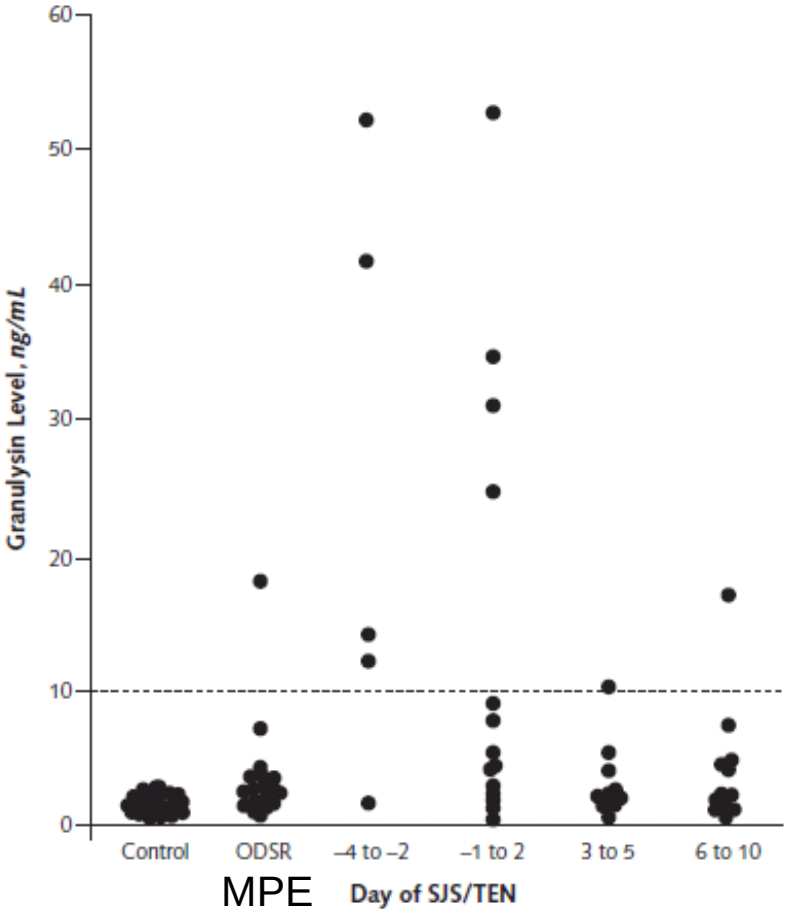
Figure. **Granulysin levels of healthy control participants, patients with ODSRs, and patients with SJS/TEN.**



MPE

ODSR = ordinary drug-induced skin reaction; SJS/TEN = Stevens–Johnson syndrome/toxic epidermal necrolysis.

SJS caused by mortrin?

Comparison of *in vitro* tests to detect the causative drugs for the type IV (delayed type) drug hypersensitivity

Test	Method	Limitation
1. lymphocyte transformation test (LTT)	Measure the cell proliferation	Radioactivity; expensive equipment
		Need well-trained technicians
		Sensitivity for the detection of causality of MPE: 57%-78% **Sensitivity for the detection of causality of SJS/TEN: <20%**
2. Detection of CD69 expression on **Th1** cell surface	Flow cytometry	CD69: a marker of early T cells activation, is associated with Th1 T cell differentiation. Only 0.5-3% T cells expressing CD69
3. Cytokine expression and secretion (IL2, IL5, IFNγ)	ELISA	These cytokines are not specific for delayed-type drug hypersensitivity Poor results of the sensitivity and specificity

Nyfeler et al, 1997; Luque et al, 2001 Pichler et al, 2004, Fu and et al, 2012

Cytokines& chemokines in SJS/TEN

SJS or TEN related cytokines and chemokines.

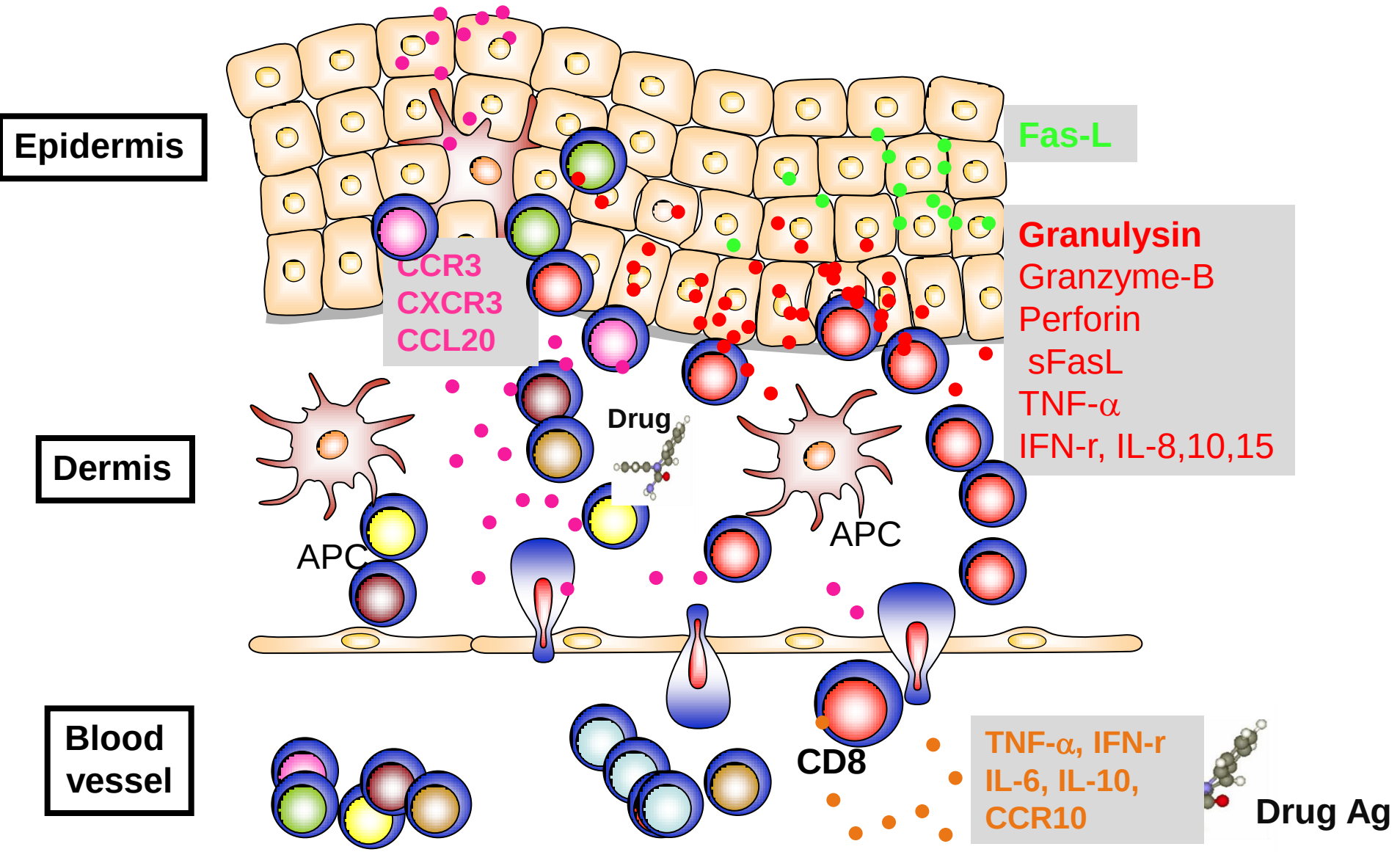
Cytokines/chemokines	Functions	Blister cell	Blister fluid	Skin tissue	PBMC	Serum
TNF-α [55,60,62]	Inflammation, apoptosis	NS[a]	+	+	+	
IFN-γ [55,60,62]	Activation of immune cells	+	+	+	+	
IL-2 [55,62]		NS	NS	+	+	
IL-5 [62]	Acute phase response			+		
	Proinflammatory cytokine					
	Antiinflammatory cytokine					
IL-6 [58,59,62]	Acute phase response			+	+	+
	Pro-inflammatory and anti-inflammatory cytokine					
IL-10 [59,60]	Anti-inflammatory cytokine	NS	+		+	+
IL-12 [60]	Activation of NK and CTL		NS			
IL-13 [62]				+		
IL-15 [60]	Regulation of T and NK cell activation and proliferation		+/–			
IL-18 [60]	Stimulation of the growth of T lymphocytes		++			
CCR3 [62]	Binding chemokines (eotaxin, MCP-3, MCP-4, and RANTES)			+		
CXCR3 [62]	Regulation of leukocyte trafficking			+		
CXCR4 [62]	Chemotactic activity for lymphocytes			NS		
CCR10 [61]	Trafficking leukocytes				+	

TNF-α, tumor necrosis factor-alpha; IFN-γ, interferon-gamma; IL, interleukin; CCR, C–C chemokine receptor; CXCR, CX chemokine receptor; +, positive; –, negative.

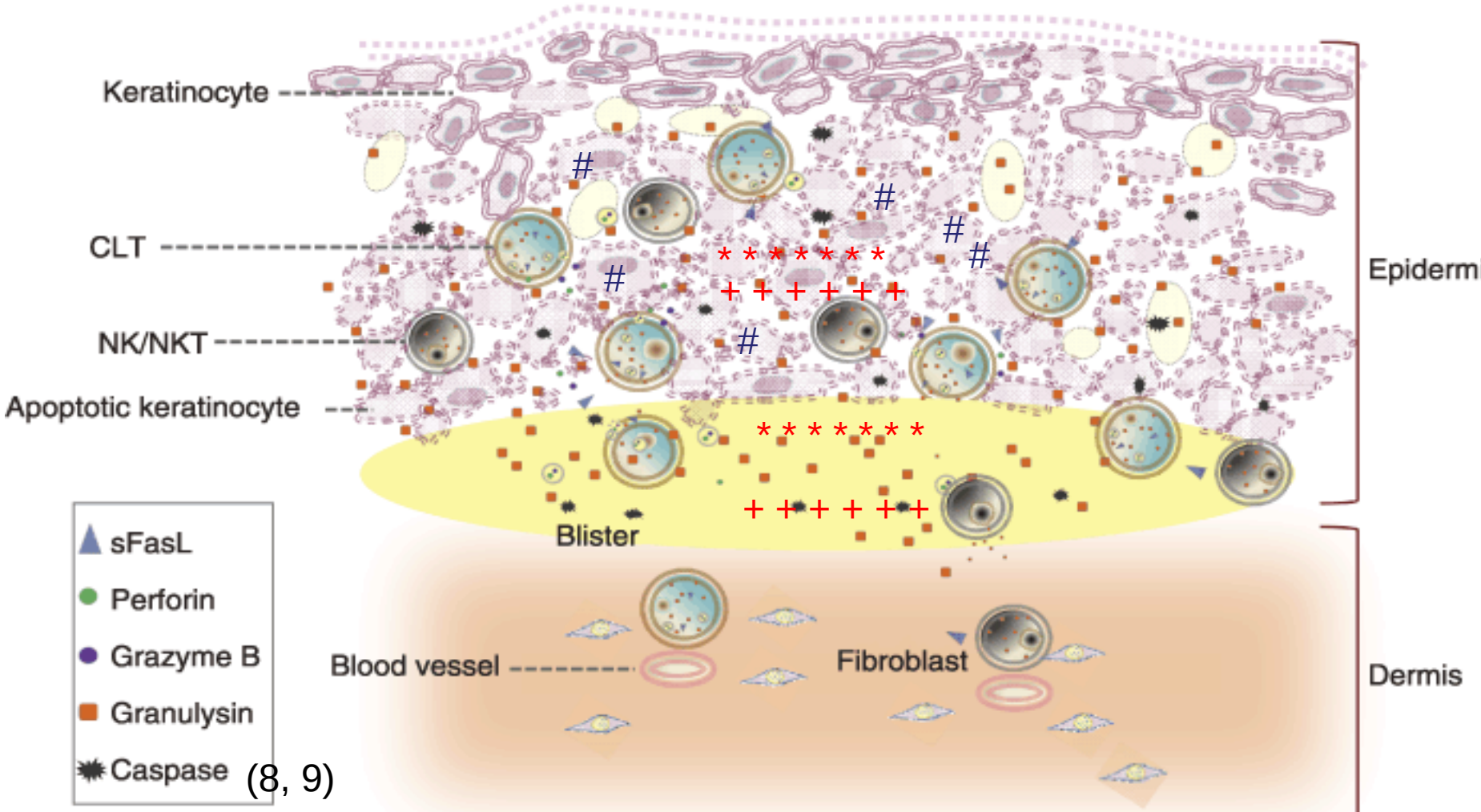
[a] NS, not significant.

Chung WH et al, J Dermatol Sci, 2012

Immune mechanism in SJS/TEN



Cell death mechanisms in SJS/TEN



(8, 9)

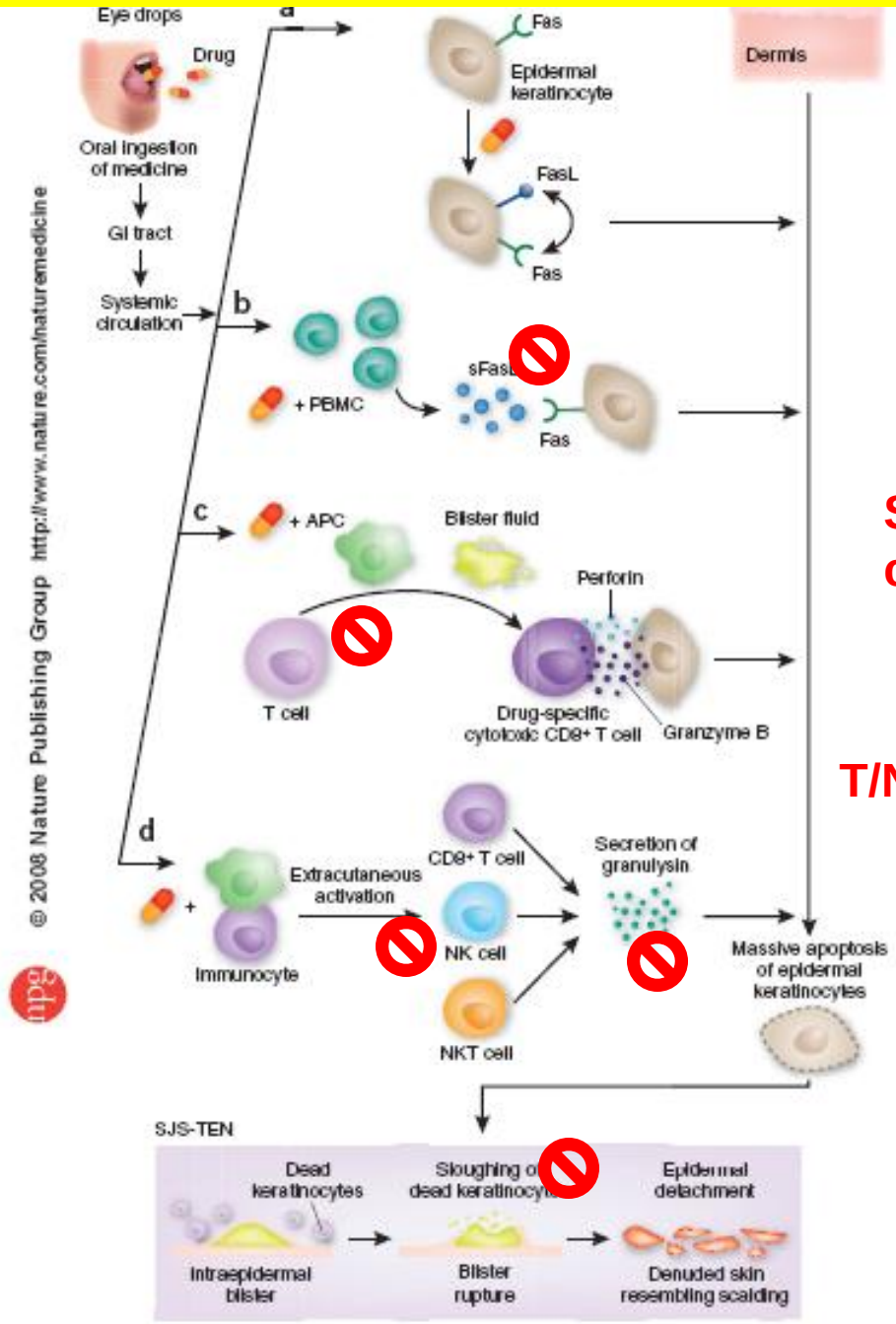
* TNF-α & IFN-γ => increase iNOS & Fas L in keratinocytes

\+ Annexin A1=> formyl peptide recptor 1 induced Necroptosis of Keratinocytes

\# miR-18a-5p inhibits BCL2L10 of keratinocytes

Chung WH, Allergol Int. 2010; Viard-Leveugle J Invest Dermatol. 2013; Nao Saito et al.,*Sci Transl Med 16 July 2014*
Ichihara A et al, J Allergy Clin Immunol. 2014

From pathomechanism to therapeutic strategy for SJS/TEN



IVIG?

Systemic corticosteroids?

T/NK cell activation inhibitors

Neutralizing antibodies

Apoptosis inhibitor

Acknowledgements

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