DILI as a polygenic disease. International Consortia and DILI initiatives to strengthen knowledge development

Ann K. Daly
Institute of Cellular Medicine
Newcastle University
UK

To be considered

- iDILIC and other networks
- HLA associations with DILI relating to flucloxacillin and related penicillins
- Non-HLA genes and their relevance to DILI
  - PTPN22
- Anti-TB drug-related DILI
  - Genetics still not well understood

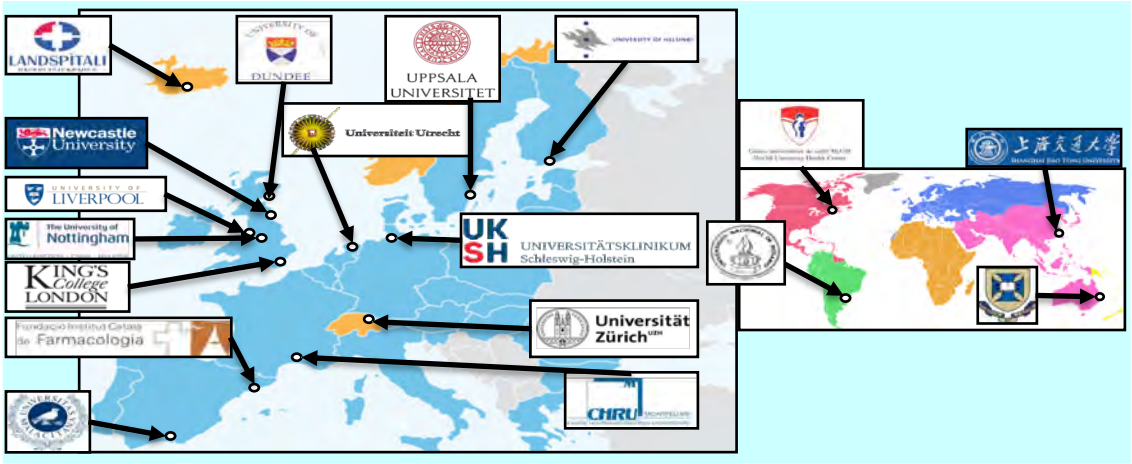
Global genetic studies on DILI susceptibility

- **DILIGEN (UK-based)**
  - Primarily concerned with DILI due to amoxicillin-clavulanate, flucloxacillin, anti-TB agents, diclofenac
  - Now complete
- **iDILIC (worldwide)**
  - Any hepatotoxic licensed drug
  - Data analysis ongoing
- DILIN (US-based), Eudragene, Spanish DILI
  - Collaboration with DILIGEN and iDILIC
- **Indian anti-TB DILI**
- ProEuroDILI
  - New multiomics prospective study

Newcastle University
UK | Malaysia | Singapore

iDILIC study

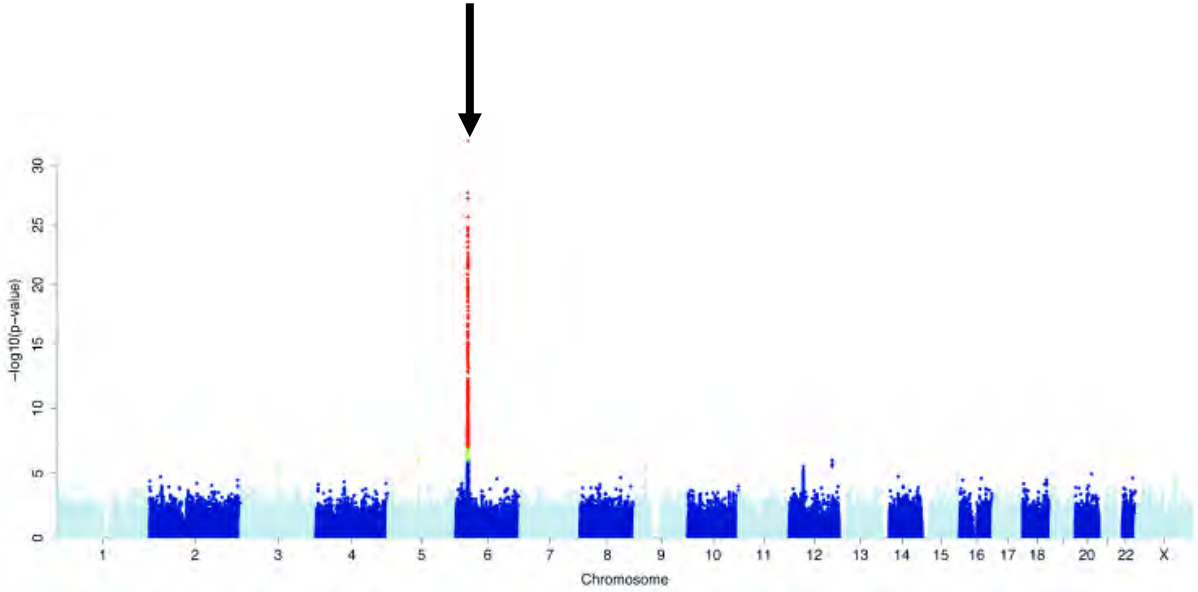
Worldwide study on idiosyncratic DILI genetics-Ann Daly and Guru Aithal-co-PIs



Flucloxacillin

- Beta-lactamase resistant penicillin with isoxazolyl ring
- Used widely, especially in UK, Australia and Sweden, in treatment of staphylococcal infections
- Well established cause of liver injury
- A leading cause of DILI in the UK

Flucloxacillin DILI GWAS study-strong effect for *HLA B*57:01*



- Strongest signal with SNP in HCP5 gene which tags HLA-*B*57:01* ($p=8.7 \times 10^{-33}$)
- Only needed 51 cases to see this strong effect

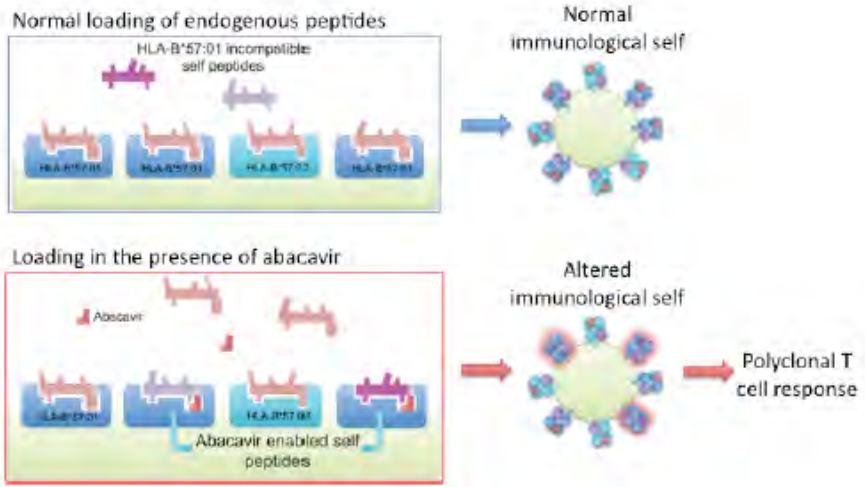
Daly et al., *Nature Genetics* 2009; 41:816-822

diligen

Abacavir hypersensitivity

- Approx. 5% of patients develop hypersensitivity reactions which resolve on discontinuation
  - Rechallenge results in more severe reaction
- Up to 100% of proven hypersensitivity cases have one or two HLA *B*57:01* alleles
  - Not all patients with this genotype will show detectable reaction (~50%)

Abacavir-B*57:01 interaction

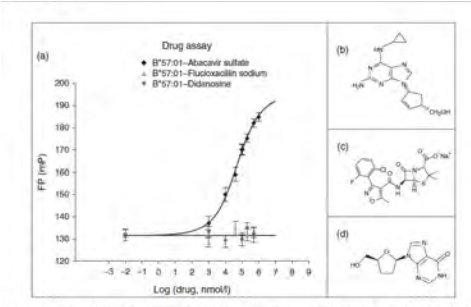


Abacavir binds directly to B*57:01 protein causing inappropriate immune response

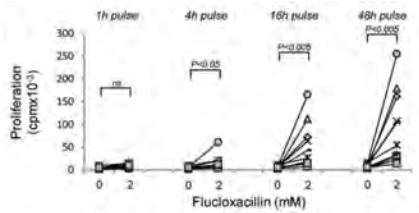
From Illing et al., Nature 2012;486:554-8

In vitro studies on flucloxacillin DILI mechanism

- Unlike abacavir, flucloxacillin does not bind directly to HLA-B*57:01 protein
- Appears to bind covalently to proteins resulting in modified self-peptides being presented to T cells

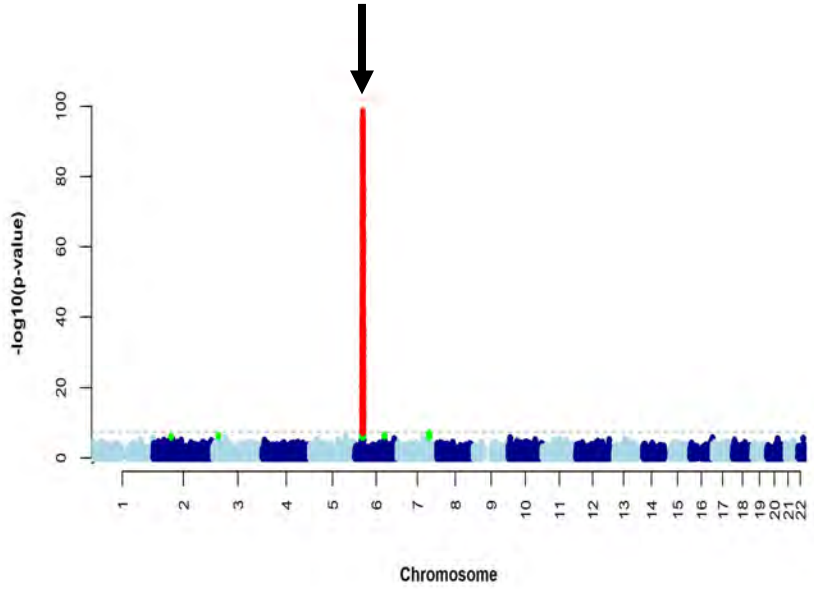


Norcross et al., 2012; AIDS 26: F21-F29



Monshi et al., *Hepatology* 2013;57:727-739

New genome-wide association study on flucloxacillin DILI: 197 cases



- HLA-B*57:01 tag: OR 36.62 (26.14-51.29), $p=2.61 \times 10^{-97}$
- Used imputation and additional exome content to increase coverage of genome
- Almost 4-fold more cases than original study

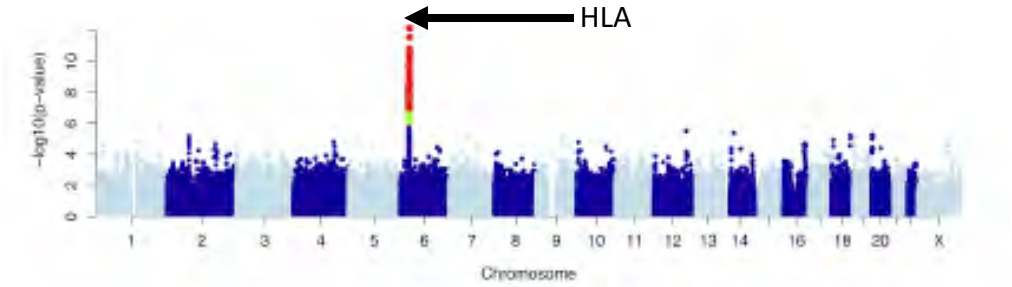
Nicoletti et al., CPT 2019

Recent findings on flucloxacillin DILI

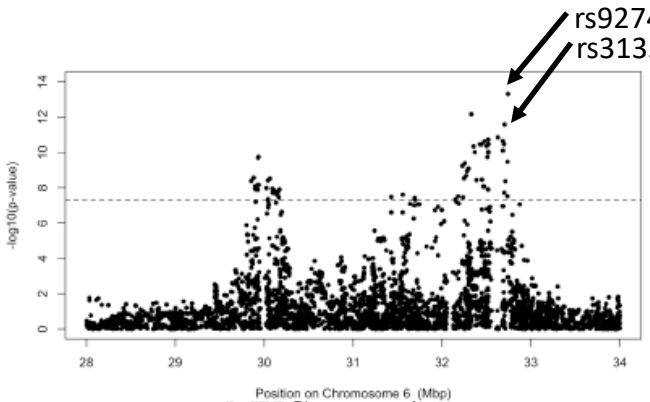
- *HLA-B*57:03* is a risk factor for flucloxacillin DILI in addition to *B*57:01*
  - Different to abacavir hypersensitivity where strong specificity for HLA-B*57:01 only
- HLA-B alleles positive for Val 97 had largest effect size
  - Position 97 sits at the bottom of peptide-binding cleft and appears critical for HLA protein conformation and folding
    - may affect both epitope presentation and surface expression
- Approx. 20% of flucloxacillin DILI cases not positive for B*57
  - Different to abacavir hypersensitivity
  - *HLA-A*02:02* enriched OR 16.57 (2.05-133.8), p = 0.008
- No evidence for non-HLA genetic risk factors in either B*57 positive or negative cases

Nicoletti et al., CPT 2019

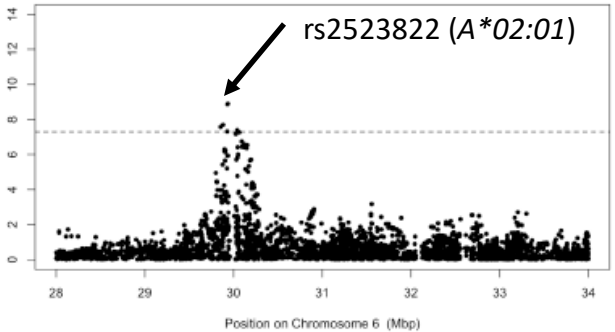
Amoxicillin-clavulanate DILI GWAS



201 cases- UK DILIGEN (n=77), US DILIN (n=56), Spanish DILI (n=49), EUDRAGENE (n=19); 532 matched controls



MHC zoom-in



MHC zoom-in, conditioned on the top SNP from HLA class II (rs9274407)

Lucena et al; Gastroenterology 2011; 141:338-347

HLA associations with DILI

Newcastle University
UK | Malaysia | Singapore

Allele	Drug	Odds ratio (allelic)	p value
HLA class I			
*A*02:01*	**Amoxicillin-clavulanate**	**2.3 (1.8-2.9)**	**$1.8x10^{-10}$**
A*33:01	**Terbinafine**	**40.5 (12.5-131.4)**	**$6.7x10^{-10}$**
	Fenofibrate	58.7 (12.3-279.8)	$3.2x10^{-7}$
	Ticlopidine	163.1 (16.2-1642)	0.00002
*A*33:03*	Ticlopidine	13.0 (4.4-38.6)	1.2×10^{-5}
*B*35:02*	Minocycline	29.6 (7.8-89.8)	$2.57x10^{-8}$
B*57:01	**Flucloxacillin**	**36.62 (26.14-51.29)**	**$2.6x10^{-97}$**
	Pazopanib	2.1 (1.3–3.60)	0.0058
*B*57:02 & B*57:03*	Anti-HIV & anti-TB comb	30.08 (3.44-263.11)	0.002
B*57:03	**Flucloxacillin**	**19.77 (3.37-116.1)**	**0.001**
HLA class II			
*DRB1*07:01*	Ximelagatran	4.4 (2.2-8.9)	$6x10^{-6}$
	Lapatinib	2.9 (1.3-6.6)	0.007
*DRB1*15:01*	Lumiracoxib	5.0 (3.6-7.0)	$6.8x10^{-25}$
	Amoxicillin-clavulanate	**2.8 (2.1-3.8)**	**$3.5x10^{-11}$**
*DRB1*16:01*	**Flupirtine**	**18.7 (2.5-140.5)**	**0.002**

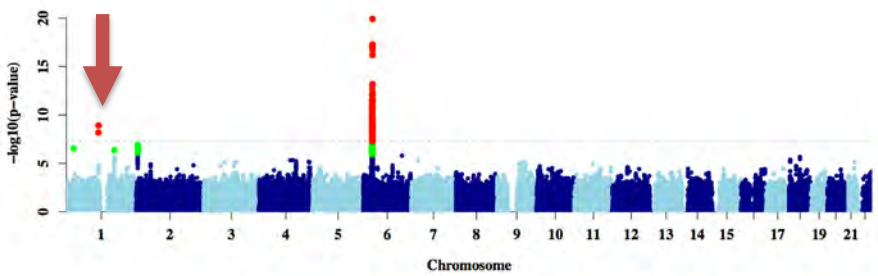
HLA associations with DILI-underlying mechanism

- Inappropriate T-cell response
- Specific HLA protein interacts with drug-peptide complex inappropriately
- Local cellular damage

Non-HLA risk factors in DILI

- Some relatively important forms of DILI clearly not HLA-associated e.g.
  - Diclofenac
  - Isoniazid
  - Statins
- Suggested mechanisms
  - Reactive metabolite formation and direct damage
  - Inappropriate innate immune response

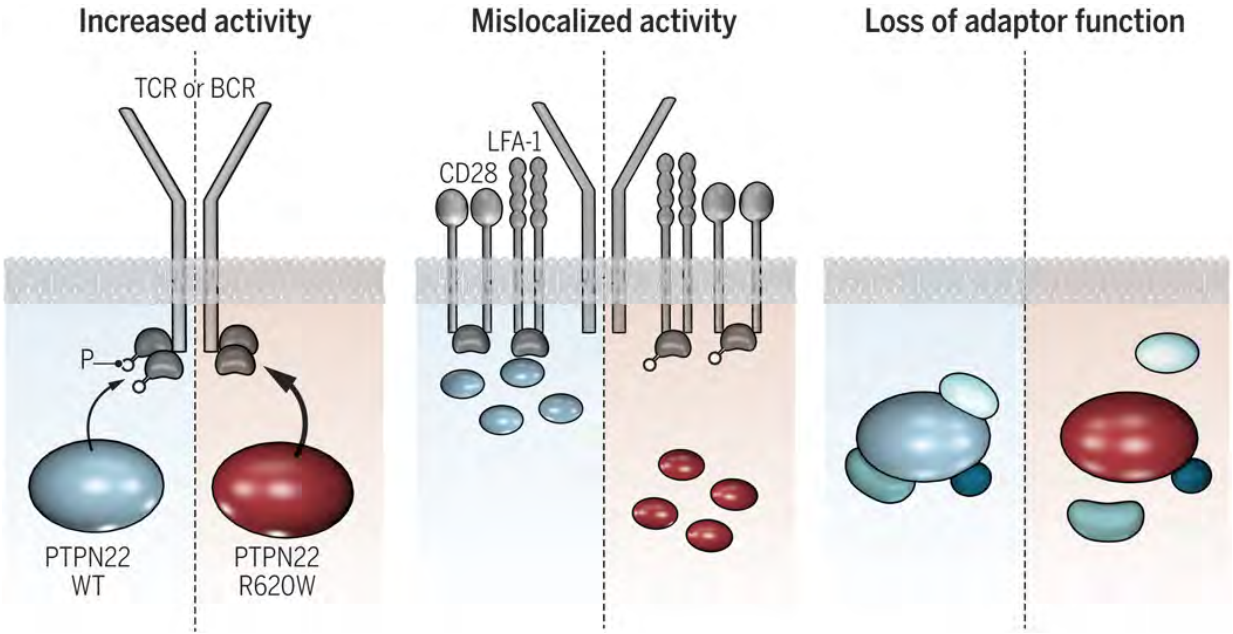
All available DILI cases- GWAS signal for *PTPN22*



- 2,048 DILI cases and 12,429 controls: European (1,806 cases), African American (133 cases) and Hispanic (109 cases)
- *PTPN22* rs2476601(Trp620Arg) OR 1.44 (95%CI 1.28-1.62) $P=1.2x10^{-9}$
  - Replicated in 113 Icelandic cases OR 1.48, (95%CI 1.09-1.99), p=0.04
- *PTPN22* codes for lymphoid-specific protein tyrosine phosphatase, nonreceptor type 22
  - Involved in T-cell and B-cell signalling
  - Established genetic risk factor for type 1 diabetes and rheumatoid arthritis

Cirulli et al., Gastroenterology, 2019

PTPN22 R620W is a switch-of-function polymorphism



Torkel Vang et al., Sci. Signal. 2018;11:eaat0936

PTPN22 and DILI: the most associated drugs

DRUGS	# Cases	OR (95% CI)	p value
Amoxicillin-clavulanic Acid	**444**	**1.62 (1.32-1.98)**	**0.000004**
Terbinafine	**15**	**3.23 (1.29-8.10)**	**0.01**
Sulfamethoxazole-trimethoprim	42	2.07 (1.16-3.71)	0.01
Methotrexate	9	3.34 (1.09-10.16)	0.03
Rofecoxib	6	4.08 (1.05-15.82)	0.04
Flupirtine	**6**	**4.43 (0.98-20.05)**	**0.05**
Valproate	16	2.43 (0.99-5.95)	0.05

Combined *HLA-PTPN22* genotypes

AMOXICILLIN/
CLAVULANIC ACID

HLA-DRB1*15:01 (P) / HLA-A*02:01 (P)/ 1:114377568 (A)

Combination	AF cases	AF ctl	OR	P
PPA	0.02	0.003	59.30	7.89E-26
APA	0.04	0.02	2.93	1.78E-06
PAA	0.03	0.008	7.11	3.99E-10
AAA	0.04	0.05	0.76	0.154
PPG	0.13	0.03	7.88	5.77E-51
APG	0.25	0.22	1.24	0.00789
PAG	0.12	0.08	1.95	1.67E-09
AAG	0.37	0.58	0.33	1.49E-41

A=PTPN22 variant

FLUCLOXACILLIN

HLA-B*57:01 (P) / 1:114377568

Combination	AF cases	AF ctl	OR	P
PA	0.05	0.003	101	3.20E-28
AA	0.07	0.08	0.709	0.10
PG	0.37	0.03	31.8	1.31E-98
AG	0.51	0.88	0.114	4.80E-62

- Overall HLA class II (DRB1*15:01) may be more associated with a PTPN22 combined effect for amoxicillin-clavulanate DILI
- Limited relevance of PTPN22 to flucloxacillin DILI

PTPN22 summary

- PTPN22 genotype is a genome-wide significant risk factor for DILI
  - Drug specificity for association suggests higher level of significance when combined with a HLA class II genetic risk factor (e.g. *DRB1*15:01* or **16:01*)
- Not a HLA gene but contributes to same T cell pathway!
  - Could also be relevant to non-HLA innate immunity mechanisms

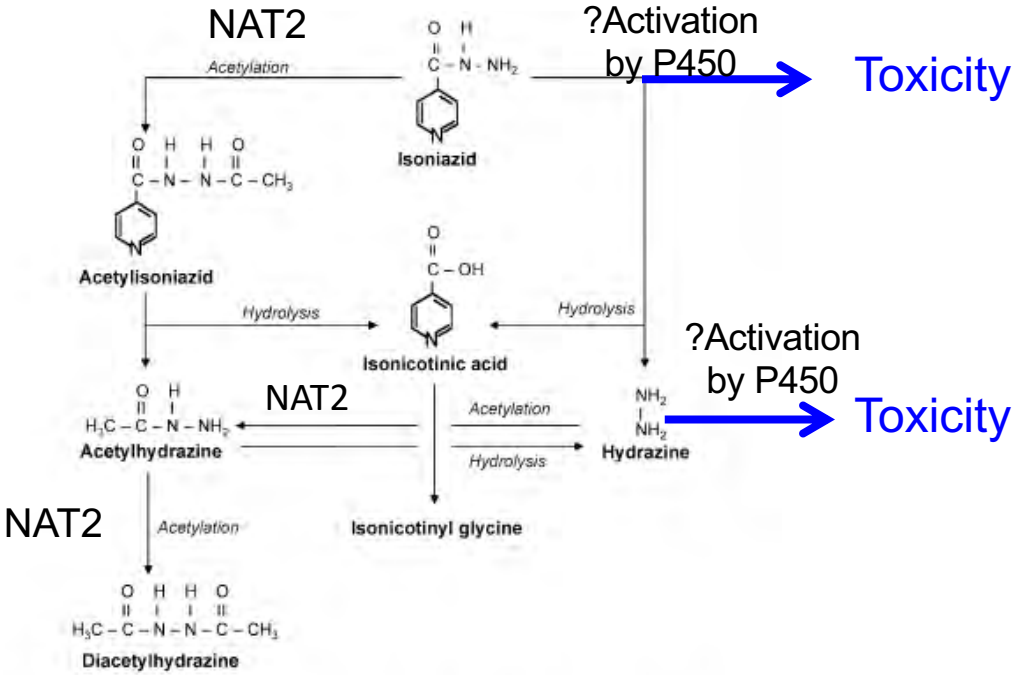
Other genetic risk factors for DILI?

- No evidence for P450 gene involvement
- Evidence for transporter polymorphism risk very limited
  - ABCC2 and BSEP still possible
- NAT2 genotype may affect risk of mild DILI due to anti-TB drugs
- Other liver disease genes
  - PNPLA3, MBOAT7, HSD17B13, TM6SF2 all negative for DILI

Anti-TB DILI

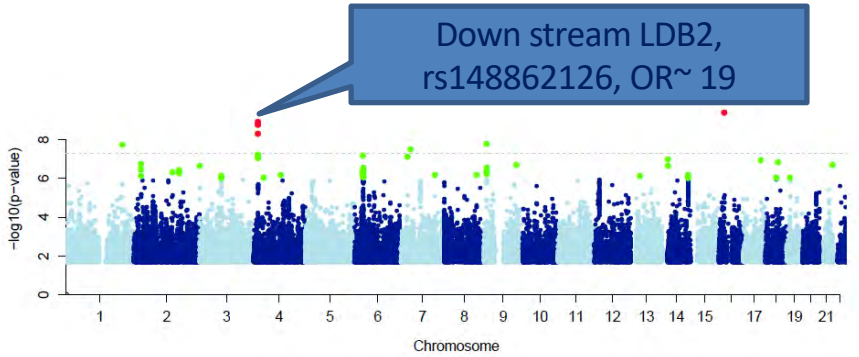
- Anti-TB drugs important cause of DILI worldwide
- No detectable HLA association
- Reports suggesting that NAT2 which codes for enzyme metabolizing isoniazid may be genetic risk factor
- Problems with this
  - Many studies are small and involve mild toxicity only
  - NAT2 only relevant to isoniazid but pyrazinamide and possibly rifampicin may also cause DILI

Isoniazid metabolism



NAT2: N-acetyltransferase 2 which is absent in slow acetylators

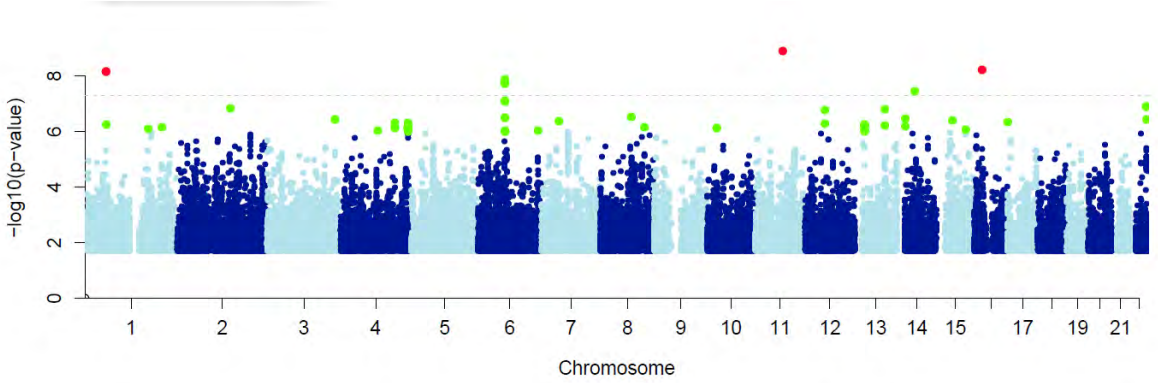
European anti-TB DILI



SNP	CHR	BP	ODDS	Pvalue	L95	U95	FreqCases	FreqControl	Freq1kg	Fisher	missing	hweALL	hweAFF	hweUNAF
rs74781707	4	16937618	19.98	4.85E-09	7.328	54.47	0.03623	0.001795	0.003	8.37E-06	1	0.04889	0.1041	1

- 69 European cases from DILIGEN, iDILIC and DILIN
- Significant signal relates to rare variant in LDB2-possible transcriptional regulator with adaptor function
- Did not replicate in small cohort

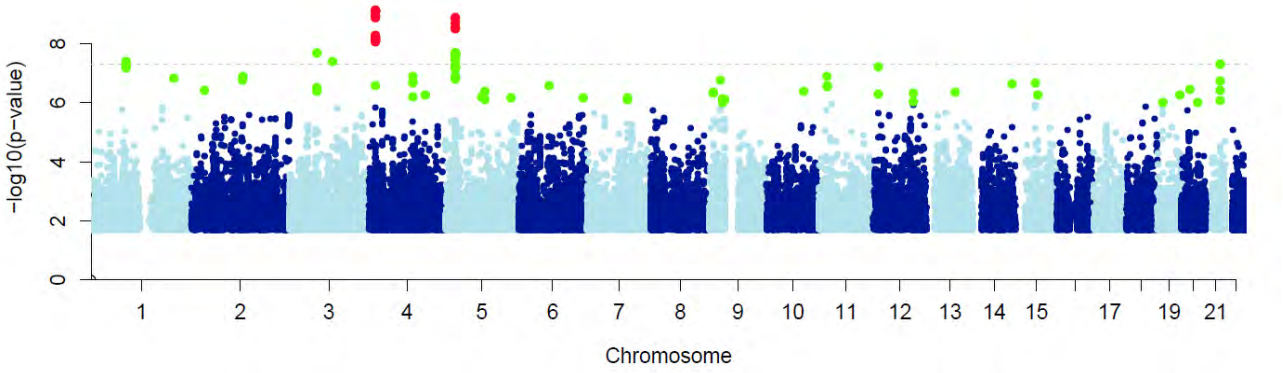
Isoniazid DILI alone



SNP	CHR	BP	ODDS	Pvalue	L95	U95	FreqCases	FreqContr	Freq1kg	Fisher
rs72929958	6	72248322	18.62	1.3E-08	6.796	51.02	0.06944	0.00432	0.007	1.91E-05

- 37 European cases
- Chromosome 6 signal not in HLA region

Combinations of anti-TB drugs



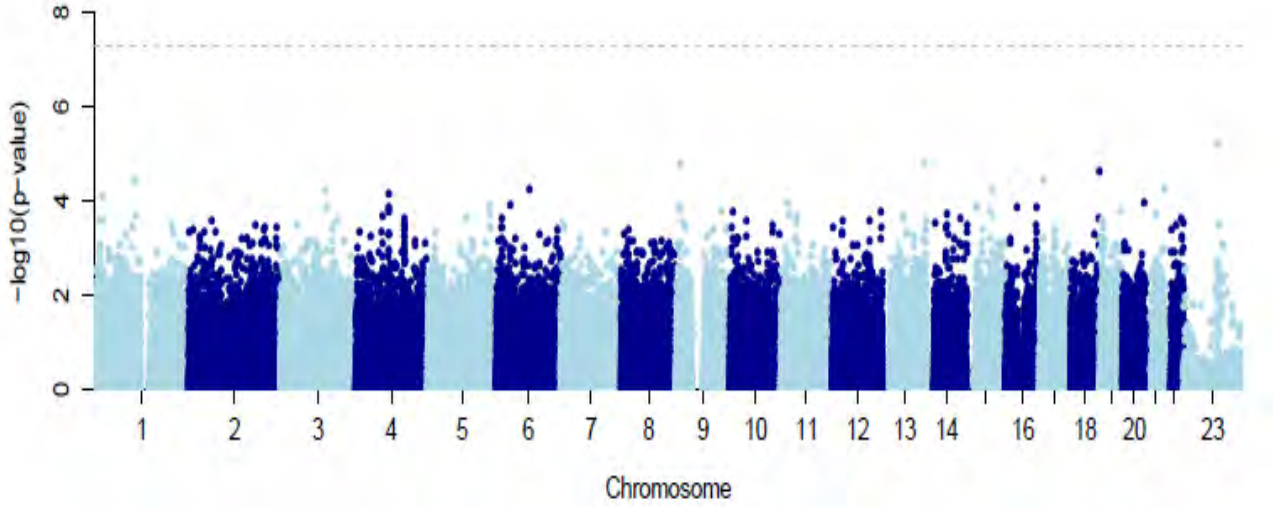
SNP	Gene	CHR	BP	ODDS	Pvalue	L95	U95	FreqCases	FreqContr	Freq1kg	Fisher
rs193130816	Intergenic	4	17009321	46.51	7.26E-10	13.71	157.8	0.0625	0.001179	0.002	1.68E-06
rs141109899	intergenic	5	24786641	25.11	1.27E-09	8.872	71.08	0.07812	0.003072	0.005	2.21E-06

- 32 European cases
- Chromosome 4 signal again downstream of LDB2

Indian anti-TB DILI cases

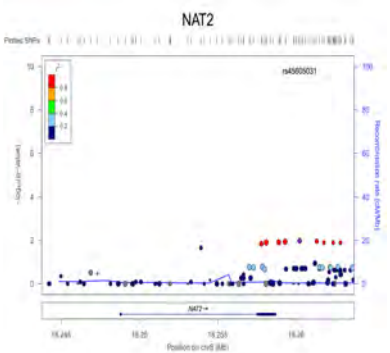
- 59 cases with adjudicated moderate/severe DILI due to anti-TB drugs
  - 111 population based controls and 109 drug-exposed controls
- GWAS on human core exome chip
- Prediction of acetylator status of N-acetyltransferase 2 (NAT2) using typed genotypes for rs1799929, rs1799930 and rs1799931 (*NAT*5, *6* and **7* alleles)

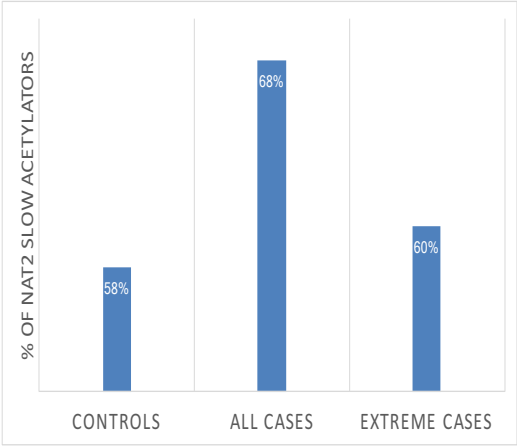
GWAS on Indian anti-TB DILI cases



No genome-wide significant signals

NAT2 analysis





OR= 1.54 (95% CI 0.84-2.84) P = 0.18

- NAT2 slow acetylators were not associated with anti-TB DILI
- Confining analysis to *6 and *7 carriers only improves significance

Anti-TB DILI summary

- Statistical power likely to be insufficient to see genome-wide significant signals for effects with odds ratio in range 1.5 to 4 approx.
- Useful to consider collaboration and meta analysis to increase number of cases

Summary and prospects for clinical implementation

- Collaborative studies have enabled detection of HLA signals especially for flucloxacillin, amoxicillin-clavulanate and terbinafine-related DILI
- Very large recent study shows role for non-HLA gene PTPN22
- None of the associations detected to date justify preprescription implementation
  - As preemptive genotyping and addition of genomic data to medical records increases, these findings may be of value in prescription decisions
- Progress to date on anti-TB DILI disappointing
  - One of the most common forms of idiosyncratic DILI requiring liver transplantation

Acknowledgements

Paola Nicoletti (New York)
Guru Aithal (Nottingham)
Sally Coulthard (Newcastle)
Julian Leathart (Newcastle)
Pete Donaldson (Newcastle)
Tom Chamberlain (Newcastle)
Munir Pirmohamed (Liverpool)
Dean Naisbitt (Liverpool)
Yufeng Shen (Columbia)
Matt Nelson (GSK)
Paul Watkins (North Carolina)
Maribel Lucena (Malaga)
Raul Andrade (Malaga)
Einar Bjornsson (Reykjavik)
Thorunn Rafnar (deCODE)
iDILIC, Indian anti-TB DILI and DILIN collaborators

Newcastle University
UK | Malaysia | Singapore