

# ROLE OF PHARMACOGENETICS IN PERSONALISED MEDICINE IN HEPATOLOGY

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# To be described

- Introduction to pharmacogenetic studies
  - Candidate gene case-control studies
  - Genome-wide association studies
  - Genome-wide sequencing
    - Exome sequencing
    - Whole genome sequencing
- Application of pharmacogenetics to hepatology
  - **Drug-induced liver injury**
  - Other liver diseases

# Pharmacogenetics, Pharmacogenomics and Personalised Medicine

- Pharmacogenetics
  - Study of unusual responses to drugs and other foreign compounds that have a hereditary basis
    - Typically involves genes relevant to drug disposition, drug response or immune responses
- Pharmacogenomics
  - Individualization of drug therapy using genomic information
  - Can use existing drugs or develop new ones
    - Up to now emphasis is on existing drugs
  - Includes Pharmacogenetics
- Personalised medicine
  - Includes application of pharmacogenetics/genomics to treatment but other factors also relevant

# Candidate gene case-control studies

- Need to select biologically plausible candidate gene and compare frequency of selected genetic polymorphism between cases and controls
  - Less obvious candidates not considered
- Easy and cheap to perform
- May work well if the obvious genes contribute with large effect size
- Successful pharmacogenetic examples
  - CYP2C9 and VKORC1 in relation to warfarin dosing
  - HLA gene associations with adverse drug reactions e.g. B\*57:01 and abacavir toxicity

# Genome-wide association studies

- Genotyping for many (500,000 to 1 million) genetic polymorphisms (SNPs) using array-based technology
  - These polymorphisms occur throughout the genome on all genes and account for most common genetic variability
- Open nature of method means most possible genetic associations screened for
- Successful implementation usually requires large numbers of samples (cases and controls)
- Pharmacogenetic applications
  - Adverse drug reactions
  - Response to drugs such as warfarin and clopidogrel

# Genome sequencing

- Exome sequencing
  - Sequencing of all exons in human genome
  - Allows analysis of rarer variants
  - Up to now most useful in study of rare genetic diseases
- Whole-genome sequencing
  - Entire genome including introns, regulatory regions and areas of unknown function sequenced
  - Expensive and difficult data analysis but great potential to understand genetic variability

# Drug-induced liver injury (DILI)

- Rare but serious idiosyncratic toxicity
- Aims of current studies
  - Develop strategies to prevent DILI reactions by genotyping patients prior to treatment and individualizing drug prescription
  - Develop screening approaches for use during drug development to screen out hepatotoxic compounds

# Genetic studies on DILI susceptibility

- **DILIGEN (UK-based)**
  - Primarily concerned with DILI due to amoxicillin-clavulanate, flucloxacillin, anti-TB agents
  - Now complete
- **iDILIC (worldwide)**
  - Any hepatotoxic licensed drug
  - Sample collection and data analysis ongoing
- **DILIN (US-based), Eudragene, Spanish DILI**
  - Collaboration with DILIGEN and iDILIC

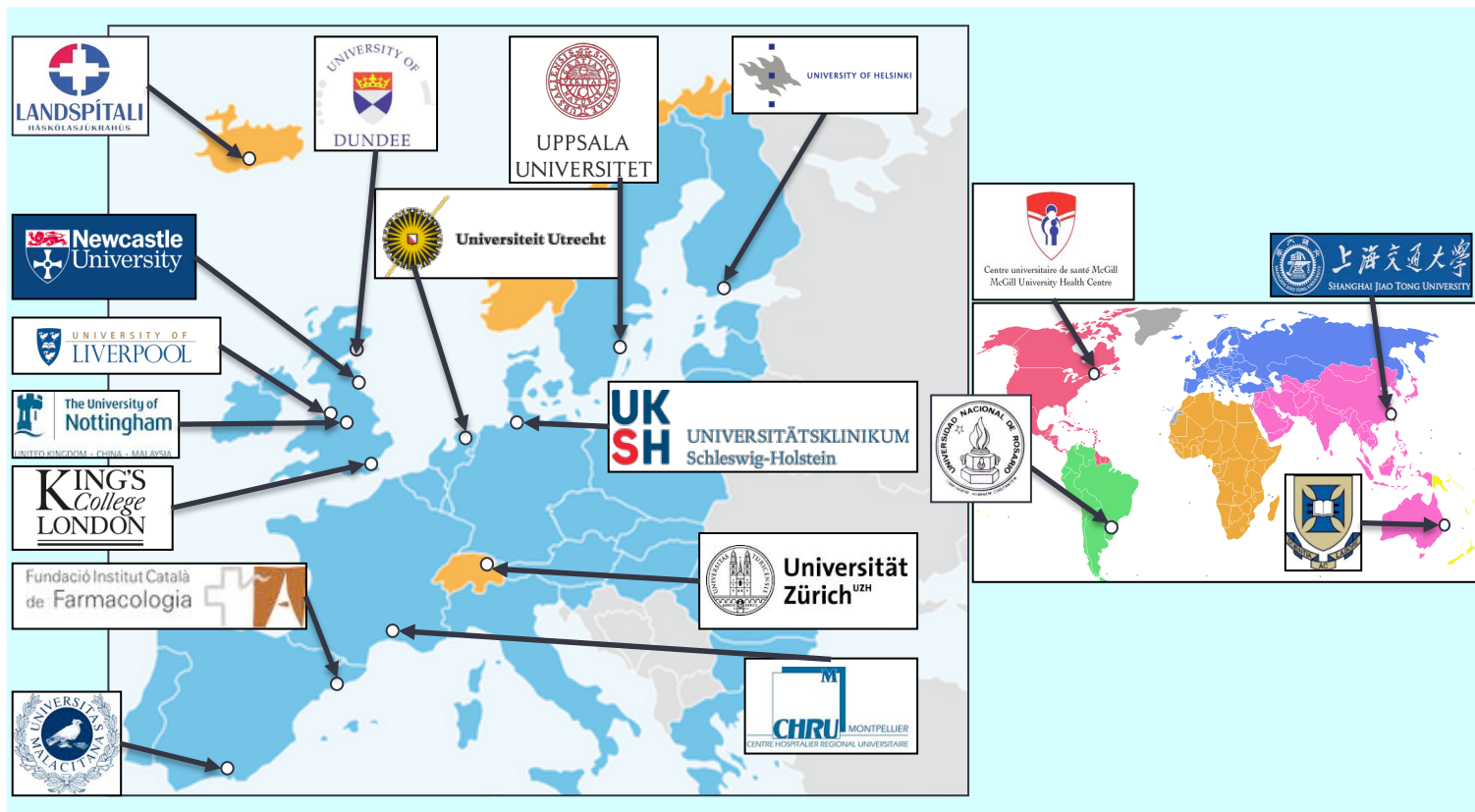


## UK-wide study on genetics of drug-induced liver injury

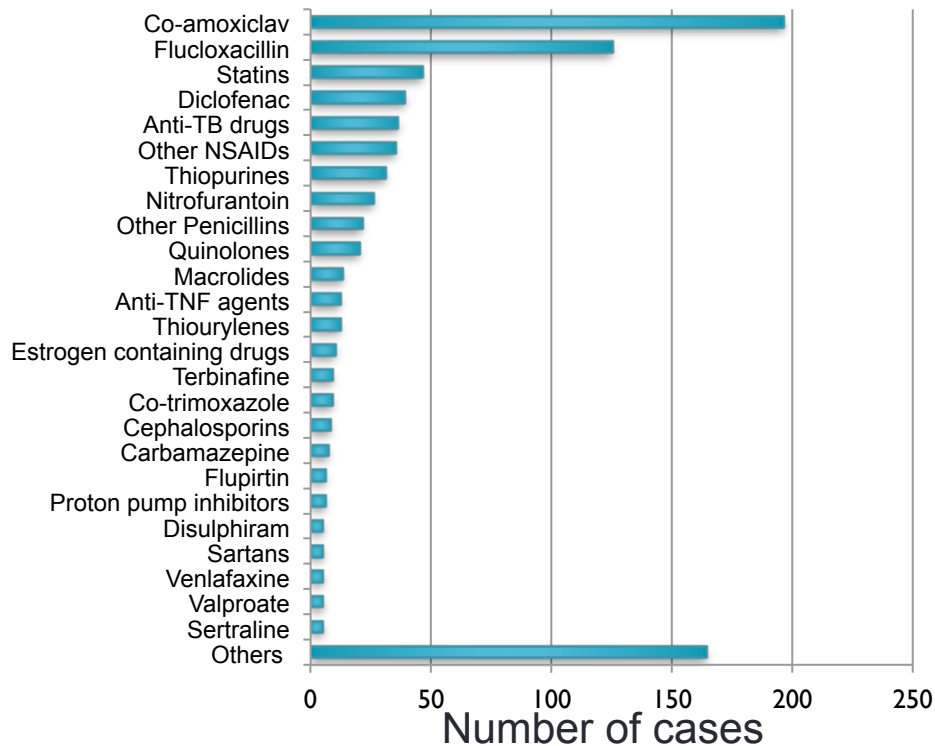
- Drugs
  - Anti-TB medication or Flucloxacillin or Co-amoxiclav
  - Others included later in study
- Final recruitment
  - 76 flucloxacillin, 78 co-amoxiclav, 26 anti-TB medication, >50 others
  - Retrospective and prospective enrolment

# iDILIC study

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



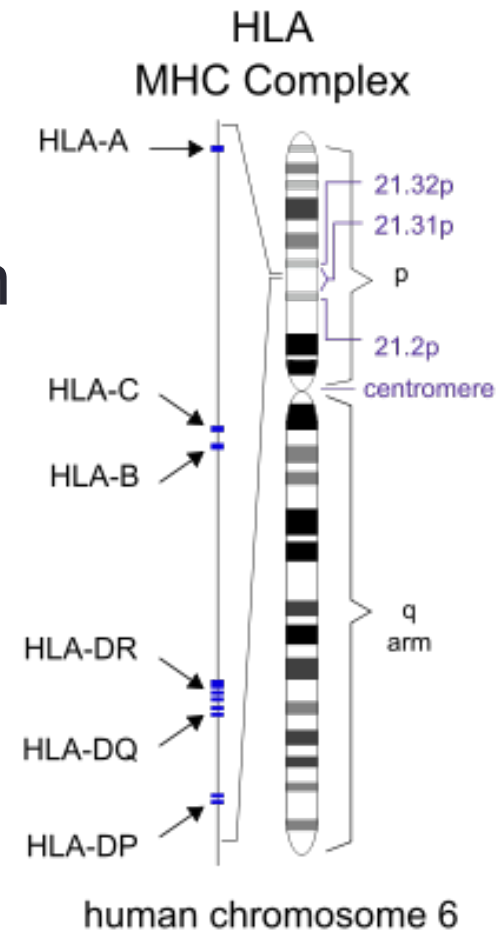
# Successfully adjudicated new cases in iDILIC



- 1053 cases recruited with 862 passing adjudication (82%)
- Cases mainly of European ethnicity
- GWAS-exome chip analysis on 747 of these cases

# HLA genes-common thread in DILI genetics

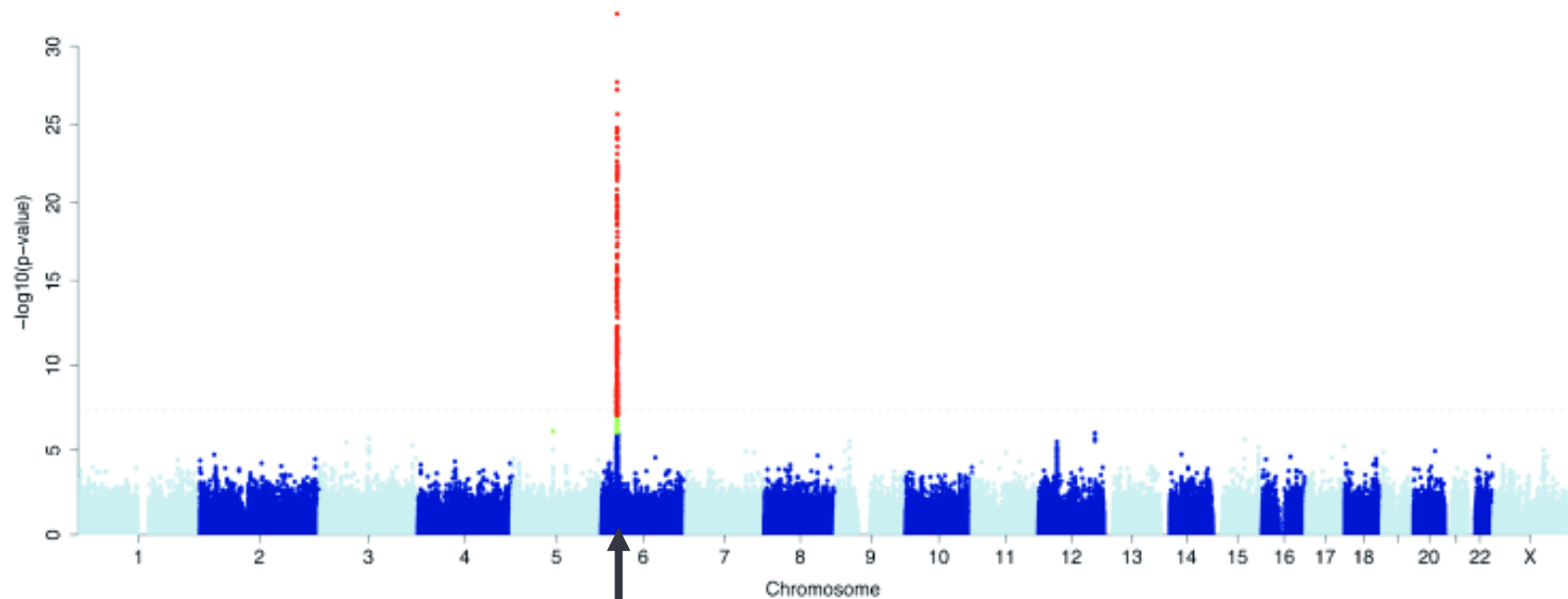
- HLA class I genes expressed on most cells
  - A, B and C genes
- HLA class II genes expressed on antigen presenting cells
  - DR, DQ, DP genes
- HLA proteins normally present peptide antigens to T cells
  - May inappropriately present drug-peptide complexes



# GWAS on liver injury due to flucloxacillin

- 51 UK cases (DILIGEN study) and population control group (n=282)
  - POPRES controls
- Illumina 1M bead chip

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



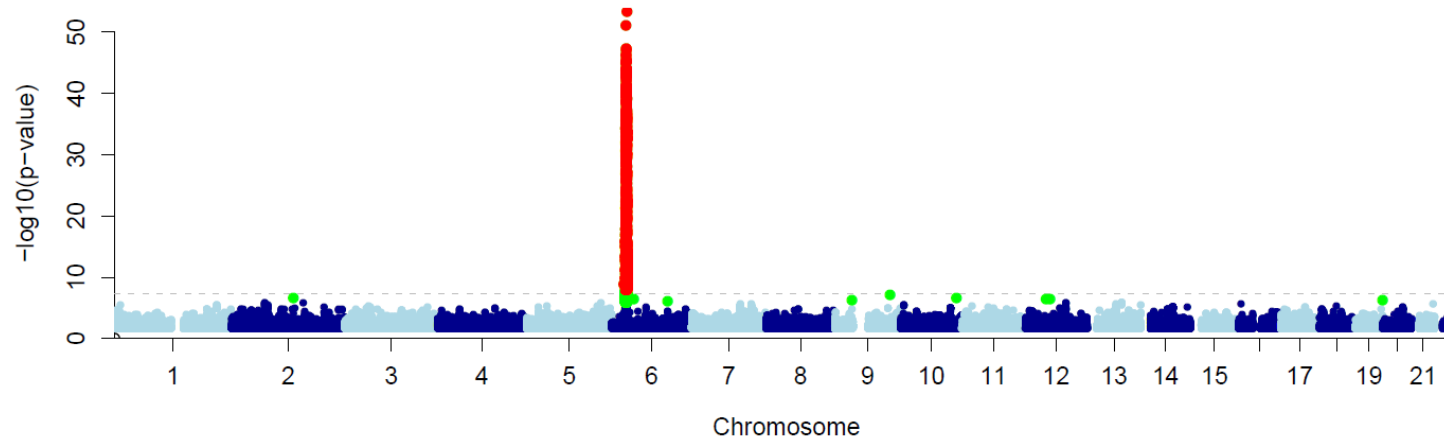
Strongest signal with SNP in  
HCP5 gene which tags  
*HLA-B\*57:01*

## *HLA-B\*57:01* genotypes of flucloxacillin cases and controls

	Positive	Negative	Odds ratio (95% CI)	P-value
Controls (n=64)	4 (6.3)	60 (93.7)		
Cases (n=51)	43(84.3)	8 (15.7)	80.6 (22.8 - 284.9)	8.97x10 <sup>-19</sup>
Replication cases (n=23)	20 (87.0)	3 (13.0)	100.0 (20.6-485.8)	6.62x10 <sup>-13</sup>

- 84% of all cases positive, with 4 homozygous mutant

# GWAS on flucloxacillin DILI: 197 cases



SNP	ODDS	UCI	LCI	Pvalue	FreqCases	FreqControls	hwe2	ALL1 M
HLA-B*57:01	30.96	43.66	21.96	$2.30E-8$ 5	0.4239	0.04181	1	0.0423

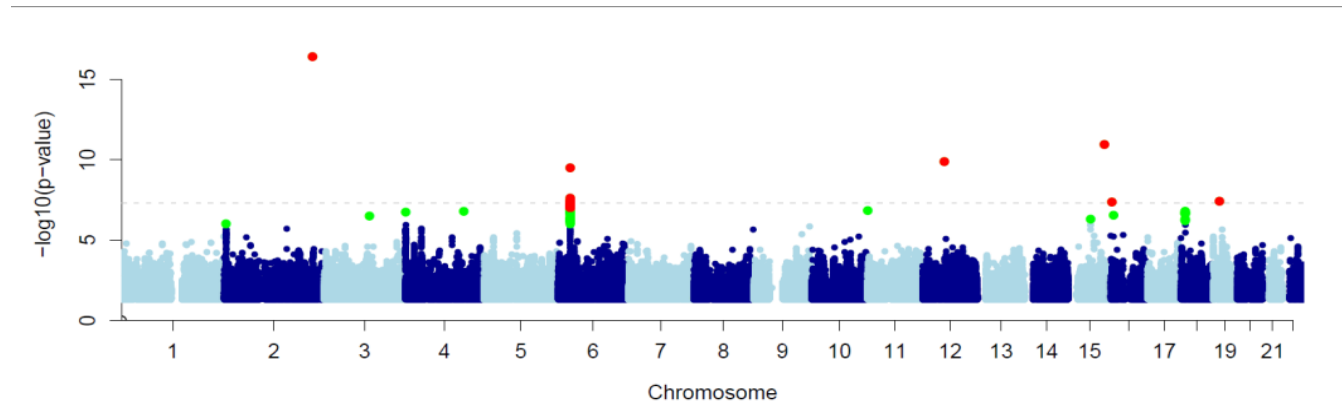
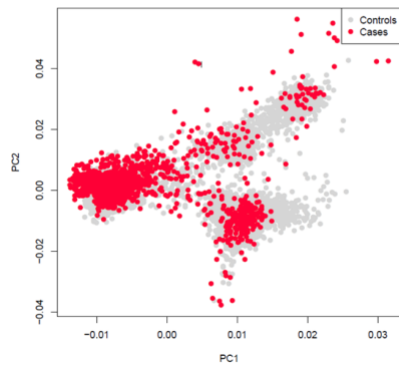
- No convincing signals other than for B\*57:01

# Flucloxacillin DILI and *B\*57:01*

- Association with HLA *B\*57:01* similar to that for abacavir hypersensitivity but may involve different mechanism
  - 84% of 197 cases now studied are *B\*57:01*-positive
- Sensitivity of *B\*57:01* genotyping as predictor of flucloxacillin DILI lower
  - Only 1 in 500 patients positive for *B\*57:01* treated with flucloxacillin develop DILI
  - Non-genetic risk factors likely to contribute to susceptibility?

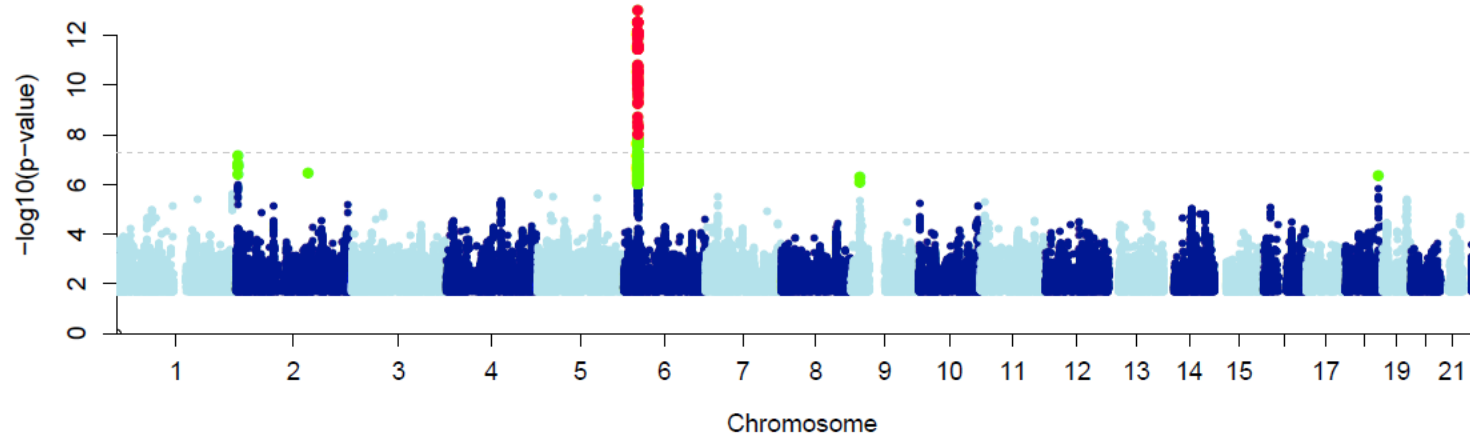
# "Omnibus" DILI study

- 1021 Caucasian cases (iDILIC, DILIGEN, DILIN, Eudragene, Spanish DILI)
- Excludes all Flucloxacillin and Co-amoxiclav cases



- HLA and other additional genotypes assigned statistically by imputation
- *HLA-A\*33:01* (OR ~2, p-value  $10^{-7}$ ) possibly associated with DILI more generally

# Omnibus study: 290 cases cholestatic and mixed only



SNP	CHR	BP	ODDS	Pvalue	L95	U95	FreqCases	FreqContr	Freq1kg
rs115651142	6	30115320	5.485	9.28E-14	3.505	8.582	0.04114	0.01089	0.014
rs114577328	6	29927282	5.469	2.81E-13	3.467	8.63	0.03956	0.01061	0.014
rs72610705	2	5254575	2.376	6.98E-08	1.735	3.254	0.07803	0.03767	0.052

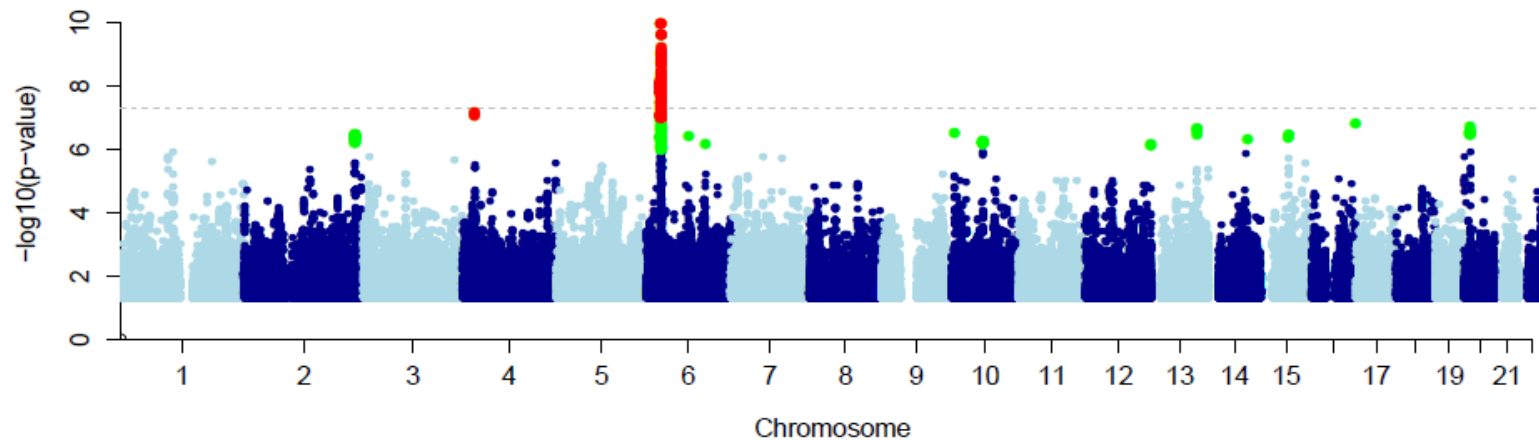
- See stronger A\*33:01 signal ( $p=10^{-14}$ )
- Don't see genome-wide significance for hepatocellular only

# "Omnibus" cases relating to specific drugs: *A\*33:01* frequency

DRUG	MAF	NCHR	MAC
TICLOPIDINE	0.4	10	4
FENOFIBRATE	0.3	10	3
ENALAPRIL	0.25	8	2
METHYLDOPA	0.25	8	2
TERBINAFINE	0.2	30	6
SERTRALINE	0.1667	12	2
CONTROLS	<b>0.012</b>	<b>18270</b>	<b>213</b>
FLUCLOXACILLIN	0.007	402	3
CO-AMOXICLAV	0.002	526	1

- *HLA-A\*33:01* possibly associated with DILI due to various drugs
- Terbinafine has the highest number of positive cases (6 heterozygotes out of 15 cases)

# Terbinafine only



SNP	ODDS	UCI	LCI	Pvalue	PvalueFist	FreqCases	FreqContr	missing	hwe2	ALL1M	ALLHCE	ALLOEX	ITA1M	ITAOEX	SPA1M	SPAHCE	NEU1M	
HLA-B*14:02	17.58	49.97	6.186	7.48E-08	4.81E-05	0.2333	0.03323		1	1	0.03286	0.03769	0.04221	0.01973	0.01433	0.05273	0.04065	0.02683
HLA-A*33:01	76.53	288.9	20.27	1.56E-10	1.28E-06	0.2	0.01166		1	0.2144	0.01136	0.01759	0.01299	0.01038	0.04221	0.02773	0.01897	0.004679
HLA-C*08:02	12.43	34.08	4.533	9.75E-07	0.00034	0.2333	0.04566		1	0.7323	0.04527	0.05653	0.03896	0.02648	0.001165	0.06659	0.06098	0.03944

r2/D	HLA-B*14:02	HLA-C*08:02	rs190865297	rs116076476
HLA-A*33:01	0.2/0.7	0.176/ 0.7	0.538/0.840	0.302/1

- 15 Caucasian cases
- *HLA-A\*33:01* risk factor (OR >76, p-value  $10^{-10}$ ) is genome-wide significantly associated with Terbinafine-DILI

# Summary of data on *A\*33:01*

- Significant association with this allele predicted from "omnibus" GWAS especially for cholestatic and mixed DILI
- Involves terbinafine and other drugs
  - No structural similarities
  - Finding for ticlopidine consistent with previous report from Japan
- HLA genotypes assigned by imputation of GWAS data
  - Confirmation by direct typing achieved for subset of samples

# HLA associations with DILI: summary

Compound	No of cases	HLA allele	Odds ratio (95% CI)	p value
Flucloxacillin	51	<i>B*57:01</i>	<b>80.6(22.8-284.9)</b>	$9 \times 10^{-19}$
Amoxicillin-clavulanate	201	<i>A*02:01</i>	2.3(1.8-2.9)	$1.8 \times 10^{-10}$
		<i>DRB1*15:01-DQB1*06:02</i>	2.8(2.1-3.8)	$3.5 \times 10^{-11}$
Lumiracoxib	41	<i>DRB1*15:01-DQB1*06:02</i>	5.0(3.6-7.0)	$6.8 \times 10^{-25}$
Lapatinib	35	<i>DRB1*07:01-DQA1*02:01</i>	2.9(1.3-6.6)	0.007
Ximelagatran	74	<i>DRB1*07:01-DQA1*02:01</i>	4.4(2.2-8.9)	$6 \times 10^{-6}$
Ticlopidine	22	<i>A*33:03</i>	13.0 (4.4-38.6)	$1.2 \times 10^{-5}$

Mainly based on GWAS but also some direct HLA typing

# HLA and related gene associations with DILI-underlying mechanism

- Specific HLA protein interacts with drug or metabolite inappropriately
- Triggers T-cell response
- Local cellular damage

# Non-HLA risk factors

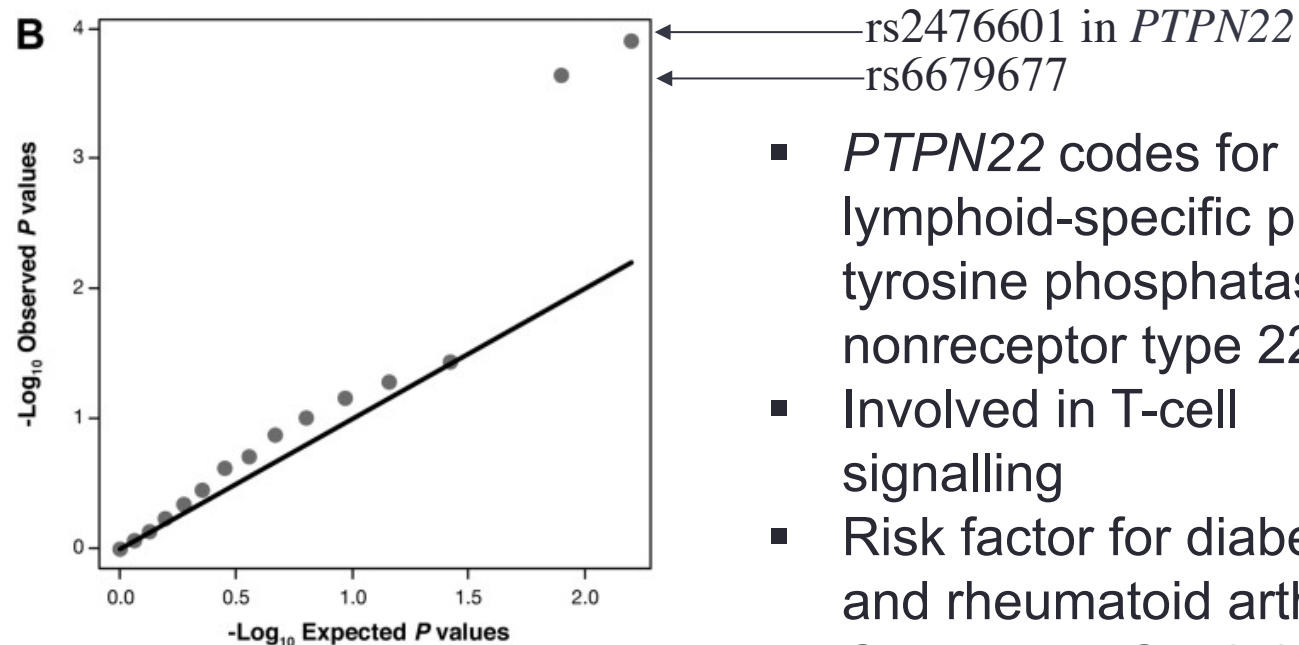
- Some relatively important forms of DILI not HLA-associated e.g.
  - Isoniazid
  - Diclofenac
- Where HLA association shown, non-HLA risk factors may also contribute e.g.
  - Amoxicillin-clavulanate

# Finding non-HLA genetic risk factors for DILI

- Genome-wide significance on GWAS
  - Challenging unless relatively large effect size (e.g. OR>3) and number of cases
- Candidate-gene approaches using GWAS or other data
  - PTPN22
  - UGT2B7
- Exome sequencing

# Amoxicillin-clavulanate DILI and non-HLA genes

- Autoimmune disease gene subset from GWAS (QQ plot)



- *PTPN22* codes for lymphoid-specific protein tyrosine phosphatase, nonreceptor type 22
- Involved in T-cell signalling
- Risk factor for diabetes and rheumatoid arthritis
- Overall risk OR 2.1 (1.5 – 3.2)  $p=1.3 \times 10^{-4}$

# ADME genes as candidates for diclofenac DILI-using GWAS data

SNP Set	SNP	P	Coordinates	Function	Nearest Gene
ADME	rs903446	0.0006	4:70336501	INTERGENIC	<i>UGT2B4</i>
	<b>rs4587017</b>	<b>0.0008</b>	<b>4:69947398</b>	<b>UPSTREAM</b>	<b><i>UGT2B7</i></b>
	rs4772131	0.0012	13:99397536	INTRONIC	<i>SLC15A1 (PEPT1)</i>
	<b>rs1523130</b>	<b>0.002</b>	<b>3:119499507</b>	<b>5PRIME_UTR</b>	<b><i>NR1I2 (PXR)</i></b>
	<b>rs7375178</b>	<b>0.0024</b>	<b>4:69969679</b>	<b>INTRONIC</b>	<b><i>UGT2B7</i></b>

- Based on 30 diclofenac DILI cases
- In agreement with our earlier UGT2B7 finding but PXR an interesting additional candidate
- UGT2B7 may affect levels of reactive acyl glucuronides

# Exome sequencing and exome chip studies on DILI

- Exome sequencing performed on 125 amoxicillin-clavulanate DILI cases
  - 66 from UK and 59 from Spain
  - All from previous GWAS
- Exome chip studies
  - 233 further amoxicillin-clavulanate DILI cases from iDILIC study and from DILIN network for replication of exome sequencing

# Sequencing + Exome Chip + Additional Genotyping

SNP	F_A	F_U	P_FISHER	OR	GENE	FUNCT	ESP MAF	ESP P
exm1381768	0.013	0.0000	4.03E-07	NA	FAM59A	Missense	0	1.30E-09
exm1621613	0.009	0.0000	4.60E-05	NA	HDAC10	SPLICE_REGION	0	1.13E-06
<b>exm1611079</b>	<b>0.009</b>	<b>0.0007</b>	<b>1.77E-04</b>	<b>13.07</b>	<b>SGSM3</b>	<b>Missense</b>	<b>0.0001</b>	<b>3.40E-06</b>
var_chr19_3767610 2_CA_C	0.025	0.0022	1.39E-03	11.84	ZNF585B	3' UTR	0.0012	3.54E-06
rs112786048	0.017	0.0000	1.83E-03	NA	FMNL1	INTRON	0.0005	2.18E-05
exm526375	0.034	0.0107	7.32E-06	3.274	ZFP57	Missense	0.0143	2.75E-05
rs73271560	0.025	0.0077	6.06E-03	3.324	IK	INTRON	0.00708	3.67E-05
rs11575960	0.030	0.0033	2.08E-04	9.431	PCDHGA9	Missense	0.00577	4.13E-05

- SGSM3: Small G Protein Signaling Modulator 3  
-6 cases heterozygous for this variant
- Extremely rare in all control groups

## Sequencing and exome chip studies on DILI: progress so far and where next?

- No consistent evidence that rare coding variants are contributing to risk of DILI due to amoxicillin-clavulanate
  - SGSM3 result of some interest in small minority of samples
- Moving towards whole genome sequencing could be valuable

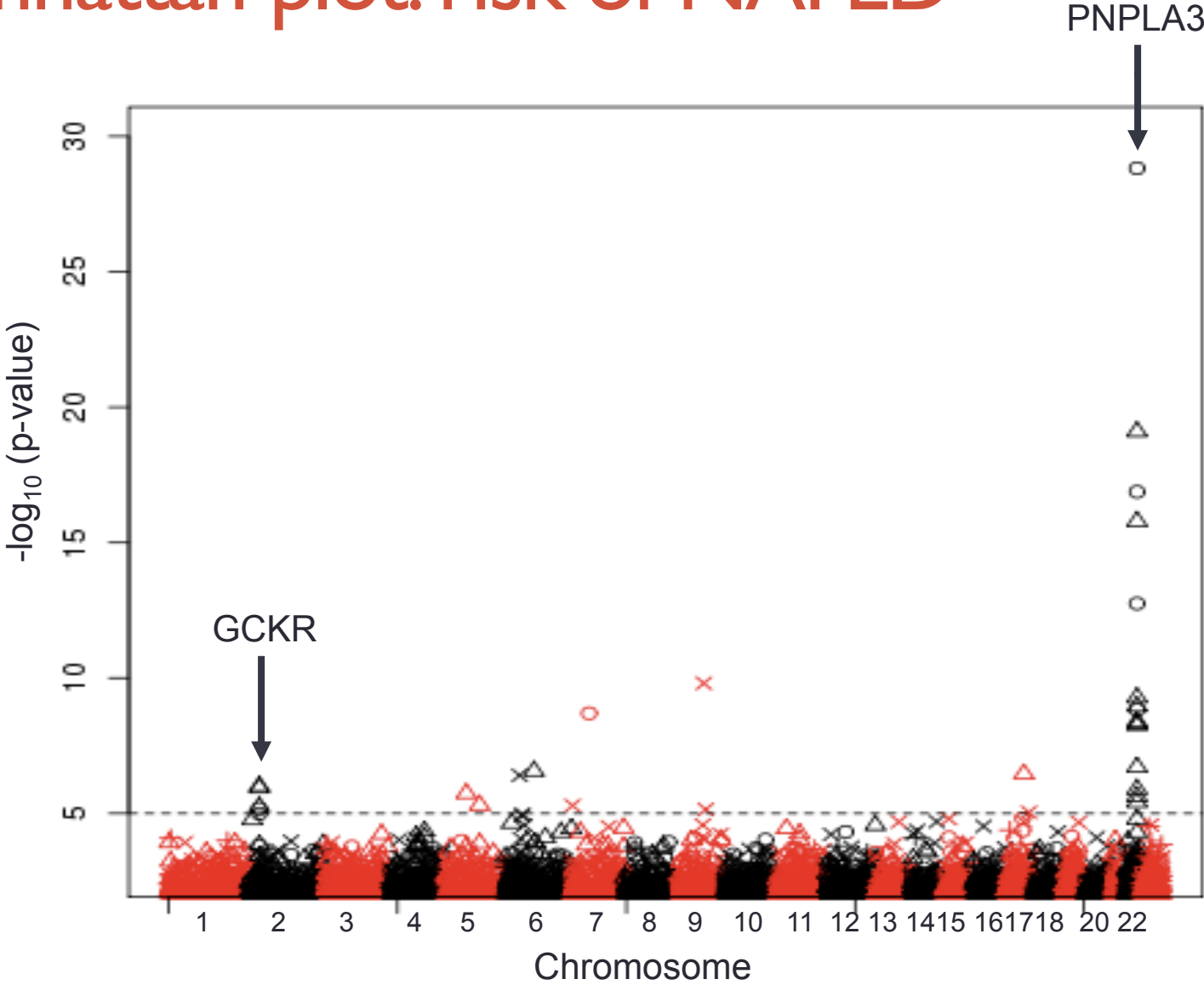
# Pharmacogenetics and DILI: summary

- No finding up to present strong enough for clinical implementation
  - Flucloxacillin-B\*57:01 association may be helpful for diagnostic purposes
- May be valuable to develop more general risk algorithms involving age, gender, genetics and concurrent drugs

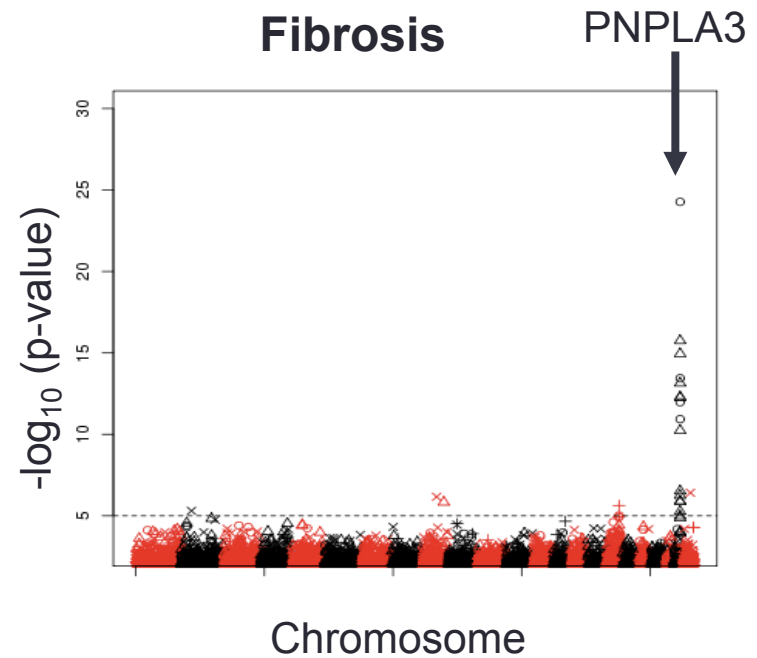
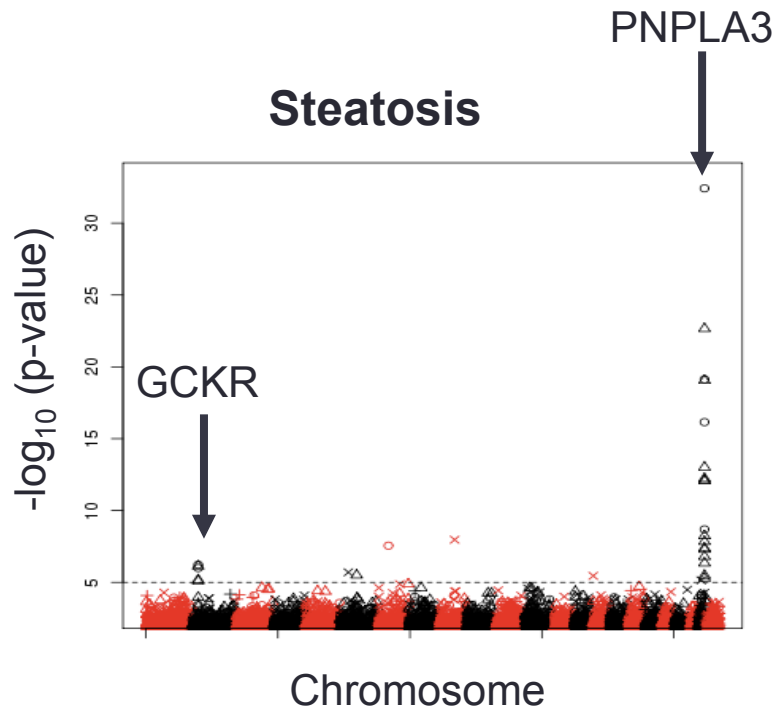
# Pharmacogenetics and other liver diseases

- Drugs and xenobiotics suggested to be important triggers for other liver diseases
  - e.g. alcoholic liver disease, primary biliary cirrhosis
- Primary biliary cirrhosis (PBC)
  - Immune system polymorphisms (including HLA) main predictors of risk
  - No genetic evidence for xenobiotic role
  - Risk genotypes may be relevant to ursodeoxycholate response?
- Alcoholic liver disease (ALD)
  - Our early candidate gene studies suggested roles for alcohol metabolism-related genes (CYP2E1, ADH3) and innate immune system (TNF-alpha, IL10)
  - Recent European GWAS suggests these are not big risk factors
    - PNPLA3, TM6SF2 show significance as alcoholic cirrhosis genes
    - Lipids important with results very similar to our NAFLD GWAS

# Manhattan plot: risk of NAFLD



# Manhattan plots for steatosis and fibrosis



Steatosis/fibrosis  $\geq 2$  against 0+1 including controls

## TM6SF2 rs58542926 INFLUENCES HEPATIC FIBROSIS PROGRESSION IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE

Yang-Lin Liu, Helen L. Reeves, Alastair D. Burt, Dina Tiniakos, Stuart McPherson, Julian B.S. Leathart, Michael E.D. Allison, Graeme J. Alexander, Anne-Christine Piguet, Rodolphe Anty, Peter Donaldson, Guruprasad P. Aithal, Sven Francque, Luc Van Gaal, Karine Clement, Vlad Ratziu, Jean-Francois Dufour, Christopher P. Day, Ann K. Daly & Quentin M. Anstee

**Table 1 | Multivariate analysis of association between TM6SF2 rs58542926 genotype and fibrosis stage F0-1 (mild) versus F2-4 (advanced).**

Variables	Discovery cohort (n = 349)		Validation cohort (n = 725)		Combined cohort (n = 1,074)	
	OR (95%CI)	P-value	OR (95%CI)	P-value	OR (95%CI)	P-value
TM6SF2 genotype	<b>2.94 (1.76-4.89)</b>	<b><math>3.44 \times 10^{-5}</math></b>	<b>1.46 (1.03-2.09)</b>	<b>0.0362</b>	<b>1.88 (1.41-2.5)</b>	<b><math>1.63 \times 10^{-5}</math></b>
PNPLA3 genotype	<b>1.57 (1.21-2.19)</b>	<b>0.0086</b>	<b>1.32 (1.05-1.66)</b>	<b>0.0183</b>	<b>1.40 (1.16-1.69)</b>	<b><math>4.84 \times 10^{-4}</math></b>
Age	<b>1.03 (1.01-1.06)</b>	<b>0.0045</b>	<b>1.02 (1.01-1.04)</b>	<b>0.0041</b>	<b>1.03 (1.01-1.04)</b>	<b><math>1.57 \times 10^{-5}</math></b>
Gender (female)	0.94 (0.57-1.56)	0.8297	<b>1.81 (1.30-2.50)</b>	<b><math>4.50 \times 10^{-4}</math></b>	<b>1.43 (1.09-1.89)</b>	<b>0.0096</b>
BMI	<b>1.05 (1.00-1.10)</b>	<b>0.0368</b>	<b>1.03 (1.01-1.05)</b>	<b><math>9.80 \times 10^{-4}</math></b>	<b>1.04 (1.02-1.05)</b>	<b><math>3.78 \times 10^{-5}</math></b>
T2DM	<b>2.39 (1.49-3.84)</b>	<b>0.0003</b>	<b>2.73 (1.93-3.88)</b>	<b><math>1.68 \times 10^{-8}</math></b>	<b>2.57 (1.95-3.39)</b>	<b><math>1.78 \times 10^{-11}</math></b>

BMI, body mass index; CI, confidence interval; OR, odds ratio; T2DM, type 2 diabetes mellitus.

Additive model including age, gender, BMI, T2DM and PNPLA3 rs738409 genotype as covariates. Discovery/validation/combined cohorts: stage F0-1 (mild) n = 198/439/637, stage F2-4 (advanced) n = 151/286/437.

- TM6SF2 is a significant predictor for risk of advanced fibrosis development (OR > PNPLA3)
- Lesser effect as steatosis predictor

## PNPLA3: risk factor for HCC

- PNPLA3 I148M (rs738409) gives odds ratio of 2.26 (95% CI 1.23-4.14;  $p=0.0082$ ) for risk of hepatocellular carcinoma development in multivariate analysis with cirrhosis as co-variate
  - Liu et al. *J Hepatol* 2014; 61:75–81
- Genotype may be useful in evaluating risk for progression to HCC relating to a variety of liver diseases

# Conclusions

- HLA alleles are key predictors for susceptibility to some forms of DILI but also other liver diseases e.g. PBC
- Overall role for pharmacogenetic polymorphisms (e.g. those in CYP genes) as predictors of disease risk limited
- Immune system and lipid-related genes appear to be key risk factors across a range of different diseases
  - May be helpful in future in personalising treatment

# Acknowledgements

## Newcastle

Chris Day

Pete Donaldson

Julian Leathart

Julia Patch

Ruth Wake

Heather Cordell

Sally Coulthard

Quentin Anstee

Yang-Lin Liu

Guru Aithal (Nottingham)

Munir Pirmohamed (Liverpool)

Yufeng Shen (Columbia)

Aris Floratos (Columbia)

Paola Nicoletti (Columbia)

Mark Daly (Broad Institute)

Jackie Goldstein (Broad Institute)

Matt Nelson (GSK)

Paul Watkins (North Carolina)

Tom Urban (North Carolina)

Maribel Lucena (Malaga)

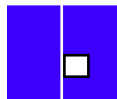
Raul Andrade (Malaga)

Camilla Stephens (Malaga)

Mariam Molokhia (London)

iDILIC collaborators

FLIP collaborators



SAE Consortium, Ltd.

