Multiple HLA B*57 alleles, sharing the amino acid residue Valine^{97}, are associated with drug-induced liver injury (DILI) due to flucloxacillin in a European population.

Guruprasad P. Aithal, Paola Nicoletti, Thomas C. Chamberlain, Sally Coulthard, Mohammad Al-Shabeeb, Raul Andrade, Einar Bjornsson, John F. Dillon, Par Hallberg, M. Isabel Lucena, Anke H. Maitland-van der Zee, Jennifer H. Martin, Mariam Molokhia, Munir Pirmohamed, Mia Wadelius, Aris Floratos, Yufeng Shen, Paul B. Watkins, Matthew R. Nelson and Ann K. Daly

- National Institute for Health Research (NIHR) Nottingham Biomedical Research Centre, Nottingham University Hospital NHS Trust and University of Nottingham, Nottingham, UK
- Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, USA
- Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK
- UGC Digestivo, Instituto de Investigación Biomédica de Málaga (IBIMA), Hospital Universitario Virgen de la Victoria, Universidad de Málaga, Málaga, Spain; Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd);
- Division of Gastroenterology and Hepatology, Department of Internal Medicine, The National University Hospital of Iceland, Reykjavik, Iceland
- Medical Research Institute, University of Dundee,Ninewells Hospital, Dundee, UK;
- Department of Medical Sciences and Science for Life Laboratory, Uppsala University, Uppsala, Sweden;
- Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht University, Utrecht, Netherlands;
- School of Medicine and Public Health, University of Newcastle, New South Wales, Australia;
- Department of Primary Care and Public Health Sciences, King's College, London, UK;
- Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK;
- The Herbert Irving Comprehensive Cancer Center, Columbia University, New York, USA
- Department of Biomedical Informatics, Columbia University, New York, USA
- UNC Eshelman School of Pharmacy, Chapel Hill, NC, USA
- Target Sciences, GSK, King of Prussia, PA, USA

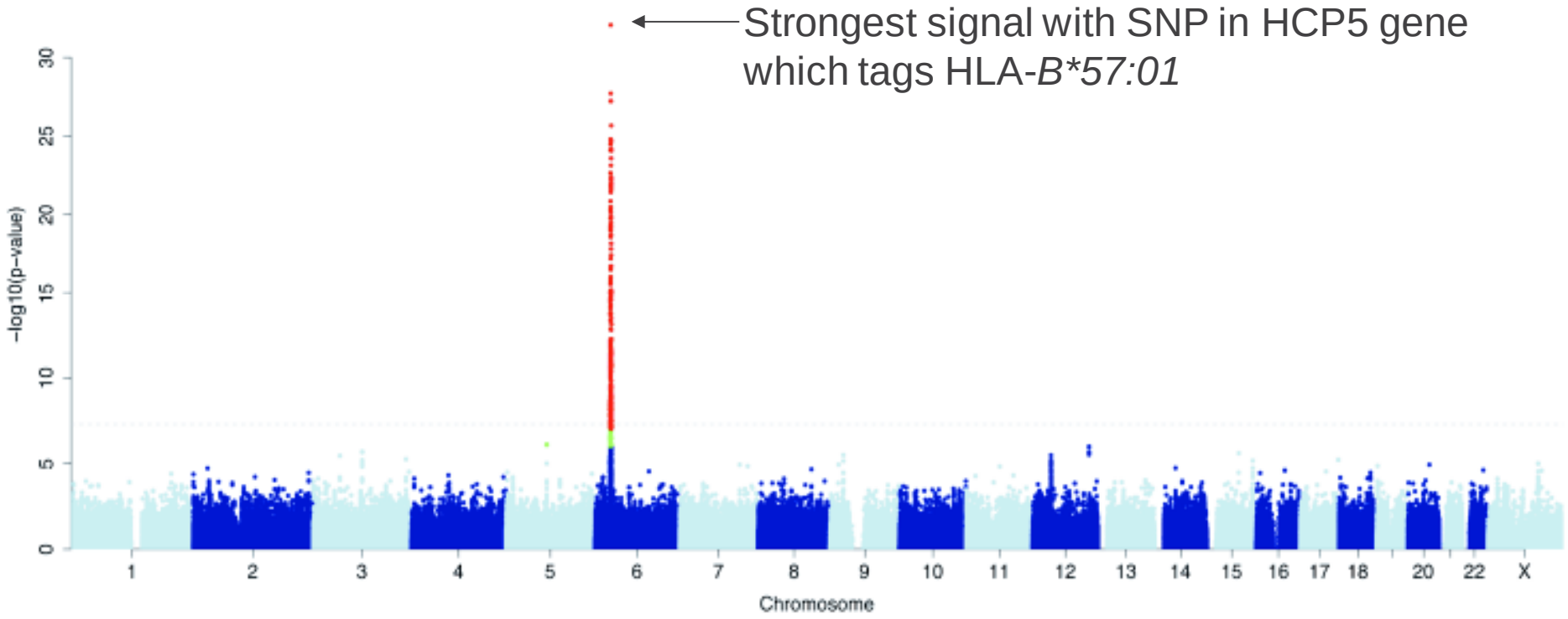
for the International Drug-induced Liver Injury consortium (iDILIC) and DILIN

Flucloxacillin and Drug-induced Liver Injury (DILI)

- Beta-lactamase resistant penicillin with isoxazolyl ring; effective in the treatment of staphylococcal infections
- Used widely especially in UK, Australia and Sweden
- DILI in 6.1 – 8.5 per 100,000 people
  - Presentation within 45 days of 1st exposure
  - 2nd most important cause of DILI in the UK
  - Rash and fever described
  - Fatality and vanishing bile duct syndrome

Li et al. *Br J Clin Pharmacol* 2009; 68:2: 269-70.

Genome-Wide Association Study (GWAS): Flucloxacillin DILI



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

Genetic studies on DILI susceptibility

- **DILIGEN (UK-based)**

  - DILI due to co-amoxiclav, flucloxacillin, anti-TB agents, diclofenac
  - Now complete

- **iDILIC (worldwide)**

  - Any licensed drug
  - Data analysis ongoing

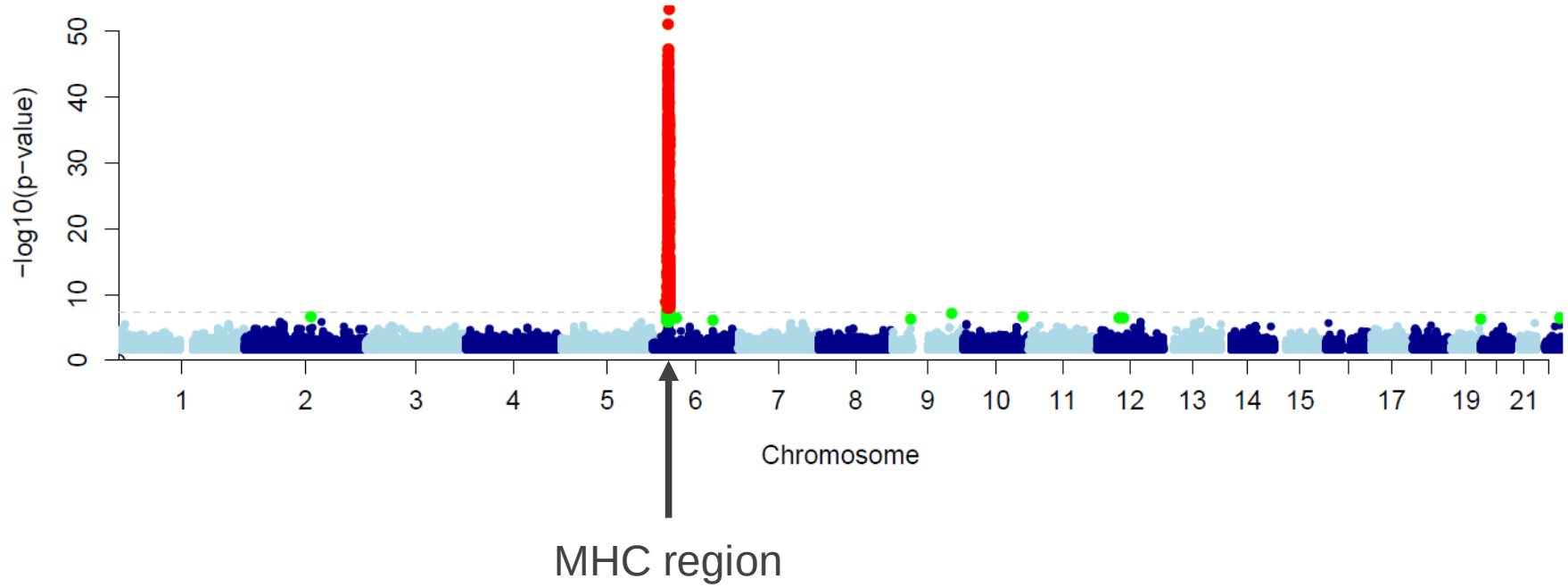
Patients and Methods

- GWAS: 197 Flucloxacillin DILI cases (2005-2013)
  - All of European ethnicity
  - 51 of these reported previously (DILIGEN)
- 6835 matched population controls
- Used Illumina HC and 1M chips for genotyping
- Performed imputation to increase data coverage
  - Four digit HLA alleles and amino acid changes were inferred using SNP2HLA

Patients and DILI characteristics

Gender (F/M)	133/64 (68% female)
Mean Age (years) (±SD)	62 ± 13
Mean Time to onset from first exposure (days) (±SD)	24 ± 18
Total days on drug (±SD)	10 ± 6
Cholestatic	74 (38%)
Hepatocellular	39 (20%)
Mixed	84 (43%)
RUCAM scores	
3-5 (possible)	22 (11%)
6-8 (probable)	90 (46%)
>8 (highly probable)	85 (43%)

New GWAS: Results

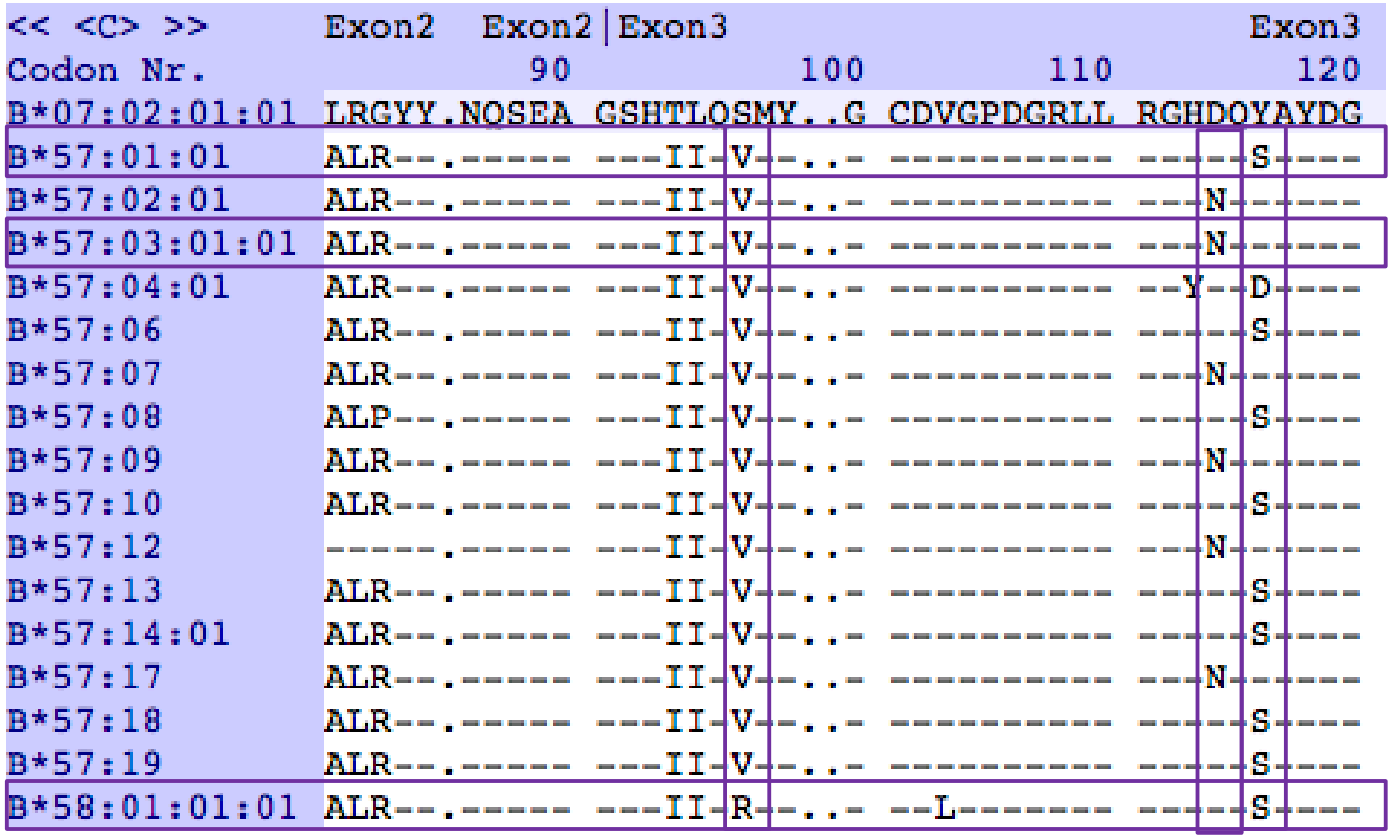


Results

- *HLA-B*57:01* major Risk factor; allelic OR=36.6; 95% CI 26.14-51.29, $p=2.67x10^{-97}$)
- Protective effect from *HLA-B*07:02, C*07:02* and *DQB1*03:01*
- *HLA-B*57:03* is also a risk factor; OR=79.21; 95% CI 13.57-462.2, $p=1.2X10^{-6}$)
  - Shown by conditioning on B*57:01
- HLA-B alleles positive for valine-97 had largest effect size; OR 38.1 (27.1-53.6) $p = 9.7x10^{-97}$)
  - Different to abacavir hypersensitivity where there is strong specificity for HLA-B*57:01 only

Amino acid sequences of HLA-B variants

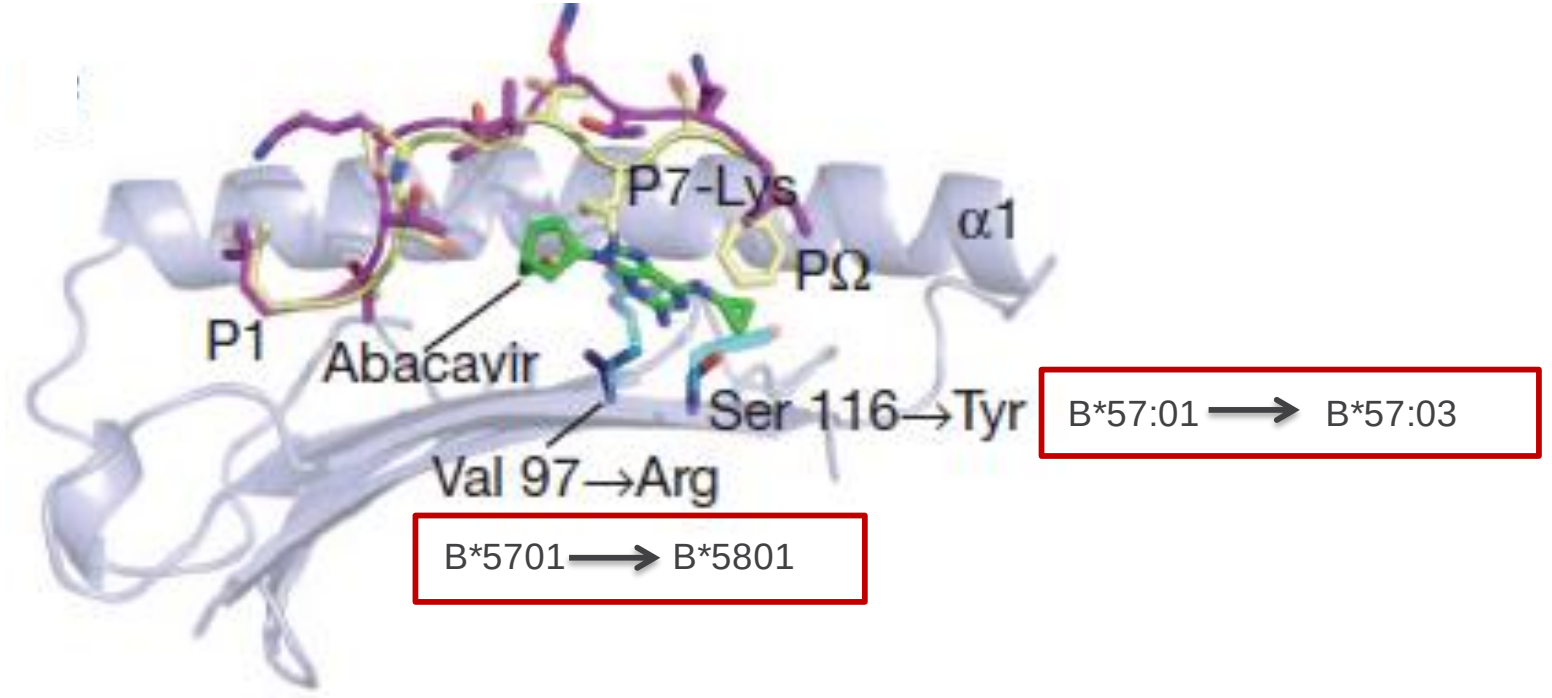
- Residues 97, 114 and 116 appear crucial
- Abacavir: B*57:01 only
- Flucloxacillin: B*57:01 and B*57:03 only
- Neither associates with B*58:01



<< <C> >>	Exon2	Exon2 | Exon3			Exon3
Codon Nr.	90	100	110	120	
B*07:02:01:01	LRGYY.NQSEA	GSHTLQSMY..G	CDVGPDGRLL	RGHDQYAYDG	
B*57:01:01	ALR--.-----	---II-V--..-	----------	-----S----	
B*57:02:01	ALR--.-----	---II-V--..-	----------	---N------	
B*57:03:01:01	ALR--.-----	---II-V--..-	----------	---N------	
B*57:04:01	ALR--.-----	---II-V--..-	----------	--Y--D----	
B*57:06	ALR--.-----	---II-V--..-	----------	-----S----	
B*57:07	ALR--.-----	---II-V--..-	----------	---N------	
B*57:08	ALP--.-----	---II-V--..-	----------	-----S----	
B*57:09	ALR--.-----	---II-V--..-	----------	---N------	
B*57:10	ALR--.-----	---II-V--..-	----------	-----S----	
B*57:12	-----.-----	---II-V--..-	----------	---N------	
B*57:13	ALR--.-----	---II-V--..-	----------	-----S----	
B*57:14:01	ALR--.-----	---II-V--..-	----------	-----S----	
B*57:17	ALR--.-----	---II-V--..-	----------	---N------	
B*57:18	ALR--.-----	---II-V--..-	----------	-----S----	
B*57:19	ALR--.-----	---II-V--..-	----------	-----S----	
B*58:01:01:01	ALR--.-----	---II-R--..-	--L-------	-----S----	

HLA-B*5701

- Serine 116 essential for binding of abacavir purine group
- Position 97 sits at the bottom of the peptide-binding cleft and is critical for HLA protein conformation and folding



Illing *et al.* *Nature* 2012; 486:554-58.

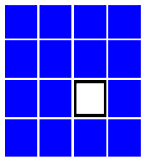
Results

- Arg97 and Ser97 have significant protective effects (OR= 0.43 P= $5.13x10^{-14}$ and OR= 0.53, P=$9.82x10^{-7}$)
- Approx. 20% of flucloxacillin DILI cases not positive for B*57
  - *HLA-A*02:02* enriched in these cases (OR 16.57, (2.05-133.8) p = 0.008)
  - Two other class I alleles, *A*30:01* and *B*13:02*, also show significant associations (P=0.009 and 0.017)
- Amoxicillin and other isoxazolyl penicillin DILI cases don't share HLA alleles seen in either the B*57 positive or negative flucloxacillin DILI cases
  - Suggests that there isn't a common genetic risk factor relating to T cell response to the beta lactam structure

Summary

- Strong association of Flucloxacillin DILI with HLA B*57:01 confirmed in an enlarged cohort
- Novel association with *B*57:03* detected
- No evidence for non-HLA genetic risk factors
- Abacavir hypersensitivity is associated only with *B*57:01* and appears to have a different mechanism from flucloxacillin DILI
- 83% of DILI patients and 6% normal with HLA-B*5701/03; Genetic factors can support the diagnosis of DILI in specific scenarios

Nottingham University Hospitals
NHS Trust



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