Recent Research Advances in Drug Induced Liver Injury

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October 1, 2009

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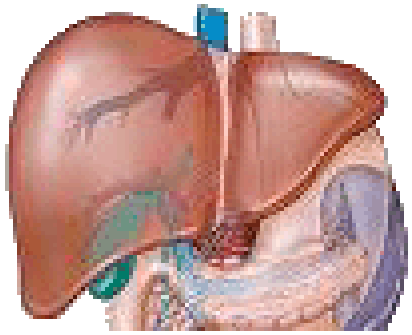
Drug Induced Liver Injury (DILI)

Background

- Drug-induced hepatotoxicity has been the most frequent cause of safety-related post-marketing withdrawals for the past 50 years*
- Most drugs withdrawn for severe DILI have rates for death and transplantation of ≤1 per 10,000
- Drugs that show severe DILI generally do not show signs of hepatotoxicity in animal models



**from FDA Draft Guidance document on DILI, October 2007*

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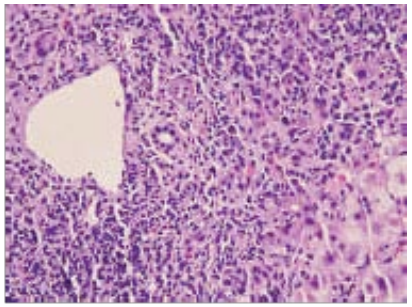
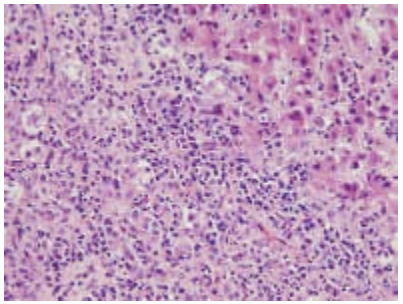
Drug Induced Liver Injury (DILI)

Background (Cont'd)

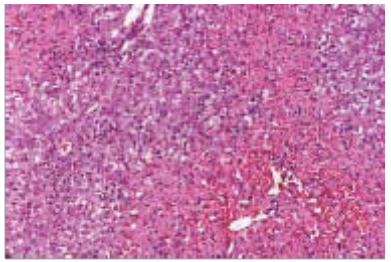
- Laboratory measures of elevated aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), are used as the criteria for defining potential hepatotoxicity
  - AST and ALT levels >3x upper limit of normal (ULN) suggest potential mild hepatocellular injury, while >5x, >10x, and >15x ULN suggest increasing severity in hepatotoxicity
- Hy's law: >3x ULN ALT/AST **and** >2x ULN serum bilirubin
  - It is estimated that ~10% Hy's law cases progress to death or liver transplantation



Atorvastatin *



Minocycline*



Acetaminophen*

** R Ramachandran and S Kakar. J. Clin Pathol 2009*

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Drugs Associated with Hepatocellular Injury Pattern

Acetaminophen (FHF)
Acarbose (FHF)
Allopurinol
Amiodarone
Amoxicillin, Ampicillin
Anti-HIV: (Didanosine, Zidovudine, protease inhibitors)
Asparaginase
Bentazepam
Chlormethizole (FHF)
Diphenytoin
Disulfiram (FHF)
Ebrotidine (FHF), withdrawn (Spain, Peru)
Fluoxetine, Paroxetine
Flutamide (FHF)
Halothane
Isoniazid (FHF)
Ketoconazole, Mebendazole, Albendazole, Pentamidine (FHF)
Mesalazine
Methotrexate
Minocycline
Nitrofurantoin
Nefazodone (FHF)
NSAIDs: e.g. aspirin, ibuprofen, diclofenac, indomethacin
withdrawn: bromfenac, benoxaprofen, ibufenac (FHF)

Omeprazole
Penicillin G
Pemoline (FHF), withdrawn
Pyrazinamide
Risperidone
Ritodrine
Statins:Lovastatin, Pravastatin, Simvastatin, Atorvastatin
Sulfasalazine
Telithromycin
Terbinafine (FHF)
Tetracycline (FHF)
Tolcapone (FHF), withdrawn (Australia)
Topiramate
Trazodone
Troglitazone (FHF), withdrawn
Trovafloxacin (FHF), withdrawn
Valproic acid
Venlafaxine
Verapamil
Vitamin A
Ximelagatran (FHF), withdrawn

FHF = Fulminant Hepatic Failure has been reported

Adapted from: Andrade RJ et al . Drug-induced hepatotoxicity in clinical practice. World J Gastroenterol, 2007

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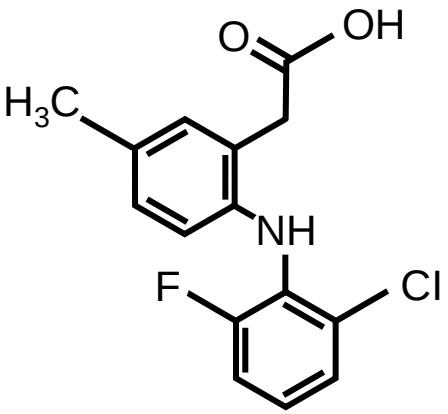
Lumiracoxib Background

- Mechanism of Action
  - Selective COX-2 inhibitor
  - Lumiracoxib has a favorable distribution (Rheumatoid Arthritis), with higher concentrations in the synovial fluid versus plasma 3 hours after dosing
- Efficacy
  - Efficacious for knee and hip osteoarthritis (OA, 100 mg qd)
  - Highly efficacious in acute pain (e.g. dental pain, acute gout, dysmenorrhea, 400 mg qd)
- Safety Benefits
  - Favorable safety profile compared to non-selective COX-1/2 inhibitors:
    - GI benefit in non-aspirin users compared to ibuprofen or naproxen at 12 months
    - Effects on BP comparable to naproxen and slightly better than ibuprofen
  - Incidence of CV events similar to NSAIDs (naproxen and ibuprofen)
- Hepatic Safety
  - Associated with reversible transaminase elevations and Hy's Law events during the development program
  - Product labeling in countries where lumiracoxib was approved thus recommended monthly hepatic monitoring for chronic use
  - Cases of liver failure and death associated with lumiracoxib were observed during post-marketing surveillance
  - Lumiracoxib was not approved in the US

lumiracoxib



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Therapeutic Arthritis Research and Gastrointestinal Events Trial (TARGET)

- **52-week gastrointestinal clinical safety study in subjects with osteoarthritis (OA) to determine the risk of developing complicated ulcers on treatment with lumiracoxib (400 mg qd) compared to NSAIDs (naproxen 500 mg bid and ibuprofen 800 mg tid)**
  - Comprised of two trials CCOX189 0117 and CCOX189A2332 with identical study design using different comparator therapies (ibuprofen & naproxen)
  - 18,244 patients randomized (9,117 to lumiracoxib, 4,730 to naproxen, 4,397 to ibuprofen)
  - 10,057 consented DNA samples obtained
- **Trial Results:**
  - 79% reduction in upper GI ulcer complications was observed with lumiracoxib as compared to studied NSAIDs in non-aspirin population
  - No statistical difference in incidences of CV events between lumiracoxib and NSAIDs at 12 months
  - Higher proportion of >3xULN ALT/AST elevations seen for lumiracoxib-treated patients (2.6%) compared to NSAIDS (0.6%)

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Pharmacogenetic Approach to Understanding the Risk for Severe Liver Toxicity

- Hepatic Toxicity was the main issue with approval of lumiracoxib (US) and for withdrawal from markets where approved
- Given the otherwise favorable safety profile and efficacy, could a marker for susceptibility to hepatotoxicity be identified?
- The TARGET study provided a unique opportunity to study genetic associations with hepatotoxicity based on sample size (over 10,000 consented DNA samples available) and ~40 subjects with moderate severity Liver Function Test (LFT) abnormality (>5x ULN)
- Approach: Multistage analysis of pharmacogenetic association

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TARGET Pharmacogenetic DNA collections by country

10,057 consented DNA samples collected at randomization (55% ascertainment)

Country	Total randomized	DNA samples	Percent
Argentina	1336	796	59.6
Belgium	200	178	89.0
Brazil	326	0	0.0
Canada	701	519	74.0
Chile	123	76	61.8
China	653	0	0.00
Colombia	360	0	0.00
Czech Republic	278	252	90.7
Ecuador	312	199	63.8
Estonia	33	30	90.9
Finland	213	176	82.6
Germany	1877	1351	72.0
Guatemala	150	89	59.3
Hungary	62	28	45.2
Italy	695	185	26.6
Malaysia	44	0	0.0

Country	Total randomized	DNA samples	Percent
Mexico	881	572	64.9
Netherlands	324	0	0.0
Peru	399	193	48.4
Poland	347	313	90.2
Russian Federation	1250	533	42.6
Singapore	29	0	0.0
South Africa	449	334	74.4
Spain	640	157	24.5
Sweden	168	73	43.5
Switzerland	144	84	58.3
Taiwan	126	14	11.1
Turkey	291	142	48.8
United Kingdom	1209	775	64.1
United States of America	4503	2885	64.1
Uruguay	58	0	0.0
Venezuela	143	103	72.0

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Stage 1: Genome-wide Association Study Design
>5xULN ALT/AST patients from TARGET

- Case/control design
  - Initial pharmacogenetic analysis of 41 lumiracoxib recipients with ALT/AST > 5xULN (with DNA and informed consent)
  - Selected 176 controls matched ~4:1 to cases based on clinical trial, sex, race, age (±2 years), and, where possible, country
  - Patients that were adjudicated by the liver safety committee to not be drug-related were removed from the analysis

- Genome-wide analysis
  - Analyzed only SNPs on Affymetrix array 6.0
- Statistical methods
  - Association test performed individually for each SNP using Cochran-Mantel-Haenszel (CMH) test
  - Permutation test applied to adjust for multiple testing across SNPs
  - PLINK software (Shaun Purcell, MGH)

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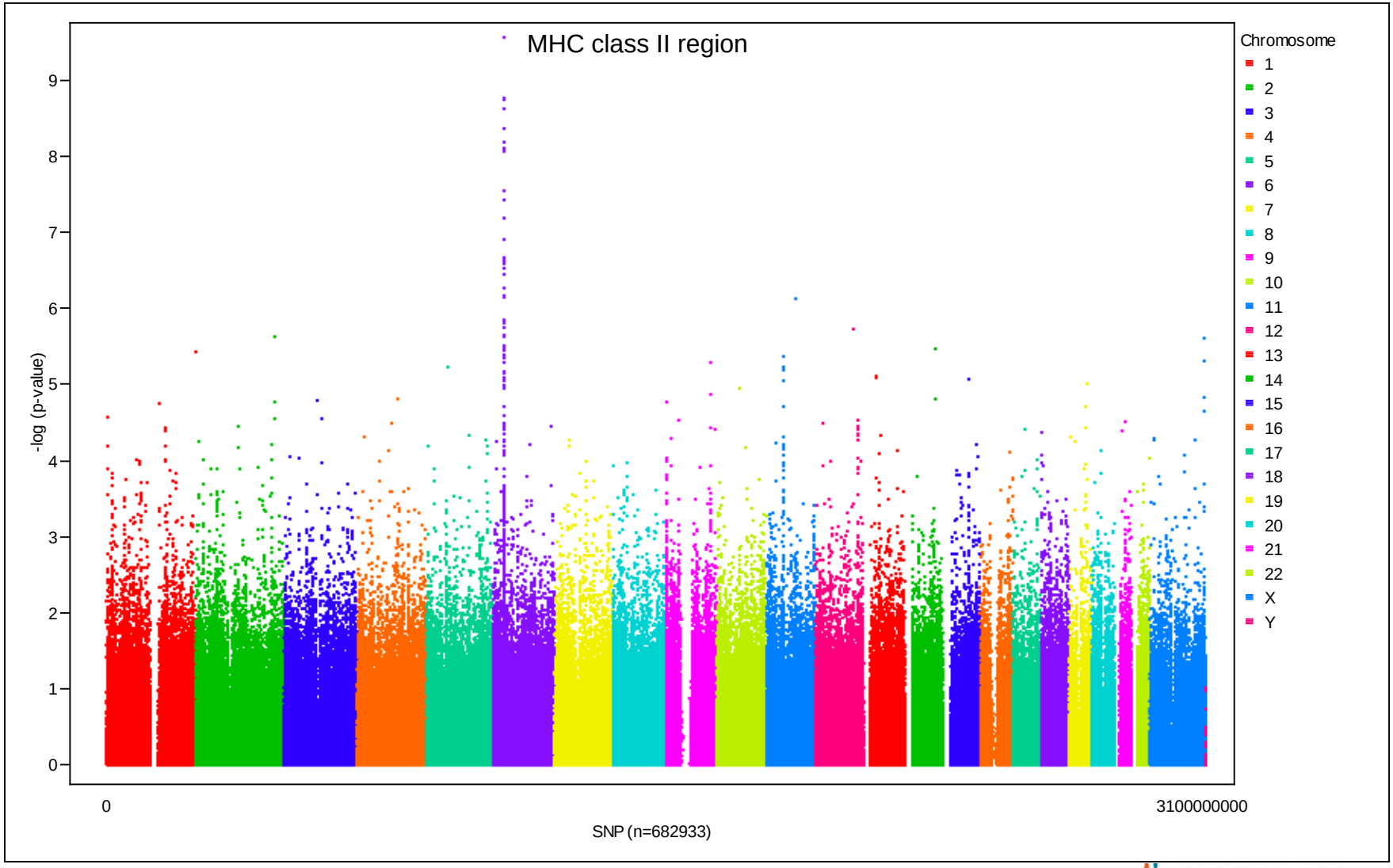
Genome-wide Association Results for All SNPs

p-values Plotted by Genomic Location; >5xULN ALT/AST



Genome-wide Results >5xULN ALT/AST Patients

- Significant findings from the exploratory genome-wide association study after multiple testing corrections

rs number	Chromosome	Region	Position	Nominal p-value	Study-wide p-value
rs9270986	6	MHC	32682038	2.8×10^{-10}	0.0075
rs3129900	6	MHC	32413957	1.8×10^{-9}	0.022
rs3132943	6	MHC	32416443	1.9×10^{-9}	0.023
rs3129934	6	MHC	32444165	2.5×10^{-9}	0.026
rs3135365	6	MHC	32497233	4.5×10^{-9}	0.038
rs3129932	6	MHC	32444105	6.5×10^{-9}	0.047
rs910049	6	MHC	32423705	6.6×10^{-9}	0.047

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Stage 2: Pharmacogenetic Replication Study Design

- **Additional TARGET trial samples used**
- **Case/control design**
  - Analyzed 98 independent lumiracoxib patients with ALT or AST >3xULN (with DNA and consent)
  - Selected 405 controls matched ~4:1 to cases based on clinical trial, sex, race, age (±2 years), and, where possible, country.
- **Evaluated 13 SNPs in replication analysis (most from the MHC region)**
  - 6 SNPs are from the MHC class II region, 2 from a distal MHC region, 1 from a drug metabolizing enzyme, 2 from the top non-MHC SNPs, and 2 from other chromosomes
- **Secondary analysis: recipients of comparator therapy**
  - Analyzed 18 ibuprofen recipients and 9 naproxen recipients with ALT or AST >3xULN (with DNA and consent)
  - Selected control samples for each comparator therapy
    - Controls matched ~4:1 to cases based on above criteria

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Stage 2: Replication Study Results

Data from independent samples

rs number	Gene/ region	p-value	Carrier[1] case frequency	Carrier[1] control frequency	MAF[2] cases	MAF[2] controls
3129900	MHC	**4.4×10^{-12}**	61.2%	27.3%	36.7%	14.8%
3129934	MHC	**4.9×10^{-11}**	61.1%	27.6%	36.3%	14.9%
3135365	MHC	**6.3×10^{-10}**	66.3%	33.8%	39.8%	18.8%
9270986	MHC	**1.0×10^{-9}**	59.8%	26.9%	34.5%	14.8%
9275772	MHC	**2.6×10^{-6}**	58.2%	35.7%	36.2%	20.7%
3130952	MHC	**4.5×10^{-4}**	36.5%	21.9%	21.4%	11.9%
2517451	MHC	0.0018	27.8%	16.6%	15.5%	8.5%
2517538	MHC	0.08	69.5%	62.0%	45.8%	38.6%
10509681	CYP2C8	0.099	26.0%	18.1%	14.1%	9.8%
2123139	chr. 11	0.48	8.4%	10.9%	4.2%	5.5%
2577302	FN1	0.47	8.2%	6.4%	4.1%	3.2%
7131977	ALDH1L2	0.56	14.4%	11.9%	7.2%	6.2%
9659646	chr. 1	0.66	2.1%	1.5%	1.0%	0.8%

[1]Carriers are defined as having either 1 or 2 copies of the allele. [2]MAF= minor allele frequency

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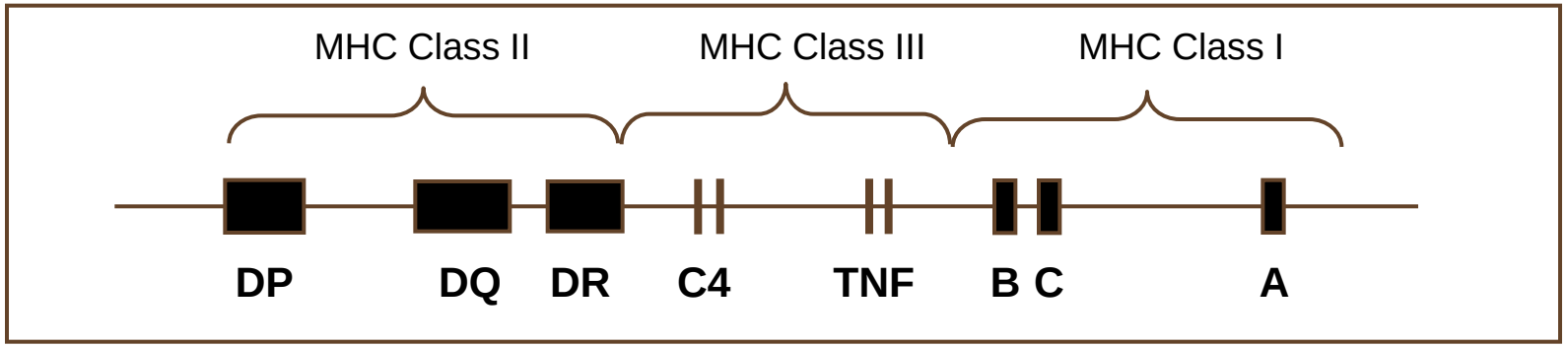
Stage 2: Replication Study Results Summary

- Top Stage 1 SNPs in MHC Class II region were highly significant in Stage 2 (best nominal p= 4.4×10^{-12})
  - Provides clear evidence of replication
- No SNPs in other genes showed evidence of association
- No SNPs showed significant evidence of association in ibuprofen, naproxen, or combined NSAID arms
  - Sample sizes are too small for definitive conclusions for comparator arms

Stage 3: HLA[1] Allele Identification

- Evaluate whether top SNP(s) may be tagging a potentially causative classical MHC[2] allele/haplotype
  - May improve predictive capability
  - May provide insight into mechanism
- Case/control design using all >3xULN patients (137 cases & 577 controls) from TARGET for which DNA was available
- HLA genes evaluated (all from MHC Class II region)
  - DRB1, DRB3, DRB4, DRB5, DQA1, and DQB1



[1]HLA=Human Leukocyte Antigens, [2]MHC=Major Histocompatibility Complex

HLA Background

- MHC class II complexes are heterodimers (α & β subunits)
  - HLA-DR complex: HLA-DRA & HLA-DRB(1,3,4,5)
  - HLA-DQ complex: HLA-DQA1 & HLA-DQB1
  - HLA-DP complex: HLA-DPA1 & HLA-DPB1
- Function: present peptides to T cells (T Cell Receptor)
- MHC Class II molecules present peptides to CD4+ helper T cells and are expressed by antigen presenting cells (dendritic cells, B lymphocytes, macrophages)
- MHC region is the most polymorphic in the human genome
- Large haplotype blocks make it difficult to ascertain definitive gene/allele causality via genetic association studies.

Human MHC class II Polymorphisms

Gene	Number		
	Alleles	Proteins	Null Alleles
HLA-DRA	3	2	0
HLA-DRB	697	559	8
HLA-DQA1	34	25	1
HLA-DQB1	95	69	1
HLA-DPA1	27	16	0
HLA-DPB1	132	116	3

http://www.anthonynolan.org.uk/research/hlainformaticsgroup/

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HLA Results for All TARGET Hepatotoxicity Cases

(with DNA available)

Gene/allele	p-value
DRB1*1501	6.8×10^{-25}
DQB1*0602	1.1×10^{-22}
DRB5*0101	1.6×10^{-20}
DQA1*0102	1.2×10^{-18}

- DRB1*1501-DQA1*0102-DQB1*0602-DRB5*0101 are part of a very well-characterized haplotype
  - Also associated with an increased risk for developing multiple sclerosis
  - Associated with amoxicillin-clavulanate induced hepatotoxicity

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Performance Characteristics of HLA-associated Alleles

Lumiracoxib-treated patients with >3xULN ALT/AST

Gene/allele	Sensitivity	Specificity	PPV	NPV	RR	Allele frequency case/control
DRB1*1501	64.2%	80.8%	8.3%	98.82%	7.0	35.4%/10.5%
DQB1*0602	62.0%	80.8%	8.0%	98.74%	6.4	34.3%/10.5%
DRB5*0101	64.2%	80.1%	8.0%	98.81%	6.7	32.1%/10.0%
DQA1*0102	73.7%	69.2%	6.1%	**98.98%**	6.0	42.7%/17.4%

Patients carrying at least one copy of the risk allele are considered to have the risk genotype.
Overall TARGET lumiracoxib-treated study event-free rate for >3xULN is **97.37%**

RR= relative risk
PPV= positive predictive value
NPV= negative predictive value

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Performance Characteristics of HLA-associated Alleles

Lumiracoxib-treated patients with >5xULN ALT/AST

Gene/allele	Sensitivity	Specificity	PPV	NPV	RR	Allele frequency case/control
DRB1*1501	77.0%	80.8%	4.9%	99.64%	13.5	41.0%/10.2%
DQB1*0602	72.1%	80.0%	4.4%	99.56%	9.9	38.5%/10.8%
DRB5*0101	77.0%	79.6%	4.6%	99.63%	12.5	38.5%/10.2%
DQA1*0102	83.6%	68.2%	3.3%	**99.69%**	10.6	48.4%/17.6%

Patients carrying at least one copy of the risk allele are considered to have the risk genotype.
Overall TARGET lumiracoxib-treated event-free rate for >5xULN is **98.74%**
RR= relative risk, PPV= positive predictive value, NPV= negative predictive value

- The DQA1*0102 allele performs the best as a predictive marker and was thus further characterized

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DQA1*0102 Marker Sensitivity Improves with Increasing Thresholds of ALT/AST Elevation

Sensitivity and number of cases with DQA1*0102 allele

ALT/AST (fold >ULN)	Sensitivity	Number of cases with DQA1*0102 allele	Total number of genotyped cases
>3x	73.7%	101	137
>5x	83.6%	51	61
>8x	90.9%	30	33
>10x	91.7%	22	24
>15x	93.8%	15	16
>20x	100%	8	8

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HLA Genotypes for Hy's Law Cases

Hy's law case	DRB1*1501	DQA1*0102	DRB5*0101	DQB1*0602
Patient 1	Carrier	Carrier	Carrier	Carrier
Patient 2	Carrier	Carrier	Carrier	Carrier
Patient 3	Carrier	Carrier	Carrier	Non-carrier (carrier for *0601 allele)

- DNA samples are being sought for 6 additional Hy's Law cases in the TARGET study

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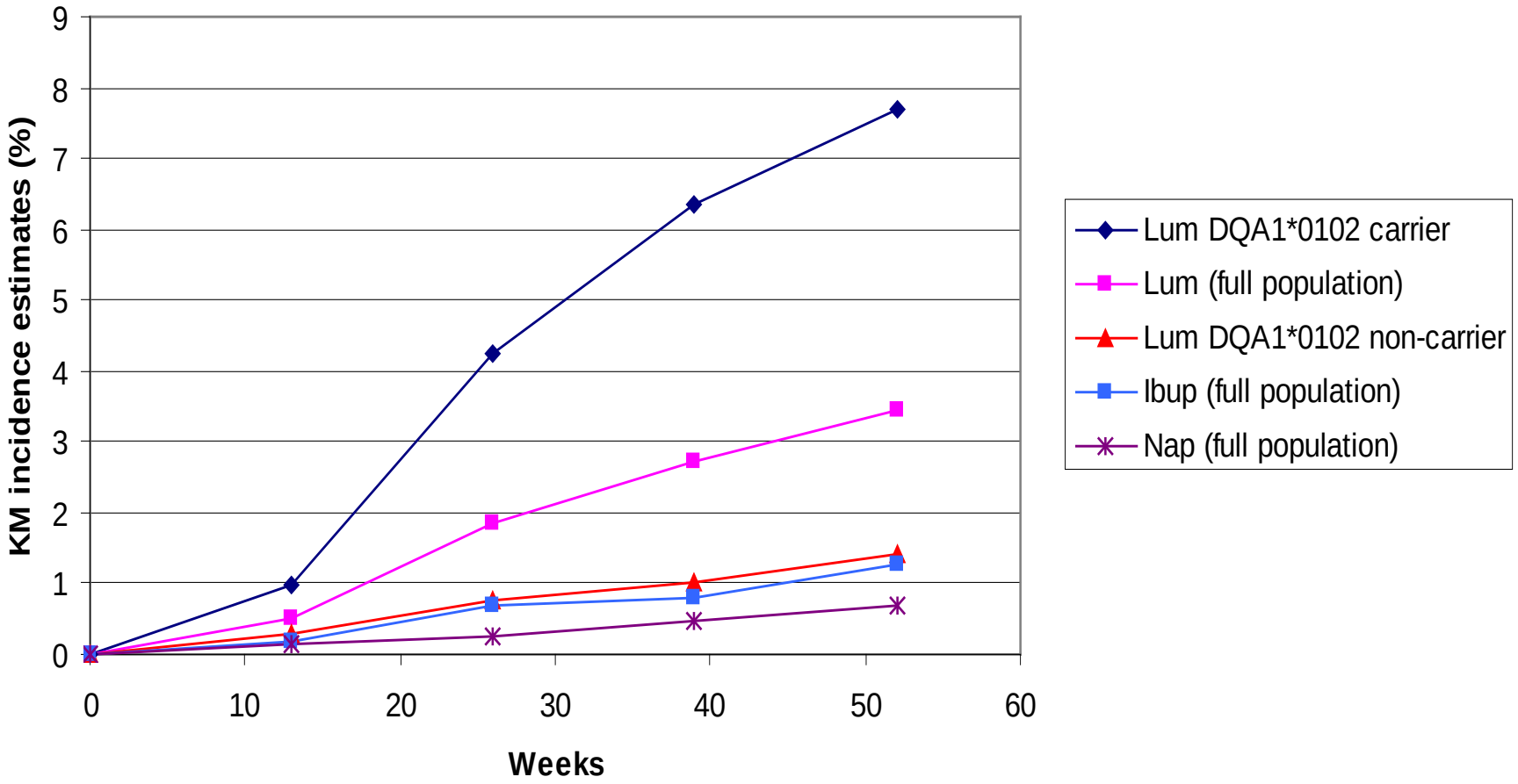
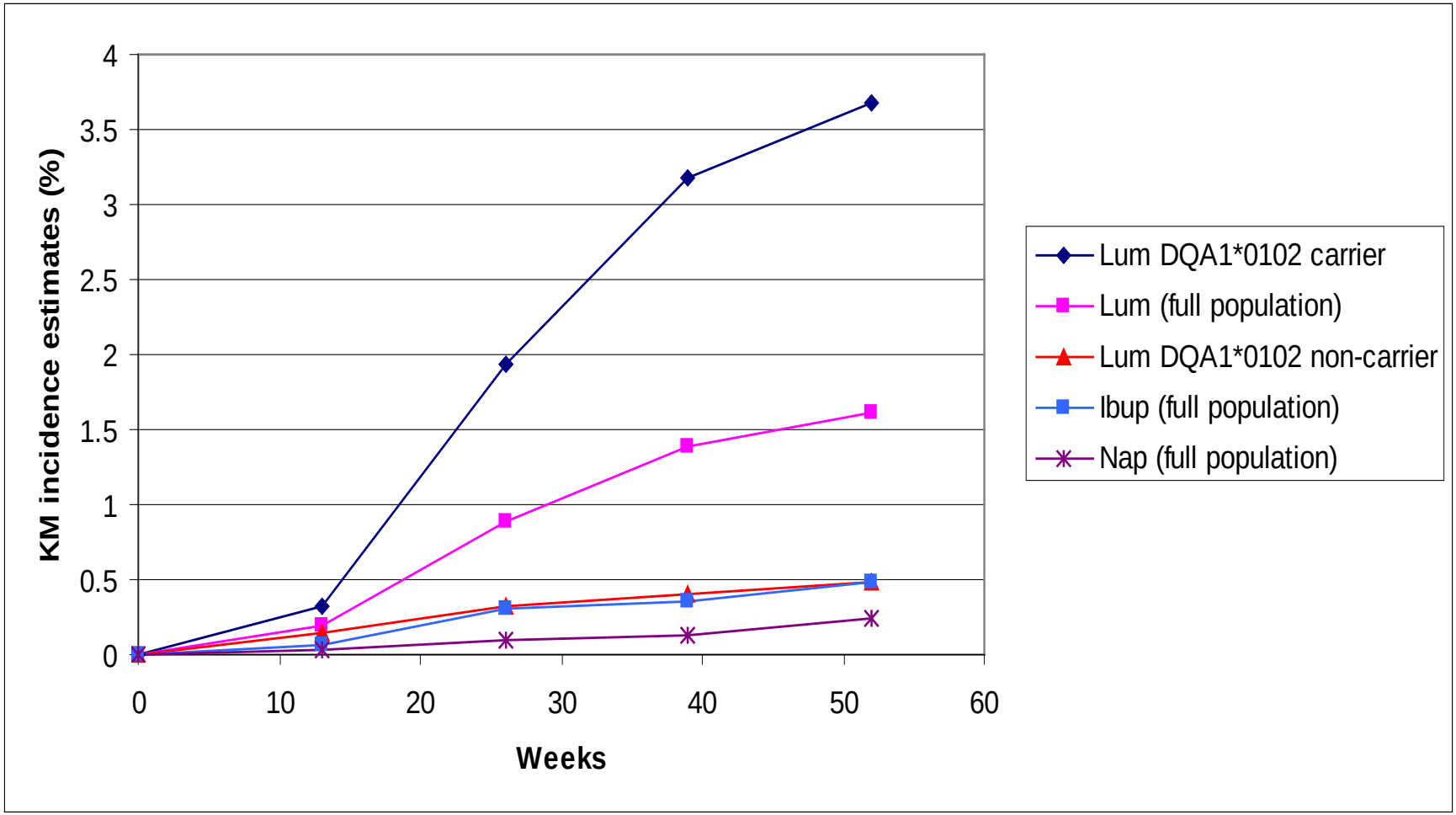
Kaplan-Meier Incidence Estimates (%) for >3xULN ALT/AST Elevations (weeks 13, 26, 39, and 52) for TARGET



Carriers are defined as having either 1 or 2 copies of the DQA1*0102 allele

Kaplan-Meier Incidence Estimates (%) for >5xULN ALT/AST Elevations (weeks 13, 26, 39, and 52) for TARGET



Carriers are defined as having either 1 or 2 copies of the DQA1*0102 allele

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Summary of Results

- **Genome-wide association study of 41 cases (>5xULN ALT/AST) and 176 matched controls identified a significant association to the MHC class II region (top SNP p=2.8x10^{-10}, genome-wide p=0.0075)**
- **Findings were replicated in an independent set of 98 cases (>3xULN ALT/AST) and 405 matched controls (top SNP p=4.4x10^{-12})**
- **HLA fine mapping identified a highly significant association to a well characterized haplotype (>3xULN ALT/AST cases n=137, controls n=577)**

Gene/allele	p-value
DRB1*1501	6.8x10^{-25}
DQB1*0602	1.1x10^{-22}
DRB5*0101	1.6x10^{-20}
DQA1*0102	1.2x10^{-18}

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Summary of Results 2 of 2

- DQA1*0102 allele used as a predictive marker would have a sensitivity of 73.7% and an NPV of 98.98% for >3xULN ALT/AST (overall population event-free rate is 97.37%)
- The DQA1*0102 marker sensitivity improves with increasing ULN thresholds, and 3 of 3 Hy's law cases carry the risk allele
- Kaplan-Meier estimates show that lumiracoxib 400mg qd DQA1*0102 non-carrier patients have similar incidence estimates for both >3xULN and >5xULN ALT/AST as ibuprofen-treated patients
- Further exploratory analysis failed to clearly identify additional hepatotoxicity markers

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Drug-related SAEs Associated with HLA Genes

- HLA-B*1502 with Stevens-Johnson syndrome and toxic epidermal necrolysis after carbamazepine treatment (Asians)
- DRB1*07/DQA1*02 is associated with hepatotoxicity after ximelagatran (Exanta) treatment
- DRB1*1501-DQA1*0102-DQB1*0602-DRB5*0101 is associated with amoxicillin-clavulanate induced hepatotoxicity
- HLA-B*5701 is associated with abacavir hypersensitivity and hepatotoxicity due to flucloxacillin
- DRB1*1302 and DQB1*0604 was found to be associated with ticlopidine hepatotoxicity (Japanese)

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Acknowledgements

- C Paulding, S Lewitzky, J Singer, N Nirmala, J Meyer - Biomarker Development
- L Klickstein -Translational Medicine
- Gabriel Helmlinger - Modeling and Simulation
- Patrice Matchaba – Drug Safety and Epidemiology
- The Prexige Global Project Team
- Participants and Investigators of the TARGET study

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QUESTIONS?

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Distribution map of allele frequencies DQA1*0102 (indigenous populations)

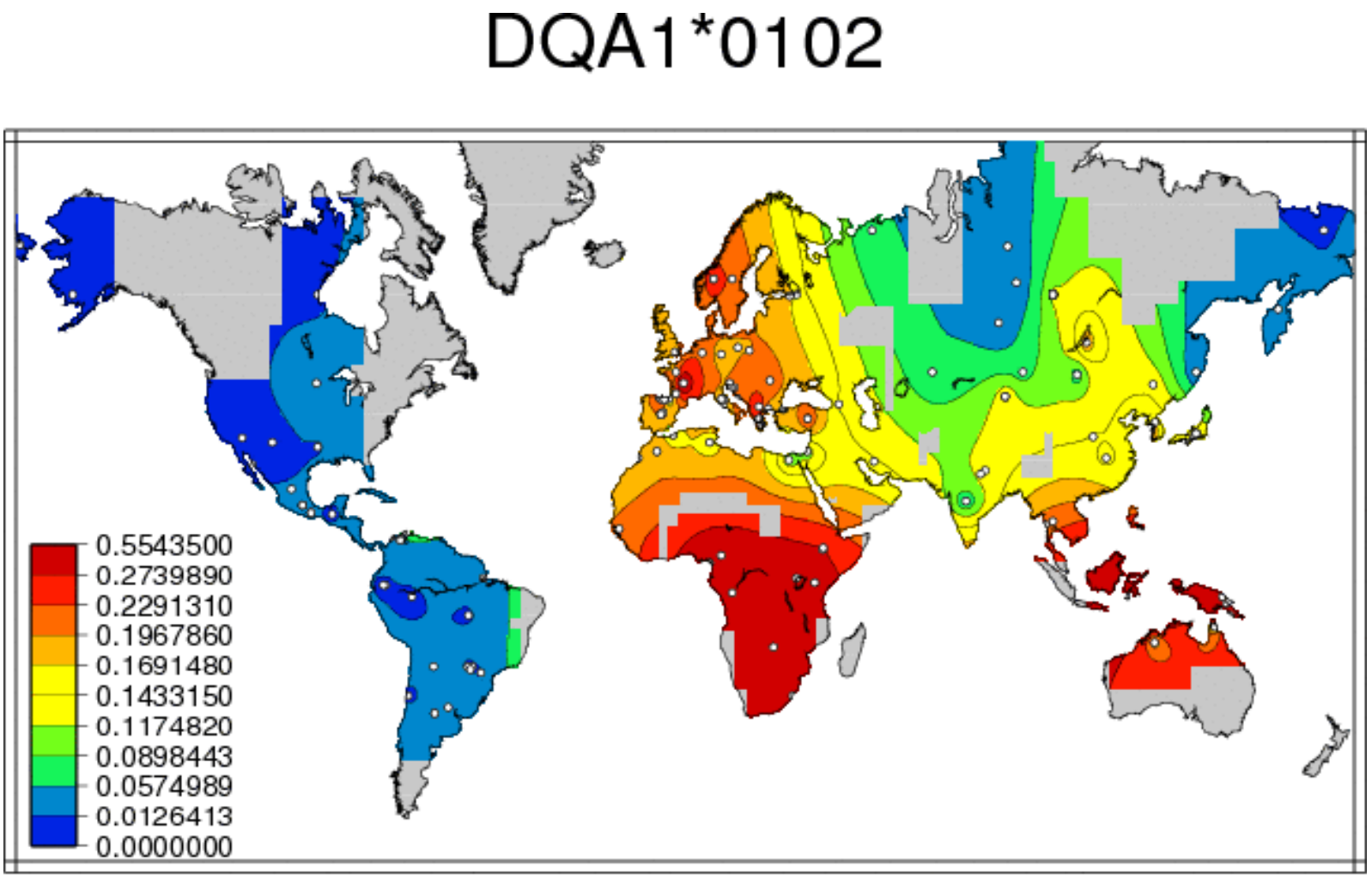

Image from Solberg et al. (2008) – see www.pypop.org/popdata for more info.

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Distribution map of allele frequencies DRB1*1501 (indigenous populations)

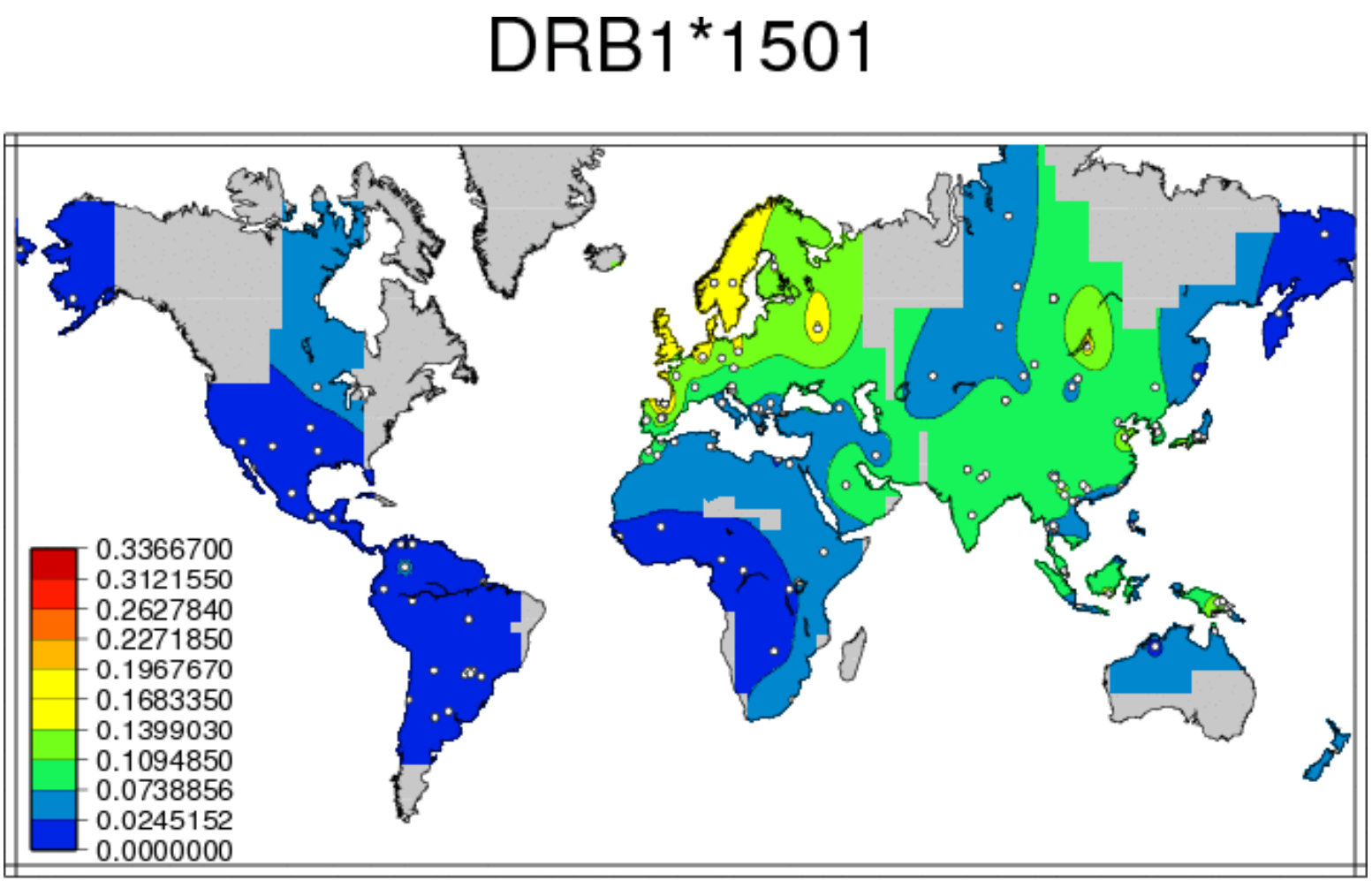

Image from Solberg et al. (2008) – see www.pypop.org/popdata for more info.

Distribution map of allele frequencies DQB1*0602 (indigenous populations)

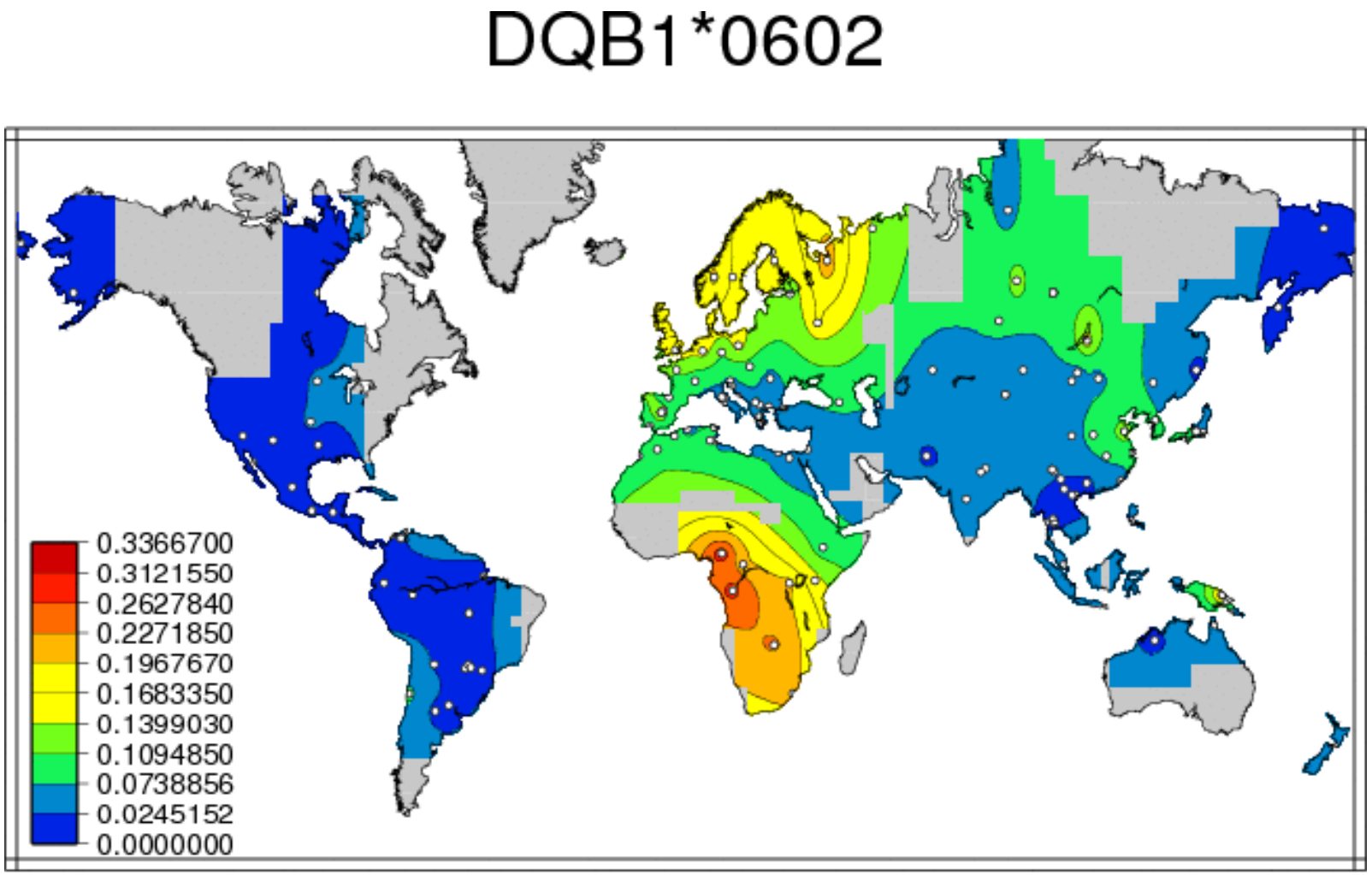

Image from Solberg et al. (2008) – see www.pypop.org/popdata for more info.

Human Genome Variability

- 3 billion nucleotides (A, T, G, or C) and ~20-25,000 genes
- For any 2 people 99.9% of those nucleotides are identical
- Remaining differences 0.1% are responsible for the genetic contribution to inter-individual differences:
  - appearance, abilities, response to environment, disease susceptibility, drug responses, etc.

- ~10 million common **Single Nucleotide Polymorphisms (SNPs)** exist in the human population
- SNPs frequently occur in haplotypes: contiguous blocks of SNPs inherited together
- SNPs are the most common type of variation; however, other types also exist: insertion/deletions, copy number variation, short tandem repeats

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Advances in SNP Array Technology

~100x resolution enhancement in the past 5 years

- Affymetrix 1.5K Array
- 10K *(113 kb median intermarker distance)* 2003*
- 100K, 2005*
- 500K *(2.5 kb median intermarker distance)* 2006*
- SNP Array 5.0; 500K SNPs, 400K copy number probes
- SNP Array 6.0; >900K SNPs, 940K copy number probes *(<700 bases median intermarker distance)* 2008*

* First publications using these Arrays

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