MOLECULAR, CELLULAR, AND IMMUNOLOGICAL BASIS FOR SEVERE ADVERSE DRUG REACTIONS

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12/13/2012
ADVERSE DRUG REACTIONS (ADRS)

- Minor
- Severe
  - 6.2-6.7% hospitalized patients in USA
  - over 2 million hospitalized patients
  - similar findings in Europe and Australia
  - tens of billions of dollars cost burden

LEADING CAUSES OF DEATH IN USA IN 1994

<table>
<thead>
<tr>
<th>Cause</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart disease</td>
<td>743,460</td>
</tr>
<tr>
<td>Cancer</td>
<td>529,904</td>
</tr>
<tr>
<td>Stroke</td>
<td>150,108</td>
</tr>
<tr>
<td>SADRs</td>
<td>106,000</td>
</tr>
<tr>
<td>Pulmonary disease</td>
<td>101,077</td>
</tr>
<tr>
<td>Accidents</td>
<td>90,523</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>75,719</td>
</tr>
<tr>
<td>Diabetes</td>
<td>53,894</td>
</tr>
</tbody>
</table>

*Lazarou et al., JAMA, 279, 1208 (1998)*
<table>
<thead>
<tr>
<th>SEVERE DRUG-INDUCED DISEASES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic</td>
</tr>
<tr>
<td>Cardiac</td>
</tr>
<tr>
<td>Skin</td>
</tr>
<tr>
<td>Renal</td>
</tr>
<tr>
<td>Pulmonary</td>
</tr>
<tr>
<td>Neurological</td>
</tr>
<tr>
<td>Lupus</td>
</tr>
</tbody>
</table>
DRUG WITHDRAWN IN USA

- Azaribine, psoriasis, **blood clots**, 1976
- Ticrynafen, blood pressure, **liver injury**, 1980
- Benoxaprofen, NSAID, **liver injury**, 1982
- Zomepirac, NSAID, **anaphylaxis**, 1983
- Nomifensine, anti-depressant, **hemolytic anemia**, 1986
- Suprofen, NSAID, **kidney failure**, 1987
- Temafloxacin, antibiotic, **kidney failure**, 1992
- Fenfluramine, appetite suppression, **heart valve disease**, 1997
- Terfenadine, anti-histamine, **fatal arrhythmia**, 1998
- Bromfenac, NSAID, **liver injury**, 1998
- Mibefradil, blood pressure, **muscle damage and fatal arrhythmia**, 1998
DRUG WITHDRAWN IN USA

- Etretinate, psoriasis, birth defects, 1999
- Grepafloxacin, antibiotic, fatal arrhythmia, 1999
- Astemizole, antihistamine, fatal arrhythmia, 1999
- Cisapride, heartburn, fatal arrhythmia, 2000
- Troglitazone, diabetes, liver toxicity, 2000
- Cerivastatin, cholesterol reduction, muscle damage leading to kidney failure, 2001
- Trovafloxacin, antibiotic, liver toxicity, 2001
- Rofecoxib, NSAID, heart attack, stroke, 2004
- Valdecoxib, NSAID, skin disease, 2005
- Pemoline, ADHD, liver toxicity, 2005
- Levomethadyl, opiate dependence, fatal arrhythmia, 2008
- Avandia, diabetes, heart attack, 2010
- Darvon, analgesic, fatal arrhythmia, 2010
TYPE A ADRs

- 80% of ADRs
- Relatively frequent and often predictable
- Excessive or diminished pharmacologic effects
- Drug-drug interactions and polymorphisms in metabolizing enzymes and transporters
- Mild to severe ADRs
- Often uncovered preclinically

EXAMPLES OF TYPE A ADRS

- Drowsiness from antihistamines
- Hypotension and fainting from antihypertensive therapy
- Excess bleeding from warfarin
- Acetaminophen
TYPE B ADRs

- 20% of ADRs
- Rare, unpredictable, and highly host-dependent
- Mild to severe ADRs
- Rarely uncovered preclinically in animals or in clinical trials
- Mechanisms often unknown but may be due to:
  
  Allergic Reactions
  Rare Polymorphisms
  Imbalance in Cellular Homeostasis
  Environmental Factors
HAPTON HYPOTHESIS AND DRUG-INDUCED ALLERGIC REACTIONS

Drug or Metabolite + B and T Cell Responses

Toxicity

B and T Cell Responses
Drug Protein Conjugate Formed in a Cell

Injured Cell

Immature Dendritic Cell

Ag Processing

B Cell

IgG, IgE, IgA

Ag Presentation by MHC Class I and II

Regulatory T Cells

Migration to Periphery

Block Drug Allergy

HMGB-1, HSPs, Uric Acid, ssRNA, dsRNA, CpG DNA, Lipopeptides

Migrate to Lymph Nodes

ImmuneT Immunization of T Cells

IL-10, PGE_2, TGF-β

Migrate to Lymph Nodes

Tolerization of T Cells

CD4^+ CD8^+

Migration to Periphery

Block Drug Allergy

CD4^+ CD8^+
THREE WAYS T CELLS MAY BE ACTIVATED
BY DRUG-HLA COMPLEXES

Camous et al., Current Opinion in Immunology, 24, 730 (2012)
DRUG-INDUCED SKIN DISEASE
MILD FORM OF CUTANEOUS TOXICITY
STEVENS JOHNSON SYNDROME (SJS) AND TOXIC EPIDERMAL NECROLYSIS (TEN)
<table>
<thead>
<tr>
<th><strong>Table 1</strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs commonly associated with developing SJS and TEN</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Antibiotics</em></td>
<td><em>Anticonvulsants</em></td>
<td></td>
</tr>
<tr>
<td>Aminopenicillins</td>
<td>Carbamazepine</td>
<td></td>
</tr>
<tr>
<td>Antimalarials</td>
<td>Lamotrigine</td>
<td></td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>Phenobarbital</td>
<td></td>
</tr>
<tr>
<td>Imidazole antifungals</td>
<td>Phenytoin</td>
<td></td>
</tr>
<tr>
<td>Macrolides</td>
<td>Valproic acid</td>
<td></td>
</tr>
<tr>
<td>Quinolones</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfonamides (esp. TMP-SMX)</td>
<td><em>Miscellaneous</em></td>
<td></td>
</tr>
<tr>
<td>Tetracyclines</td>
<td>Allopurinol</td>
<td></td>
</tr>
<tr>
<td>NSAIDs (esp. oxicam derivatives)</td>
<td>Chlormezanone</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nevirapine</td>
<td></td>
</tr>
</tbody>
</table>

*Borchers, et al., Autoimmunity Reviews, 7, 598 (2008)*
Roychowdhury and Svensson,
AAPS J., 7, E834 (2005)
Chung and Hung, Allergology Internat. 59, 325 (2010)
Genome-wide association study identifies HLA-A*3101 allele as a genetic risk factor for carbamazepine-induced cutaneous adverse drug reactions in Japanese population

Takeshi Ozeki1, Taisei Mushiroda1, Amara Yowang1, Atsushi Takahashi2, Michiaki Kubo3, Yui Shirakata4, Zenro Ikezawa5, Masafumi Iljima6, Tetsuo Shihohara7, Koji Hashimoto4, Naoyuki Kamatai8 and Yusuke Nakamurai1,8

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An anticonvulsant, carbamazepine (CBZ), is known to show incidences of cutaneous adverse drug reactions (cADRs) including Stevens–Johnson syndrome (SJS), toxic epidermal necrolysis (TEN) and drug-induced hypersensitivity syndrome (DIHS). To identify a gene(s) susceptible to CBZ-induced cADRs, we conducted a genome-wide association study (GWAS) in 53 subjects with the CBZ-induced cADRs, including SJS, TEN and DIHS, and 882 subjects of a general population in Japan. Among the single nucleotide polymorphisms (SNPs) analyzed in the GWAS, 12 SNPs showed significant association with CBZ-induced cADRs, and rs1633021 showed the smallest P-value for association with CBZ-induced cADRs (P = 1.18 × 10⁻¹⁵). These SNPs were located within a 430 kb linkage disequilibrium block on chromosome 6p21.33, including the HLA-A locus. Thus, we genotyped the individual HLA-A alleles in 61 cases and 376 patients who showed no cADRs by administration of CBZ (CBZ-tolerant controls) and found that HLA-A*3101 was present in 60.7% (37/61) of the patients with CBZ-induced cADRs, but in only 12.5% (47/376) of the CBZ-tolerant controls (odds ratio = 10.8, 95% confidence interval 5.9–19.6, P = 3.64 × 10⁻⁶), implying that this allele has the 60.7% sensitivity and 87.5% specificity when we apply HLA-A*3101 as a risk predictor for CBZ-induced cADRs. Although DIHS is clinically distinguished from SJS and TEN, our data presented here have indicated that they share a common genetic factor as well as a common pathophysiological mechanism. Our findings should provide useful information for making a decision of individualized medication of anticonvulsants.

Ozeki, et al., Human Molecular Genetics, 20, 1034 (2011)
DRUG REACTION WITH EOSINOPHILIA AND SYSTEMIC SYMPTOMS (DRESS)

- Skin pathology, mild to severe
- Fever
- Liver, kidney, lung, heart, and CNS
- Lymphocytosis, eosinophilia, and lymphadenopathy
- Human herpesvirus 6 reactivation
- Carbamazepine, phenytoin, phenobarbital, zonisamide, lamotrigine, sulfalazine, and allopurinol
- 10% fatality

Cacoub, et al., The American Journal of Medicine, 124, 588 (2011)
Tohyama and Hashimoto, J. of Dermatology., 38, 222 (2011)
DRUG-INDUCED LIVER DISEASE
DRUG-INDUCED LIVER INJURY REMAINS A MAJOR HEALTH PROBLEM

• It can cause acute liver failure.

• Halt preclinical development of drugs.

• Stop clinical trials, lead to postmarketing withdrawals, and Black Box Warnings on package inserts of drugs.
THE DILEMMA

• It is impossible to predict accurately which new drugs will cause liver injury and which patients will be susceptible to this disease.

• This is due in large part to the idiosyncratic nature of most cases of DILI and the lack of animals where the mechanisms can be defined and potential risk factors can be identified.
Preclinical Strategy to Reduce Clinical Hepatotoxicity Using In Vitro Bioactivation Data for >200 Compounds

Melanie Z. Solcia,1,2 Melinda J. Rese,2 Andrew W. Harrell,1 Maxine A. Taylor,1 Ian A. Baines,1
Lianghe Chen,2 Jackie C. Bloomer,2 Eric Y. Yang,2 Hannah M. Ellman,2 Jeffrey L. Ambrose,2
Cerys A. Lovatt,1 Andrew D. Ayton,1 and Stephen E. Clarke1

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ABSTRACT: Drug-induced liver injury is the most common cause of market withdrawal of pharmaceuticals, and thus, there is considerable need for better prediction models for DILI early in drug discovery. We present a study involving 223 marketed drugs (51% associated with clinical hepatotoxicity, 49% non-hepatotoxic) to assess the concurrence of in vitro bioactivation data with clinical hepatotoxicity and to use these data to develop a decision tree to help reduce late-stage candidate attrition. Data to assess Phase I metabolism-dependent inhibition (MDI) for all common drug-metabolizing P450 enzymes were generated for 196 compounds. MDI data for 200 compounds, relevant binding data obtained for 53 compounds, and clinical data obtained for all compounds. Individual data for all 223 compounds were presented here and interpreted to determine what level of an alert to consider termination of a compound. The analysis showed that 74% of drugs with a daily dose <100 mg were non-hepatotoxic (p<0.0001). Drugs with a daily dose of ≥100 mg or with OATP1B1/2B1 uptake, marked P450 MDI, or relevant binding ≥200 gmoL/g were more likely to be hepatotoxic (55% vs. 54% in each case). Combining these data with other parameters increased the accuracy significantly (80–100%; p<0.0001). These analyses were then used to develop the decision tree, and the tree tested using 16% of the compounds with sufficient data (49% hepatotoxic; 51% non-hepatotoxic). This model demonstrated high accuracy using the tree to predict hepatotoxicity of the compounds evaluated. The tree was recommended for inclusion in the preclinical stage. An independent set of 16 GST compounds with known clinical hepatotoxicity status were also assessed using the tree, with similar results.
ACETAMINOPHEN LIVER INJURY

- Glucuronide
- Sulfate
- NAPQI
- S-protein
- Mercapturic Acid
- Cell Death
SIGNALING PATHWAY IN AILI

OTHER DRUGS MAY CAUSE LIVER INJURY BY DAMAGING THE MITOCHONDRIA

- Troglitazone
- Diclofenac
- Nimesulide
- Mefenamic acid
- Tolcapone
- Valproic acid
- Leflunomide
- Amiodarone
- Trovafloxacin

- Simvastatin
- Perhexiline
- Isoniazid
- Dantrolene
- Sulindac
- Lamivudine
- Stavudine
- Fialuridine

Genetic Variations in \textit{TXNRD1} as Potential Predictors of Drug-Induced Liver Injury

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\^1Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Korea
\^2Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center, Seoul, Korea
\^3DNA Link Inc., Seoul, Korea
\^4Department of Internal Medicine, Hanyang University College of Medicine, Seoul, Korea
\^5Department of Internal Medicine, Ewha University College of Medicine, Seoul, Korea
\^6Department of Internal Medicine, Dankook University College of Medicine, Cheonan, Korea
\^7Department of Life Science, Pohang University of Science and Technology, Pohang, Korea
\^8Department of Allergy and Clinical Immunology, Ajou University School of Medicine, Suwon, Korea

\textbf{Purpose:} Drug-induced liver injury (DILI) is the most common adverse drug reaction; however, it is not easily predicted. We hypothesize that DILI has a common genetic basis. Based on the findings of previous animal studies on toxic hepatitis, we selected the thioredoxin reductase 1 gene (\textit{TXNRD1}) as a candidate marker of DILI for this genetic association study. \textbf{Methods:} Records from 118 patients with DILI were extracted from the database of the Adverse Drug Reaction Research Group in South Korea. Causative drugs included antibiotics drugs (n=68, 57.8%), antibiotics (n=22, 18.6%), antiepileptic drugs (n=7, 5.9%), non-steroidal anti-inflammatory drugs (n=6, 4.2%), and others (n=16, 13.7%). Seven single nucleotide polymorphisms (SNPs) in \textit{TXNRD1} (rs1073593, rs4984287, rs495619, rs10861201, rs11111997, rs4246270, and rs4246271) were scored in 118 DILI patients and in 128 drug-matched controls without liver injury. \textbf{Results:} No differences were found between the frequencies of any of the 7 SNPs in the cases and controls; however, a significant association was found between a TTA haplotype composed of rs1073593, rs4984287, and rs495619 and DILI using an allele model (odds ratio, 1.79; 95% confidence interval, 1.18-2.73; P=0.008; Bonferroni corrected P=0.024). \textbf{Conclusions:} These results suggest that genetic variations in \textit{TXNRD1} favor the development of DILI, although a larger confirmatory study is needed.

\textbf{Key Words:} Drug-induced liver injury; genetic association study; genetic polymorphism; single nucleotide polymorphisms; thioredoxin reductase 1
Mitochondrial Superoxide Dismutase and Glutathione Peroxidase in Idiosyncratic Drug-Induced Liver Injury

M. Isabel Lucena,1,11* Elena García-Martín,2,12 Raúl J. Andrade,3,11 Carmen Martínez,4,12 Camilla Stephens,1,11 Jhon D. Ruiz,4,12 Eugenia Uzurrutia,1,11 M. Carmen Fernandez,5 Manuel Romero-Gomez,5,11 Augustin Castiella,7 Ramon Planas,8,11 José Antonio Durán,9 Ana Melcón De Dios,9 Carlos Guarnier,10,11 German Soriano,10,11 Yolanda Borraz,1,11 and José A. G. Agunce4,12*

Drug-induced liver injury (DILI) susceptibility has a potential genetic basis. We have evaluated possible associations between the risk of developing DILI and common genetic variants of the manganese superoxide dismutase (SOD2 Val16Ala) and glutathione peroxidase (GPX1 Pro200Leu) genes, which are involved in mitochondrial oxidative stress management. Genomic DNA from 185 DILI patients assessed by the Council for International Organizations of Medical Science scale and 270 sex- and age-matched controls were analyzed. The SOD2 and GPX1 genotyping was performed using polymerase chain reaction restriction fragment length polymorphism and TaqMan probed quantitative polymerase chain reaction, respectively. The statistical power to detect the effect of variant alleles with the observed odds ratio (OR) was 98.2% and 99.7% for bilateral association of SOD2 and GPX1, respectively. The SOD2 Ala/Ala genotype was associated with cholestatic/mixed damage (OR = 2.3; 95% confidence interval [CI] = 1.4-3.8; corrected P [Pc] = 0.0058), whereas the GPX1 Leu/Leu genotype was associated with cholestatic injury (OR = 5.1; 95%CI = 1.6-16.0; P = 0.0112). The presence of two or more combined risk alleles (SOD2 Ala and GPX1 Leu) was more frequent in DILI patients (OR = 2.1; 95%CI = 1.4-3.0; P = 0.0006). Patients with cholestatic/mixed injury induced by mitochondria hazardous drugs were more prone to have the SOD2 Ala/Ala genotype (OR = 3.6; 95%CI = 1.4-9.3; P = 0.02). This genotype was also more frequent in cholestatic/mixed DILI induced by pharmaceuticals producing quinone-like or epoxide metabolites (OR = 3.0; 95%CI = 1.7-5.5; P = 0.0008) and S-oxides, dioxines, nitrosoamine radicals, or iminium ions (OR = 16.0; 95%CI = 1.8-146.1; P = 0.009). Conclusion: Patients homozygous for the SOD2 Ala allele and the GPX1 Leu allele are at higher risk of developing cholestatic DILI. SOD2 Ala homozygotes may be more prone to suffer DILI from drugs that are mitochondria hazardous or produce reactive intermediates. (Hepatology 2010;52:303-312)
CLINICAL FINDINGS SUGGEST THAT THE INNATE AND ADAPTIVE IMMUNE SYSTEMS HAVE A ROLE IN DILI

- Fever and skin rash often associated with DILI.
- Hepatic lesions often contain neutrophils, eosinophils and/or lymphocytes.
- Onset is often more rapid on re-exposure.
- Specific HLA associations has been found.
- Drug-protein adducts, drug metabolites and parent drugs have been found to activate T cells in the blood.
- Serum antibodies have been shown to react with drug-protein adducts, and unaltered carrier proteins of reactive metabolites.
DAMPS RELEASED FROM INJURED CELLS CAN ACTIVATE INNATE IMMUNE CELLS

<table>
<thead>
<tr>
<th>MOLECULE</th>
<th>RECEPTOR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATP</td>
<td>P2X7</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>Unknown</td>
</tr>
<tr>
<td>Defensins</td>
<td>CCR6, TLR4</td>
</tr>
<tr>
<td>Galectins</td>
<td>CD2</td>
</tr>
<tr>
<td>HMGB1</td>
<td>TLR4, RAGE</td>
</tr>
<tr>
<td>Heat Shock Proteins</td>
<td>TLR4, CD14, CD91</td>
</tr>
<tr>
<td>Hyaluronic Acid</td>
<td>TLR2, TLR4</td>
</tr>
<tr>
<td>Mitochondrial DNA</td>
<td>TLR9, NLRP3</td>
</tr>
<tr>
<td>Nuclear DNA</td>
<td>TLR9</td>
</tr>
<tr>
<td>S100 Proteins</td>
<td>RAGE</td>
</tr>
<tr>
<td>Thioredoxin</td>
<td>Many Proteins</td>
</tr>
<tr>
<td>Uric Acid Crystal</td>
<td>NLRP3</td>
</tr>
</tbody>
</table>

*Kubes and Mehal, Gastroenterology 2012*
PAMPS CAN ALSO ACTIVATE CELLS OF THE INNATE IMMUNE SYSTEM

- Bacterial tri- and diacylated lipopeptides can activate TLR1/2 and TLR2/6, respectively
- Bacterial LPS can activate TLR4
- Bacterial flagellin can activate TLR5
- Bacterial unmethylated CpG DNA can activate TLR9
- Viral dsRNA can activate TLR 3
- Viral ssRNA can activate TLR7 and 8

Seki and Brenner, Hepatology, 48, 322 (2008)
INNATE IMMUNE CELLS AND WHAT THEY HAVE BEEN SHOWN TO DO IN DILI MODEL STUDIES

Cells: Dendritic cells, neutrophils, NK and NKT cells, macrophages, Kupffer cells, and even hepatocytes

Protoxicant Factors: ROS and RNS, IFN-γ, IL-1β, IL-17, IL-18, osteopontin, MIF, IL-6, and chemokines

Protective Factors: IL-4, IL-6, IL-10, IL-13, COX-2, and Nrf2

The impact of eosinophilia and hepatic necrosis on prognosis in patients with drug-induced liver injury

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Accepted 29 March 2007

SUMMARY

Background
Drug-induced liver injury may be immunologically mediated or metabolically induced. Peripheral eosinophilia and liver eosinophilia in suspected drug-induced liver injury generally supports the role of drug aetiology.

Aim
To assess the importance of eosinophilia and hepatic necrosis on outcome in patients with suspected drug-induced liver injury.

Methods
We performed search of MEDLINE for case reports on drug-induced liver injury associated with: amoxicillin/clavulanic acid, carbamazepine, diclofenac, disulfiram, erythromycin, flucloxacinil, halothane, isoniazid, phenytoin, sulindac and trimethoprim/sulfametoxazol.

HALOTHANE-INDUCED LIVER INJURY

$ : B; F: C8

#A8B\#<A: =8; >B1 F8 A8B8

$ ?@<A B: CD'1 88P B A.@@?C8'2 8E9<CE8E

#<I @C>
HALOTHANE CAUSES PERIVENOUS LIVER INJURY IN FEMALE BALB/CJ MICE 24 HOURS AFTER HALOTHANE

A

![Graph showing changes in ALT levels over time](image)

B

![Histological images](image)
FLOW CYTOMETRY REVEALS THAT EOSINOPHILS IN ADDITION TO NEUTROPHILS INFILTRATE THE LIVER AFTER HALOTHANE
HISTOLOGY OF EOSINOPHILS AND NEUTROPHILS PURIFIED BY CELL SORTING 24 HOURS AFTER HALOTHANE
IMMUNOHISTOCHEMICAL STAINING OF EOSINOPHIL MAJOR BASIC PROTEIN IN PERIVENOUS REGIONS OF LIVER INJURY

A

<table>
<thead>
<tr>
<th>Time</th>
<th>H&amp;E</th>
<th>MBP</th>
</tr>
</thead>
<tbody>
<tr>
<td>12h HAL</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td>24h HAL</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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</tbody>
</table>
CLINICAL FEATURES SUGGEST THAT THE INNATE AND ADAPTIVE IMMUNE SYSTEMS HAVE A ROLE IN DILI

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- Serum antibodies have been shown to react with drug-protein adducts, and unaltered carrier proteins of reactive metabolites.
# HLA ASSOCIATIONS IN DILI

Table 1. HLA associations in DILI detected by genotyping.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Allele</th>
<th>Drug</th>
<th>Type of study</th>
<th>Ethnicity of subjects</th>
<th>Replication</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A</td>
<td>*3303</td>
<td>Ticlopidine</td>
<td>Candidate gene</td>
<td>Japanese</td>
<td>No</td>
<td>(Hirata et al., 2008)</td>
</tr>
<tr>
<td>HLA-B</td>
<td>*5701</td>
<td>Flucloxacillin</td>
<td>GWAS</td>
<td>European (UK)</td>
<td>Yes</td>
<td>(Daly et al., 2009)</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>*1501</td>
<td>Amoxicillin-clavulanate</td>
<td>Candidate gene and GWAS</td>
<td>European (UK, U.S. and Spanish)</td>
<td>Yes</td>
<td>(Hautekeete et al., 1999; O’Donchue et al., 2000; Donaldson et al., 2010; Lucena et al., 2011)</td>
</tr>
<tr>
<td>HLA-A</td>
<td>*0201</td>
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<td>GWAS</td>
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<td>HLA-B</td>
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<tr>
<td>HLA-DRB1</td>
<td>*1501</td>
<td>Various</td>
<td>Candidate gene</td>
<td>European (Spanish)</td>
<td>No</td>
<td>(Andrade et al., 2004)</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>*1501</td>
<td>Lumiracoxib</td>
<td>GWAS</td>
<td>Various</td>
<td>Yes</td>
<td>(Singher et al., 2010)</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>*0701</td>
<td>Ximelagatran</td>
<td>GWAS/candidate gene</td>
<td>Various</td>
<td>No</td>
<td>(Kindmark et al., 2008)</td>
</tr>
<tr>
<td>HLA-DRB1</td>
<td>*01</td>
<td>Nevirapine</td>
<td>Candidate gene</td>
<td>European</td>
<td>No</td>
<td>(Martin et al., 2005; Yuan et al., 2011)</td>
</tr>
<tr>
<td>HLA-DQA1</td>
<td>*0201</td>
<td>Lapatinib</td>
<td>Candidate gene</td>
<td>Various</td>
<td>Yes</td>
<td>(Spraggs et al., 2011)</td>
</tr>
<tr>
<td>HLA-DQB1</td>
<td>*0201</td>
<td>Isoniazid</td>
<td>Candidate gene</td>
<td>Indian</td>
<td>No</td>
<td>(Sharma et al., 2002)</td>
</tr>
</tbody>
</table>

REGULATORY T CELLS HAVE A ROLE IN TOLERANCE

Alpdogan and van den Brink, Semin. Oncol, 39, 629 (2012)
T CELL INHIBITORY MOLECULES CTLA-4 AND PD-1 HAVE ROLES IN TOLERANCE

Figure 2. T cell–antigen-presenting cell (APC) interaction with costimulatory molecules. CTLA-4, cytotoxic T-lymphocyte antigen 4; TCR, T-cell receptor; MHC, major histocompatibility complex; ICOS, inducible T-cell costimulator; PD-1, programmed death-1; PD1-L, programmed death-1 ligand.

Alpdogan and van den Brink, Semin. Oncol, 39, 629 (2012)
CARDIAC DISEASE CAUSED BY DRUG-INDUCED LONG QT SYNDROME
ECG tracing of a normal heart rhythm.

In atrial fibrillation, the tracing shows tiny, irregular "fibrillation" waves between heartbeats. The rhythm is irregular and erratic.
DRUG-INDUCED-LONG QT SYNDROME AND TORSADES DE POINTES

Table 2
Examples of drugs with QT interval prolonging potential (Haverkamp et al., 2000; www.Torsades.org, 2004; Tristani-Firouzi et al., 2001; Recanatini et al., 2005)

<table>
<thead>
<tr>
<th>Category</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antiarrhythmics</td>
<td></td>
</tr>
<tr>
<td>class IA</td>
<td>disopyramide, procainamide, quinidine</td>
</tr>
<tr>
<td>class III</td>
<td>amiodarone, doxetilide, ibutilide, sotalol</td>
</tr>
<tr>
<td>Antipsychotics</td>
<td>chlorpromazine, droperidol, haloperidol, pimozide, sertindole, thioridazine</td>
</tr>
<tr>
<td>Antidepressants</td>
<td>amitriptyline, clomipramine, desipramine, doxepin, imipramine, nortriptyline</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>fluoroquinolones (gatifloxacin, grepafloxacin, moxifloxacin, sparflaxacin)</td>
</tr>
<tr>
<td></td>
<td>macrolide antibiotics (clarithromycin, erythromycin)</td>
</tr>
<tr>
<td></td>
<td>azole antifungal agents (fluconazole, ketoconazole)</td>
</tr>
<tr>
<td></td>
<td>antimalarials (chloroquine, halofantrine, mefloquine, quinine)</td>
</tr>
<tr>
<td>Antihistamines</td>
<td>astemizole, terfenadine</td>
</tr>
<tr>
<td>GI stimulants</td>
<td>cisapride, domperidone</td>
</tr>
<tr>
<td>Opioid agonists</td>
<td>levacetylmethadol, methadone</td>
</tr>
<tr>
<td>Antianginals</td>
<td>bepridil, lidoflazine, prenylamine</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>arsenic trioxide, bupidine, terodiline</td>
</tr>
</tbody>
</table>

* The list is not intended as exhaustive.

RISK FACTORS FOR DRUG-INDUCED TORSADES DE POINTES

- Hypokalemia and hypomagnesemia
- Bradycardia
- Cardiac hypertrophy and congestive heart failure
- High drug serum concentrations
- Female
- Polymorphisms

A Large Candidate Gene Survey Identifies the KCNE1 D85N Polymorphism as a Possible Modulator of Drug-Induced Torsades de Pointes

Stefan Kääb, MD, PhD*; Dana C. Crawford, PhD*; Moritz F. Sinner, MD*; Elijah R. Behr, MD*; Prince J. Kannankeril, MD; Arthur A.M. Wilde, MD, PhD; Connie R. Bezzina, PhD; Eric Schulze-Bahr, MD; Pascale Guicheney, PhD; Nanette H. Bishopric, MD; Robert J. Myerburg, MD; Jean-Jacques Schott, PhD; Arne Pfeufer, MD, MSc; Britt-Maria Beckmann, MD; Eimo Martens, MD; Taifang Zhang, PhD; Birgit Stallmeyer, PhD; Sven Zumhagen, MD; Isabelle Denjoy, MD; Abdennasser Bardai, MD; Isabelle C. Van Gelder, MD; Yalda Jamshidi, MD, PhD; Chrysoula Dalageorgou, BSc; Vanessa Marshall, MD; Steve Jeffery, PhD; Saad Shakir, FRCP; A. John Camm, MD; Gerhard Steinbeck, MD; Siegfried Perz, MSc; Peter Lichtner, PhD; Thomas Meitinger, MD, MSc; Annette Peters, MSc, PhD; H.-Erich Wichmann, MD, PhD; Christiana Ingram, BS; Yuki Bradford, MS; Shannon Carter, RN; Kris Norris, RN; Marylyn D. Ritchie, PhD; Alfred L. George, Jr, MD; Dan M. Roden, MD

Background—Drug-induced long-QT syndrome (diLQTS) is an adverse drug effect that has an important impact on drug use, development, and regulation. We tested the hypothesis that common variants in key genes controlling cardiac electric properties modify the risk of diLQTS.

Methods and Results—In a case-control setting, we included 176 patients of European descent from North America and Europe with diLQTS, defined as documented torsades de pointes during treatment with a QT-prolonging drug. Control samples were obtained from 207 patients of European ancestry who displayed <50 ms QT lengthening during initiation of therapy with a QT-prolonging drug and 837 control subjects from the population-based KORA study. Subjects were successfully genotyped at 1424 single-nucleotide polymorphisms (SNPs) in 18 candidate genes including 1386 SNPs tagging common haplotype blocks and 38 nonsynonymous ion channel gene SNPs. For validation, we used a set of cases (n=57) and population-based control subjects of European descent. The SNP KCNE1 D85N (rs1805128), known to modulate an important potassium current in the heart, predicted diLQTS with an odds ratio of 9.0 (95% confidence interval, 3.5–22.9). The variant allele was present in 8.6% of cases, 2.9% of drug-exposed control subjects, and 1.8% of population control subjects. In the validation cohort, the variant allele was present in 3.5% of cases and in 1.4% of control subjects.

Conclusions—This high-density candidate SNP approach identified a key potassium channel susceptibility allele that may be associated with the rare adverse drug reaction torsades de pointes. (Circ Cardiovasc Genet. 2012;5:91-99.)
RESEARCH ARTICLE

HEART ARRHYTHMIA

Suppression of Phosphoinositide 3-Kinase Signaling and Alteration of Multiple Ion Currents in Drug-Induced Long QT Syndrome

Zhongju Lu,1 Chia-Yen C. Wu,1 Ya-Ping Jiang,1 Lisa M. Ballou,1 Chris Clausen,1 Ira S. Cohen,1,* Richard Z. Lin1,2,*

Many drugs, including some commonly used medications, can cause abnormal heart rhythms and sudden death, as manifest by a prolonged QT interval in the electrocardiogram. Cardiac arrhythmias caused by drug-induced long QT syndrome are thought to result mainly from reductions in the delayed rectifier potassium ion (K') current $I_{Kr}$. Here, we report a mechanism for drug-induced QT prolongation that involves changes in multiple ion currents caused by a decrease in phosphoinositide 3-kinase (PI3K) signaling. Treatment of canine cardiac myocytes with inhibitors of tyrosine kinases or PI3Ks caused an increase in action potential duration that was reversed by intracellular infusion of phosphatidylinositol 3,4,5-trisphosphate. The inhibitors decreased the delayed rectifier K' currents $I_{Kd}$ and $I_{Ks}$, the L-type calcium ion (Ca') current $I_{Ca,L}$, and the peak sodium ion (Na') current $I_{Na}$ and increased the persistent Na' current $I_{NaP}$. Computer modeling of the canine ventricular action potential showed that the drug-induced change in any one current accounted for less than 50% of the increase in action potential duration. Mouse hearts lacking the PI3K p110α catalytic subunit exhibited a prolonged action potential and QT interval that were at least partly a result of an increase in $I_{NaP}$. These results indicate that down-regulation of PI3K signaling directly or indirectly via tyrosine kinase inhibition prolongs the QT interval by affecting multiple ion channels. This mechanism may explain why some tyrosine kinase inhibitors in clinical use are associated with increased risk of life-threatening arrhythmias.

Science Translational Medicine, 4, (2012)
ORIGINALL ARTICLE

Novel rare variants in congenital cardiac arrhythmia genes are frequent in drug-induced torsades de pointes

AH Ramirez¹, CM Shaffer², JT Delaney¹, DP Sexton², SE Levy³,⁴, MJ Rieder⁵, DA Nickerson⁵, AL George Jr⁴,⁶ and DM Roden¹,⁶

Marked prolongation of the QT interval and polymorphic ventricular tachycardia following medication (drug-induced long QT syndrome, diLQTS) is a severe adverse drug reaction (ADR) that phenocopies congenital long QT syndrome (cLQTS) and is one of the leading causes for drug withdrawal and re-labeling. We evaluated the frequency of rare non-synonymous variants in genes contributing to the maintenance of heart rhythm in cases of diLQTS using targeted capture coupled to next-generation sequencing. Eleven of 31 diLQTS subjects (36%) carried a novel missense mutation in genes with known congenital arrhythmia associations or with a known cLQTS mutation. In the 26 Caucasian subjects, 23% carried a highly conserved rare variant predicted to be deleterious to protein function in these genes compared with only 2–4% in public databases (P<0.003). We conclude that the rare variation in genes responsible for congenital arrhythmia syndromes is frequent in diLQTS. Our findings demonstrate that diLQTS is a pharmacogenomic syndrome predisposed by rare genetic variants.

The Pharmacogenomics Journal advance online publication, 15 May 2012; doi:10.1038/tpj.2012.14

Keywords: adverse drug reaction; next-generation sequencing; sudden cardiac death
MODELS FOR STUDYING PATHOLOGY AND DRUG SENSITIVITY TO TORSADES

• *In vitro* assays with cells expressing the K ion channel have been used.
• Cardiomyocytes derived from human embryonic and induced pluripotent stem cells have also been used.
• The best cardiomyocytes for these tests would be from diseased individuals.

*Braam, et al., Stem Cell Research 4, 107 (2010)*
Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture

Anna L. Lahti¹,²*, Ville J. Kujala¹,²*, Hugh Chapman³, Ari-Pekka Koivisto⁴, Mari Pekkanen-Mattila¹,², Erja Kerkelä¹,⁴, Jari Hyttinen²,⁵, Kimmo Kontula⁶, Heikki Swan⁷, Bruce R. Conklin⁸, Shinya Yamanaka⁹,¹⁰, Olli Silvennoinen¹,²,¹¹ and Katriina Aalto-Setälä¹,²,¹¹

SUMMARY

Long QT syndrome (LQTS) is caused by functional alterations in cardiac ion channels and is associated with prolonged cardiac repolarization time and increased risk of ventricular arrhythmias. Inherited type 2 LQTS (LQT2) and drug-induced LQTS both result from altered function of the hERG channel. We investigated whether the electrophysiological characteristics of LQT2 can be recapitulated in vitro using induced pluripotent stem cell (iPSC) technology. Spontaneously beating cardiomyocytes were differentiated from two iPSC lines derived from an individual with LQT2 carrying the R176W mutation in the KCNH2 (hERG) gene. The individual had been asymptomatic except for occasional palpitations, but his sister and father had died suddenly at an early age. Electrophysiological properties of LQT2-specific cardiomyocytes were studied using microelectrode array and patch-clamp, and were compared with those of cardiomyocytes derived from control cells. The action potential duration of LQT2-specific cardiomyocytes was significantly longer than that of control cardiomyocytes, and the rapid delayed potassium channel (I_{K1}) density of the LQT2 cardiomyocytes was significantly reduced. Additionally, LQT2-derived cardiac cells were more sensitive than controls to potentially arrhythmogenic drugs, including sotalol, and demonstrated arrhythmogenic electrical activity. Consistent with clinical observations, the LQT2 cardiomyocytes demonstrated a more pronounced inverse correlation between the beating rate and repolarization time compared with control cells. Prolonged action potential is present in LQT2-specific cardiomyocytes derived from a mutation carrier and arrhythmias can be triggered by a commonly used drug. Thus, the iPSC-derived, disease-specific cardiomyocytes could serve as an important platform to study pathophysiological mechanisms and drug sensitivity in LQT2.
Summary

• Drug-drug interactions are the major cause of ADRs, but are often predictable and avoidable.
• Most SADRs are rare, highly host-dependent, difficult to predict, and likely involve multiple genetic and environmental factors and the innate and adaptive immune systems.
• It is anticipated that future animal model studies will be based on whole genome sequencing and whole proteome and metabolome analyses and will lead to the discovery of useful biomarkers, susceptibility factors, and safer drugs.
• Currently, the best way to avoid SADRS is to design new drugs that are not metabolized to reactive metabolites and are very potent.