Phenotyping in Studies of Epilepsy Genetics

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May 2010
Broad research goals

- Understand the genetic influences on common forms of human epilepsy
  - Epilepsy susceptibility genes
- Understand the genetic influences on anti-epileptic drug (AED) response
  - Pharmacogenomics
Road Map

- Epilepsy classification overview
- Phenotyping
  - Carbamazepine (CBZ) and Stevens-Johnson Syndrome (SJS)
  - Childhood absence epilepsy (CAE)
  - Non-acquired epilepsy with affected sibs
  - Non-acquired epilepsy
Epilepsy

- Recurrent unprovoked seizures
- So diverse, the word “epilepsy” means nothing
FIG. 1. Schematic diagram of the International Classification of Epilepsies and Epileptic Syndromes.
Neurobiological spectrum of the epilepsies

Genetic

Channelopathies
expression of ion channels carrying mutation

Polygenic inheritance

Single gene

Acquired cause trauma, hypoxia, vascular etc.

Structural/Metabolic
### Anti-epileptic drug (AED) options

<table>
<thead>
<tr>
<th>Focal epilepsy (with or without 2\textsuperscript{nd} gen)</th>
<th>Focal and generalized epilepsy</th>
<th>Syndrome-specific use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbamazepine</td>
<td>Clobazam</td>
<td>Ethosuximide – absence seizures</td>
</tr>
<tr>
<td>Gabapentin</td>
<td>Lamotrigine</td>
<td>Adrenocorticotropic hormone – infantile spasms</td>
</tr>
<tr>
<td>Oxcarbazepine</td>
<td>Levetiracetam</td>
<td>Vigabatrin – infantile spasms</td>
</tr>
<tr>
<td>Phenobarbital</td>
<td>Rufinamide</td>
<td>Prednisone – infantile spasms</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>Topiramate</td>
<td></td>
</tr>
<tr>
<td>Pregabalin</td>
<td>Valproate</td>
<td></td>
</tr>
<tr>
<td>Lacosamide</td>
<td>Zonisamide</td>
<td></td>
</tr>
<tr>
<td>Lacosamide</td>
<td>Felbamate</td>
<td></td>
</tr>
</tbody>
</table>
Stevens-Johnson Syndrome (SJS)
- Severe hypersensitivity reaction to a drug
- Idiosyncratic
  - Not dose-related
  - It just happens

Carbamazepine (CBZ)
- Widely prescribed for focal epilepsy, pain, bipolar disorder, mood stabilization
Central Bile Aciddosis and SJS

- Taiwan - Han Chinese
  - 44 patients with CBZ-induced SJS
  - 101 patients taking CBZ without adverse reaction
  - 93 normal subjects

Chung et al, Nature 2004;428:486
Han Chinese

- 19% of world’s population
  - 92% of People’s Republic of China
  - 98% of Taiwan
  - 95% of Hong Kong
  - 75% of Singapore
  - Large segments of Thailand, Indonesia, Vietnam, Malaysia, and Philippines
China: Ethnolinguistic Groups
Human Leukocyte Antigen (HLA)
### Table 1 Frequency of HLA alleles in patients with Stevens–Johnson syndrome

<table>
<thead>
<tr>
<th>HLA allele</th>
<th>CBZ–SJS</th>
<th>CBZ-tolerant</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>B*1502</td>
<td>44 (100%)</td>
<td>3 (3%)*</td>
<td>8 (8.6%)†</td>
</tr>
<tr>
<td>Cw*0801</td>
<td>41 (93.2%)</td>
<td>17 (16.8%)</td>
<td>13 (14%)</td>
</tr>
<tr>
<td>A*1101</td>
<td>36 (81.8%)</td>
<td>51 (50.5%)</td>
<td>53 (57%)</td>
</tr>
<tr>
<td>DRB1*1202</td>
<td>33 (75%)</td>
<td>12 (11.9%)</td>
<td>18 (19.4%)</td>
</tr>
<tr>
<td>B<em>1502, Cw</em>0801</td>
<td>41 (93.2%)</td>
<td>3 (3%)</td>
<td>7 (7.5%)</td>
</tr>
<tr>
<td>B<em>1502, A</em>1101</td>
<td>36 (81.8%)</td>
<td>2 (2%)</td>
<td>6 (6.5%)</td>
</tr>
<tr>
<td>B<em>1502, DRB1</em>1202</td>
<td>33 (75%)</td>
<td>1 (1%)</td>
<td>5 (5.4%)</td>
</tr>
<tr>
<td>B<em>1502, Cw</em>0801, A<em>1101, DRB1</em>1202</td>
<td>29 (66%)</td>
<td>0 (0%)</td>
<td>3 (3.2%)</td>
</tr>
</tbody>
</table>

Frequencies (by number and percentage) of individual or combined loci of the B*1502 ancestral haplotype are shown in patients with carbamazepine-induced Stevens–Johnson syndrome (CBZ–SJS; n = 44), and in carbamazepine-tolerant (n = 101) and normal subjects (n = 93). For methods, see supplementary information.

*Odds ratio (CBZ–SJS/CBZ-tolerant): 2.504 (95% CI, 1.26–49.522); corrected P value $P_c = 3.13 \times 10^{-27}$.

†Odds ratio (CBZ–SJS/normal): 895 (95% CI, 50–15,869); $P_c = 1.38 \times 10^{-23}$. 

Chung et al, Nature 2004;428:486
HLA-B*1502 allele
- Positive predictive value: 93.6% for CBZ-induced SJS
- Negative predictive value: 100%

- Sensitivity: 100%
- Specificity: 97%
Replication studies

- Hong Kong
- Europe

Man et al, Epilepsia 2007;48:1015-1018
Lonjou et al, Pharmacogenomics 2006;6:265-268
WARNINGS

SERIOUS DERMATOLOGIC REACTIONS AND HLA-B*1502 ALLELE

SERIOUS AND SOMETIMES FATAL DERMATOLOGIC REACTIONS, INCLUDING TOXIC EPIDERMAL NECROLYSIS (TEN) AND STEVENS-JOHNSON SYNDROME (SJS), HAVE BEEN REPORTED DURING TREATMENT WITH TEGRETOL. THESE REACTIONS ARE ESTIMATED TO OCCUR IN 1 TO 6 PER 10,000 NEW USERS IN COUNTRIES WITH MAINLY CAUCASIAN POPULATIONS, BUT THE RISK IN SOME ASIAN COUNTRIES IS ESTIMATED TO BE ABOUT 10 TIMES HIGHER. STUDIES IN PATIENTS OF CHINESE ANCESTRY HAVE FOUND A STRONG ASSOCIATION BETWEEN THE RISK OF DEVELOPING SJS/TEN AND THE PRESENCE OF HLA-B*1502, AN INHERITED ALLELIC VARIANT OF THE HLA-B GENE. HLA-B*1502 IS FOUND ALMOST EXCLUSIVELY IN PATIENTS WITH ANCESTRY ACROSS BROAD AREAS OF ASIA.

PATIENTS WITH ANCESTRY IN GENETICALLY AT-RISK POPULATIONS SHOULD BE SCREENED FOR THE PRESENCE OF HLA-B*1502 PRIOR TO INITIATING TREATMENT WITH TEGRETOL. PATIENTS TESTING POSITIVE FOR THE ALLELE SHOULD NOT BE TREATED WITH TEGRETOL UNLESS THE BENEFIT CLEARLY OUTWEIGHS THE RISK (SEE WARNINGS AND PRECAUTIONS, LABORATORY TESTS).
Diversity of HLA-B*1502

- Prevalance of HLA-B*1502 allele
  - 15% or more in Hong Kong, Thailand, Mayalasia, Philippines
  - 10% in Taiwan
  - 4% in North China
  - 2-4% in India
  - < 1% in Japan and Korea
  - Caucasians, African Americans, Hispanics and Native Americans
    - “largely absent”
    - Possibly 1-2%
Lessons

- A clear phenotype matters
  - SJS
- Ethnic background and geographical location can be critical
- Some genetic associations can be found with relatively small numbers of patients
Phenotype # 2 - CAE

- Childhood absence epilepsy (CAE)
Why Childhood Absence Epilepsy (CAE)?

- Common
  - 10-17% of childhood onset epilepsy
- Homogeneous
  - More or less
- Variable drug response
  - Ethosuximide, Valproate, Lamotrigine
- EEG allows for clear diagnosis and outcome assessment
- “Wolf in sheep’s clothing”
24
Lamotrigine
Ethosuximide
Valproic Acid

Eligibility Assessment
Randomization

Double Blind Phase

Lamotrigine
Ethosuximide
Valproic Acid

Rx Success
Continue Double Blind Lamotrigine

Rx Failure*

Ethosuximide
Valproic Acid
Lamotrigine

Open Label Phase

Randomization (Rx Failure Only)

Ethosuximide
Valproic Acid
Lamotrigine

4 Month Evaluation

N= 446 (32 sites)

T. Glauser, P. Adamson, A. Cnaan (PIs)
Primary hypothesis

- A single AED will be at least 20% more **effective** than either of the other two AEDs

- Effectiveness
  - Efficacy
  - Tolerability
Ethosuximide, Valproic Acid, and Lamotrigine in Childhood Absence Epilepsy

Tracy A. Glauser, M.D., Avital Cnaan, Ph.D., Shlomo Shinnar, M.D., Ph.D., Deborah G. Hirtz, M.D., Dennis Dlugos, M.D., David Masur, Ph.D., Peggy O. Clark, M.S.N., Edmund V. Capparelli, Pharm.D., and Peter C. Adamson, M.D., for the Childhood Absence Epilepsy Study Group*
There were no significant differences among the three drugs with regard to discontinuation because of adverse events. Attentional dysfunction was more common with valproic acid than with ethosuximide (in 49% of the children vs. 33%; odds ratio, 1.95; 95% CI, 1.12 to 3.41; P = 0.03).

CONCLUSIONS
Ethosuximide and valproic acid are more effective than lamotrigine in the treatment of childhood absence epilepsy. Ethosuximide is associated with fewer adverse attentional effects. (ClinicalTrials.gov number, NCT00088452.)
Secondary objectives

- Pharmacogenetics
- Endophenotypic predictors of outcome
  - Pre-treatment seizure semiology
  - Pre-treatment EEG features
  - Neuropsychologic profile
Enrollment details

- 453 subjects enrolled and randomized
  - 6 determined to be ineligible soon after randomization
    - 3 of 6 ineligible by EEG criteria
  - 1 withdrew consent after randomization, but before treatment
- 446 subjects
Subjects: Age

Figure 4. Recruitment to DB by Age

![Bar chart showing recruitment by age groups](chart.png)
Subjects: Gender

- 57% female
- 43% male
Subjects: Race

- 72% White
- 22% Hispanic
- 19% Black or African-American
Baseline EEG

1 hour pre-treatment video-EEG
- Standardized protocol
- Hyperventilation (HV)
- Photic stimulation

Seizure
- Generalized spike and wave discharge lasting 3 seconds or longer
- With or without clinical signs
Baseline EEG – confirming eligibility

- **Time to 1\textsuperscript{st} seizure (n = 446)**
  - 7.1 minutes (mean)
  - 0-58.5 minutes (range)

- **Circumstances**
  - Pre-HV: 42%
  - 1\textsuperscript{st} HV trial: 50%
  - After 1\textsuperscript{st} HV trial: 8%
Pre-treatment: # seizures/hour

Number of seizures per hour (n = 263)

- Mean = 7.2
- Std. Dev = 5.90
- N = 263.00
Pre-treatment: Mean seizure duration

Mean seizure duration in seconds (n=263)

Std. Dev = 6.94
Mean = 13.5
N = 263.00
Pre-treatment: Seizure duration

- 40% of subjects had at least 1 seizure longer than 20 seconds
Pre-treatment: % seizure time per hour

% Seizure time per hour (n = 263)
CAE EEG phenotype conclusions to date

- 1 hour EEG allows for clear diagnosis and outcome assessment
- Cohort is homogeneous with some outliers
- An untreated CAE patient spends an average of 2.5% of a waking hour having seizures
- Prolonged seizures (> 20 sec) are common
FIG. 1. Schematic diagram of the International Classification of Epilepsies and Epileptic Syndromes.
# Genetic Association Studies in Common Epilepsy

<table>
<thead>
<tr>
<th>Positive</th>
<th>Positive + Replication</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>KCNJ10</strong></td>
<td><strong>KCNJ10</strong></td>
<td>IL-1B META is ++</td>
</tr>
<tr>
<td>IL-1B</td>
<td>IL-1B</td>
<td>ABCB1</td>
</tr>
<tr>
<td>ABCB1</td>
<td>ABCB1</td>
<td>MOR</td>
</tr>
<tr>
<td>MOR</td>
<td></td>
<td>CACNA1A</td>
</tr>
<tr>
<td>CACNA1A</td>
<td></td>
<td>GABAR2B3</td>
</tr>
<tr>
<td>GABAR2B3</td>
<td></td>
<td>CHRNA4</td>
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<td>BDNF, GABR2G2,</td>
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<td>GABAR2A5, GRIK1,</td>
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<tr>
<td>GABR2G2</td>
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<td>GLUR5, 7 &amp; 8, GLRA3,</td>
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<tr>
<td>Prodynorphin</td>
<td></td>
<td>GLRB, SCN2A, SCN2B,</td>
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<tr>
<td>Sz susceptibility</td>
<td></td>
<td>KCNK9, KCNJ6, KCNJ3</td>
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<tr>
<td>Pharmacoresistance</td>
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<td>KCNQ3, KCNN3, PAX6,</td>
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<td>CACNA1A4, SLC12A6,</td>
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<tr>
<td></td>
<td></td>
<td>MAO-A, 5HT2C, NCAM,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ATP1A2, HLA-DR13…</td>
</tr>
</tbody>
</table>
Phenotype # 3 – GE and FE

- Generalized epilepsy (GE)
- Focal epilepsy (FE)
  - Non-acquired
  - Developmentally normal (more or less)
  - MRI - normal

- Must have a full sibling with non-acquired epilepsy
FIG. 1. Schematic diagram of the International Classification of Epilepsies and Epileptic Syndromes.
Epilepsy Phenome Genome Project (EPGP)

- Phenotyping of:
  - 750 sibling pairs with GE
  - 750 sibling pairs with non-acquired focal epilepsy
  - 1500 unrelated healthy controls

www.epgp.org
Phenotype # 4 – GE and FE

- Generalized epilepsy (GE)
- Focal epilepsy (FE)
  - Non-acquired
  - Developmentally normal (more or less)
  - MRI - normal

- No affected sibling required
Phenotype # 4 – GE and FE

- Cohort collection as of 2010
- 1000 patients with epilepsy from 7 sites
  - 60% with GE
  - 40% with FE
- 3000 Controls
Genome Wide Association (GWA):

CHOP Center for Applied Genomics
Hakon Hakonarson, MD, PhD

- Scan 550,000 SNPs in each of 1000 epilepsy patients (600 GE, 400 FE) and 3000 controls
- Identify SNPs that are segregating dependent on phenotype
- Statistical issues…
- Hypothesis generating…confirming
GWA and Epilepsy Classification

- Analyze both GE and FE together
  - Epidemiology and animal models suggest an overlap between syndromes
- Analyze GE and FE separately
- Analyze GE subgroups
  - Including specific epilepsy syndromes
GWAS Summary

- Focal and Generalized combined
  - (n = 1000, 3000 cts; 2.2 billion data points)
- 19 SNPs reach genome wide significance $10^{-7}$, 40 SNPs reach $p = 10^{-6}$
- Top hit is ZF gene $p = 10^{-20}$
  - Too good to be true! Caution!
- Five linked markers on Chr 6 at $p = 10^{-7}$
- Top hits are in developmental genes
  - NOT ion channels, neurotransmission
  - Establishing networks and pathways!
GWAS Summary

- **Copy Number Variation**
  - Deletions/Duplications
    - More duplications in cases compared to controls
    - \( p = 0.0001 \)

- **REPLICATION**
  - Local cohort being gathered
  - Compare results with other GWA studies
    - Dr. Thomas Sander, Berlin
    - \( n=1500 \) GE, 2000 cts
Conclusions

- A clinically relevant advance has been made in epilepsy pharmacogenetics
  - CBZ-induced SJS and HLA-B*1502
- Phenotype can be narrow or broad, but must be clear
- Ethnic background may matter tremendously
- Multiple strategies are needed for future progress
  - Sib pairs and singletons
  - Specific epilepsy syndromes
  - Broader epilepsy types
- GWA is current strategy of choice
  - Replication is essential
  - Methodological challenges are not trivial
  - Full genome sequencing is coming fast
Thanks

◆ Russ Buono, Tom Ferraro, Hakon Hakonarson
◆ Tracy Glauser, Tuli Cnaan, Shlomo Shinnar, Eli Mizrahi, Nico Moshe
◆ Dan Lowenstein
◆ Samantha Hagopian, Yong Collins
◆ Calley Levine, Cate Bakey