Choosing the Gene & Polymorphism

1. Topic
   - Concussions

Concussions

- 1.6 - 3.8 million per year
  - (Langlois, 2006)
- Accounted for $16.5 billion in hospitals
  - (Thurman, 2001)
- Could lead to adverse effects later in life
  - (Grabowski et al., 2007; Mastroti, Barth, Litterfield, 1998; Tysvaer & Lochen)

Concussion Signs & Symptoms (S/S)

- Difficulty thinking clearly
- Feeling slowed down
- Difficulty concentrating
- Difficulty remembering new information
- Headache
- Nausea or vomiting (early on)
- Balance problems
- Dizziness
- Fuzzy or blurry vision
- Feeling tired, having no energy
- Sensitivity to noise or light
- Irritability
- Sadness
- More emotional
- Nervousness or anxiety
- Sleeping more than usual
- Sleeping less than usual
- Trouble falling asleep
Concussion Recovery

- Recovery rates
  - Rapid
    - Less than 7 days
  - Normal
    - 7 days to 10 days
  - Prolonged/Long
    - Still undefined
      - Over 10, 14, or 21
- Most athletes recover within 7 to 10 days
- 20% of athletes take longer to recover

Concussions Pathomechanics

- Force causes
  - acceleration/deceleration
- Neuron strain
- Alters cells environment

Intrinsic Factors

- Age
- Gender
- Migraine Hx
- ADHD Hx
- Dizziness
- GENETICS

Extrinsic factors

- Concussion Hx
- Magnitude of impact
- Location of injury
- Sport

Neurometabolic Cascade

- Force causes
  - acceleration/deceleration
- Neuron strain
- Alters cells environment

Choosing the Gene & Polymorphism

1. Topic
   - Concussions
2. Evidence there will be an observable phenotype
   - Recovery
3. What proteins are important for the phenotype
   - Neurometabolic Cascade
     - NMDA Channel (NR2A subunit)
Choosing the Gene & Polymorphism

1. Topic
   - Concussions
2. Evidence there will be a observable phenotype
   - Recovery
3. What proteins are important for the phenotype
   - Neurometabolic Cascade
     • NMDA Channel (NR2A subunit)
4. What gene codes for this protein
   - GRIN2A

N-methyl-D aspartate Channel

- NMDA contains glutamate receptors
  - NR1 and NR2A and NR2B
- Subunits produced via the GRIN1 and GRIN2A and GRIN2B genes
- When activated allows Ca^{2+} into the cell

Genes

- Basic physical & functional unit of heredity
  (Gelehrter, Collins, Ginsburg, 1998)
- 4 Regions of a gene
  - Exon
  - Intron
  - Promoter
  - Terminator
  - (Roth, 2007)

Choosing the Gene & Polymorphism

1. Topic
   - Concussions
2. Evidence there will be a observable phenotype
   - Recovery
3. What proteins are important for the phenotype
   - Neurometabolic Cascade
     • NMDA Channel (NR2A subunit)
4. What gene codes for this protein
   - GRIN2A
5. What polymorphism could cause an observable change in the phenotype
   - GT repeat in the promoter regions

Genes

- Each region has a set of codons
- Codon
  - 1 AA coded from 3 nucleotides

Polymorphisms

- Mutations
  - < 1% in population
- Polymorphisms
  - > 1% in population
- Allele
  - E.g., Nucleotides T and C at position 471 on APOE gene
- Homozygous and Heterozygous
  - TT, TC, CC
SNPs

<table>
<thead>
<tr>
<th>Protein Function</th>
<th>DNA Sequence</th>
<th>Amino Acid Sequence</th>
<th>Cell Response to Stress Influence</th>
<th>Influence on Phenotype</th>
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</thead>
<tbody>
<tr>
<td>Ca+ Channel x</td>
<td>TAA TTA GCG GCC</td>
<td>Iso Ile Gly Arg</td>
<td>Increased Ca+ influx</td>
<td>Normal Concussion Recovery</td>
</tr>
<tr>
<td>Ca+ Channel x</td>
<td>TAA TCA GCG GCC</td>
<td>Iso Thr Gly Arg</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Polymorphisms cause changes in AA

- GRIN2A (GT)n Repeat Polymorphism

- Repeat Polymorphism
  - Referred to as variable nucleotide repeats (VNTR)
  - Repetitive stretches of short DNA sequences — e.g., GTGTGTGTGT
  - Vary among individuals — Different number and sites — (Roth, 2007)
  - Promoter VNTR within GRIN2A has shown to alter transcription — (Ozekawa et al., 2003; Iwayama-Shigeno et al., 2005)

- GRIN2A Promoter Fragment

- Genetic Association to Concussion
  - **APOE**
    - Association with concussion history and carrying all 3 rare alleles — (Tierney et al., 2010)
    - No association between rare alleles and concussion risk — (p = 0.09; Terrell et al., 2008)
  - **NEFH**
    - No association between concussion susceptibility and genotype — (Ozekawa et al., 2003)
Problem Statement

- Athletes vary in their concussion recovery
- Genetic variation may be a factor in regulating glutamate binding and therefore cell recovery time
- No research performed on the association of (GT)n repeat polymorphism and concussions

Methods

- Research Design
  - Between subjects design
- Independent Variable
  - (GT)n repeat polymorphism
- Dependent Variable
  - Recovery time

Purpose Statements

- Aim 1: To assess if GRIN2A (GT)n genotype is associated to concussion recovery time.

Hypotheses

- There will be a significant association between carrying the GRIN2A (GT)n genotype and duration of recovery.

Independent Variables

- Genetic Models
  - Dominant
    - LL + LS vs. SS
  - Recessive
    - LL vs. LS + SS
  - Codominant
    - LL vs. LS vs. SS

(ITOKAWA et al., 2003; IWATA et al., 2006)
Dependent Variable

- Primary
  - Recovery time
    - Prolonged recovery > 20 days
    - Normal recovery ≤ 20 more days

DNA Extraction

- Pure DNA needed
- Extract DNA from buccal cells
- Final elution was 200 ml
- DNA stored at 4 °C

Concussion Assessment Instrumentation

- Vestibular ocular exam
- BESS test
- ImPACT neurocognitive exam
- Using a multi tool approach sensitivity exceeds 90%
  - (Broglio et al., 2007)

DNA Collection

- DNA collected using saliva samples
- Collected at the end of concussion assessment
- Transported and stored at Jayne Haines Center
- Coded according to IRB approved protocol

DNA Estimation

- Quantified fluorometrically
- 96-black well plate
- 2 sets of DNA standards

Polymerase Chain Reaction (PCR)

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Length, bp</th>
<th>Tm, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>FWD</td>
<td>5'-GAAGGAAGCATGTGGGAAATGCAG-3'</td>
<td>24</td>
<td>64.6</td>
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<tr>
<td>5' FAM-FWD</td>
<td>5'-FAM-GAAGGAAGCATGTGGGAAATGCAG-3'</td>
<td>24</td>
<td>64.6</td>
</tr>
<tr>
<td>REV</td>
<td>5'-gtttctGTGCTGGTATCTCCCT-3'</td>
<td>28</td>
<td>67.5</td>
</tr>
</tbody>
</table>

(Itokawa et al., 2003)
Validation of PCR Protocol

- Genetic analysis of VNTR is greatly facilitated by PCR
- However can be problematic
  - Addition of terminal dA nucleotides
  - Create additional peaks
    - Slipped strain mispairing
  - Reverse primer contained gttctt
    - Reduces heterogeneity
- Establish method of (GT)n genotyping performed PCR optimization

pCR4-TOPO Plasmid

Electropherogram

Alignment of PCR Fragment SW48

Electropherogram
**Pedigree Analysis**

- = Female
- = Male

**Procedures**

- Patients referred to Center
- Researcher explained the study
- If patient agrees to participate, they signed appropriate IRB forms
- Patient went through concussion battery exam
- DNA was extracted
- Estimated & Amplified Genotyped

**Participants**

- 51 Concussed Athletes
  - 38 M, 13 F
  - Age 18.69 ± 6.65
- Temple University Sport Concussion and Athletic Neurotrauma Program
- Exclusionary criteria
  - Any non sports related concussions (e.g., fall, car accident)

**Data Analysis**

- Primary Hypothesis
  - Chi square
  - Fisher Exact
  - Regression Analysis
  - Estimate extent of variability is explained by repeat polymorphism in this study

- Pearson correlations were run on potential covariates
  - Days between assessment, age, number of previous concussion, gender, race
    - R > 0.60
    - No covariates met threshold
- SPSS 21.0 (IBM SPSS INC., Armonk, NY) used for all analysis
- Alpha level set to p < .05

**Participants Descriptive Data by Recovery**
Participants Descriptive Data by Genotype

<table>
<thead>
<tr>
<th>Variable</th>
<th>Genotype</th>
<th>Sex n (%</th>
<th>Race n (%)</th>
<th>Migraine n (%)</th>
<th>ADHD n (%)</th>
<th>Age M(SD)</th>
<th>Prev Concussion M(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>11 (22)</td>
<td>27 (53)</td>
<td>17 (33)</td>
<td>33 (63)</td>
<td>6 (12)</td>
<td>19.3 (7.5)</td>
<td>1.3 (1.4)</td>
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<tr>
<td>Female</td>
<td>3 (6)</td>
<td>10 (20)</td>
<td>5 (10)</td>
<td>5 (10)</td>
<td>2 (4)</td>
<td>18.5 (6.4)</td>
<td>1.0 (0.9)</td>
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<tr>
<td>Race</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
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<td>17 (33)</td>
<td>10 (20)</td>
<td>20 (40)</td>
<td>3 (6)</td>
<td>19.3 (7.5)</td>
<td>1.3 (1.4)</td>
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<tr>
<td>African American</td>
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<td>5 (10)</td>
<td>2 (4)</td>
<td>4 (8)</td>
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<td>2 (4)</td>
<td>4 (8)</td>
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<tr>
<td>No Hx</td>
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<td>10 (20)</td>
<td>20 (40)</td>
<td>3 (6)</td>
<td>19.3 (7.5)</td>
<td>1.3 (1.4)</td>
</tr>
<tr>
<td>Hx</td>
<td>1 (2)</td>
<td>2 (4)</td>
<td>2 (4)</td>
<td>4 (8)</td>
<td>1 (2)</td>
<td>18.5 (6.4)</td>
<td>1.0 (0.9)</td>
</tr>
<tr>
<td>ADHD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Hx</td>
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<td>32 (63)</td>
<td>64 (128)</td>
<td>3 (6)</td>
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<td>1.3 (1.4)</td>
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<tr>
<td>Hx</td>
<td>1 (2)</td>
<td>5 (10)</td>
<td>5 (10)</td>
<td>10 (20)</td>
<td>1 (2)</td>
<td>18.5 (6.4)</td>
<td>1.0 (0.9)</td>
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<tr>
<td>Dizziness</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No Hx</td>
<td>6 (12)</td>
<td>14 (27)</td>
<td>14 (27)</td>
<td>28 (56)</td>
<td>5 (10)</td>
<td>19.3 (7.5)</td>
<td>1.3 (1.4)</td>
</tr>
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<td>22 (43)</td>
<td>44 (88)</td>
<td>10 (20)</td>
<td>18.5 (6.4)</td>
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</table>
| Association Between Allele & Recovery

<table>
<thead>
<tr>
<th>Recovery</th>
<th>Long Allele</th>
<th>Short Allele</th>
<th>Total</th>
<th>x^2</th>
<th>p</th>
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<tbody>
<tr>
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<td>25</td>
<td>46</td>
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<td></td>
</tr>
<tr>
<td>Prolonged</td>
<td>34</td>
<td>22</td>
<td>56</td>
<td>2.80</td>
<td>.094</td>
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</table>

Association Between Recovery & Genetic Models

<table>
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<tr>
<th>Model</th>
<th>Normal</th>
<th>Prolonged</th>
<th>x^2</th>
<th>p</th>
</tr>
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<tbody>
<tr>
<td>Recessive</td>
<td>4.37</td>
<td>0.032*</td>
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<tr>
<td>LL</td>
<td>3</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS + LS</td>
<td>20</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dominant</td>
<td>2.05</td>
<td>0.152</td>
<td></td>
<td></td>
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<tr>
<td>LL + LS</td>
<td>17</td>
<td>6</td>
<td></td>
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<tr>
<td>SS</td>
<td>27</td>
<td>3</td>
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<tr>
<td>Codominant</td>
<td>2.54</td>
<td>0.280</td>
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<tr>
<td>LL</td>
<td>4</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>6</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LS</td>
<td>13</td>
<td>14</td>
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<td></td>
</tr>
</tbody>
</table>

Univariate Regression of Risk Factors for Prolonged Recovery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wald x^2</th>
<th>p</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.07</td>
<td>0.300</td>
<td>1.06</td>
<td>0.95-1.18</td>
</tr>
<tr>
<td>Sex</td>
<td>3.18</td>
<td>0.075</td>
<td>0.27</td>
<td>0.06-0.13</td>
</tr>
<tr>
<td>Race</td>
<td>2.69</td>
<td>0.100</td>
<td>2.64</td>
<td>0.83-8.39</td>
</tr>
<tr>
<td>Migraine</td>
<td>0.04</td>
<td>0.843</td>
<td>1.13</td>
<td>0.33-3.92</td>
</tr>
<tr>
<td>ADHD</td>
<td>1.21</td>
<td>0.272</td>
<td>0.37</td>
<td>0.06-2.21</td>
</tr>
<tr>
<td>Num Prev</td>
<td>2.22</td>
<td>0.135</td>
<td>1.43</td>
<td>0.89-2.30</td>
</tr>
<tr>
<td>Acute Dizz</td>
<td>1.14</td>
<td>0.284</td>
<td>4.31</td>
<td>0.17-16.7</td>
</tr>
<tr>
<td>Recessive</td>
<td>4.01</td>
<td>0.045*</td>
<td>4.31</td>
<td>1.03-18.04</td>
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<tr>
<td>Dominant</td>
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<td>0.163</td>
<td>2.91</td>
<td>0.65-13.40</td>
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<td>Codominant</td>
<td>1.04</td>
<td>0.307</td>
<td>0.71</td>
<td>0.36-1.37</td>
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Multivariate Regression for Risk Factors of Prolonged Recovery

<table>
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<tr>
<th>Parameter</th>
<th>Wald x^2</th>
<th>p</th>
<th>Odds Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td>0.696</td>
<td>1.03</td>
<td>0.91-1.16</td>
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<tr>
<td>Sex</td>
<td>2.54</td>
<td>0.111</td>
<td>0.20</td>
<td>0.03-1.44</td>
</tr>
<tr>
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<td>2.64</td>
<td>0.104</td>
<td>4.25</td>
<td>0.74-24.32</td>
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<tr>
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<td>0.542</td>
<td>0.60</td>
<td>0.11-1.35</td>
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<td>0.790</td>
<td>1.39</td>
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<tr>
<td>Num Prev</td>
<td>1.32</td>
<td>0.250</td>
<td>1.34</td>
<td>0.81-2.21</td>
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<tr>
<td>Acute Dizz</td>
<td>4.35</td>
<td>0.036*</td>
<td>5.4</td>
<td>0.17-16.7</td>
</tr>
<tr>
<td>Recessive</td>
<td>6.29</td>
<td>0.012*</td>
<td>0.60</td>
<td>0.27-1.31</td>
</tr>
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</table>

Stepwise Regression of Risk Factors of Prolonged Recovery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>Std Error</th>
<th>Wald x^2</th>
<th>p</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-1.61</td>
<td>0.77</td>
<td>4.31</td>
<td>0.037*</td>
<td>0.04-0.91</td>
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<td>Recessive</td>
<td>1.74</td>
<td>0.77</td>
<td>5.15</td>
<td>0.023*</td>
<td>1.27-25.56</td>
</tr>
</tbody>
</table>

significance at p < .05
Discussion

- First study to investigate genetic associations by utilizing the known pathophysiology events
- Prolonged recovery is 4 times greater with those carrying the long allele genotype
- Clinically, those carrying long allele genotype may be predisposed to prolonged recovery

Discussion

- Risk factors for prolonged recovery
  - Sex
    • Females
      - Head neck strength, estrogen levels, honest
  - Current study found trend (p = 0.0544)
  - Males were more likely to have prolonged recovery
  - Age
  - Pediatric Population
  - Developing brain, musculature, skull sutures
  - There was no association found between age and recovery

Discussion

- Significant association between long allele genotype (LL) and prolonged recovery
- LL was associated with prolonged recovery
  - Decreased transcription of NR2A
- NR2A may be necessary for NMDA function
- GRIN2A produces NR2A
  - Previous findings suggest GT VNTR within this gene contribute to poor outcomes

Discussion

- Risk factors for prolonged recovery
  - Dizziness
    • Predictor within HS football athletes that took more than 21 days to recover
    - (Sass et al., 2010; Inoue, Yamada, Nakamura-Sigeno et al., 2002; Hoshino, Yamada, Yoshitaga et al., 2003; Nakamura-Sigeno et al., 2005)
  - Current study agrees that dizziness is associated with long recovery
  - Previous hx of concussions
    • Athletes that reported concussion hx are 2-5 times likely to sustain a subsequent concussion
    - (Bruce & Chermakova, 2004; Colvin et al., 2009; Guskiewicz et al., 2000; Iverson et al., 2004; Schultz et al., 2004)
    - This study does not support this notion

Discussion

- A novel study investigated association between VNTR and concussion recovery and severity
  - Collected data and followed participants prospectively through their recovery
  - Developed a DNA collection, estimation & genotyping protocol
Conclusion

- Statistical analyses demonstrated an association between genetic polymorphism in the promoter of GRIN2A and recovery
  - Prolonged recovery was 4 times greater for those carrying LL
- Gender and dizziness at time of injury were also trending toward significant
  - Both were more likely to have prolonged recovery
- Prospective genotyping could improve monitoring and concussion management

Future Research

- Larger prospective study
- Control for
  - Concussion history, severity, age, athletic exposure, head impact exposure
- Consider additional GT VNTR and other genes/polymorphisms

Thank You

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Thank you EATA for your support. I would also like to thank Dr. Tierney, Dr. Krynetskiy, Dr. Torg, and Ms. Phillips for their guidance and support on this study.