Genetic Variation and Individualized Medicine

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Translational research uses laboratory results to develop and test practical applications for clinicians. Clinical researchers are increasingly collaborating with basic scientists to improve clinical outcomes by examining the mechanisms underlying injuries and responses to treatments. For example, pharmaceutical companies are investing resources to determine optimal drug doses for individuals based on genotype (the genetic code of an individual). Translational research can be applied toward various clinical applications (eg, the use of therapeutic exercise, modalities, and injury-prevention strategies). Knowledge of how an individual will respond to specific treatment protocols could make rehabilitation more efficient and effective. Furthermore, genetic data may be used to understand and identify risk factors that predispose individuals to injuries or prolonged recoveries.\(^1\)\(^-\)\(^3\) The objective of this article is to present a model that incorporates genetic laboratory methodology to better understand a clinical phenomenon and present pilot data that we have collected using this model.

A basic concept in human anatomy and physiology is that structure determines function. For example, proteins help determine the structure and function of cells, and cells express certain proteins and not others depending on their purpose.\(^4\) Ultimately, response to stressors such as exercise or modalities is determined by proteins. As basic scientists identify genetic variations that influence function at the protein level, this may ultimately provide clinicians additional insight into treating or preventing problems on an individualized basis.

Deoxyribonucleic acid (DNA) contains the basic genetic material of a cell that is unique in all individuals except identical twins. Two main functions of DNA are to carry and replicate genetic information, as well as provide instructions for making new proteins. DNA is composed of 2 intertwining complementary strands of nucleotides. Nucleotides are represented as letters (ie, A = adenine, C = cytosine, G = guanine, and T = thymine). A person’s genetic code provides blueprints for amino acid arrangements for generating proteins. Although individuals typically
make the same proteins, the proteins may vary in structure or quantity because of differences in DNA instructions among individuals.

Variations in nucleotide and amino acid sequences across the population are normal and may result from environmental factors or evolutionary processes. A single nucleotide polymorphism (SNP) is the most common type of DNA sequence variation, with millions in the genome across the population. An SNP is characterized by the possibility of two nucleotides (or alleles) being found at the same DNA sequence location in different individuals (Table 1). There are several different types of SNPs that have direct influences on protein structure and expression (ie, amount made), which may or may not influence an individual’s phenotype (products of gene expression). For example, a missense SNP occurs when a variant or less common allele in the DNA coding sequence alters the amino acid sequence and changes a protein’s structure (Table 1). A research goal is to determine which SNPs influence clinically relevant phenotypes (eg, response to exercise or stress, increased risk of injury). This knowledge may allow clinicians to employ individualized prevention and rehabilitation strategies, ultimately reducing the time and money spent treating specific injuries or illnesses (eg, sports-related concussion).

Approximately 1.6 to 3.8 million sports-related concussions occur each year at an estimated treatment cost of $17 billion. Concussions may be an example of how genetic knowledge can enhance patient care. Long-term consequences of concussions may include depression, emotional problems, and an increased risk of chronic traumatic encephalopathy. Soccer, football, and ice hockey are among the sports at highest risk for concussion. Additional long-term risks in soccer athletes include an elevated risk for neurophysiologic impairments and the manifestation of signs and symptoms caused by repeated low loads associated with soccer heading and secondary influences (eg, alcohol consumption).

A concussion is caused by acceleration forces occurring directly via a blow to the head, face, or neck or indirectly through an impulsive force transmitted to the head. These events can then create tensile, compressive, or shear forces on the brain, producing cell deformation. After cell strain, a neurometabolic cascade (eg, potassium ion efflux and glutamate release) may trigger the manifestation of

<table>
<thead>
<tr>
<th>Patient</th>
<th>Nucleotide sequence with corresponding amino acid</th>
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<tr>
<td>A</td>
<td>ATG tyrosine TTT lysine CTC glutamic acid</td>
</tr>
<tr>
<td>B</td>
<td>ATG tyrosine TGT threonine CTC glutamic acid</td>
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</table>

DNA nucleotide sequence or “instructions” for linking amino acids (AA) in a specific sequence. In the second nucleotide triplet (that codes for a specific AA) there is a missense SNP. Specifically, two nucleotides or alleles (T and G) occur at that DNA location commonly in the population. People carrying a T allele will express a protein with the AA lysine in that sequence location, and people carrying the G allele will express a protein with the AA threonine in that sequence location (ie, different protein structures). The allele more commonly found in the population can be referred to as the normal or reference allele, and the less common allele can be referred to as the rare or variant allele.
concussive signs and symptoms immediately or hours postimpact. A concussion threshold has not been established, but researchers have reported a range of acceleration values for concussive and nonconcussive impacts. A threshold is difficult to establish because the acceleration required to cause injury may vary depending on extrinsic factors (eg, impact magnitude, direction, and location) and intrinsic factors (eg, head position on impact, number and severity of prior impacts, genetic predisposition, or other individual characteristics of the athlete). When extrinsic factors are controlled, variable clinical outcomes still occur, supporting a theory that the athlete’s intrinsic characteristics are important factors.

Recent research has focused on a possible genetic link (eg, apolipoprotein E gene [APOE]) to individual responses to head impacts. ApoE is a plasma lipoprotein produced by astrocytes and microglia in the brain that helps transport lipids to damaged neurons for repair and healing. Human ApoE contains 229 amino acid residues with 3 isoforms distinct at 2 positions, E2 (Cys112, Cys158), E3 (Cys112, Arg158), and E4 (Arg112, Arg158). These variations are brought about by genetic polymorphisms rs429358 (471T → C) and rs7412 (609C → T). Moreover, a polymorphic site in the promoter region of the apolipoprotein gene (rs405509, –219G → T) has been found to be strongly associated with alterations in APOE expression.

APOE E4 has been associated with poor short- and long-term outcomes including poor scores on neuropsychological assessments, cognitive assessments, and the Glasgow Outcome Scale (a measure of brain-injury severity). In addition, carrying the APOE E4 variant allele is associated with increased risk of fatigue, chronic traumatic brain injury, cerebral amyloid angiopathy, and a trend toward lower cerebral blood flow. The risk of chronic traumatic brain injury is greatest in high-exposure boxers possessing the E4 allele, suggesting a gene–environment interaction.

An SNP in the promoter region of a gene does not affect the amino acid sequence directly; however, it can change the level, location, or timing of gene expression. APOE promoter G-219T TT genotype has been associated with a higher risk of more severe concussions, poor outcome after traumatic brain injury, and an increased amyloid beta deposition. The APOE promoter rare allele when combined with the APOE E4 rare allele has been associated with altered ApoE brain concentrations and identified as one of several significant risk factors for clinical deterioration after brain injury. Although most evidence suggests an association between APOE genotype and poor brain-injury outcome, some researchers have reported that having the E4 rare allele is not associated with poor outcomes and may be associated with positive outcomes.

Preliminary Data From an in Vivo Model to Assess Genetic Influence of Head-Impact Response

Methods

We have begun to use genetic methodology to better understand clinical outcomes of head impacts. We hope to use this translational research model to help identify...
causative factors present in athletes who respond poorly to head impacts to provide better head-injury prevention and treatment strategies. Methodology for our pilot work and preliminary findings are outlined herein.

Fourteen male and female soccer athletes (age 21 ± 2 y, height 171 ± 14 cm, weight 72 ± 20 kg) were randomly assigned to an experimental (3 men, 5 women; age 21 ± 2 y, height 168 ± 17 cm, weight 69 ± 22 kg) or control group (3 men, 3 women; age 21 ± 2 years, height 171 ± 16 cm, weight 72 ± 12 kg) before commencement of the study via a computer-generated random-number table. On the first day, potential participants met with the investigators, and the purpose and procedures of the study were explained during a familiarization session. The participants completed the informed-consent form, health history and activity questionnaire, and Health Insurance Portability and Accountability Act form. On review of this paperwork, participants who met the study inclusion criteria had a custom-made mouthpiece molded as previously described. On day 2, participants reported their concussion signs and symptoms on a checklist before head-impact data collection. This assessment is necessary to identify any changes from baseline after the soccer heading task. The checklist assessed 18 signs and symptoms: blurred vision, dizziness, drowsiness, fatigue, feeling “in a fog,” feeling “slowed down,” headache, loss of consciousness, loss of orientation, memory problems, nausea, poor balance, ringing in the ears, seeing stars, sensitivity to light, sensitivity to noise, sleep disturbances, and vomiting. Responses on this checklist were later used to calculate the number of signs and symptoms the participants experienced. Antecubital blood draws were then performed. Blood was collected in 7-mL EDTA Vacutainer tubes (BD Diagnostics, Franklin Lakes, NJ), and the plasma was stored at 4°C until DNA was extracted and genotyped. Researchers were blinded to the status of APOE genotype.

After the blood draw, participants performed a neck warm-up (15 s each of clockwise and counterclockwise neck rotations) and neck stretching (2 repetitions of 15-s stretches for neck flexors and extensors). A custom-made mouthpiece with a triaxial accelerometer affixed to the hard palate was placed in the participant’s mouth to assess peak and total (sum across trials) linear head-impact acceleration (LHIA). Participants assigned to the experimental group were instructed to head a soccer ball forward in the air as if heading a shot on goal. Ten linear soccer headers were performed with a 450-g ball (size 5) inflated to 8 PSI directed toward them at 25 miles/h (11.2 m/s) at a distance of 11 m by a Jugs (JPS Sports, Tualatin, OR) soccer machine. Investigators ensured that the ball made contact with the head in a consistent region using a force-sensitive resistor (Noraxon USA Inc, Scottsdale, AZ) that was positioned on the forehead and functioned as an on–off switch activated by a force application greater than 1 Newton. Participants in the control group performed 10 simulated headers (ie, heading motion with no ball contact). Posttest measurements of signs and symptoms were assessed 1, 24, and 48 hours after the 10 trials. All data were recorded on a data-collection form for future analysis.

**DNA Purification and Genotyping**

DNA was extracted from the blood samples using a DNA-purification kit (Gentra Puregene; Qiagen, Germantown, MD) within 24 hours of blood draws according to manufacturer guidelines. Purified DNA was quantified using a Quant-iT PicoGreen
dsDNA assay kit (Invitrogen, CA) according to the manufacturer’s instructions with modifications. Briefly, the sample DNA was diluted to the final volume with 100 μl of 1XTE buffer mixed with 100 μl of Picogreen dsDNA reagent buffer in a 96-well plate. Fluorescence data were collected by a Spectramax M2 (Molecular Devices, CA) fluorescence plate reader (excitation 480 nm, emission 520 nm) and quantified against the calibration curve prepared from DNA standards provided with the Quant-iT PicoGreen dsDNA assay kit.

Genotyping was performed using premade TaqMan SNP genotyping assays C___905013_10 (rs405509), C___3084793_20 (rs429358), and C___904973_10 (rs7412; Applied Biosystems, CA)and an ABI 7300 real-time PCR instrument (Applied Biosystems, CA) according to the manufacturer’s instructions with modifications. Briefly, 5 to 20 ng (11.2 μl) DNA was added to each well of the 96-well reaction plate containing 13.8 μl reaction mix (12.5 μl 2xTaqMan Universal PCR master mix, no AmpErase UNG, and 1.25 μl 20XSNP genotyping assay mix), along with negative (no template control) and positive (DNA samples with known genotype) controls in a final volume of 25 μl. An allelic discrimination assay was performed under the following parameters: Step 1 was incubation at 92°C for 10 minutes, and step 2 was 40 cycles (incubation at 95°C for 15 s followed by incubation at 60°C for 1 min). Control DNA samples with known genotypes (Coriell, New Jersey) were used for assay validation.

Analysis of DNA sequence variation was performed using Statistical Analysis Software Genetics Package (NV, USA). Concussion signs and symptoms and LHIA data were analyzed with descriptive statistics using SPSS 15.0. Descriptive analysis was used to describe LHIA and genetic factors associated with reported concussion signs and symptoms.

Results

This section includes the preliminary findings from a limited sample. APOE alleles, LHIA (peak and total), and the number of reported signs and symptoms across time (ie, pretest and 1, 24, and 48 h posttest) for the individual participants are presented in Table 2.

Six of the 8 experimental participants and 2 control participants reported at least 1 concussion sign or symptom during the 48 hours after the head impacts. All 6 experimental participants who reported signs and symptoms carried the promoter rare allele. Five of these 6 still reported at least 1 sign or symptom 48 hours posttest, and 50% (3 of 6) reported 3 or more at that time. Of the 6 experimental participants reporting signs and symptoms, 4 had both the APOE promoter and E4 rare alleles. The E4 allele has been associated with poor outcome after a head injury, and the promoter allele is thought to increase protein expression. If a person carries the E4 and promoter rare alleles, it is reasonable to think that this may result in a poor outcome after impacts.

One participant possessed the E2, E4, and promoter rare alleles and experienced the greatest LHIA (peak and total). The E2 rare allele has been found to have protective qualities; however, in the current study, this participant also reported the highest number of signs and symptoms at 48 hours posttest. One of the experimental participants carried no APOE rare alleles (ie, carried 2 E3 alleles), experienced the lowest total LHIA, and did not report any concussion signs and symptoms.
Table 2  Participant History, Signs and Symptoms, LHIA, and Genotype Data

<table>
<thead>
<tr>
<th>Gr</th>
<th>Gen</th>
<th>Prev MTBI</th>
<th>Yr exper</th>
<th>s-spr</th>
<th>s-s01</th>
<th>s-s24</th>
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</table>

LHIA indicates linear head-impact acceleration; Gr, group (E = experimental, C = control); Gen, gender (F = female, M = male); Prev MTBI, previous history of concussion (Y = yes, N = no); Yr exper, number of years playing soccer; s-spr, s-s01, s-s24, and s-s48, sum of the number of signs and symptoms reported for that time period (s-spr = pretest, s-s01 = 1 h posttest, s-s24 = 24 h posttest, s-s48 = 48 h posttest); LHIAT, total (sum) LHIA from all trials (g = gravitational units of acceleration); LHIAP, peak LHIA; E2, presence of apolipoprotein E E2 variant (+ = has variant, – = does not have variant); Prom, presence of APOE promoter variant; E4, presence of APOE E4 variant.
Discussion

Our objective with this article was to present a model that incorporates genetic laboratory methodology to better understand whether response to head trauma is related to genotype. In addition, we present interesting pilot data that we have collected using this model. With this limited sample size we cannot draw any firm conclusions but can only suggest specific areas for new and continued study that seem warranted based on these pilot data. Future translational studies in this area may help identify individuals who are more at risk for concussions and help prevent and treat them.

Our results suggest that carrying 1 or more of the APOE rare alleles may influence individual responses to head impacts. Seven of 8 experimental participants possessed either the APOE E4 and promoter rare alleles or the promoter allele alone, and 6 of those 7 reported at least 1 concussion sign or symptom after the head impacts. Three of the 7 promoter carriers also reported 3 or more signs and symptoms at 48 hours. Previous literature has indicated that carrying an APOE E4 or promoter rare allele is associated with poor outcome after head trauma. This could be because the E4 or promoter rare alleles alter the structure and function of the production of APOE, respectively. ApoE helps transport lipids to damaged neurons for repair. If the structure or amount of this protein is altered by these genetic causes, it may take longer for a neuron to repair itself postinjury. Clinically, this might necessitate longer wait to return to play based on an athlete’s genotype. Further research is needed to determine whether this is truly occurring.

In the current study, 1 participant carried all 3 SNPs (ie, E4, promoter, and E2) and experienced the greatest peak and total LHIA. Note that this participant also reported the greatest number of signs and symptoms at 48 hours. A previous study determined that boxers experiencing greater head-impact exposure and carrying the E4 rare allele reported worst outcomes on a chronic-brain-injury scale. The experimental participant who never reported any signs and symptoms carried no APOE rare alleles and experienced the lowest total LHIA. These results are interesting because the APOE genotype that can greatly alter the protein structure and function, in combination with the greatest amount of LHIA, yielded the worst clinical outcome 48 hours after head impact. In contrast, the individual possessing the normal ApoE in combination with the lowest amount of LHIA yielded the best clinical outcome at 48 hours. This indicates that the interaction between genetics and environmental factors (eg, exposure to concussion and subconcussive impacts) may influence clinical outcome.

Two of the 6 control participants reported signs and symptoms after simulated headers. One of these reported an unrelated illness after the testing session; however, there was no explanation for the second. Although head impacts that occurred outside the study cannot be completely ruled out because participants were not monitored, members of both groups were instructed not to participate in any additional soccer heading during the study. Monitoring of possible head impacts outside of the testing protocol could strengthen the study design.

Conclusion

This translational research model offers a unique ability to control some extrinsic factors (eg, ball speed, direction of impact) to assess the role of intrinsic factors (eg, genotype). Using this model with a larger sample size may enable investigators to
examine SNPs in various athletic populations. Additional concussion-assessment tools (eg, balance and neuropsychological testing) could be used to strengthen the head-impact outcomes assessment. Similar models that control for external factors should be developed to evaluate the influence of genetic variation on other aspects of clinical practice (eg, the use of therapeutic exercise or modalities). Future directions of translational research may also include combining in vivo and in vitro research methodologies to determine whether genetic variations influence the response to similar trauma.

A translational research model can be used to provide basic-science evidence to improve patient care. For example, if it is determined that patients who carry the E4 and/or the promoter rare alleles are more susceptible to experiencing poor outcome after a head impact, clinicians may then determine that more extensive baseline testing or more stringent return-to-play guidelines are warranted. Perhaps knowing their genotype may enable athletes to make more informed choices regarding their individual risk in sport participation. Ultimately, the understanding of possible genetic influences of head injury may lead to better bedside care by clinicians because of better patient education, explanation of injury, and intervention strategies.

Acknowledgment

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References