



BDNF Val⁶⁶Met Positive Players Demonstrate Diffusion Tensor Imaging Consistent With Impaired Myelination Associated With High Levels of Soccer Heading: Indication of a Potential Gene-Environment Interaction Mechanism

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The purpose of this study was to examine the potential effect modifying role of the BDNF Val⁶⁶Met polymorphism on the association of soccer heading with white matter microstructure. We studied 312 players enrolled in the ongoing Einstein Soccer Study, a longitudinal study of amateur soccer player in New York City and surrounding areas. At enrollment and 2 years later, total heading in the prior 12 months (12-mo.) was estimated using an established self-report instrument and diffusion tensor imaging (DTI) was performed. Generalized Estimating Equations (GEE) logistic regression models were employed to test effect modification by the BDNF Val⁶⁶Met polymorphism on the association between 12-mo. heading exposure and DTI. We identified a significant interaction of 12-mo heading*BDNF Val⁶⁶Met genotype on the presence of low Radial Diffusivity, a DTI marker associated with myelination. Only Met (+) players demonstrated significantly reduced odds of low RD [OR (95 % CI): -2.36 (-3.53, -1.19)] associated with the highest vs. lowest quartile of 12-mo heading exposure. BDNF Val⁶⁶Met (+) soccer players with long-term exposure to high levels of heading exhibit less low Radial Diffusivity, suggesting impaired re-myelination may be a substrate of the previously reported association between heading and poor functional outcomes in soccer players.

Keywords: BDNF Val⁶⁶Met, diffusion tensor imaging, myelination and re-myelination, soccer heading, mild traumatic brain injury

INTRODUCTION

The game of soccer is a global phenomenon played by over 265 million people worldwide (1). Since the early 1980's, there has been growing awareness regarding the potential adverse neurological consequences associated with repetitive heading of the ball in soccer (2–4). As described in Maher et al.'s review article (5), the literature examining the effects of soccer heading on functional impairment is variable. While some studies have demonstrated no association between heading and poor neuropsychological performance (6, 7), a growing body of evidence suggests that heading in soccer is associated with lasting functional impairments (8–12); Given the apparent idiopathic response, it is essential to understand factors that may contribute to individual differences in vulnerability to heading, including potential genetic modifiers of outcomes.

Diffusion Tensor Imaging (DTI) is an MRI modality capable of detecting changes to the white matter microstructure following Traumatic Brain Injuries (TBIs) (13). There is now evidence to suggest that subconcussive head injury is associated with aberrations in brain tissue microstructure (8, 14–16). Radial Diffusivity (RD) is a DTI marker which reflects myelination wherein high RD is associated with myelin loss following injury and low RD is associated with myelination (17).

Brain Derived Neurotrophic Factor (BDNF) is a protein most well-known for facilitating neuronal maturation and synaptic plasticity (18). Recent animal studies have shown that BDNF also promotes re-myelination after a brain lesion (19, 20). The BDNF Val⁶⁶Met polymorphism represents a substitution of valine to methionine in the BDNF pro-peptide, which decreases secretion of BDNF into the synapse (18). Recent studies have demonstrated that presence of the Met allele is associated with impaired cognition (21) and more emotional symptoms (22) in mild TBI patients.

Understanding genetic modifiers of the association of soccer heading with brain microstructure can lead to identification of high risk individuals as well as provide insight into the underlying mechanism(s) of the microstructural consequences of heading. Because no study to date has examined this role for the BDNF Val⁶⁶Met polymorphism, the goal of the present study was to explore the effect modifying role of the Met allele on the association between long-term heading and DTI. We hypothesized that Met (+) players who head the most would demonstrate a more adverse profile on DTI outcomes, consistent with axonal injury and/or impaired re-myelination, compared to Met (-) players.

METHODS

Participants: Soccer players enrolled in the Einstein Soccer Study from November 2013 through August 2018 were included in this analysis. Recruitment and study procedures are described in detail elsewhere (9, 23, 24). In brief, adult amateur soccer players in the New York City and surrounding areas were recruited via print, internet advertisement and through soccer leagues, clubs, and colleges. A research team member contacted

qualifying individuals, confirmed eligibility and willingness to participate in the full longitudinal study, and invited enrollment. Players were eligible if they were aged 18–55, played soccer for more than 5 years, were currently playing soccer more than 6 months per year, and were fluent in English. Exclusion criteria included a self-reported diagnosis of schizophrenia, bipolar disorder, a known neurologic disorder (e.g., stroke or transient ischemic attack), or illicit drug use within 30 days based on urine toxicology. At enrollment, the initial study visit participants completed: (1) Written Informed Consent, (2) A web-based demographic questionnaire (e.g., gender, race, years of education), (3) HeadCount-12m (described below), (4) Venipuncture to obtain blood for genotyping (described below), and (5) a DTI scan. At the final visit, 2 years after enrollment, subjects repeated a DTI scan. All study procedures were approved by the Institutional Review Board at the Albert Einstein College of Medicine.

Healthy subjects, that were used as a reference group for imaging analysis (see below), consisted of non-athletes ages 18–50 years old. Exclusion criteria for healthy subjects included a history of a head injury, a psychiatric illness (schizophrenia, bipolar disorder, anxiety, depression), diabetes, hypertension, heart disease, or contraindication to MRI. All soccer players and controls provided written informed consent that was approved by the Institutional Review Board at the Albert Einstein College of Medicine.

HeadCount-12mo. Questionnaire: HeadCount-12mo is a questionnaire that estimates their total number heads in the prior 12-months [refer to Levitch et al. (9) and Rubin et al. (14) for a detailed explanation of Head Count-12mo]. In brief, participants are asked a series of questions pertaining to their soccer play during practice and competition in indoor and outdoor settings: (1) the number of months per year active in each setting; (2) the average number of competitive soccer games per week; (3) the average number of headers per game; (4) the average number of practices per week; (5) the average number of headers per practice. Total heading in the past year was estimated by multiplying average headers in each setting by number of sessions per week in each setting, converted to month, and then multiplying by the number of months of play per year. Subtotals in each setting were summed to obtain an estimate of total 12-mo. heading. HeadCount-12m also asks participants to report the number of years they have been playing soccer at a similar frequency and their lifetime concussion history. Subjects were instructed to consider a concussion any head injury for which they sought or were asked to seek medical attention.

DIFFUSION TENSOR IMAGING (DTI)

Details of image acquisition and processing procedures have been previously described (14).

In brief, imaging was performed using a 3.0T Philips Achieva TX scanner (Philips Medical Systems, Best, The Netherlands) with a 32-channel head coil. T1-weighted 3D magnetization-prepared rapid acquisition of gradient echo imaging was

performed with TR/TE/TI = 9.9/4.6/900 ms, flip angle 8°, 1 mm³ isotropic resolution, 240 × 188 × 220 matrix. Diffusion tensor imaging was performed using 2D single-shot EPI with 32 diffusion encoding directions, b-value = 800 s/mm², TR = 10 s, TE = 65 ms, 2 mm³ isotropic resolution, 128 × 120 matrix, 70 slices. Image processing was performed using a high-performance computing system running the Community Enterprise Operating System (CentOS) Linux distribution which incorporates the FSL package (25). The 32 diffusion-weighted image sets (32 b = 800 s/mm² images) were corrected for head motion and eddy current effects by using an affine registration algorithm, with the b = 0 s/mm² image as the target volume. Brain extraction was performed using Brain Extraction Tool (BET), and a white matter mask that was generated using FAST was applied to limit data analysis to white matter only. All images were reviewed by a board-certified neuroradiologist for structural abnormalities or evidence of prior trauma including hemorrhage. Trained reviewers checked the raw and processed images for image quality, artifacts and aberrant results of processing. Problematic or suboptimal images were excluded from the analysis. Diffusion parameter images for each control participant were transformed to match each soccer player using Subject-based registration (SURE-Quant), which has been shown to minimize potential registration errors (26). A voxel-wise analysis of covariance (VANCOVA) was performed to identify subject-specific abnormalities in DTI parameters including Fractional Anisotropy (FA), Radial Diffusivity (RD), Axial Diffusivity (AD), and Mean Diffusivity (MD). As previously described (26, 27), this is a method that permits us to identify clusters (e.g., a grouping of voxels) of DTI abnormalities by comparing each individual subject to a group of normative controls. In the present study, DTI parameter image volumes from each soccer player were compared to those of 110 healthy controls to identify subject-specific abnormalities in DTI parameters. To guard against Type I errors, we only retained clusters comprising at least 100 voxels that were all significant at $p \leq 0.01$ (8, 26). For each subject, we extracted total volume (mm³) of abnormally low and abnormally high FA, RD, AD, and MD, yielding 8 summary measures from each player for subsequent statistical analysis. All imaging analyses were adjusted for effects of age and gender.

Whole blood (5 ml) was obtained via venipuncture at enrollment. The BDNF Val⁶⁶Met polymorphism (rs6265, G > A) was genotyped using the Global Screening Array-24.v1.0 (GSA) chip and genotype data was analyzed with the Golden Helix SVS software. Data quality control (QC) was performed at both “per-marker” and “per-individual” level (28). The rs6265 variant had a call rate of 98.7% (meaning only 1.3% of the individuals had a missing genotype for this variant), which is above the $\geq 95\%$ genotype call rate cutoff. The Hardy Weinberg Equilibrium (HWE) predicts the expected genotype frequencies from the observed allele frequencies in a population and genotyping errors often lead to departure from HWE (29). No significant deviation from Hardy-Weinberg Equilibrium (HWE) was observed for rs6265 ($P = 0.68$). At the “per-individual” level, QC samples with an overall genotyping failure rate of >10% on the GSA chip were removed from analysis.

STATISTICAL ANALYSIS

All analyses were conducted using STATA 15.0. Baseline demographic group differences between Met (–) vs. Met (+) players were assessed using Student’s *t*-test or Wilcoxon Rank sum test for continuous variables and chi-squared or Fischer’s exact test for categorical variables. Our 12-months heading predictor variable demonstrated extreme positive skewness, based on visual inspection of a histogram; therefore, this variable was categorized into quartiles, yielding groups approximately equal in size. To determine if the presence of the Met allele modified the association between 12-mo. heading and presence of low or high DTI parameters, we utilized Generalized Estimating Equations (GEE) logistic regression models with independent covariance structure. GEE is an approach that models data from different visits as independent while accounting for within subject correlation (30). Gender, age, race, years of education, maximum number of alcoholic beverages consumed in a week, years playing soccer at a similar frequency and number of lifetime concussions (0,1, 2+) were considered as possible confounder. A backward-stepwise regression approach with $p < 0.05$ as criterion for retention was used to determine the final models. To correct for multiple comparisons, we applied a False Discovery Rate (FDR) correction at $p = 0.10$ (31). In the event of a significant interaction, *post-hoc* analyses stratified by Met status were conducted.

RESULTS

Our final sample consisted of 312 soccer players and 422 DTI scans obtained from the baseline and two-year visits. Seventy-three (73%) percent of the participants were men. The average age of players in this analysis was 25.9 (SD = 7.5). **Table 1** demonstrates that Met (–) and Met (+) players differed significantly with regards to gender and therefore this variable was used as a covariate in all subsequent GEE analyses. The results of the multivariate logistic regressions estimating the odds of high FA ($p = 0.33$), low FA ($p = 0.86$), high AD ($p = 0.16$), low AD ($p = 0.05$), high MD ($p = 0.10$), low MD ($p = 0.22$), and high RD ($p = 0.16$) were not significant after FDR correction. However, there was a significant interaction ($p = 0.02$) of 12-mo. heading*Met allele on the odds of low RD, aka abnormality in RD compared to a group of controls, after correction for multiple comparisons. As demonstrated in **Table 2**, only Met (+) players demonstrated significantly reduced odds of low RD associated with the highest vs. lowest quartile of 12-mo heading exposure (**Table 2**).

DISCUSSION

To our knowledge, this study is the first to demonstrate an effect of genotype on microstructural tissue outcomes from sub-concussive heading in soccer. We have found that compared to Met (–) players, the odds of low RD associated with high levels of 12-mo. heading is reduced by 89% in players with at least one copy of the BDNF Met allele.

TABLE 1 | Baseline characteristics in BDNFVal⁶⁶Met (-) vs. BDNFVal⁶⁶Met (+) players (N = 312).

		Met (-) players (N = 221)	Met (+) players (N = 91)	p
Variable (continuous)				
Age, years		24 (21–29)	23 (21–27)	0.32
Education, years		15.5 (3.0)	15.8 (2.47)	0.43
Years playing at similar frequency ^a		11 (7–16)	10 (5–16)	0.50
Variable (categorical)				
Gender	Male	167 (76)	58 (64)	0.03
Race	White	148 (67)	66 (72)	0.34
Past/Present Smoker	Yes	67 (30)	23 (25)	0.37
Alcoholic drinks/week	0	60 (27)	17 (19)	0.07
	1–2	99 (45)	34 (37)	
	3–7	47 (21)	32 (35)	
	8–14	14 (6)	8 (9)	
	14+	1 (1)	0 (0)	
Lifetime concussion(s) ^a	0	149 (68)	48 (54)	0.053
	1	31 (14)	20 (22)	
	2+	38 (18)	21 (24)	

Continuous variables are reported as mean (sd) for education and median (IQR) for age and years at similar frequency. Categorical variables are reported as frequency (%).

^aData missing in five subjects.

TABLE 2 | Odds Ratio and 95% Confidence intervals (CI) from regression models estimating the association of 12-mo heading on the presence of low Radial Diffusivity on DTI imaging.

	BDNF Val ⁶⁶ Met stratified analyses ⁺			
	Met (-) players ^a		Met (+) players ^b	
	OR (95% CI)	P	OR (95% CI)	P
Heading exposure (range of heading)				
Q1 (0–174)	—	—	—	—
Q2 (178–548)	-0.00 (-0.73, 0.73)	0.99	-0.76 (-1.84, 0.32)	0.17
Q3 (556–1459)	-0.08 (-0.79, 0.63)	0.82	-0.89 (-2.09, 0.30)	0.14
Q4 (1460–22828)	-0.24 (-0.96, 0.48)	0.51	-2.36 (-3.53, -1.19)	<0.001*

⁺ P-value for Met*Heading interaction from GEE model: p = 0.02.

^aModel adjusted for gender and years of education.

^bModel adjusted for gender.

*p < 0.05.

There is still much debate regarding the long-term consequences of exposure to repetitive soccer heading; however, understanding the distinct pathological mechanisms underlying exposures may help to elucidate the basis for heterogeneous outcomes. Prior studies combining histology and DTI have demonstrated that high RD specifically reflects myelination and lower RD is associated with myelin loss following experimental injury (32–34). Moreover, preclinical studies have demonstrated that BDNF promotes re-myelination following de-myelinating brain lesions (19, 20). One possible mechanism of this observation is that BDNF regulates autophagy (35), which in turn is essential for proper myelination (36). In the context of this evidence, our results suggest that impaired

re-myelination, due to presence of the Met allele, is a potential pathological substrate of functional deficits, which are associated with high levels of sub-concussive heading (8, 9). However, it must be noted that RD is indeed a surrogate marker for myelination as there is no myelin-specific imaging measure available at present. Another potential explanation is that our findings reflect a phenomenon in which high-heading negates neuroplastic changes, such as enhanced myelination, that are conferred by physical activity and training. Finally, there is a risk of chance despite our adjustment for multiple comparisons.

Several limitations to this preliminary study must be considered. First, this a candidate gene association study that always requires replication in a different and larger sample. Number of lifetime concussions was not determined as a significant confounder in the models; however, Met (+) players demonstrated a borderline trend toward a greater number of lifetime concussions; a fact that may have contributed to our results. Likewise, we have previously reported gender differences in DTI outcomes from soccer heading (14). Though we adjusted for gender in our analyses, we were not powered to detect a heading*gender* BDNF Val⁶⁶Met interaction; which limits our ability to address the combined effect of the Met allele and gender on white matter abnormalities from soccer heading. Moreover, we estimate exposure level based on self-report and therefore we cannot entirely dismiss potential bias due to reporting or recall error. However, our prior validation of HeadCount, treatment of exposure as categorical variable and the consistency of HeadCount across multiple timeframes and domains of measurement make reporting error unlikely to bias our findings. The method of exposure assessment does not permit us to address the role of biomechanical features of individual impacts. Our imaging analysis utilized a voxel-wise approach, that examines the aggregate abnormalities across the whole brain (26); thus we cannot identify specific abnormal white matter tracts in soccer players. Finally, our cohort of young, healthy amateur soccer players is generally reflective of a large segment of soccer participants worldwide, but differences from other populations and age groups may limit generalization of our findings to other populations.

The maximum population benefit protecting against the potential adverse effects of soccer heading will accrue when injury can be detected prior to overt clinical dysfunction. Our preliminary findings point to impaired re-myelination, associated with the BDNF Met allele, as a pathological mechanism underpinning the adverse consequences of soccer heading. Further study of the role of BDNF in the exposure-response relationship, in both human and preclinical studies of repetitive sub-concussive head impacts are warranted and may serve as the basis for assessment of player risk and motivate development and testing of remyelination treatments.

DATA AVAILABILITY STATEMENT

This manuscript contains previously unpublished data. Request to access the dataset should be directed to ML (michael.lipton@einstein.yu.edu).

ETHICS STATEMENT

This study involving human participants was reviewed and approved by Albert Einstein College of Medicine Institutional Review Board. The participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LH: study concept and design, analysis and interpretation of data, and critical revision of manuscript for intellectual contents. RF: acquisition, analysis and interpretation of imaging data,

and critical revision of manuscript for intellectual content. YF-H, PD, MK, WS, and RL: interpretation of data and critical revision of manuscript for intellectual content. ML: study concept and design and interpretation of data, and critical revision of manuscript for intellectual content and study supervision.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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