DNA methylation on PPARG and its relation to sports injury and pain in male football (soccer) players at all levels.

For correspondence:
chris.collins@muhdo.com

Christopher Collins

Muhdo Health Ltd. Columba House, Adastral Park, Ipswich, IP5 3RE, UK.

ABSTRACT

Injuries in English football are a cause of great financial burden to clubs and cause distress to players and coaching staff, therefore novel interventions to help prevent injuries should be considered to aid this burden. 287 male players across multiple levels of the English football leagues were tested using saliva for methylation levels across the gene PPARG, a known inflammation modulation gene. Player injury status and pain scale for each player was analysed at the point of testing to compare against the PPARG methylation status. The average methylation across PPARG on non-injured players was 0.508 and on those injured (n=78) was 0.4488, the difference between player/s indicating a pain score of 10 against the players indicating a pain score of 0 was +0.2437 towards the lower pain result. Therefore, it is theorised that PPARG hypomethylation occurs in response to injury or/and overtraining to aid in the inflammation processes of the body, this study will require further investigation to establish this link. Follow-up analysis on the same players that indicated injury and a high pain

score to see if PPARG methylation returns to normative values upon physical improvement is also a logical step for further analysis.

INTRODUCTION

On average English premier league teams lost 45 million GBP due to player injury across a season[1], therefore novel interventions to help prevent injuries should be considered to aid this financial burden on clubs, improve performance of players and maintain the safe playing and training of players. The following research project is completed on 287 male players across multiple levels of the English football leagues from amateur players (193), Semiprofessional/professional up to English football league 2 (59), and professional players which are rated from English league 2 and above (35). Injury data was gathered for players regarding injury status and current pain level, and this is correlated against the methylation levels on the gene PPARG.

PPARG

The gene PPARG (Peroxisome Proliferator-Activated Receptor Gamma) provides instructions for making a protein that is part of a group of proteins known as nuclear receptors. These receptors are involved in regulating the expression of various genes, which in turn affects different biological processes in the body. The role of the PPARG gene suggests it may impact muscle inflammation and repair processes, especially post musculoskeletal injury due to its ability regulate inflammation. Activation or expression of PPARG is associated with anti-inflammatory processes and the modulation of pro-inflammatory genes[2].

DNA methylation

DNA methylation is a chemical modification of DNA that plays a crucial role in gene regulation and genome stability. It involves the addition of a methyl group (-CH3) to the DNA molecule,

specifically at the carbon 5 position of cytosine residues in CpG dinucleotides (where C represents cytosine and G represents guanine). This modification is catalysed by enzymes called DNA methyltransferases[3].

DNA methylation is known as an epigenetic mechanism that can influence gene expression without altering the underlying DNA sequence. Methylation of DNA typically leads to the suppression of gene expression by blocking the binding of transcription factors and other regulatory proteins to the DNA, known as hypermethylation[3].

DNA methylation patterns are established during early development and are then faithfully maintained throughout cell divisions[4]. Studies have shown DNA methylation patterns can also undergo changes in response to various environmental factors[5,6], developmental processes, and diseases. DNA methylation patterns have been associated with various diseases, including cancer. In cancer cells, abnormal DNA methylation can lead to the silencing of tumour suppressor genes, allowing uncontrolled cell growth and proliferation[7].

DNA methylation analysis can be performed using various types of biological samples with many studies opting for tissue specific samples, however this incurs disadvantages such as being invasive and lowering the number of recruited participants[8]. One less/non-invasive method to achieve DNA methylation is the usage of saliva. Saliva contains cells from the oral cavity, which can provide DNA for methylation analysis, the sample from saliva is aimed to reproduce systemic DNA methylation.

METHOD

The aim of the of the study was to analyse if the average methylation across 53 CpG sites on the gene PPARG is indicative of player injury status. Injury status was established with:

1. The player injury records at the time of testing. (Any soft tissue injury that led to missed games or/and training at the time of testing).

2. A current pain score of 0 – 10 at the time of testing.

287 anonymised male soccer players (Ages 19-38) were analysed during a period of 21days over the 2018-19 English football season (appendix 1):

193 amateur players (unpaid players, but still play in some form of league which includes university/college leagues)

59 semi-professional or professional players (players which are paid on a part time basis or must supplement their football salary with other work)

35 professional players (players where football is their full-time career)

The study employed the Muhdo Health Epigenetic database and tag.bio analytics software. Players were tested with saliva and used the Illumina Infinium MethylationEPIC V1 array covering 850k~CpG sites, samples were analysed on the iScan high throughput system with the aid of Eurofins. The average beta value was taken from the raw data for each player on the required CpG sites for PPARG, this measurement on each site is between 0 – 1, 0 indicating hypomethylation and 1 being hypermethylation. The data was ran through the Muhdo Health algorithm tools and players utilised the Muhdo Health mobile phone application to fill out relevant questions.

The 53 CpG sites analysed across PPARG:

cg00057836
cg01412654
cg02430720
cg02605957
cg02726798
cg04702010
cg04908300
cg05563966
cg05671501
cg06340600
cg06573644
cg07424807
cg07556134

cg07676920
cg07895576
cg08288126
cg09405169
cg09663755
cg09702791
cg10237171
cg10441555
cg10499651
cg11176018
cg12880856
cg13369760
cg13518792
cg14113891
cg15722404
cg15938746
cg16197186
cg16340410
cg16470128
cg16827534
cg17369845
cg17819501
cg18063278
cg18303782
cg18861661
cg18887186
cg19046290
cg19182242
cg21859053
cg21946299
cg23514324
cg24271538
cg25845026
cg25911248
cg25929976
cg26197008
cg26364899
cg26741988
cg27051533

cg27095527

Table 1. Analysed CpG sites.

The pain scale utilised was from 0 – 10:

0 = No pain

1 = No real pain, hardly noticeable

2 = minor pain on exertion

3 = minor pain on exertion/may affect daily activities

4 = minor pain with or without exertion that affects daily activity

5 = minor pain that rises to moderate pain on exertion
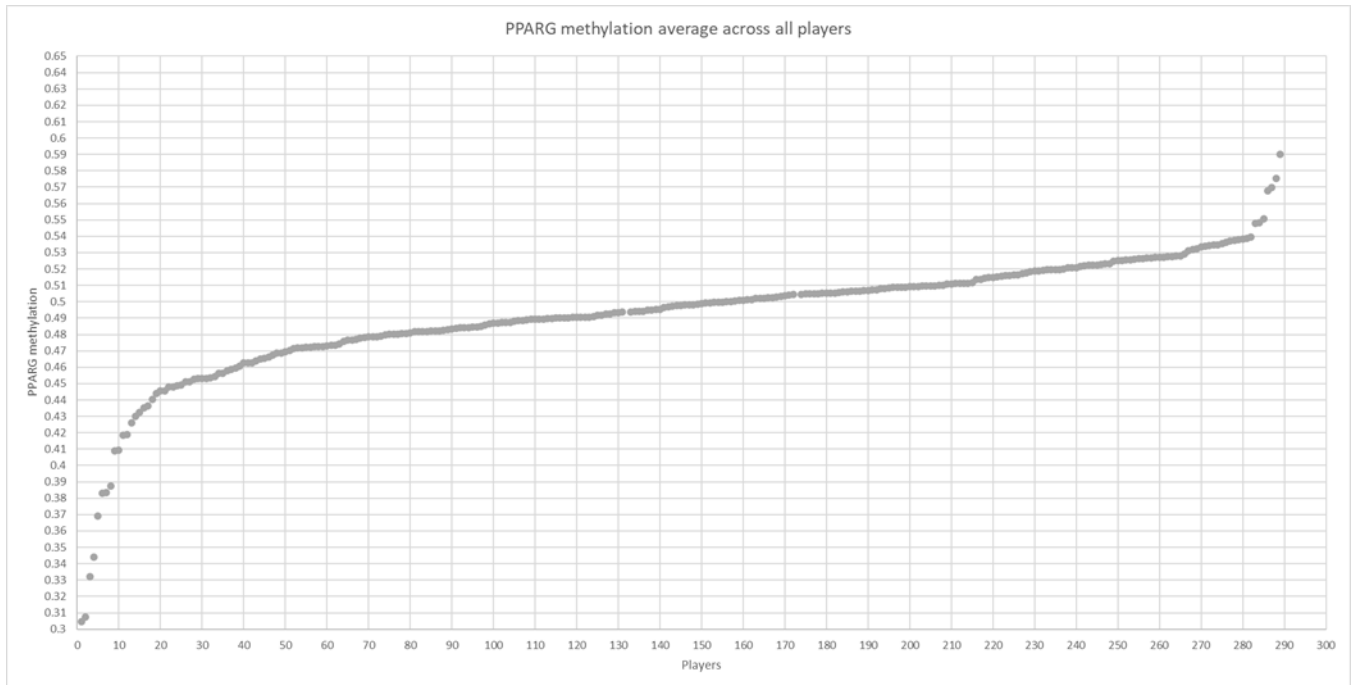
6 = constant moderate pain

7 = constant moderate pain that significantly impacts daily activity

8 = constant moderate pain rising to significant pain on exertion

9 = constant significant pain

10 = unbearable pain/no pain worse

Results

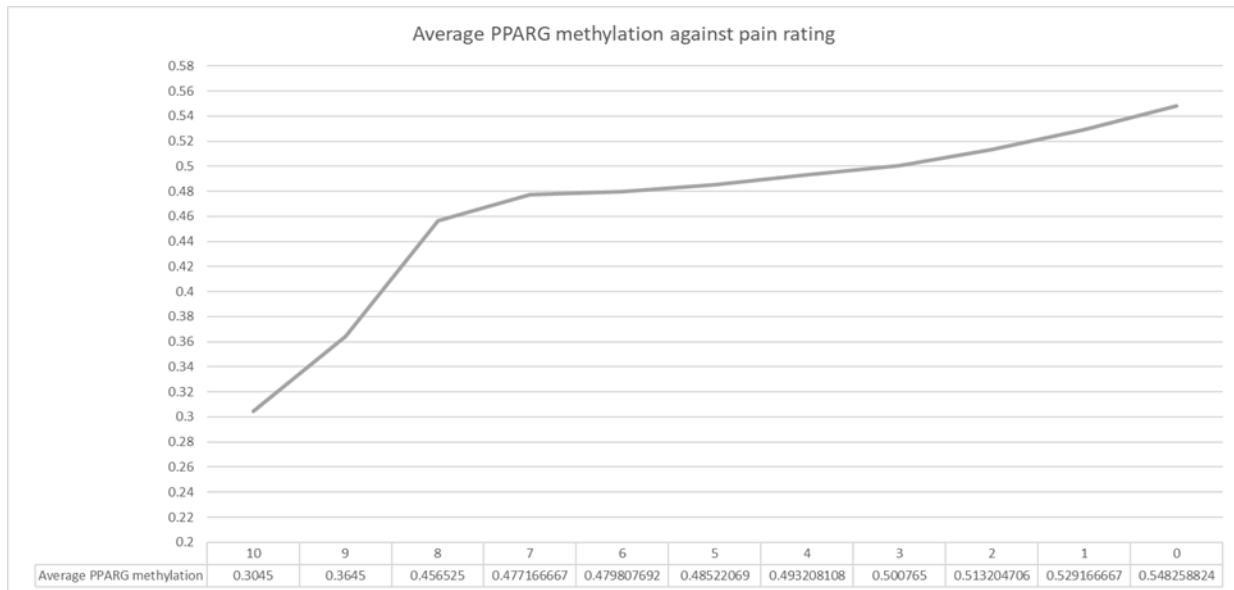Graph 1. Average PPARG methylation across all players.

Across all players the average methylation for all CpG sites was 0.4921, graph 1 shows how most players fall within this range of methylation and this shows some correlation with the Muhdo Health epigenetic database norm based on 6000~ subjects of 0.51 across the same CpG site averages.
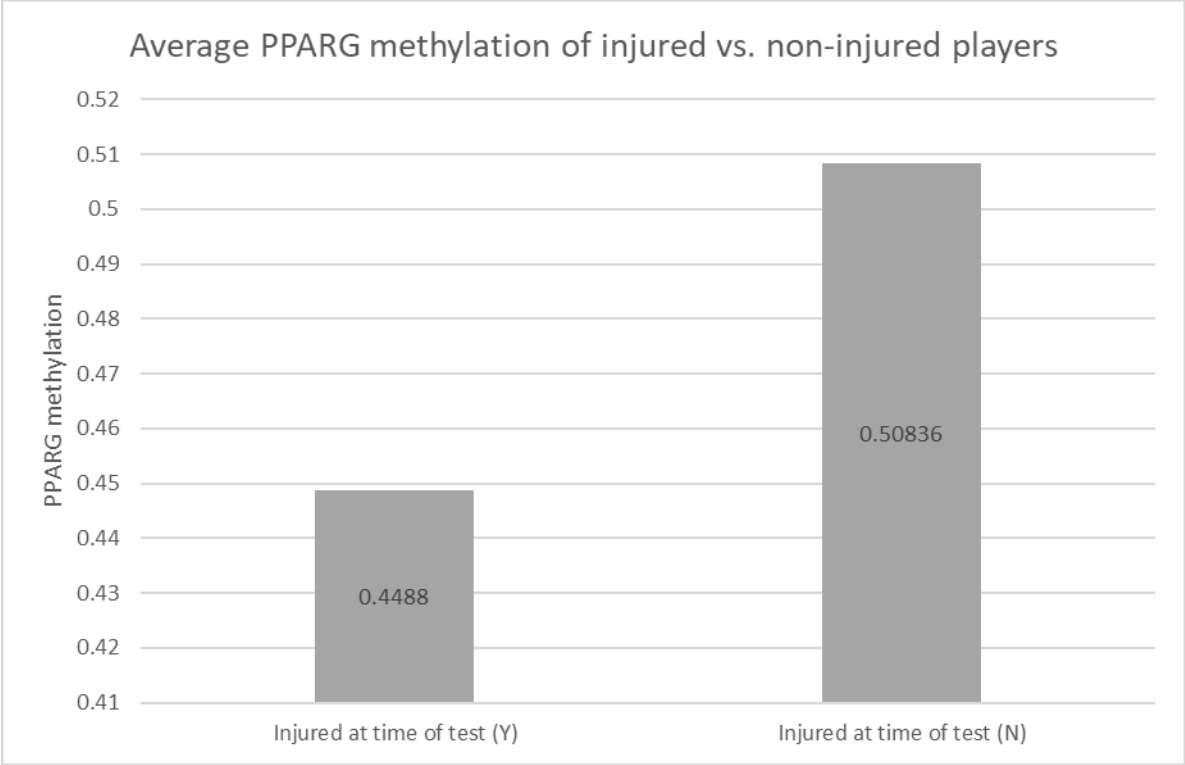
Graph 2. Average PPARG methylation of injured vs. non-injured players.

78 players were indicated to have a soft tissue injury that prevented them from play or training at the time of testing, with the remaining 209 indicated to have no injury at time of testing. Graph 2 shows that the average methylation across PPARG on non-injured players was 0.508 and on those injured was 0.4488.

Graph 3. Average PPARG methylation against pain rating.

	10	9	8	7	6	5	4	3	2	1	0
Average PPARG methylation	0.3045	0.3645	0.456525	0.477166667	0.479807692	0.48522069	0.493208108	0.500765	0.513204706	0.529166667	0.548258824

1 player stated they had a pain rating of 10, 8 players stated they had a pain rating of 9, 56 players stated the had a pain rating of 8, 3 players stated they had a pain rating of 7, 13 players stated they had a pain rating of 6, 29 players stated they had a pain rating of 5, 37 players stated they had a pain rating of 4, 20 players stated had a pain rating of 3, 85 players stated they had a pain rating of 2, 18 players stated they had a pain rating of 1 and 17 players stated they had a pain rating of 0. Graph 3 shows that on average those with an indicated pain rating of 5 and greater had a lower PPARG methylation when compared to the overall average PPARG methylation level of 0.4921. The difference between the 1 player indicating a pain score of 10 against the players indicating a pain score of 0 was +0.2437 towards the lower pain result. The average methylation of players indicating a pain score of 0-5 was 0.516920661, the average methylation of players indicating a pain score of 5+ was 0.427953341, a difference of +0.088 towards the average lowest pain scores.

Discussion

The results indicate that lower PPARG methylation is associated with increased indicated pain levels and a higher incidence of injury. Lower levels of methylation are associated with heightened gene expression[9]. As PPARG is associated with anti-inflammatory properties and the modulation of inflammatory markers[2,10] it would be theorised that lower methylation should be associated with superior pain outcomes and lower injury incidence due to superior expression. However, acute PPARG activation may occur in response to inflammation, injury or illness[11]. PPARG methylation may alter based on a need to modulate pro-inflammation genes, therefore a rapid hypomethylation may occur in a response to injury or illness, research does show that methylation may alter rapidly[12]. In adipocytes increased methylation across PPARG showed to be the earliest indicator of insulin resistance, whilst tissue specific, this may show acute changeable characteristics of PPARG methylation[13].

This study did not analyse other parameters which may have been affected by PPARG such as diabetes risk, activation of PPARG is known to have blood sugar lowering affects[14] and this may correlate with lower methylation levels across the gene. Compared with the Muhdo Health epigenetic database (6000~) which indicated an average PPARG result of 0.51 the players which indicated the highest pain ratings had a lower PPARG methylation average which backs up a theory that PPARG shows hypomethylation in response to pain causing pathology to help modulate pro-inflammatory genes. Three players had no indication of injury stopping their play, but their specified pain scale rating was 6 and their methylation level across PPARG was similar to that of players with indicated injury, this increased perceived pain level would in theory result in worsened performance[15] therefore acute hypomethylation changes in PPARG may be an early warning sign of overtraining or/and low-level injury. One limiting factor to the study could be proposed that the usage of saliva is not as specific as a direct muscle cell methylation pattern, however due to invasiveness this would be impossible given the researcher resources and possible harm to the players. Saliva methylation patterns have

shown to be highly correlated to blood methylation patterns[16] and therefore both are legitimate and interchangeable for reproducing this study in other athlete groups. Other variable data was acquired during the study; however, it was not analysed for these results, these variables which include diet, supplements and training schedule may impact PPARG methylation patterns but were not considered during in the analysis.

Follow up from this study aims to analyse player PPARG methylation upon reduction of pain levels or/and change to injury status in players with these indications.

Conclusion

This study shows that there may be a link between PPARG methylation levels and injury status and pain perception in male football players, it is unlikely that PPARG methylation is a causative factor of pain and/or injury due to its inflammatory modulation effects. Therefore, it is theorised that PPARG hypomethylation occurs in response to injury or/and overtraining to aid in the inflammation processes of the body, this study will require further investigation to establish this link and follow-up analysis on the same players that indicated injury and a high pain score to see if PPARG methylation returns to normative values upon physical improvement. If a link can be adequately established, periodic testing of players for clubs with the financial means to do so may benefit in early prediction of potential injury outcomes and may better monitor players who are falling into overtraining syndrome/s.

Contributions

Illumina for providing epigenetic analysis array technology.

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