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Review

Epigenetics of concussion: A systematic review

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ABSTRACT

Background: Concussion is the most common neurological disorder affecting millions of people globally each year. Identifying epigenetic mechanisms influencing concussion incidence, severity and recovery could provide diagnostic and prognostic insight into this injury.
Objectives: This systematic review aims to identify the epigenetic mechanisms underpinning concussion.
Methods: Seven electronic databases; PubMed, MEDLINE, CINAHL, Cochrane library, SPORTDiscus, Scopus and Web of Science were searched for studies that investigated the epigenetic mechanisms of concussion and its underlying neuropathology.
Results: Based on inclusion and exclusion criteria, 772 titles were independently analysed by two of the authors to a final list of 28 studies that totaled 3042 participants. We observed separate associations between sncRNAs, methylation, histone modification and concussion. Overall, 204 small non-coding RNAs were significantly dysregulated between concussed participants and controls or between concussion participants with no post-concussive symptoms and those with post-concussive symptoms. From these, 37 were reported in more than one study and 23 of these were expressed in a consistent direction with at least one further study. Ingenuity pathway analysis identified 10 miRNAs known to regulate 15 genes associated with human neurological pathologies. Two studies found significant changes in global methylation in concussed participants and one study found a decrease in H3K27Me3 in the context of DNA damage and concussion.
Conclusions: The review findings suggest that epigenetic mechanisms may play an important role in the pathophysiological mechanisms that could influence outcome, recovery, and potential long-term consequences of concussion for individuals.

Key Points

Epigenetic mechanisms may play an important role in the pathophysiological mechanisms that could influence risk, outcome, recovery, and potential long-term consequences of concussion for individuals.

A better understanding of epigenetic mechanisms that contribute to the neuropathology of concussion is essential to improve understanding and subsequent management of this form of traumatic brain injury and to provide directions for future study.

1. Introduction

Traumatic brain injury (TBI) is the most common neurological disorder affecting 50–69 million people globally each year (Dewan et al., 2018; Lefevre-Dognin et al., 2021). The severity of TBI varies from severe to mild, with the latter accounting for most cases (70–95 %) presented at hospital (Maas et al., 2022; Maas et al., 2017). In addition, incidence is underestimated as up to 50 % of concussions may go undiagnosed (Broglio et al., 2017; Meehan et al., 2013; McCrea et al., 2004). Concussion is a subgroup of TBI also termed as mild-TBI (mTBI)

Abbreviations: TBI, Traumatic brain injury; mTBI, mild-TBI; GWAS, Genome-wide-association study; SNPs, Single nucleotide polymorphisms; miRNAs, microRNAs; piRNAs, PIWI-interacting RNAs; siRNAs, small interfering RNAs; snoRNAs, small nucleolar RNAs; GCS, Glasgow Coma Score; NOS, Newcastle-Ottawa Scale; CSF, cerebral spinal fluid.

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however, within the related literature a wide variety of definitions are used which has implications for study comparison. Sustaining a concussion elicits altered brain function via complex pathophysiological processes that can have both short and long-term consequences (Barkhoudarian et al., 2016). Most individuals recover from concussion within 2–4 weeks post-concussive event, however 10 % of patients experience persisting symptoms after concussion for 1–3 months or longer (Willer and Leddy, 2006). The potential effects of sustaining a concussion may have long-lasting functional and structural changes to the frontal and temporal regions of the brain, as observed in certain individuals who sustained a concussion 20 years prior (June et al., 2020). In addition, sustaining repetitive concussions may be a risk factor for developing progressive neurodegenerative diseases such as chronic traumatic encephalopathy and tauopathies (Buckland et al., 2022;13:1107.; Nowinski et al., 2022; Mckee and Daneshvar, 2015; Russell et al., 2022; Bieniek et al., 2020).

Several environmental risk factors contribute to concussion risk such as, behaviour, rules of the sport, nutrition, quality of sleep and prior history of concussion (Ponsford et al., 2000; Abrahams et al., 2014; Iverson et al., 2017; Raikes et al., 2019; Lust et al., 2020). Additionally, genetic risk factors may affect incidence and outcomes related to concussion (Panenka et al., 2017; Antrobus et al., 2021). A recent genome-wide-association study (GWAS) identified 2 novel SNPs (*SPATA5* rs144663795 and *PLXNA4* rs117985931) associated with concussion (Kim et al., 2020). Currently the heritability of concussion is unknown. Based on other observations of substantial genetic contributions to inter-individual variability in most human traits, it is likely that a substantial genetic component applies to concussion (Antrobus et al., 2021). However, these genetic variants only partially explain the inter-individual variability of incidence and outcomes related to concussion. In addition, epigenetic mechanisms are likely to also contribute to the neuropathophysiology of concussion.

Epigenetics refers to functional changes to the genome that regulate gene expression in response to environmental stimuli, without changing the primary DNA base sequence (Waddington, 1942). The primary epigenetic mechanisms are DNA methylation, histone modifications, and the post-transcriptional regulation by small non-coding RNAs (sncRNAs, which include microRNAs (miRNAs), PIWI-interacting RNAs (piRNAs), small interfering RNAs (siRNAs), and small nucleolar RNAs (snoRNAs)). DNA methylation occurs by the addition of a methyl group to the C-5 carbon atom position of cytosine bases to form 5′ methyl-cytosine which alters gene expression by recruiting proteins involved in gene repression or by inhibiting transcription factor(s) binding to DNA (Moore et al., 2013). Histone acetylation, phosphorylation, and methylation are epigenetic modifications that are associated with altered gene transcription (Bannister et al., 2011). Small non-coding RNAs are highly abundant molecules that regulate essential cellular functions with a wide variety of roles. For example, miRNAs directly control transcription by recruiting the machinery for gene silencing of gene promoter in various neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease (Li et al., 2022). Similarly, piRNAs silence gene expression through transcription and post-transcriptional processes and within the brain can play a role in neuronal differentiation and development of neurodegenerative diseases (Sato et al., 2023). siRNAs regulate the expression of genes through the process of RNA interference (Dana et al., 2017) and finally snoRNAs stabilise the structure of rRNA through modifying rRNA with 2′-O-methylation and pseudouridylation of nucleotides (Stepanov et al., 2015;2015.).

To date, there is little current evidence delineating the role of epigenetic mechanisms in concussion. Therefore, we have systematically reviewed studies which have investigated the epigenetic mechanisms of concussion. A better understanding of epigenetic mechanisms that contribute to the neuropathology of concussion is essential to improve understanding and subsequent management of this form of TBI and to provide directions for future study.

2. Methods

A systematic review assessed the evidence for epigenetic mechanisms of concussion and how they contribute to the neuropathology of concussion. This systematic review was developed in accordance with the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al., 2020). A review protocol was registered with PROSPERO on 15th September 2022.

2.1. Search terms and databases

Keywords were selected by agreement of all authors and drafted into a PubMed search strategy. The search strategy was reviewed and revised using the CADTH Peer Review Check-list for Search Strategies according to the PRESS 2015 Guideline Statement, and then tested to ensure key studies were retrieved (McGowan et al., 2015). The PubMed search (Table 1) was adapted for all other databases searched in this review (MEDLINE, CINAHL, Cochrane library, SPORTDiscus, Scopus and Web of Science). Results were filtered to include studies in peer-reviewed journals (no date restriction) and were limited to English language articles only. The search was conducted on 20th May 2024.

Table 1. Schematic to represent 2-level search strategy.

2.2. Study selection criteria and data extraction

Studies were included if they (i) Were primary research; (ii) Included concussion participants (those of any age or sex, diagnosed with a concussion, occurring from any sport, activity, combat, accident, or life event) diagnosed according to a form of diagnosis e.g. GCS $\geq$ 13 or self-reported history of concussions; and (iii) Contained epigenetic mechanism (DNA methylation, histone modification and sncRNAs) study in humans only. Studies were excluded if they contained the following: (i) Participants with a moderate or severe TBI, or mixed population of mild, moderate or severe traumatic brain injury or penetrating head injury; (ii) Intervention (use of drugs alone or with physical exercise, intervention not clear); (iii) Animal based studies; and (iv) design (reviews, letters to the editor, case reports, commentaries, editorials, expert opinion, theses or dissertations). Initially, citations were independently screened by pairs of authors according to the inclusion and exclusion criteria. When required, a third reviewer adjudicated disagreements between authors. Study title and abstracts were screened for suitability and full text articles were retrieved. Data was extracted by one author and reviewed by a second author to ensure accuracy and completeness.

2.3. Assessment of risk of bias

For each included study the risk of bias was independently rated by two authors using the Newcastle-Ottawa Scale (NOS) in line with previous studies (Bannister et al., 2011; Li et al., 2022), with a third reviewer adjudicating disagreements between authors. A score of nine stars deemed a study to be of good quality and at low risk of bias; studies

Table 1
Schematic to represent 2-level search strategy.

Operator		Search terms
	#1	epigen* OR DNA methylation OR S-Adenosylmethionine OR hypermethylation OR hypomethylation OR CpG Islands OR Histone OR acetylation OR demethylation OR methylation OR phosporylation OR ubiquination OR modification OR adenosylmethionine OR CpG OR microRNA* OR miRNA OR mirna biomarker* OR non-coding RNA* OR gene modification OR gene expression
AND	#2	mild traumatic brain injur* OR MTBI OR concussion* OR brain concussion* OR post-concussion* OR sports-related concussion*
NOT		Animal* NOT rat* NOT mouse NOT murine NOT dog* NOT canine NOT cat* NOT feline

that scored eight or seven stars were deemed at medium risk of bias; while those that scored below seven were at high risk of bias.

2.4. Ingenuity pathway analysis

Qiagen's Ingenuity Pathways Analysis (IPA, v23.0) was used to identify pathways based on post-concussion miRNA expression profiles. To reduce the number of miRNA-mRNA interactions to a workable number, we employed filters for miRNA confidence (experimentally observed), disease (neurological diseases), pathways (neuronal system), species (human), and tissue (nervous system).

3. Results

3.1. Literature search Results

The search of the databases identified 735 articles (Fig. 1). After duplicates were removed and each article's reference list was hand searched for other relevant articles, 575 records were screened and a total of 28 were included in this review. The NOS scores of the case-control studies ranged from 6 to 8, with a mean score of 7.6. The NOS scores of the cohort studies ranged from 7 to 8, with a mean score of 7.6. The overall mean NOS score across all studies was 7.6 (Table 2). Each of the 28 studies were critically reviewed by at least two investigators using a standard review form.

3.2. Characteristics of the included studies

The characteristics and key findings are summarised including; participant characteristics, concussion diagnosis, epigenetic mechanism and main findings (Table 3). Of the 28 included studies 16 were case-control studies and the remaining 12 were cohort studies. The total number of participants across all studies was 3042, ranging from 18 to 538 participant sample sizes, with a median sample size of 55. The mean age range of participants in the selected articles was 12.8–54.6 years, seven studies (25 %) included children (<18 years). Twenty-two studies (79 %) included both male and female participants and only 6 (21 %) studies included only male participants. Only one study (4 %) had white only participants. Fifteen studies (54 %) used a participant sample of mixed ethnic groups, and twelve studies (43 %) did not report the ethnicity of participants.

Five studies included military personnel, four studies included only participants under the age of 18 years old, two studies focused on professional rugby union athletes, one study included Australian Football League athletes, one study contained professional contact athletes from a variety of sports, four studies included professional American football athletes, three studies contained collegiate athletes from a mix of sports and finally eight studies focused on the general population (some of which included participants under the age of 18 years old). Most studies used whole blood collection (17), nine studies utilised saliva, one study collected cerebral spinal fluid (CSF) and one collected human brain tissue. A variety of methods were utilised to measure the epigenetic response of concussion with six studies using miRNA from saliva, six studies used miRNA from serum, four studies used miRNA from plasma, two studies utilised miRNA from plasma and extracellular vesicles, two studies used miRNA from exosomal plasma, two studies measured DNA methylation at 850,000 CpG sites, one study measured global DNA methylation (5-mC%), one study used miRNA from CSF, one study measured histone modification from brain tissue, with the remaining three studies measuring small nuclear RNA/miRNA and sncRNA from blood and saliva, respectively.

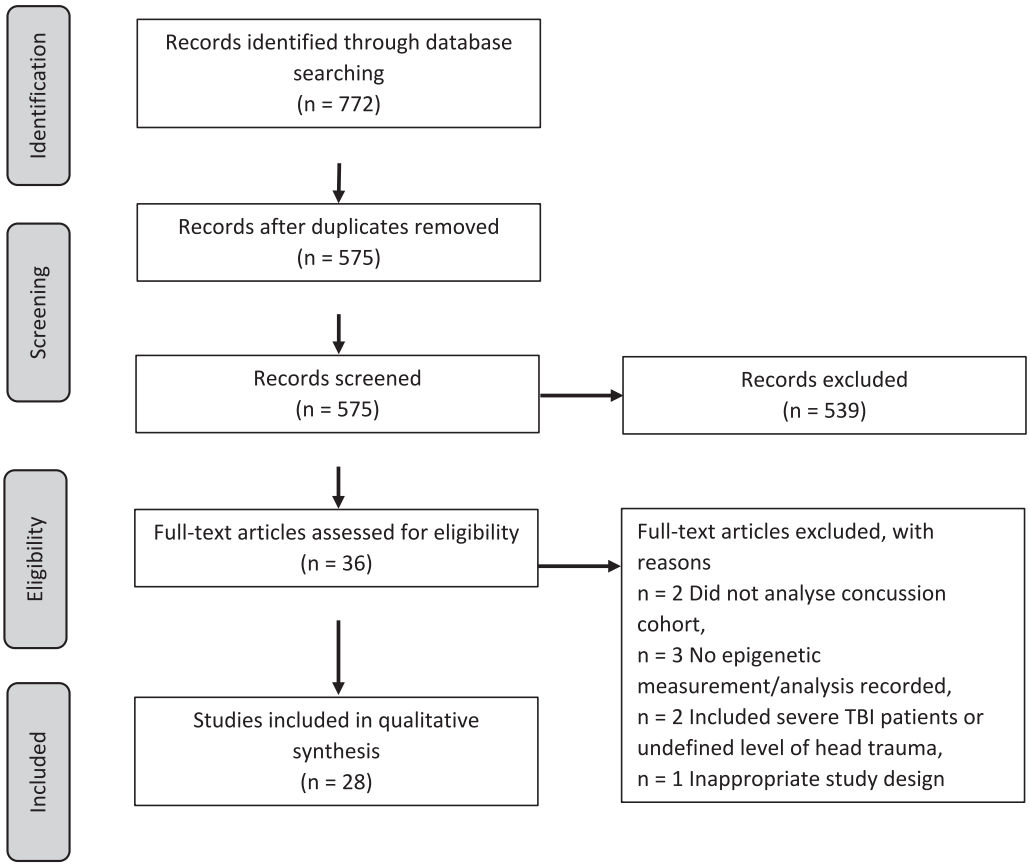

Fig. 1. PRISMA flow diagram.

Table 2
Quality assessment of studies.

Study	Study design	Newcastle-Ottowa score			
		Selection	Comparability	Outcome/ exposure	Total
Devoto *et al.* (Devoto et al., 2022)	CC	****	**	**	8
Shultz *et al.* (Shultz et al., 2022)	CH	****	*	***	8
Di Pietro *et al.* (Di Pietro V, O'Halloran P, Watson CN, Begum G, Acharjee A, Yakoub KM, et al. Unique diagnostic signatures of concussion in the saliva of male athletes: the Study of Concussion in Rugby Union through MicroRNAs (SCRUM). Br J Sports Med [Internet]. Br J Sports Med;, 2021)	CH	***	*	***	7
Duan *et al.* (Duan et al., 2021)	CC	****	**	**	8
Fedorchak *et al.* (Fedorchak et al., 2021)	CC	***	*	***	7
Guedes *et al.* (Guedes et al., 2021)	CH	***	**	***	8
Hicks *et al.* (Hicks et al., 2021)	CC	****	**	**	8
Lusardi *et al.* (Lusardi et al., 2021)	CC	****	**	**	8
Papa *et al.* (Papa et al., 2021)	CH	***	*	***	7
Vorn *et al.* (Vorn et al., 2021)	CC	****	*	*	6
Bahado-Singh *et al.* (Bahado-Singh et al., 2020)	CC	****	*	**	7
Ghai *et al.* (Ghai et al., 2020)	CC	****	**	**	8
Hicks *et al.* (Hicks et al., 2020)	CC	****	*	**	7
Lee *et al.* (Lee et al., 2020)	CC	****	**	**	8
Polito *et al.* (Polito et al., 2020)	CC	****	**	**	8
Davies *et al.* (Davies et al., 2019)	CC	****	**	**	8
Papa *et al.* (Papa et al., 2019)	CH	***	*	**	6
Schwab *et al.* (Schwab et al., 2019)	CC	****	**	**	8
Svingos *et al.* (Svingos et al., 2019)	CH	***	**	***	8
Di Pietro *et al.* (Di Pietro et al., 2018)	CC	****	*	**	8
Johnson *et al.* (Johnson et al., 2018)	CH	***	**	***	8
Pasinetti *et al.* (Pasinetti et al., 2012)	CC	****	*	**	7
Hicks *et al.* (Hicks et al., 2020)	CC	****	**	**	8
Miller *et al.* (Miller et al., 2022)	CH	***	**	***	8
Mitra *et al.* (Mitra et al., 2022)	CH	***	**	***	8
Mitra *et al.* (Mitra et al., 2023)	CH	***	**	***	8
Tas *et al.* (Tas et al., 2020)	CH	***	**	***	8
Wyczechewska *et al.* (Wyczechowska et al., 2023)	CH	***	**	***	8

CC, case-control; CH, cohort.

3.3. Epigenetic mechanisms associated with concussion

Across the 28 studies identified, 24 of these investigated sncRNAs. There was a large variation in the expression of sncRNAs in concussed patients between studies (Table 3). Specifically, there was a total of 204 sncRNAs that were significantly up-or down-regulated between concussed patients and controls or between concussion patients with no post-concussive symptoms and those with post-concussive symptoms (Table 3). From these, 37 were reported in more than one study and 23 of these miRNAs were reported to be differentially expressed in a consistent direction with at least one further study. Table 4 summarises the miRNAs identified in multiple studies. Twenty-two miRNAs were identified in two or more studies which could have potential diagnostic roles in the management of concussion (Fig. 2). Thirty-four miRNAs were identified in two or more studies which could have potential prognostic roles in the management of concussion (Fig. 2).

For the remaining four studies, three investigated DNA methylation. Lee *et al.* identified higher blood global methylation ratio (5-mC%, percentage of methylated cytosines in total DNA) in concussed cases (4.42 ± 0.43) than in controls (3.93 ± 0.03) (Lee et al., 2020). Bahado-Singh et al. found 449 CpG sites were significantly methylated with some methylation changes > 10 % in concussed youth compared to youth controls (Bahado-Singh et al., 2020). However, Duan *et al.* did not identify any significant global methylation changes between a concussion group and controls (Duan et al., 2021). Finally, Schwab *et al.* examined histone modification in human brain tissue, finding cases with a history of concussion had a decrease of H3K27Me3 in the context of DNA damage (Schwab et al., 2019).

To investigate the potential contribution of concussion-associated candidate miRNAs, ingenuity pathway analysis (IPA) was performed employing the 37 miRNAs to identify putative miRNA targets relevant to the nervous system and neuronal disease (Fig. 2). This resulted in identification of 10 miRNAs known to regulate 15 genes (*ESR1*, *NR3C2*, *PGR*, *CREB1*, *PDPK1*, *PRKCA*, *AP2A1*, *STX1A*, *KRAS*, *ADCY6*, *ERBB4*, *GRIA2*, *SLC6A4*, *GNAI2*, *RAC1*) that play a role in human neurological pathologies (Fig. 3). Based on the post-concussion directions of change reported in Table 4, we decided to build a consensus prediction model, i.e. a miRNA is said to be upregulated (coloured in red, let-7a-5p, miR-142-3p, miR-135b-5p, and miR-372-3p) if it is upregulated in a majority of papers. The opposite is true for downregulated miRNAs (coloured in green, miR-30e-5p, miR-181a-5p, miR-27a-3p, and miR199a-3p). Where a consensus cannot be reached because there is an even number of inconsistent findings, the miRNA is not coloured (miR-182-5p and miR-20a-5p). The phenotypes predicted by IPA based on the consensus approach, show an activation of brain concussion, neuronal cell death, long-term synaptic depression of brain cells, and long-term potentiation of brain. Conversely, there is an inhibition of long-term synaptic depression of neurons, inhibition of proliferation of neuronal cells, and inhibition of neuron density.

4. Discussion

This systematic review identified 28 studies suitable for establishing our present understanding of the epigenetic mechanisms associated with concussion (Table 2 and 3). The methods adopted across these studies included case studies, cohort studies, clinical assessments, self-reporting of concussion, neuroimaging and autopsy. Separate associations between sncRNAs, methylation, histone modification and concussion were observed within the selected studies. The earliest published study in this review dates back only to 2012 suggesting that there is a relatively nascent but growing literature on epigenetic mechanisms that may play an important role in the pathophysiological mechanisms that influence outcome, recovery and potential long-term consequences following concussion for individuals.

Table 3
Characteristics and key findings of epigenetics of concussion studies.

Study	Participants	Sex	Ethnicity	Age (mean) [Years]	Concussion Diagnosis	Epigenetic mechanism	Tissue type	Main Findings
	N							
Devoto *et al.* (Devoto et al., 2022)	152 military service members and veterans with a history of concussion, 35 controls without history of concussion.	163 males, 24 females	Not reported	38.0	Modified OSU mTBI-ID, VCU rCDI) (mean time since last concussion ranged between 8.0–12.2 years for all groups).	miRNAs (exosomal plasma)	Whole blood	23 miRNAs associated with pathways involved in neuronal function, chronic inflammatory processes and neuronal death were upregulated in the blast-related concussion group compared to controls (hsa-let7e-5p, hsa-miR-1233-3p, hsa-miR-1268b, hsa-miR-1304-3p, hsa-miR-18a-5p, hsa-miR-197-5p, hsa-miR-204-5p, hsa-miR-3190-3p, hsa-miR-324-3p, hsa-miR-326, hsa-miR-361-3p, hsa-miR-3615, hsa-miR-372-3p, hsa-miR-376b-3p, hsa-miR-4792, hsa-miR-5001-5p, hsa-miR-5010-3p, hsa-miR-516a-5p, hsa-miR-567, hsa-miR-615-5p, hsa-miR-619-3p, hsa-miR-631, hsa-miR-767-5p) and 1 downregulated (hsa-miR-139-5p).
Shultz *et al.* (Shultz et al., 2022)	28 AFL athletes with a history of concussion, 27 AFL athlete controls with no history of concussion.	39 males, 16 females	Not reported	24.0	GCS (samples were collected pre-season, 2-, 6-, and 13-days post-concussion).	miRNA (plasma)	Whole blood	miR-221-3p levels were decreased at 6- and 13-days post-concussion, and miR-27a-3p levels were decreased at 6-days post-concussion, when compared to baseline. Both miR-27a and miR-221-3p levels were inversely correlated with concussion symptom severity. miR-221 plays a role in neuronal cell differentiation vascular homeostasis, miR-27a has been related to regulation of neuroinflammation.
Di Pietro *et al.* (Di Pietro V, O'Halloran P, Watson CN, Begum G, Acharjee A, Yakoub KM, et al. Unique diagnostic signatures of concussion in the saliva of male athletes: the Study of Concussion in Rugby Union through MicroRNAs (SCRUM). Br J Sports Med [Internet]. Br J Sports Med;, 2021)	106 rugby athletes with concussion, 50 rugby athletes for who concussion was ruled out, 102 non-injured control rugby athletes.	258 males	Mixed ethnicities	26.7	HIA and SCAT-5 (samples were collected in-match, post-match and 36–48 h post-match).	miRNA (salivary)	Saliva	14 miRNAs associated with neuroinflammation and roles in AD and depression, could differentiate concussed athletes from all other athlete groups, immediately after a match (let-7a-5p, miR-143-3p, miR-103a-3p, miR-34b-3p, RNU6-7, RNU6-45, snora57, snoU13.120, tRNA18Arg-CCT, U6-168, U6-428, U6-1249, Uco22cjg1, YRNA-255).
Fedorchak *et al.* (Fedorchak et al., 2021)	32 youth participants with a persistent post-concussion symptoms, 80 youth control participants with non-persistent post-concussion symptoms history of concussion	63 males, 49 females	Mixed ethnicities	15.9	GCS scores (within 24 h post-injury)	ncRNA (salivary)	Saliva	16 ncRNAs demonstrated prognostic utility for persisting symptoms after concussion (7 miRNAs; hsa-miR-486-5p, hsa-miR-1246, hsa-miR-92b-3p, hsa-miR-203a-5p, hsa-miR-148a-5p, hsa-miR-100-5p, hsa-miR-148a-5p; 8 wiRNAs; wiRNA-48, wiRNA-176, wiRNA-1500, wiRNA-9924,

(*continued on next page*)

Table 3 (*continued*)

Study	Participants	Sex	Ethnicity	Age (mean) [Years]	Concussion Diagnosis	Epigenetic mechanism	Tissue type	Main Findings
	N							
								wiRNA-7971, wiRNA-1385, wiRNA-7876; 1 snoRNA; SNORD81).
Guedes *et al.* (Guedes et al., 2021)	71 military service members and veteran with a history of concussion and no PTSD, 45 military service members and veteran with a history of concussion and PTSD, 28 controls with no history of concussion and no PTSD.	125 males, 19 females	Not reported	37.0	GCS scores, VCU rCDI) (mean time since last concussion was 9 years).	miRNA (plasma and extracellular vesicles)	Whole blood	12 miRNAs associated with neurodegenerative and inflammatory processes were differentially regulated between groups; concussion history and no PTSD group vs controls (hsa-miR-139–5p, hsa-miR-204–5p, hsa-miR-372–3p, hsa-miR-509–3-5p, hsa-miR-615–5p, hsa-miR-1277–3p); concussion history and PTSD group vs controls (hsa-miR-3190–3p, hsa-miR-615–5p, hsa-miR-1185–1-3p, hsa-miR-3196, hsa-miR-372–3p, hsa-miR-139–5p).
Hicks *et al.* (Hicks et al., 2021)	75 participants with concussion, 97 control participants with no history of concussion.	107 males, 65 females	Mixed ethnicities	20.4	GCS scores (within 24 h post-injury)	miRNA (salivary)	Saliva	miR-27a-5p was the most accurate miRNA for differentiating concussed and non-concussed participants. A logistic regression model using a ratio of miR-27a-5p and miR-30a-3p provided the highest accuracy for differentiating concussion status.
Lusardi *et al.* (Lusardi et al., 2021)	45 male military veterans with a history of concussion, 18 male military control veterans without a history of concussion, 52 male civilian controls a without history of concussion.	115 males	Mixed ethnicities	33.1	Clinical history via semi-structured interview (mean time between sample collection and the last concussion experienced was 5 years).	miRNA (CSF)	CSF	10 miRNAs were identified with differential expression between veterans with history of concussion and civilian controls (miR-502-3p, miR-362-3p, miR-191-5p, miR-197-3p, miR-30c-5p, miR-140-5p, miR-30b-5p, miR-20a-5p, miR-548a-3p, miR-20b-5p). 8 miRNAs were identified with differential expression between veterans with no history of concussion and veterans with a history of concussion (miR-191-5p, miR-152-3p, miR-132-3p, miR-362-5p, miR- 548c-3p, miR-125a-5p, miR-130a-3p, miR-411-5p).
Papa *et al.* (Papa et al., 2021)	14 collegiate American Football athletes with a history of concussion, 30 non-athlete controls with no history of concussion.	29 males, 15 females	Not reported	26.5	Heads Up Centres for Disease Control'sAcute Concussion Evaluation tools (samples were collected within 24 h of injury).	miRNA (serum)	Whole blood	3 miRNAs that play a role in neurocognitive functioning (miR-505, miR-30d, miR-92a) were associated with lower fractional anisotropy values of white matter dorsal (posterior) cervical spinal tracts in athletes.
Vorn *et al.* (Vorn et al., 2021)	29 participants with a history of concussion, 11 control participants with no history of concussion.	19 males, 21 females	Mixed ethnicities	24.4	Self-reported history of concussion diagnosed using ACRM guidelines (mean time between sample collection and the last concussion experienced was 4 years).	miRNAs (exosomal plasma)	Whole blood	25 significantly dysregulated miRNAs associated with neurological disease, organismal injury and psychological disorders were identified in concussion group compared to controls (upregulated; hsa-miR-520e, hsa-miR-499b-3p, hsa-miR-520b, hsa-miR-4488) (downregulated; hsa-miR-

(*continued on next page*)

Table 3 (*continued*)

Study	Participants	Sex	Ethnicity	Age (mean) [Years]	Concussion Diagnosis	Epigenetic mechanism	Tissue type	Main Findings
	N							
								625-5p, hsa-miR-421, hsa-miR-664a-3p, hsa-miR-28-3p, hsa-miR-125a-5p, hsa-miR-222-3p, hsa-miR-140-5p, hsa-miR-98-5p, hsa-miR-148a-3p, hsa-miR-423-5p, hsa-miR-107, hsa-miR-181a-5p, hsa-miR-374a-5p, hsa-miR-340–5, hsa-miR-29b-3p, hsa-miR-191-5p, hsa-miR-199a-3p, hsa-miR-126-3p, hsa-miR-23a-3p, hsa-miR-142-3p, hsa-miR-223-3p).
Ghai *et al.* (Ghai et al., 2020)	27 military veterans with a history of concussion, 11 military control veterans without a history of concussion, 31 civilian controls without a history of concussion.	69 males	Mixed ethnicities	31.9	Clinical examinations and semi-structured interviews (time lapse since the last concussion and sample collection was 1.6–7.7 years).	miRNA (plasma and extracellular vesicles)	Whole blood	32 miRNAs associated with pathways involved in neuronal function, vascular remodelling, blood–brain barrier integrity, and neuroinflammation in plasma were significantly changed in the chronic concussion veterans group compared with control groups (let-7f-1-5p, miR-103a-1-3p, miR-103b-1-5p, miR-106a-5p, miR-106b-5p, miR-1246-5p, miR-132-5p, miR-142-3p, miR-144-3p, miR-144-5p, miR-148a-5p, miR-15a-5p, miR-15b-5p, miR-16–1-5p, miR-17-5p, miR-182-5p, miR-183-5p, miR-184-3p, miR-18a-5p, miR-19a-3p, miR-20a-5p, miR-20b-5p, miR-223-3p, miR-2355-3p, miR-32-5p, miR-3613-5p, miR-374b-5p, miR-411-5p, miR-4440-3p, miR-484-5p, miR-490-3p, miR-96-5p).
Hicks *et al.* (Hicks et al., 2020)	13 American Football athletes with history of concussion, 18 aged-match controls, 310 controls with mixed history of concussion	239 males, 102 females	Mixed ethnicities	47.0	Diagnosis method of concussion history not reported.	miRNA (salivary)	Saliva	3 miRNAs (miR-28-3p, miR-339-3p, and miR-361-5p) differentiated between the older former professional athletes and younger individuals with prior concussion. In the younger group the same 3 miRNAs differentiated between participants with a history of concussion and those without.
Polito *et al.* (Polito et al., 2020)	20 participants with a history of concussion, 10 matched 'normal' controls.	15 males, 15 females	Not reported	37.8	GCS (samples were collected at 0 h, 24 h and 48 h post-concussion).	miRNA (serum)	Whole blood	10 miRNAs postulated to be involved in the regulation of genes associated with the neurometabolic cascade (miR-151-5p, miR-20a, miR-362-3p, miR-486, miR-505, miR-499, miR-625, miR-638, miR-381, miR-142-3p, miR-30d, miR-328, miR-27b, miR-92) were up-regulated in concussed individuals and progressive reduction at 24 h and 48 h post-concussion was observed.
Davies *et al.* (Davies et al., 2019)	41 athletes with a history of concussion, 15	45 males,	Not reported	25.1	Rugby Football Union HIA and SCAT-5.	miRNA (serum)	Whole blood	miR-502 implicated in the modulation of pathology in extra cranial diseases was

(*continued on next page*)

Table 3 (*continued*)

Study	Participants	Sex	Ethnicity	Age (mean) [Years]	Concussion Diagnosis	Epigenetic mechanism	Tissue type	Main Findings
	N							
	controls without history of concussion.	10 females						downregulated for 7 days post-concussion compared to controls.
Papa *et al.* (Papa et al., 2019)	23 collegiate American Football athletes (9 athletes had a history of concussion and 2 in season concussions reported), 30 non-athlete controls with no history of concussion.	38 males, 15 females	Not reported	25.5	SAC-C (samples were collected pre- and post-season).	miRNA (serum)	Whole blood	Post-season 6 miRNAs (miR-195, miR-92a, miR-30d, miR-505*, miR-151-5p, and miR-362-3p) were elevated in the 2 athletes that sustained concussions over the season, only miRNA-92a was significantly higher post-season compared to non-concussed athletes. Higher levels of 4 miRNAs (miR-20a, miR-505*, miR-195, and miR-151-5p) were associated with lower neurocognitive scores in athletes compared to controls post-season.
Svingos *et al.* (Svingos et al., 2019)	27 collegiate athletes with a history of concussion.	11 males, 16 females	Mixed ethnicities	18.8	SCAT-3, SAC-C, (samples were collected pre-season and as soon as possible post-concussion (mean time of 6.8 h)).	miRNA (serum)	Whole blood	3 miRNAs (miR153–3p, miR223–3p, miR-let-7a-5p) associated with central nervous system dysfunction were upregulated post-concussion in male and female athletes.
Di Pietro *et al.* (Di Pietro et al., 2018)	28 rugby athletes with history of concussion, 16 rugby athlete controls with no history of concussion	44 males	Not reported	27.3	SCAT-3 (samples from concussed players were collected 48, 72 and > 120 h post-concussion).	miRNA (salivary)	Saliva	5 miRNAs (miR-27b-3p, let-7i-5p, miR-142-3p, miR-107, miR-135b-5p) were significantly upregulated in concussed athletes.
Johnson *et al.* (Johnson et al., 2018)	22 youths with acute concussion symptoms, 30 youth participants with prolonged concussion symptoms.	30 males, 22 females.	Mixed ethnicities	14.0	GCS and SCAT-3 within 14 days of injury.	miRNA (salivary)	Saliva	Levels of miRNAs (miR-320c-1, miR-133a-5p, miR-769-5p, let-7a-3p, and miR-1307-3p) associated with neuronal regulatory pathways were able to identify individuals with prolonged concussion symptoms compared to those with acute symptoms. Additionally, 3 miRNAs were associated with specific symptoms 4 weeks post-concussion; miR-320c-1 was associated with memory difficulty, miR-629 was associated with headaches, and let-7b-5p was associated with fatigue.
Pasinetti *et al.* (Pasinetti et al., 2012)	9 military veterans with history of concussion, 9 military control veterans without history of concussion.	13 males, 5 females	Mixed ethnicities	30.7	VAT-BIST (mean time between last deployment and recruitment to the study was 3.5 years).	small nucleolar RNA and miRNA (peripheral blood mononuclear cell isolation)	Whole blood	12 small nucleolar RNA (ACA48, ENSG199411, HBII-239, HBII-289, U15B, U27, U35A, U55, U56, U58B, U83A, U91) and 1 miRNA (hsa-miR-671-5p) were downregulated in veterans with a history of concussion compared to veterans with no history of concussion. 3 small nucleolar RNAs (HBII-289, ENSG199411 and U35A), could distinguish concussion cases in veteran participants.
Hicks *et al.* (Hicks et al., 2020)	251 participants with diagnosed concussion, 287	331 males,	Mixed ethnicities	18.3	Clinical assessments carried out < 14 days post-injury using the	ncRNA (salivary)	Saliva	A four-miRNA model (miR-34a-5p, miR-192-5p, miR-27a-5p, miR-4510) could

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Table 3 (*continued*)

Study	Participants	Sex	Ethnicity	Age (mean) [Years]	Concussion Diagnosis	Epigenetic mechanism	Tissue type	Main Findings
	N							
	controls with no recent history of concussion.	207 females			Zurich Concussion in Sport Group guidelines (samples collected < 3 days, 4–7 days, 8–14 days, 15–30 days and 31–60 days post-concussion).			differentiate concussed participants from non-concussed controls.
Miller *et al.* (Miller et al., 2022)	60 participants with diagnosed concussion.	32 males, 28 females	Mixed ethnicities	14.3	Clinical assessments carried out < 14 days post-injury using the Zurich Concussion in Sport Group guidelines, SAC-C and daily SCAT-5 (samples collected < 7 days, 7–14 days and 28 days post-concussion).	miRNA (salivary)	Saliva	13 miRNA levels (hsa-miR-95-3p, hsa-miR-301a-5p, hsa-miR-626, hsa-miR-548y, hsa-miR-203a-5p, hsa-miR-548e-5p, hsa-miR-585-3p, hsa-miR-378 h, hsa-miR-1323, hsa-miR-183-5p, hsa-miR-200a-3p, hsa-miR-888-5p, hsa-miR-199a-3p, hsa-miR-199b-3p) at 3 timepoints post-concussion were higher in concussed children with persisting symptoms after concussion compared to concussed children without persisting symptoms after concussion.
Mitra *et al.* (Mitra et al., 2022)	28 participants with diagnosed concussion, 30 controls with no history of concussion.	28 males, 30 females	Not reported	31.0	GCS (samples were collected at 0 days, 7 days and 28 days post-concussion).	miRNA (plasma)	Whole blood	At 0 days miR-32-5p was higher in concussed participants compared to controls. In concussed participants reporting higher symptom severity scores, at 7 days miR-32-5p was lower and at 28 days miR-142-3p and miR-223-3p were higher, compared to concussed participants who reported lower symptom severity scores.
Mitra *et al.* (Mitra et al., 2023)	75 participants with diagnosed concussion, 44 controls with no history of concussion.	65 males, 54 females	Not reported	31.5	GCS (samples were collected at < 6 hrs of injury).	miRNA (plasma)	Whole blood	miR423-3p was higher among concussed participants < 6 hrs post-injury compared to controls.
Tas *et al.* (Tas et al., 2020)	79 participants with diagnosed concussion, 92 controls with no history of acute trauma.	134 males, 37 females	Not reported	38.8	GCS (samples were collected < 24 hrs of admission).	miRNA (plasma)	Whole blood	miR191 levels were elevated in participants with concussion compared to controls.
Wyczechewska *et al.* (Wyczechowska et al., 2023)	57 collegiate American Football athletes (10 athletes sustained concussions and 11 non-concussed control athletes)	57 males	Mixed ethnicities	20.0	SCAT-3 and clinical assessments (samples were collected 1–2 hr and 18 hrs post-injury and end of season).	miRNA (serum)	Whole blood	From combining all timepoints, miRNA Let-7c-5p was upregulated in concussed athletes and 12 miRNAs were downregulated (miR181c-5p, miR-146a-5p, miR-200c-3p, miR-22-3p, miR-17-5p, miR26a-5p, miR-154-5p, miR-210-5p, miR-19b-3p, miR-16-5p, miR29a-3p, and miR-181c-3p).
Duan *et al.* (Duan et al., 2021)	110 paediatric patients with a history of concussion, 87 controls with no history of concussion.	108 males, 89 females	Mixed ethnicities	14.9	Clinical assessments carried out 1–10 days post-injury using ACRM and the Zurich Concussion in Sport Group guidelines (samples collected 7.26 days post-concussion).	DNA methylation (850,000 CpG loci)	Saliva	Global methylation did not show significant concussion group versus control group differences. However, DNA methylation factors under the major depression pathway contributed to the prediction of the quality of life but not persistent post-concussive symptoms.

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Table 3 (*continued*)

Study	Participants	Sex	Ethnicity	Age (mean) [Years]	Concussion Diagnosis	Epigenetic mechanism	Tissue type	Main Findings
	N							
Bahado-Singh *et al.* (Bahado-Singh et al., 2020)	18 youths with a history of concussion, 18 youth controls without a history of concussion.	28 males, 8 females	White	12.8	GCS and SAC-C (8-hour time lapse after concussion and sample collection).	DNA methylation (850,000 CpG loci)	Whole blood	449 CpG sites were significantly differentially methylated (some methylation changes ≥ 10 %) in concussed youths compared to youth controls. These CpG sites have been associated with; impaired brain function, cognition, memory, neurotransmission, intellectual disability, and behavioural change disorders.
Lee *et al.* (Lee et al., 2020)	11 collegiate students with a history of concussion, 14 collegiate student controls without history of concussion.	13 males, 12 females	Mixed ethnicities	28.7	Self-reported concussions (mean time lapse since the last concussion and sample collection was 7 years).	Global DNA methylation (5-mC%)	Whole blood	Higher blood global methylation ratio (5-mC%) in concussion cases (4.42 ± 0.43) than in controls (3.93 ± 0.03).
Schwab *et al.* (Schwab et al., 2019)	38 donated professional athlete brains with a history of concussion, 10 controls (5 healthy controls with no history of concussion, 5 AD cases with no history of concussion).	48 males	Not reported	54.6	Donated brains of professional contact sport athletes (>5 years exposure to repeated concussions).	Histone modification	Human brain tissue	Individuals with a history of concussion had a decreased level of H3K27Me3 in the context of DNA damage. This marker is considered marker of cellular senescence conferring susceptibly to brain dysfunction post-concussion.

OSU mTBI-ID, Ohio State university mTBI identification method; VCU rCDI, Virginia Commonwealth University retrospective concussion diagnosis interview; HIA, Head Injury Assessment; ACRM, American Congress of Rehabilitation Medicine; SCAT-3(5), Sport Concussion Assessment Tool-3(5); SAC-C, Standardised Assessment of Concussion score; GCS, Glasgow Coma Scale; CSF; Cerebral spinal fluid; AD, Alzheimer's Disease; PTSD, Post Traumatic Stress Disorder; VAT-BIST, Veteran traumatic brain injury screening tool, AFL, Australian Football League.

4.1. sncRNAs

The most extensively examined epigenetic modifiers in the studies from this review were miRNAs. miRNAs can act as post-transcriptional regulators of pathophysiological and functional cellular processes within the brain (Coolen and Bally-Cuif, 2009; Bhalala et al., 2013). Understanding the roles and functions of miRNA are rapidly expanding. However, for many miRNAs their full role and functions remain unclear. miRNAs are well suited to provide insight to the reactions of the injured brain following concussion as they can cross the blood–brain barrier and are stable in peripheral biofluids (Chen et al., 2016; García-Romero et al., 2017). In contrast, blood brain barrier disruption may be required for protein biomarkers such as, glial fibrillary acidic protein, neurofilament light chain protein and tau to migrate into peripheral tissues (Middeldorp and Hol, 2011) and can be degraded by endogenous proteases (Bhomia et al., 2016). Moderate and severe TBIs result in cell injury, and consequently protein biomarkers can be detected in peripheral tissues which can reliably be used to differentiate the severity of TBI (Honda et al., 2010). However, for the 'mild' form of TBI (concussion) cell injury may be subtle (Gonzalez et al., 2021). Thus, miRNAs and other sncRNAs may be more sensitive for diagnostic and prognostic purposes when compared to protein biomarkers, as their dysregulated expression in live cells may be more readily detected (Goldie et al., 2014).

4.1.1. Potential diagnostic miRNAs markers of concussion

It is possible that miRNAs could provide diagnostic insight to the management of concussion (Fig. 2). For example, the let-7 family and its target genes play a role in neuroinflammation modulation (Lv et al., 2018). Di Pietro *et al.* reported that the upregulated let-7f-5p was able to provide good discrimination (AUC 0.80–0.89) between elite male rugby athletes with a history of concussion (36–68 h post-concussion) compared to those without a history of concussion (Di Pietro et al., 2021). Similarly, Svingos *et al.* observed an acute (~7 h post-concussion) upregulation of let-7f-5p in male and female collegiate athletes (Svingos et al., 2019).

The complex polygenic pathophysiology of concussion reflects the involvement of multiple miRNAs (Buckland et al., 2022;13:1107.; Barter and Foster, 2018). Therefore, the combination of multiple candidate miRNA biomarkers could provide additional objective diagnostic and prognostic insight to an individual's concussion status, alongside validated assessment tools. Di Pietro *et al.* demonstrated a 16 saliva sncRNA signature (let-7a-5p, miR-143-3p, miR-103a-3p, miR-34b-3p, RNU6-7, RNU6-45, snora57, snoU13.120, tRNA18Arg-CCT, U6-168, U6-428, U6-1249, Uco22cjg1, YRNA-255) could accurately identify concussed male elite rugby athletes from non-concussed participants immediately post-match (AUC = 0.91) and 36–48 h post-match (AUC = 0.94) (Di Pietro et al., 2021). Similarly, Hicks *et al.* demonstrated a single saliva miRNA ratio of miR-27a-5p/miR-30a-3p (logit (P) = 0.155 + 0.169) was able to provide a high accuracy (AUC = 0.810, sensitivity = 82.4 % specificity = 73.3 %) for identification of concussion status (Hicks et al., 2021). We propose that a subset of markers identified from this review may provide additional utility as a diagnostic signature although this requires further exploration.

Table 4
Differentially expressed miRNAs identified in multiple studies.

miRNA	Devoto *et al.* (Devoto et al., 2022)	Shultz *et al.* (Shultz et al., 2022)	Di Pietro *et al.* (Di Pietro V, O'Halloran P, Watson CN, Begum G, Acharjee A, Yakoub KM, et al. Unique diagnostic signatures of concussion in the saliva of male athletes: the Study of Concussion in Rugby Union through MicroRNAs (SCRUM). Br J Sports Med [Internet]. Br J Sports Med;, 2021)	Fedorchak *et al.* (Fedorchak et al., 2021) [$]	Guedes *et al.* (Guedes et al., 2021)	Hicks *et al.* (Hicks et al., 2021)	Lusardi *et al.* (Lusardi et al., 2021)	Papa *et al.* (Papa et al., 2021)	Vorn *et al.* (Vorn et al., 2021)	Ghai *et al.* (Ghai et al., 2020)	Hicks *et al.* (Hicks et al., 2020)	Polito *et al.* (Polito et al., 2020)	Papa *et al.* (Papa et al., 2019)	Svingos *et al.* (Svingos et al., 2019)	Di Pietro *et al.* (Di Pietro et al., 2018)	Johnson *et al.* (Johnson et al., 2018)	Hicks *et al.* (Hicks et al., 2020)	Miller *et al.* (Miller et al., 2022) [$]	Mitra *et al.* (Mitra et al., 2022)
Let 7a-5p			↑											↑		↓				
miR-107			↑						↓						↑					
miR-1246 *			↓	↓																
miR-125a-5p*							↓		↓											
miR-126-3p*									↓	↓										
miR-1307-3p										↓						↑				
miR-135b-5p*			↑												↑					
miR-139-5p*	↓				↓					↓										
miR-140-5p*							↓		↓											
miR-142-3p									↓	↑		↑			↑					↑
miR-148a-5p*				↑						↑										
miR-148a-3p			↑						↓											
miR-151a-3p*						↓				↓										
miR-181a-5p*									↓	↓										
miR-182-5p						↓				↑										
miR-18a-5p*	↑									↑										
miR-191-5p*							↓		↓											
miR-192-5p						↓				↓						↑	↑			
miR-199a-3p*									↓	↓								↑ [$]		
miR-204-5p*	↑				↑															
miR-20a-5p							↓			↑										
miR-20b-5p							↓			↑										
miR-21-5p			↑							↓										
miR-222-3p*									↓	↓										
miR-223-3p									↓	↑				↑						↑
miR-27a-3p*		↓								↓										
miR-27b-3p										↓					↑					
miR-28-3p*									↓		↓									
miR-30d								↑				↓								
miR-30e-5p*						↓				↓										
miR-3190-3p*	↑				↑															
miR-362-3p							Mixed [#]	↑				↑								
miR-372-3p*	↑				↑															
miR-423-5p				↑					↓	↓										
miR-505*								↑				↑								
miR-615-5p*	↑				↑															
miR-92a								↑					↑							

[*] miRNA differentially expressed in a consistent direction in at least two studies.
[#] Lusardi *et al.* (Lusardi et al., 2021) miR-362-3p was found in more military concussion participants than community controls but at lower levels. [$]Fedorchak *et al.* (Fedorchak et al., 2021), all participants had a history of concussion, the miRNA differential expression is between participants showing signs of persisting symptoms after concussion and those without symptoms. Papa *et al.* (Papa et al., 2021); Davies *et al.* (Davies et al., 2019); Pasinetti et al. (Pasinetti et al., 2012) were not included in the table as their identified miRNAs were not found in an additional study.

miRNAs	Diagnostic	Prognostic
let7a-5p[37,53,55]	✓	✓
miR-107[44,54]	✓	✓
miR-1246 [37,39]	✓	✓
miR-125a-5p [42,44]	✓	✓
miR-126-3p [44.46]	✓	✓
miR-1307-3p [46,55]		✓
miR-135b-5p [37,54]	✓	
miR-139-5p[35,40,46]		✓
miR-140-5p [42,44]		✓
miR-142-3p[44,46,49,54,59]	✓	✓
miR-148a-5p [39,46]		✓
miR-148a-3p [37,44]	✓	✓
miR-151a-3p[41,46]	✓	✓
miR-181a-5p [44,46]	✓	✓
miR-182-5p [41,46]	✓	✓
miR-18a-5p [35,46]		✓
miR-191-5p [42,44]		✓
miR-192-5p[41,46,55,57]	✓	✓
miR-199a-3p[44,46,58]	✓	✓
miR-204-5p [35,40]		✓
miR-20a-5p [42,46]		✓
miR-20b-5p[42,46]		✓
miR-21-5p [37,46]	✓	✓
miR-222-3p[44,46]	✓	✓
miR-223-3p[44,46,53,59]	✓	✓
miR-27a-3p [36,46]		✓
miR-27b-3p [46,54]	✓	✓
miR-28-3p [44,47]	✓	✓
miR-30d [43,49]	✓	✓
miR-30e-5p [41,46]	✓	✓
miR-3190-3p[35,40]		✓
miR-362-3p [42,43,49]	✓	✓
miR-372-3p [35,40]		✓
miR-423-5p [39,44,46]		✓
miR-505 [43,49]	✓	✓
miR-615-5p [35,40]		✓
miR-92a [43,51]	✓	

Fig. 2. Potential diagnostic and prognostic miRNAs markers of concussion.

4.1.2. Potential prognostic miRNAs markers of concussion

In addition, it is possible that miRNAs could provide some sort of prognostic use in the management of concussion (Fig. 2). For example, increased levels of miR-1307-3p could identify youths with persisting symptoms post-concussion (4–8 weeks) (Johnson et al., 2018). Similarly, increased levels of miR-1307-3p were observed in adult military veterans 2–8 years after sustaining a concussion (Ghai et al., 2020). In recovery from concussion, Fedorchak *et al.* developed a signature using

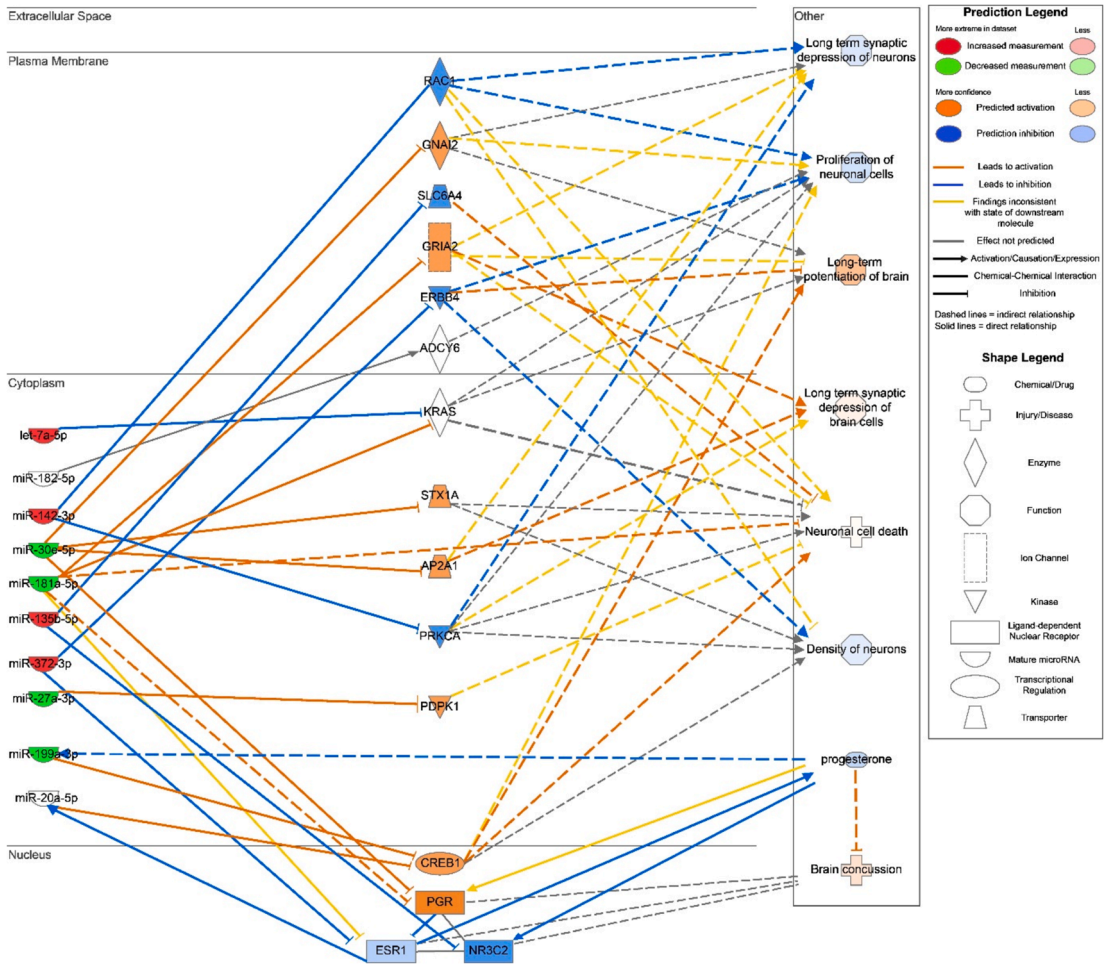

Fig. 3. Ingenuity pathway analysis network of 10 miRNAs previously associated with concussion. Red-labelled miRNAs indicate increased expression post-concussion. Green-labelled miRNAs indicate decreased expression post-concussion. White-labelled miRNAs indicate inconclusive evidence to suggest direction of expression change post-concussion.

16 saliva sncRNAs (7 miRNAs, 1 snoRNA and 8 piRNA clusters) combined with age that could better predict persisting symptoms after concussion in youths (AUC = 0.83) than cognitive and balance testing (AUC = 0.79) (Fedorchak et al., 2021). However, combining sncRNAs with cognitive and balance testing yielded the greatest predictive accuracy (AUC = 0.86). Similarly, Johnson *et al.* developed a saliva miRNA model (miR-320c-1, miR-133a-5p, miR-769-5p, let-7a-3p, and miR-1307-3p) that could accurately identify youths with prolonged concussion symptoms (AUC = 0.86) (Johnson et al., 2018). Findings indicate that some miRNAs potentially have both diagnostic and prognostic properties (Fig. 2). Whilst these findings are still in the preliminary stages, individual biomarkers or combined models of epigenetic biomarkers may yet provide a useful corroborative diagnostic and prognostic tool for concussion management.

Ingenuity pathway analysis provides a valuable tool to help interpret and understand the significance of miRNA changes, and can identify gene signatures, which may provide putative biomarkers associated with concussion. With improved understanding of these pathway perturbations, clinicians may be better prepared to innovate targeted therapeutic interventions to improve patient outcomes post-concussion. Our work shows that a subset of 37 miRNAs have been identified from multiple studies, of which 10 were identified as having network interactions with 15 neuropathology associated genes. Further replication studies are needed to provide strong supporting evidence to confirm whether this gene signature is associated with concussion and further sncRNAs may be used to identify additional key networks, causal relationships, and novel regulatory pathways that assist with the discovery of potential concussion biomarkers for diagnostic and prognostic purposes.

4.2. *DNA methylation*

Altered levels of global DNA methylation (average methylation status that occurs across the genome) have been associated with neuroinflammation and neurodegeneration such as, short-term memory loss (Tsankovaet al., 2007; Tooby et al., 2023). Lee *et al.* demonstrated that concussion had a long-term effect on the global methylation ratio (5-mC %), as college athletes with a history of concussion (seven years post-concussion) had a higher blood global methylation ratio (4.42 ± 0.43) than controls with no history of concussion (3.93 ± 0.03) (Lee et al., 2020). In contrast, Duan *et al.* did not observe significant differences in global methylation between the youth concussion group compared to the control group (Duan et al., 2021). Differences in methods employed may account for these conflicting findings. Duan *et al.* collected saliva samples at 7.26 days post-concussion which, may not have been sufficient time for global methylation changes to occur in youths (Duan et al., 2021). The methylation profile of saliva could be different to that of blood, as it is more distant from the injury site. In addition, under/over estimation of global methylation may occur between studies as different assays were used (Kurdyukov and Methylation, 2016). Indeed, Duan *et al.* used Infinium Methylation EPIC array (Duan et al., 2021), whilst Lee *et al.* used enzyme-link immunosorbent assay (Lee et al., 2020).

However, global methylation ratios could be a useful tool in the future as an epigenetic marker of concussion recovery and potential associated neurodegeneration as changes in epigenetic marks have long-lasting effects within neurons (Tsankova, 2007). In addition, using artificial intelligence techniques such as deep learning, Bahado-Singh *et al.* demonstrated that 119 CpG methylation markers provided a classifier with good diagnostic accuracy (AUC $\geq$ 0.80–0.89) for the detection of paediatric concussion (Bahado-Singh et al., 2020). In addition, combining epigenetic data with clinical data relating to concussion such as Sport Concussion Assessment Tool (SCAT) scores further increased the diagnostic accuracy of the concussion prediction model. Findings suggest that DNA methylation changes can be detected years post-concussion. Thus, providing long-term markers of concussion that could be used to monitor recovery may present target sites for therapeutic development and stratified intervention.

4.3. Histone modification

There is a paucity of data about the effects of concussion on histone modifications and the potential influences on outcomes and recovery post-concussion. Findings from Schwab *et al.* suggest that an inability to effectively repair DNA could impact the long-term effects of concussion (Schwab et al., 2019). Histone H3K27Me3 modifications are reduced in the context of DNA damage in individuals with a history of concussion (Schwab et al., 2019). Reduced expression of this histone mark in glial cells suggests activation of senescence through activation of p16 and p21 and is linked with neurodegenerative and cognitive decline (Schwab et al., 2019). Reduced expression of H3K27Me3 may also confer altered DNA damage responses potentially influencing recovery and risk of neurodegenerative disease post-concussion. It is plausible that a combination of histone modification, DNA methylation, and sncRNA expression contribute to neurodegenerative pathology and symptomology associated with concussion.

4.4. Clinical applications

In-depth neurological examination and sophisticated neuroimaging are not widely used to diagnose concussion, especially in youth and sub-elite level sports. Typically, clinicians reply upon subjective symptom reports to inform diagnosis, which could contribute to the under-diagnosis of concussions (Broglio et al., 2017; Meehan et al., 2013; McCrea et al., 2004). Individual epigenetic profiles in response to concussion could have the capacity to provide additional objective information to clinicians when diagnosing, managing recovery, and informing return to play processes in conjunction with current methods. Such profiles could be correlated against data derived from the developing use of impact markers, such as sensors placed in mouthguards (Tooby et al., 2023), to inform what level of force correlates with significant change in neurological cellular function – both within the general athletic population and individuals.

Epigenetic markers could be used to confirm that an individual has not sustained a concussion, thus providing reassurance of a negative concussion test. Another important clinical application is that miRNAs could serve as potential biomarkers to aid prognosis of concussion to identify individuals more at risk for persistent post-concussive symptoms and those who will have a typical recovery. Individualised therapy could be honed with the additional information derived from epigenetic markers, for example, early detection of markers associated with post-concussion chronic migraines could inform triptan therapy (Capi et al., 2020). Aerobic exercise reduces elevated post-TBI levels of miR-21 and facilitates cognitive improvements post-TBI in mice (Hu et al., 2015). Such findings suggest that monitoring of miRNAs as part of return to play protocols may inform the knowledge of likely recovery times for an individual. Sub-concussive head injuries are a growing concern due to the associations with neurodegenerative conditions such as chronic traumatic encephalopathy [92–93]. Future research investigating epigenetic markers sensitive enough to detect sub-concussive insults to the brain could provide a useful tool to inform protective protocols for individuals. In addition, markers of sub-concussion could provide insight into the long-term effects on brain health. However, these clinical applications are currently speculative, as findings highlighted in this review need to be sufficiently validated before future guidance for clinical care and practice.

4.5. Limitations

This review is limited by challenges in combining and comparing methods utilised such as sample tissue types, populations, mechanisms of concussion, injury diagnoses, sample collection times. The assays platforms used to measure epigenetic alterations were also non-uniform. In addition, we recognise that epigenetic modifications exhibit population differences (Barfield et al., 2014) therefore, the use of heterogeneous cohorts in studies could affect findings and may not replicate across different populations. For miRNAs that are upregulated shortly after concussion and downregulated years later, future studies could investigate whether there is a long-term compensation mechanism which occurs and remains even after reaching pre-injury baseline levels. Longitudinal studies that monitor the dynamic sncRNA responses post-concussion could be used to provide both short and long-term prognostic insight into recovery and potential neuropathological consequences of concussion. Concussion-related epigenetic responses are complex and can be influenced by many additional confounding factors outside of the injury mechanism including demographics, ethnicity, age, sex, lifestyle, comorbidities, and medications (Alegría-Torres et al., 2011). Controlling for confounding factors in relation to concussion injuries will provide further insight into the molecular mechanisms of concussion processes and improve the management of this injury.

5. Conclusion

The emerging evidence highlights complex epigenetic variation that influences concussion pathophysiology. This knowledge is contributing to our understanding of genes, pathways and biological networks involved in response to concussion. Indeed, supplementary epigenetic information has the potential to assist in identification of concussions and to assist in individualised concussion management strategies. In our opinion, salivary candidate miRNAs could prove the most practical and insightful tool in managing an individual's concussion status. As indicated by our IPA, it is likely that combining data from multiple concussion-associated miRNAs reflects the complexity of this injury and could increase the accuracy of a practical tool for concussion screening and management. However, the research is still in its infancy and significant knowledge gaps exist. Each concussion is a unique injury and is influenced by numerous genetic and environmental factors. This creates many challenges to consider when investigating epigenetic markers for future studies. However, for epigenetic markers of concussion to have a practical application, they will need to demonstrate replicability across studies with consistent findings evidenced.

CRediT authorship contribution statement

Mark R. Antrobus: Writing – original draft. **Terun Desai:** Writing – original draft. **David Young:** Writing – original draft. **Lee Machado:** Writing – original draft. **William J. Ribbans:** Writing – original draft. **Louis Y. El Khoury:** Writing – original draft. **Jon Brazier:** Writing – original draft.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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