Circulating microRNA evaluated in the early aftermath of motor vehicle collision predict widespread pain development in African Americans and provide potential pathogenic insights: results of a preliminary analysis

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Trauma RecoverY: Understanding Mechanisms & Promoting Healing

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INTRODUCTION

microRNA (miRNA) are small non-coding RNA molecules that regulate gene expression by binding target mRNA. miRNA are highly stable in blood.

During the past decade, the study of miRNA has transformed understanding of the regulation of major biological pathways and advanced understanding of the molecular pathogenesis of many common diseases. Available data suggest that miRNA may also play a critical role in the pathogenesis of many chronic pain conditions.[1]

Widespread pain (WP) is a disorder associated with substantial suffering and disability.[2] One known trigger of WP is motor vehicle collision (MVC).[3,4] A recent study found that 179/859 (21%) of European American individuals presenting to the emergency department (ED) after MVC and discharged to home after evaluation developed WP.[5]

Molecular mechanisms mediating the development of WP after MVC remain poorly understood. This lack of understanding is a major barrier to the development of preventive interventions.

OBJECTIVE

The purpose of this preliminary investigation was to evaluate whether miRNA circulating in the immediate aftermath of MVC differ in individuals who do and do not subsequently develop WP 6 weeks after MVC. This investigation was performed in an African American (AA) cohort because AAs are a high risk,[6] understudied group.

METHODS

ED evaluation included assessment of participant sociodemographic characteristics and blood sample collection (PAXgene RNA tube). Number of body regions with pain (NBRP) prior to MVC was assessed in the ED, NBRP 6 weeks after MVC was assessed via telephone/internet-based questionnaire. WP was defined by the presence of $\geq$7 body regions of pain; 2 individuals reported WP prior to MVC and were dropped from analyses.

miRNA expression was evaluated using Next Generation Sequencing (NGS). miRNA expressed at <300 counts across all samples were removed. NGS data were normalized using standard quantile methods. miRNA expressed in those with and without WP at 6 weeks were compared using the Mann–Whitney *U* test. DIANA miRPath[7] predictions were then used to identify biological pathways targeted by WP-associated miRNA. Target Scan was used to identify pain genes predicted to be targeted by differentially expressed miRNA.

FIGURE 1. Study sites and methods

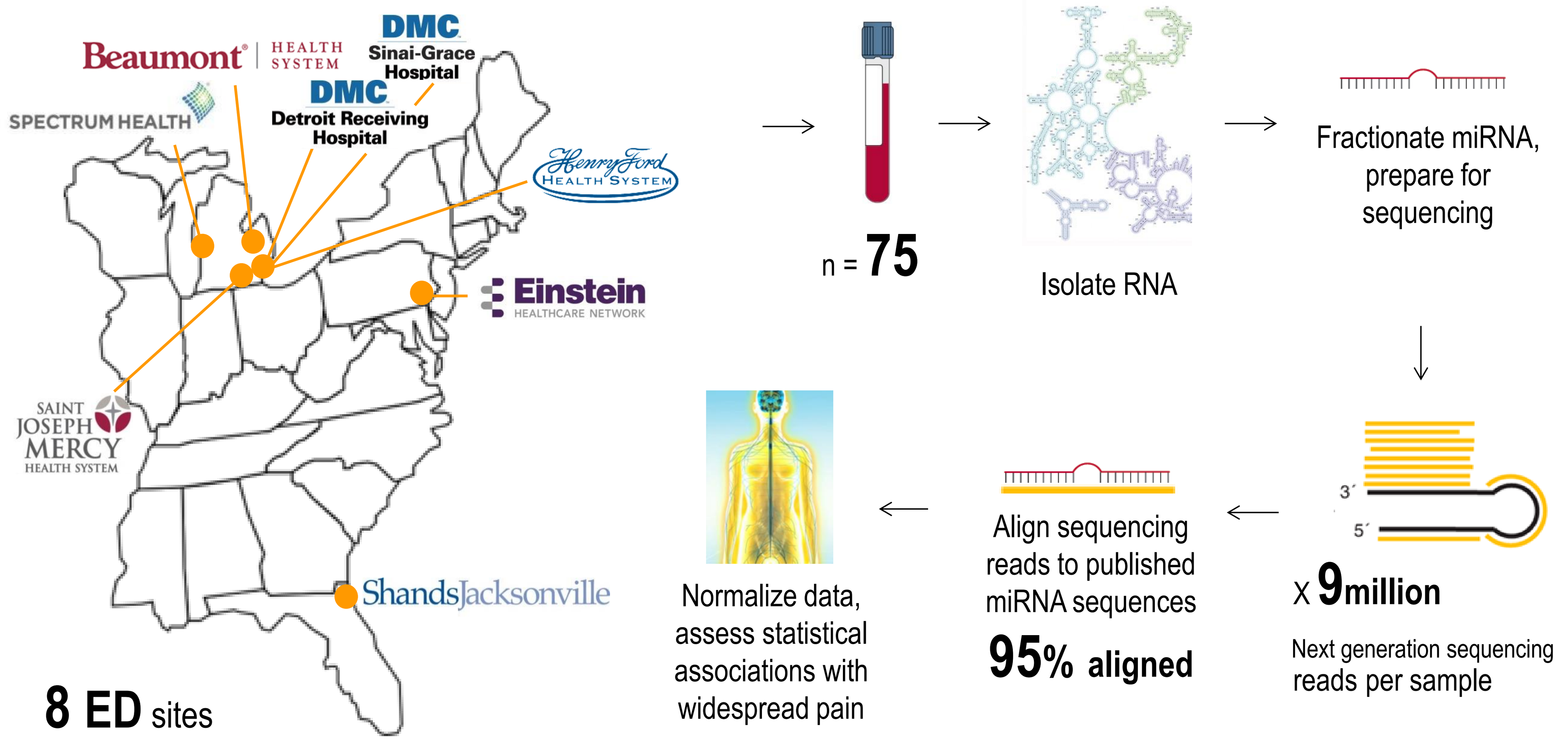

TABLE 1. Study characteristics

Characteristic	
Participants, n	**75**
Age, yrs, mean (SD)	36 (12)
Females, n (%)	44 (58)
Education, n (%)	
8-11 years	6 (8)
HS	16 (21)
Post-HS	4 (5)
Some college	33 (44)
College	12 (16)
Post-college	2 (2.7)
Time to ED, mean (SD)	1h46 (2h)

TABLE 2. miRNA whose ED expression levels differed in AAs who did (n = 24) and did not (n = 49) subsequently develop WP 6 weeks after MVC

miRNA	Fold change	P value
miR-641	-2.2	.009
miR-361-5p	-1.8	.012
miR-326/ miR-330-5p	-1.6	.023
miR-92a-2-3p	-1.7	.030
miR-484	-1.5	.032
Let7b-3p	-1.5	.037
miR-550a-3p	-1.6	.040
miR-140-3p	-1.4	.041
miR-296-5p	-1.3	.041
miR-10a-5p	-1.3	.042

TABLE 3. Ten biologic pathways most significantly enriched in targeting by miRNA differentially expressed between AA who did and did not subsequently develop WP 6 weeks after MVC

KEGG pathway	P value
Long Term Potentiation	7.0×10^{-20}
Neurotrophin signaling	3.5×10^{-15}
ErbB signaling	1.7×10^{-13}
Pancreatic cancer	1.0×10^{-11}
PI3K-Akt signaling	1.0×10^{-11}
Pathways in cancer	1.0×10^{-11}
Glioma	2.8×10^{-10}
Non-small cell lung cancer	2.9×10^{-10}
Renal cell carcinoma	6.9×10^{-10}
Focal adhesion	6.9×10^{-10}

TABLE 4. miRNA associated with WP development following MVC are predicted to target a substantial number of genes involved in pain processing[9]

296-5p	361-5p	10a-5p	550a-3p	484	140-3p	326/ 330-5p	641	92a-3p
ADRBK1	ANO1	BDNF	ADCY1	CD40	ADAMTS5	CACNA1E	ADAM11	ADM
GABBR2	BDNF	GRIK1	CACNA1B	FRMPD4	BDNF	CHRM1	ADAMTS5	CACNA1H
LEP	GUCY1B3	GRIN3A	CACNB3	PTGER4	CD274	DRD2	ADRBK2	CAMK2A
	IL10	HCN1	PPARA	PTGIR	GABBR1	EFNB1	CDK5R1	CDK5R1
	ST8SIA1	PPARA	SCN2B	SLC6A1	GRM2	GABBR2	EFNB1	CHRM2
		ST8SIA1	SLC12A5	SLC6A4	HDC	GDNF	HCN1	CNR1
		STARD13			MAPK1	GRIN1	IL6ST	PER2
					P2RY2	LMX1B	KCNA1	PRKAR1B
					RPS6KA3	MAPK1	KIF1A	PRKCE
						MMP24	KIT	PTGER4
						NGFR	LXN	RAG1
						P2RX4	MAPK10	SLC12A2
						P2RY2	NPTX1	SLC12A5
						RPS6KA3	OPRK1	SLC17A6
						SCN2B	PCSK2	SLC32A1
							PLCL1	SLC6A1
							SCN2B	STARD13
							ST8SIA1	STK39
								ZEB2
								EDNRB
								FMR1
								FSTL1
								GRM2
								HCN2
								HOXB8
								IL6ST
								ITPR1
								KCNA1
								KCND2
								KCNJ3
								KCNK3
								LYST
								MGLL
								NOX4

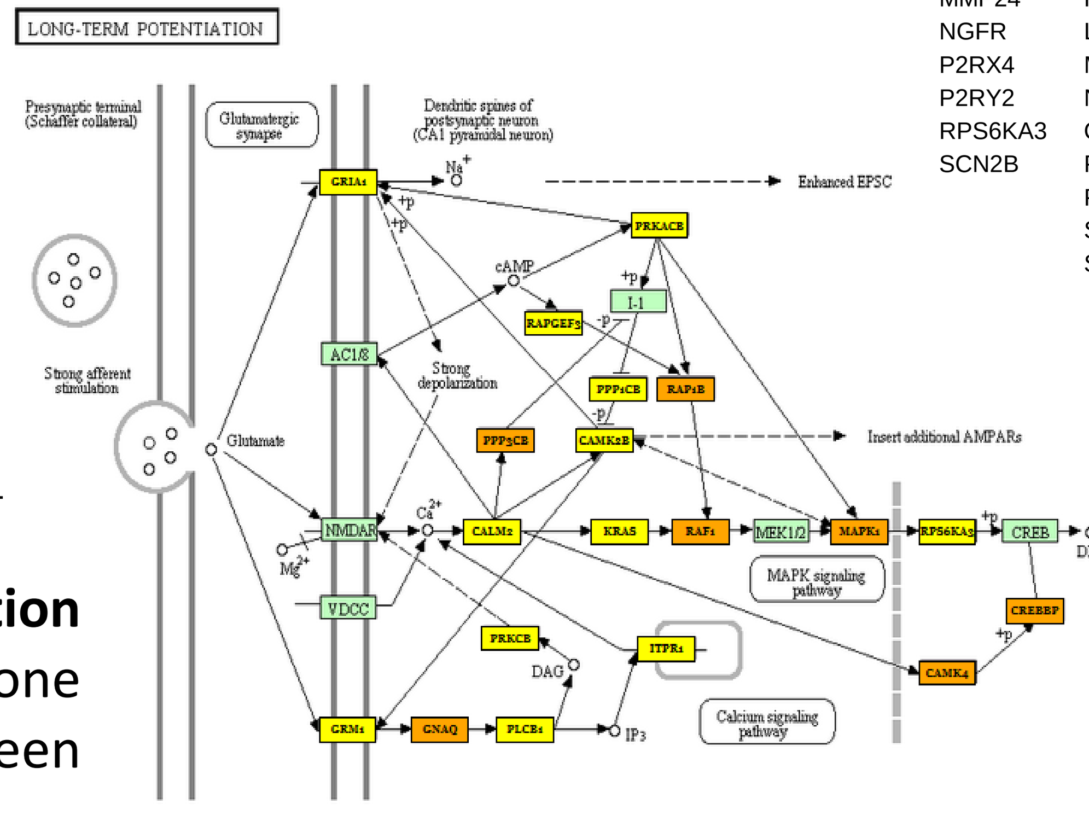

FIGURE 2. Map of miRNA targets within the Long Term Potentiation pathway. Orange boxes designate genes targeted by more than one miRNA, yellow boxes designate genes targeted by one miRNA, and green boxes designate genes targeted by no miRNA.

RESULTS

Participant characteristics are shown in Table 1. NGS resulted in an average of 9 million sequencing reads per individual, 95% of which aligned to miRNA (Figure 1).

Even in this small sample, 11 miRNA were expressed at significantly different levels in AA who subsequently did and did not develop WP 6 weeks after MVC (Table 2).

Pathway analyses (DIANA miRPath) mapped WP-associated miRNA to a number of neurological/neurotransmitter pathways (Table 3).

Long term potentiation, the biologic pathway most enriched in targeting by differentially expressed miRNA, has been shown to play a role in pain pathogenesis and is predicted to be targeted by differentially expressed miRNA at multiple pathway locations (Figure 2).

miR-92a-3p, which has been shown to exhibit increased expression after acute stress in pre-clinical models,[8] was predicted to target the most genes involved in pain processing, followed by miR-641 and miR-326/miR-330-5p (Table 4).

CONCLUSIONS

These data suggest that miRNA studies may yield novel insights into the pathogenesis of WP after traumatic events such as MVC. Further studies will expand the sample size of the study, examine additional pain outcomes, and will examine differences in miRNA isoforms, modified miRNAs, and trimmed or tailed miRNAs.

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