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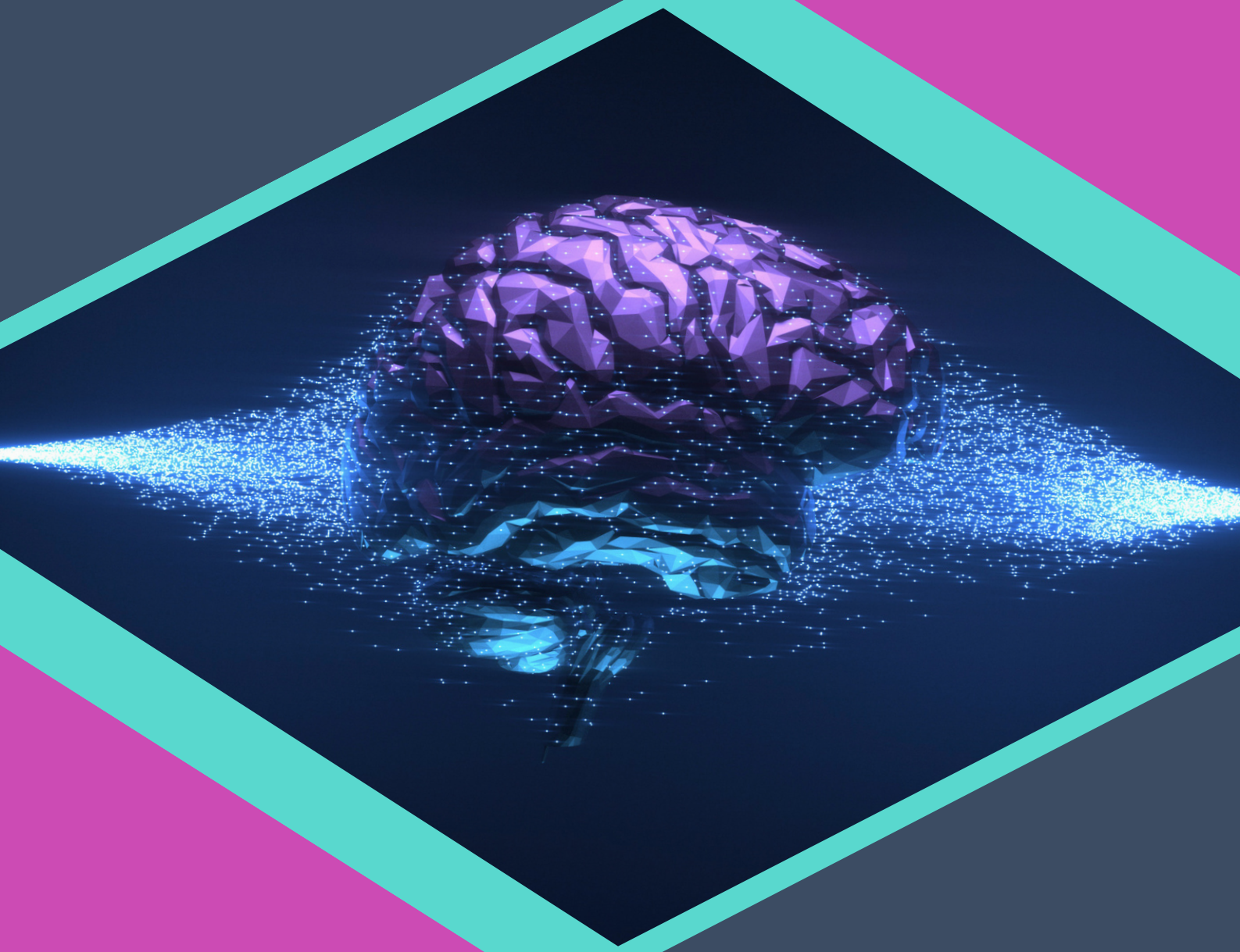

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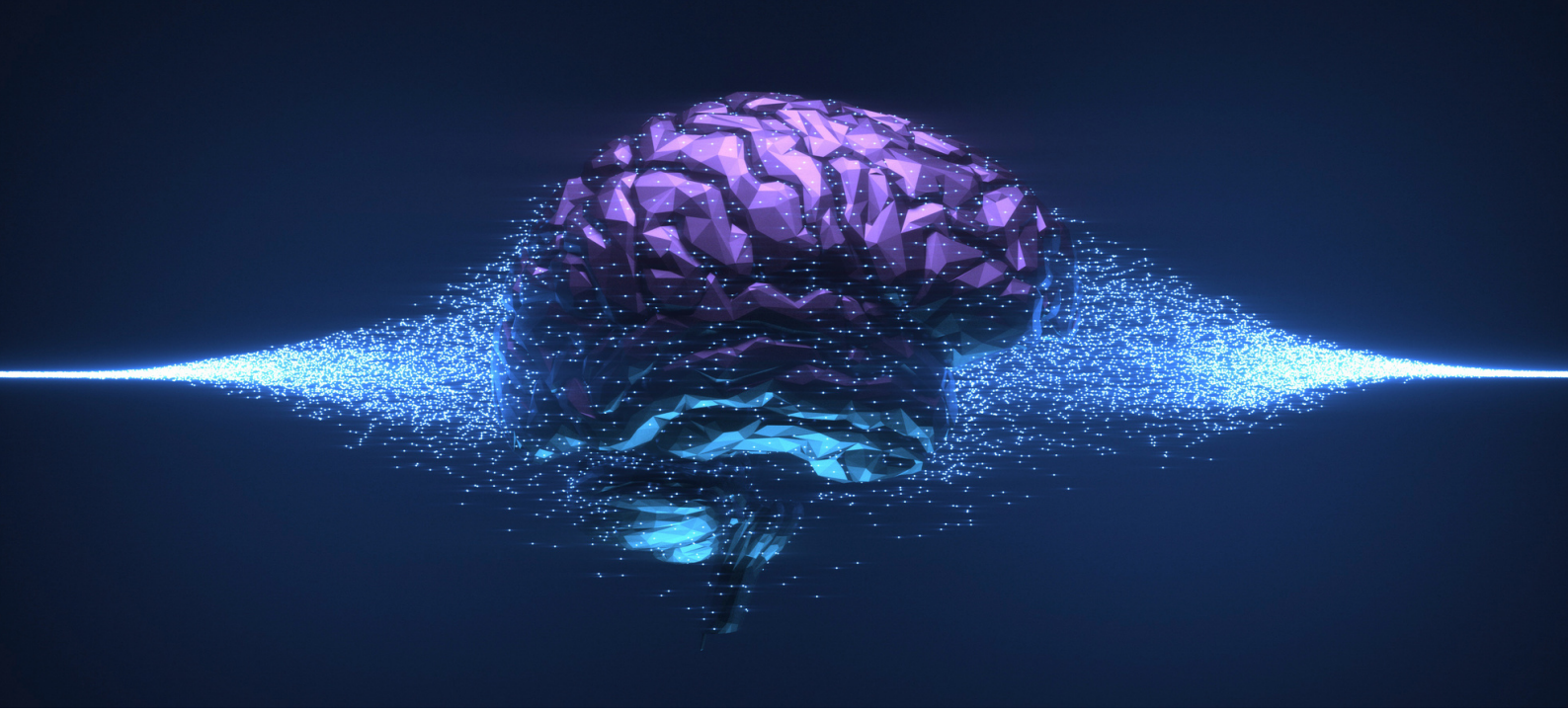

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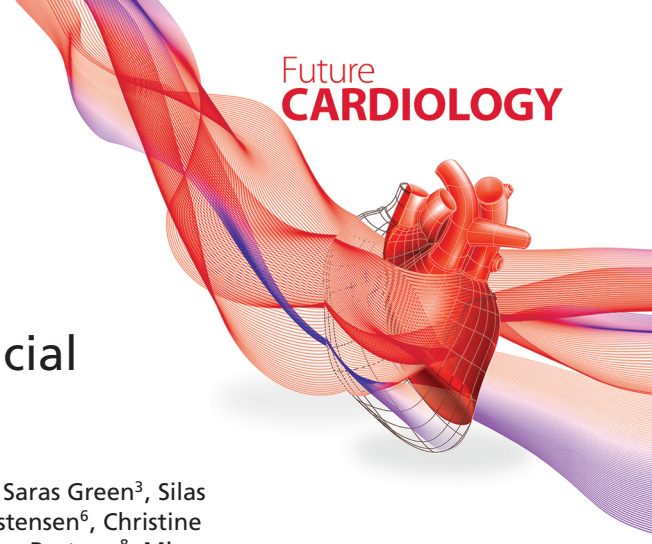

Future CARDIOLOGY

Multiomics, virtual reality and artificial intelligence in heart failure

Patrick A Gladding*[,1], Suzanne Loader[1], Kevin Smith[2], Erica Zarate[3], Saras Green[3], Silas Villas-Boas[3], Phillip Shepherd[4], Purvi Kakadiya[4], Will Hewitt[5], Eric Thorstensen[6], Christine Keven[6], Margaret Coe[6], Bahareh Nakisa[7], Tan Vuong[7], Mohammad Naim Rastgoo[8], Mia Jüllig[9], Vito Starc[10] & Todd T Schlegel[11,12]

[1]Department of Cardiology, Waitemata District Health Board, Auckland, 0620, New Zealand
[2]Clinical Laboratory, Waitemata District Health Board, Auckland, 0620, New Zealand
[3]School of Biological Science, University of Auckland, Auckland, 1010, New Zealand
[4]Grafton Genomics Ltd, Liggins Institute, University of Auckland, Auckland, 1023, New Zealand
[5]Auckland Bioengineering Institute, University of Auckland, Auckland, 1010, New Zealand
[6]Liggins Institute, University of Auckland, Auckland, 1023, New Zealand
[7]School of Information Technology, Deakin University, Victoria, 3125, Australia
[8]School of Electrical Engineering & Computer Science, Queensland University of Technology, Brisbane, QLD, 4072, Australia
[9]Paper Dog Limited, Waiheke Island, Auckland, 1081, New Zealand
[10]Faculty of Medicine, University of Ljubljana, Ljubljana, 1000, Slovenia
[11]Karolinska Institutet, Stockholm, Sweden, 171 77, Switzerland
[12]Nicollier-Schlegel Sàrl, Trélex, Karolinaka, 1270, Switzerland
*Author for correspondence: patrick.gladding@waitematadhb.govt.nz

Aim: Multiomics delivers more biological insight than targeted investigations. We applied multiomics to patients with heart failure (HF) and reduced ejection fraction (HFrEF), with machine learning applied to advanced ECG (AECG) and echocardiography artificial intelligence (Echo AI). **Patients & methods:** In total, 46 patients with HFrEF and 20 controls underwent metabolomic profiling, including liquid/gas chromatography–mass spectrometry and solid-phase microextraction volatilomics in plasma and urine. HFrEF was defined using left ventricular (LV) global longitudinal strain, EF and N-terminal pro hormone BNP. AECG and Echo AI were performed over 5 min, with a subset of patients undergoing a virtual reality mental stress test. **Results:** A-ECG had similar diagnostic accuracy as N-terminal pro hormone BNP for HFrEF (area under the curve = 0.95, 95% CI: 0.85–0.99), and correlated with global longitudinal strain ($r = -0.77$, $p < 0.0001$), while Echo AI-generated measurements correlated well with manually measured LV end diastolic volume $r = 0.77$, LV end systolic volume $r = 0.8$, LVEF $r = 0.71$, indexed left atrium volume $r = 0.71$ and indexed LV mass $r = 0.6$, $p < 0.005$. AI-LVEF and other HFrEF biomarkers had a similar discrimination for HFrEF (area under the curve AI-LVEF = 0.88; 95% CI: -0.03 to 0.15; $p = 0.19$). Virtual reality mental stress test elicited arrhythmic biomarkers on AECG and indicated blunted autonomic responsiveness (alpha 2 of RR interval variability, $p = 1 \times 10^{-4}$) in HFrEF. **Conclusion:** Multiomics-related machine learning shows promise for the assessment of HF.

Lay abstract: Multiomics is the integration of multiple sources of health information, for example, genomic, metabolite, etc. This delivers more insight than targeted single investigations and provides an ability to perceive subtle individual differences between people. In this study we applied multiomics to patients with heart failure (HF) using DNA sequencing, metabolomics and machine learning applied to ECG echocardiography. We demonstrated significant differences between subsets of patients with HF using these methods. We also showed that machine learning has significant diagnostic potential in identifying HF patients more efficiently than manual or conventional techniques.

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Keywords: artificial intelligence • metabolomics • multiomics • volatilomics

Future Medicine

New diagnostic and management tools are needed for the emerging epidemic of heart failure (HF). While the introduction of blood-based biomarkers, such as N-terminal pro hormone BNP (NTproBNP), has improved diagnosis of HF in the community, more work is needed to identify the causes of HF, stratify the syndrome into its subtypes for targeted therapies and identify patients at higher risk for adverse events, such as ventricular arrhythmia. As containment of healthcare costs has become paramount, increased efficiency must also be achieved with often diminishing resources, and with a strong emphasis on portability and accessibility. The emergence of low cost sensors, ubiquitous computing and the internet of things, as well as artificial intelligence (AI) applied to hospital data hold promise for addressing both individual and population scale diagnostic and treatment gaps [1].

Deep phenotyping with multiomics, combined with AI applied to wearable devices and existing clinical data, holds considerable promise in identifying novel low-cost biomarkers and intermediate endophenotypes for early disease stratification and prognostication [2]. Deep learning, using convolutional neural networks, applied to digital ECG is one of the more promising applications of AI in HF [3,4]. However it lacks the transparency and explainability required to stratify patients and identify disease mechanisms. We have shown that a machine learning model applied to digital 12L ECG can identify moderate to severe left ventricular systolic dysfunction (LVSD) [5] with a similarly high degree of accuracy. As the method is transparent, it also reports a number of well validated ECG biomarkers of arrhythmia including the spatial QRS-T angle [6]. Stratification of HF patients by the spatial QRS-T angle and other discrete parameters identifies those at higher risk for HF readmission, implantable cardioverting defibrillator (ICD) implantation and death [6]. Deep learning applied to echocardiography has also been shown to both accurately classify views and LV ejection fraction (LVEF), chamber volumes, LV mass, global longitudinal strain (GLS) and diagnoses [7–9]. Furthermore AI has been shown to also assist echocardiography image acquisition by probe guidance [10]. With these tools, AI-enabled unskilled users could potentially use point of care ultrasound (POCUS) to diagnose HF and its most likely cause.

Wearable devices have the advantage of gathering longitudinal data from continuous monitoring rather than from episodic hospital encounters. The LINK-HF study showed that personalized AI modeling applied to data from a single lead ECG patch accurately identified patients 6–7 days prior to readmission with HF [11]. The advantage of wearable sensors is that a wide range of activities are captured within the context of life events including, for example, walking pace, sleep, exercise and stress. Mental stress is of considerable interest as not only has it been shown to have a significant negative impact on cardiovascular health, for example, Takotsubo syndrome, but similar to an exercise stress test mental stress can be applied in a standardized fashion. Integrating multiomics data across multiple domains such as metabolomics, advanced ECG (AECG), echocardiography and mental stress testing with wearable devices is complex but achievable.

We therefore undertook an investigation of the utility of multiomics and deep phenotyping in patients with HF with reduced EF (HFrEF) using AI and machine learning applied to standard clinical data. In a subset of HF patients and controls, we also applied a standardized virtual reality mental stress test (VR-MS), using validated methods, to evaluate stress biometrics from a wearable device and arrhythmic biomarkers identified by AECG. The primary objective was to evaluate the diagnostic accuracy of AECG and echocardiography AI (Echo AI) in patients with HF. The secondary objective was to explore the biophysical response to a standardized VR-MS. We hypothesized that advanced diagnostic tools utilizing machine learning would accurately discriminate HF from healthy controls, and demonstrate the proarrhythmic effects of mental stress in patients with HF.

Patients & methods

Patients

The NanoHF study was approved by the Northern B Health and Disability Ethics Committee (16/NTB/115) (#16/680) and Waitematā District Health Board's IRB (#RM13458). Patients with HFrEF were identified from an echocardiography database, >18 years of age, able to provide written informed consent, and had previously documented signs and symptoms of HF with an EF from 20 to 45% on echocardiography. Exclusion criteria included: diabetes mellitus (Type 1, Type 2 on insulin and/or last available HbA1c ≥65 mmol), chronic renal impairment (estimated glomerular filtration rate [eGFR] <50 ml/min), chronic lung disease (e.g., chronic obstructive airways disease [COPD] and asthma) and/or hospital admission within 3 months of enrolment related to exacerbation of HF. HF was defined as a clinical syndrome with biochemical (NTproBNP >212 pmol/l at any age; normal <35 pmol/l), mechanical (LVEF <50% or GLS <18%) or electrical (using a validated AECG score [12]) evidence

for HFrEF. Enrolment was enriched for patients with devices (ICD and cardiac resynchronization therapy). Controls were self-reported volunteers who also underwent ECG and echocardiography. Recovered HF (HFrec) was defined biochemically, NTproBNP <35 pmol/l, or mechanically GLS ≥18% or LVEF ≥50%.

Biomarkers & genomics

Blood was collected using ethylenediaminetetraacetic acid tubes. After centrifugation at 3000 × *g* for 5 min, plasma was stored at -80°C before being shipped on dry ice to core lab facilities for testing. NTproBNP was measured using a Siemens Dimension Vista assay. First morning urinary levels of titin-N-terminal fragments (U-TTN) were measured by a highly sensitive sandwich ELISA (#27900 Titin N-Fragment Assay Kit, Immuno-Biological Laboratories, Gunma, Japan) system [13]. To avoid effects of concentration or attenuation of urine, the value of titin N-fragment concentration was corrected by the value of creatinine, and expressed by the following creatinine ratio: (U-TTN/Cr; pmol/μmol/l), as previously described [13].

Metabolomics

Plasma and urine samples underwent gas chromatography–mass spectrometry analysis using a methyl chloroformate derivatization, and solid-phase microextraction (SPME) volatilomics using an Agilent 7890A gas chromatograph coupled to a 5975C inert mass spectrometer. Plasma samples were analyzed using targeted liquid chromatography–mass spectrometry. A metabolomics approach was used to analyze plasma samples from HFrEF patients and controls via an AbsoluteIDQ p400 kit (Biocrates Life Sciences AG, Innsbruck, Austria) using a Thermo Q-Exactive Orbitrap liquid chromatography–mass spectrometry. SPME results were validated using a Ketoscan mini (Sentech, Gyeonggi-Do, Korea) in a sample of cardiac inpatients and outpatients.

DNA sequencing

DNA was extracted from buffy coat and underwent sequencing of 174 genes associated with inherited cardiac disease using the Cardiac Trusight panel on an Illumina MiSeq (Grafton Genomics, Auckland, New Zealand). Cardioclassifier (https://www.cardioclassifier.org/ [Imperial College London, 2017]) was used for variant calling.

Advanced ECG

ECGs were recorded using a Cardiax machine (Imed, Budapest, Etele, Hungary). AECG analyzed parameters included those derived from the conventional scalar 12-lead ECG, as well as from signal averaging of all adequately cross-correlated QRS and T complexes by using software originally assembled at NASA [12,14] to generate results for: several spatial (derived vectorcardiographic or 3D) ECG parameters including the spatial mean and peaks QRS-T angles, the spatial ventricular gradient, and various spatial waveform azimuths, elevations and time-voltages [12,15]; parameters of QRS and T-waveform complexity derived by singular value decomposition including the principal component analysis ratio [14], the dipolar and nondipolar voltage equivalents [16] of the QRS and T waveforms, and a parameter describing the shape of the T wave via measurement of the spatial allocation of equivalent dipoles that uses an error function to minimize the difference between measured and equivalent dipoles-reconstructed potentials, known as the root-normalized mean square error of the T wave (RNMSE_T) [17]. Data from the 5-min ECGs were also processed for multiple measures of both beat-to-beat RR and QT interval variability [18]. All AECG parameters have been described in previous publications [14,15,19]. We utilized a previously validated AECG score for using a validated multivariate logistic regression based on larger dataset of patients with known LVSD [5,12].

Echocardiography

A brief 5-min echocardiography protocol was used by a sonographer using a GE E95 to obtain standard measures such as LVEF using Simpson's biplane method. LVSD by echocardiography was considered present when LVEF <50%. LV GLS was also measured using EchoPAC (GE, IL, USA). DICOM files were fed into an AI pipeline to classify, segment and analyze each image. A convolutional neural network, described elsewhere [7], was used to label each view into one of 23 classes. The area-length formula was used to calculate AI-generated LV volumes (LVEDV/LVESV) and EF (AI-LVEF). AI-generated indexed LA volume and indexed LV mass were also compared with manual measurements (M). This pipeline is part of the integrated cardiac and modeling and analysis platform developed at the Auckland Bioengineering Institute (Integrated Cardiovascular Project, NSBRI Foundation, NASA Grant NCC 9-58) [20].

Table 1. Baseline characteristics.

	Heart failure (n = 46)	Controls (n = 20)	p-value
Age (years), mean (SD)	68 (8)	52 (9)	5×10^{-9}
Males, n (%)	41 (89)	10 (50)	0.0006
European, n (%)	29 (63)	16 (80)	0.18
AF, n (%)	10 (22)	0 (0)	N/A
HTN, n (%)	21 (46)	0 (0)	N/A
T2DM, n (%)	9 (20)	0 (0)	N/A
ACEi/ARB, n (%)	37 (80)	0 (0)	N/A
β-blocker, n (%)	39 (85)	0 (0)	N/A
MRA, n (%)	14 (30)	0 (0)	N/A
Statin, n (%)	29 (63)	0 (0)	N/A
Frusemide, n (%)	10 (22)	0 (0)	N/A
EF bp, mean (SD)	39% (10)	57% (5)	8×10^{-9}
GLS, mean (SD)	-13% (0.04)	-21% (0.05)	3×10^{-8}
NTproBNP (pmol/l), mean (SD)	115 (124)	8 (10)	0.0002

ACEi: Angiotensin converting enzyme inhibitor; AF: Atrial fibrillation; ARB: Angiotensin receptor blocker; EF bp: Ejection fraction by Simpson's biplane; GLS: Global longitudinal strain; HTN: Hypertension; MRA: Mineralocorticoid receptor antagonist; NTproBNP: N-terminal pro hormone BNP; SD: Standard deviation; T2DM: Type 2 diabetes.

Mental stress testing & wearable devices

A 3D VR-MS was created using validated content to evoke mental stress in a subset of participants with continuous AECG recording [21–23]. The content was based on a social trier stress test, serial subtraction, agoraphobic and other environmental stressors, designed to cause episodic mental stress, that have been associated with LV dysfunction [23] and arrhythmia [24], over a 5-min interval (Supplementary Video 1). VR epochs are listed in Supplementary Table 1. A Samsung Gear VR headset with a Galaxy S8 was used with content run in Oculus. VR-MS participants wore an Empatica E4 on their dominant wrist, measuring skin temperature, electrodermal activity (EDA) and pulse photoplethysmography (PPSG). AECG was recorded 5-min prior and during VR-MS. A further 12 participants wore a radial pulse wave tonometer built by Microsoft Research, which included a single-lead 5-min ECG at baseline. EDA and PPSG signals were fed to an AI pipeline involving feature extraction from each channel consisting of mean, median, standard deviation, and min and max of data. Subsequently, all the extracted features from each channel (EDA, PPSG and combined signals) were concatenated into a single vector feature. The feature vector from these signals were fed into two classifiers, long short-term memory and support vector machine to distinguish between groups and within groups, before and during VR-MS.

Statistics

Univariate analysis was performed using the Student's *t*-test for continuous parametric variables, a Mann–Whitney *U*-test for nonparametric and chi-square test for categorical variables. Receiver operating characteristic curve analysis was used to assess performance of diagnostic biomarkers by c-statistic. All tests were two-tailed with $p < 0.05$ deemed statistically significant, except where tests for multiplicity were applied. Metaboanalyst (Version 4.0, Alberta, Canada) was used for pathway and multivariate analysis which was adjusted for multiplicity to reduce the false discovery rate (FDR). Medcalc software version 16.8.4 was used to analyze the data. The data output from mental stress testing were analyzed using GraphPad Prism 8 (version 8.4.3), comparing slopes, intercepts and elevations using simple linear regression.

Data availability

The materials, data, code and associated protocols are available to readers with application to the corresponding author.

Results

Three hundred and sixty two patients were screened for inclusion/exclusion criteria. Sixty six participants (46 with documented diagnosis of HF and 20 self-reported healthy volunteers) were enrolled in the study, with written informed consent. Baseline characteristics are outlined in Table 1. Within HF patients 27 (59%) had an ischemic cardiomyopathy and 19 (41%) had either an ICD (n = 14) or cardiac resynchronisation therapy defibrillator

Table 2. Dilated cardiomyopathy pathogenic mutations.

Gene	Titin band	Coding HGVS	Genomic position	Zygosity	Variant type
Titin	A-band	c.96904+2T>A	chr2:179407794	Het	Splice donor variant
Titin	A-band	c.50296C>T	chr2:179476842	Het	Nonsense
Titin	I-band	c.43382delA	chr2:179497350	Het	Frameshift
Titin	M-band	c.101689G>T	chr2:179399653	Het	Nonsense
Desmoplakin	–	c.6805_6824delAAACAGAAGCTTGGCATTTA	chr6:7584298	Het	Frameshift

chr: Chromosome; del: Deletion; HGVS: Human Genome Variation Society.

therapy (CRTD; n = 5). Ten (71%) of the ICDs were implanted for primary prevention. HF patients were older and had a higher percentage of males than controls. Mean NYHA status was II.

Although patients were screened according to the criteria noted above based on historic data, a number of patients with HFrEF had recovered (HFrec) either spontaneously or with medical interventions. Seventeen (36%) had an NTproBNP <35 pmol/l and were defined as biochemical HFrec. Seven (15%) had mechanical HFrec, defined as GLS ≥18% and 7 (15%) had LVEF ≥50%.

Biomarkers & genomics

28 metabolites across all diagnostic definitions of heart failure were identified by GCMS, which met false discovery rate (FDR). Numerous of these were either directly part of or indirectly linked to the citric acid cycle and mitochondrial metabolism. By univariate analysis, isocitric acid had the highest AUC 0.84, 95% CI 0.73 to 0.92 to discriminate HF. 35 metabolites were identified by LCMS which fulfilled the FDR. Most notably these included symmetric dimethyl arginine, creatinine, arginine and kynurenine, as well as numerous phosphatidylcholines, sphingomyelins, lysophosphatidylcholines, two cholesteryl esters and one triglyceride (55:9). Only one volatile, acetone, reached significance by the stringent FDR used, however several common VOCs were identified in both plasma and urine (t-test, P<0.05) which have previously been associated with heart failure. These included pentane, 2-butanone, and 2-pentanone. Breath acetone was validated as a heart failure biomarker (n = 61) using a commercially available device (Ketoscan mini, Sentech, Gyeonggi-Do, Korea) with AUC of 0.8, 95% CI 0.61 to 0.92. Five (11%) patients had pathogenic mutations associated with dilated cardiomyopathy (Table 2), with four (9%) having *Titin* gene (*TTN*) truncations (*TTN*tv). U-*TTN*/Cr concentrations were statistically higher in patients with prior history of HF compared with controls (median 542 vs 360 pmol/µmol/l; difference 95% CI: 62–368; $p < 0.005$). In *TTN*tv carriers, U-*TTN*/Cr was not significantly different, though NTproBNP was substantially higher than in *TTN* wild-type HF patients (mean 347 vs 95 pmol/l; 95% CI: 144–359; $p < 0.0001$). Both kynurenine and hexanal, an aldehyde bioproduct of lipid peroxidation, were different ($p < 0.05$) in *TTN*tv carriers, but neither exceeded the statistical FDR.

Advanced ECG

The AECG LVSD score correlated with GLS (r = -0.77, $p < 0.0001$) as also previously demonstrated [6]. Moreover, it discriminated HF at baseline (area under the curve [AUC]: 0.95, 95% CI: 0.85–0.99) independent of NTproBNP (Figures 1 & 2), which itself correlated with cardiac energetics, not mechanics. QT variability index was higher in ischemic versus nonischemic cardiomyopathy (p = 0.003), especially in those with an ICD (p = 0.0004). Biochemical HFrec, defined by NTproBNP <35 pmol/l, was best discriminated by GLS (AUC: 0.84; 95% CI: 0.68–0.94; $p < 0.0001$), urine creatinine (AUC: 0.81; 95% CI: 0.67–0.93; $p = 1 \times 10^{-5}$) and plasma acetone (AUC: 0.79; 95% CI: 0.65–0.92; p = 0.001), whereas mechanical HFrec defined by LVEF ≥50% was best discriminated by the AECG LVSD score (AUC: 0.94; 95% CI: 0.85–0.99; $p = 5 \times 10^{-5}$). Various AECG parameters relating to R-R interval variability (RRV) and QT interval variability (QTV) differed between controls and HF patients at baseline (Figure 3). Alpha 2, a fractal parameter of RRV, was increased at baseline in HF patients versus controls, but further increased with mental stress only in the controls (Figure 3, top). On the other hand, the RNMSE_T and the root mean square of beat-to-beat QT interval variability in lead II, were not only relatively increased at baseline in the HF patients, but also even more notably increased (further clinically deteriorated) during mental stress in HF patients compared with controls (Figure 3, bottom).

The Empatica E4 output showed both heart rate (HR) and EDA increased with VR-MS in controls (Figure 4); however, only HR rose in HFrEF patients (p = 0.01) with accompanying increased QT variability index (QTVi) in

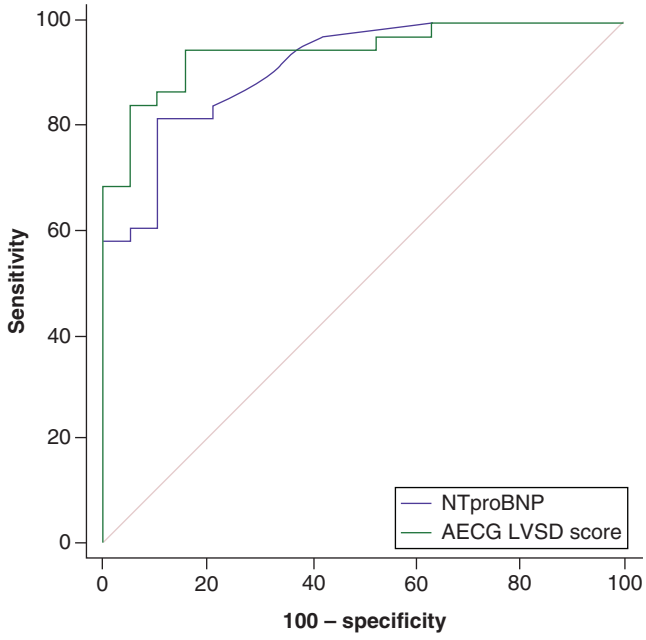

Figure 1. Receiver operating characteristic comparing N-terminal pro hormone BNP and advanced ECG left ventricular systolic dysfunction score for echocardiographic heart failure ejection fraction <50%.
AECG: Advanced ECG; LVSD: Left ventricular systolic dysfunction.

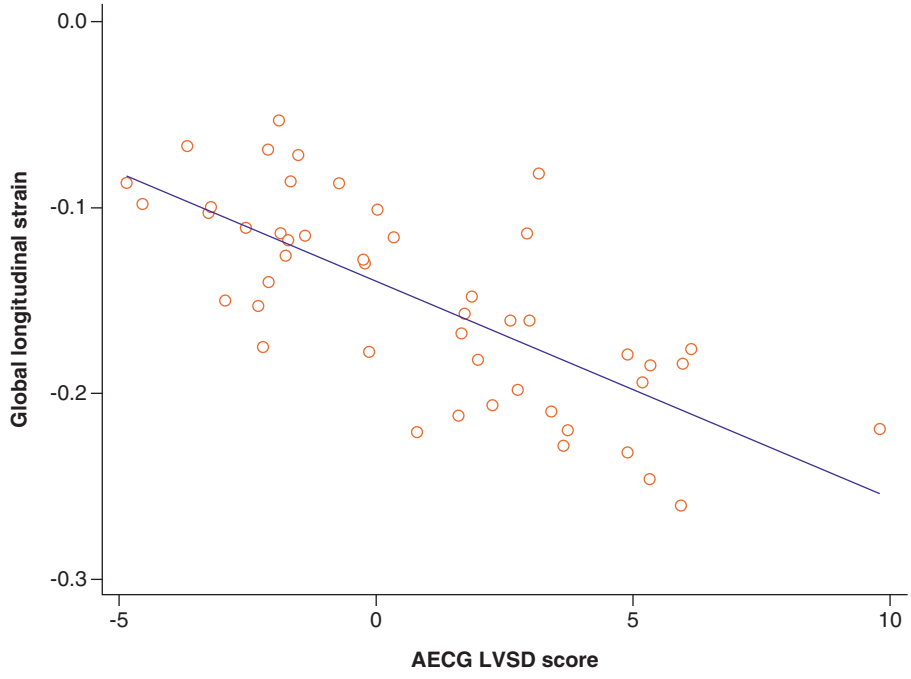

Figure 2. Pearson correlation between global longitudinal strain and advanced ECG left ventricular systolic dysfunction score.
AECG: Advanced ECG; LVSD: Left ventricular systolic dysfunction.

those with ICDs (p = 0.04). Analysis of the Empatica E4 EDA and PPSG signal with a long short-term memory classifier discriminated between HF and controls prior to and during VR-MS with 81.3 and 73.9% accuracy, respectively. Pulse tonometry analysis was confounded by the presence of atrial fibrillation, including in three of the four *TTN*tv carriers. However, in HFrEF patients without atrial fibrillation compared with controls, central dicrotic height was higher, pulse pressure lower and median time between the arrival of the pulse at the artery (the wave foot) and the anacrotic notch (reflected wave arrival) was longer in HFrEF.

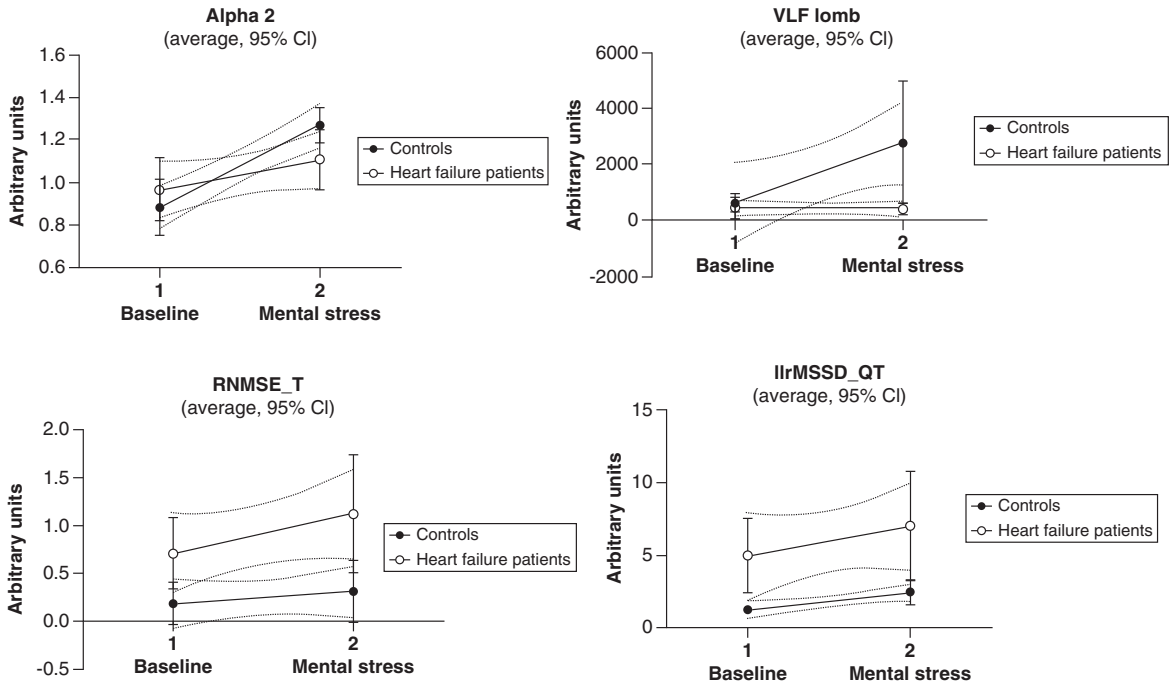

Figure 3. Advanced ECG parameters at baseline and with mental stress. Alpha 2 and VLF power (by Lomb periodogram) of RR interval variability (top panels), and RNMSE_T together with root mean square of beat-to-beat QT interval variability in lead II (IIrMSSD-QT) (bottom panels), before and after mental stress in controls versus heart failure patients. Significantly higher baseline alpha 2 in heart failure patients, along with relatively blunted alpha 2 (and VLF power) responses to mental stress (top panels), suggests relative cardiac sympathetic saturation with depleted cardiac sympathetic reserve at baseline in the heart failure patients. At the same time, both increased baseline and more notable deterioration (increases) in RNMSE_T and IIrSSD_QT with mental stress suggests reduced electrical coherence in repolarisation with potentially increased ventricular arrhythmic propensity in heart failure patients versus controls (bottom panels).
IIrMSSD-QT: Root mean square of beat-to-beat QT interval variability in lead II; RNMSE_T: Root-normalized mean square error of the T wave; VLF: Very low frequency.

Echo AI

Compute time using was < 10 s for classification, segmentation and analysis using a single graphics processing unit (GPU). A total of 11 (18%) nonphysiological AI-ESV and associated AI-LVEF were excluded versus two (3%) manual-LVEF ($\chi 2 = 7$; 95% CI: 3–27; $p = 0.008$). AI generated measurements correlated well with manual measures: LVEDV $r = 0.77$, LVESV $r = 0.8$, LVEF $r = 0.71$, indexed LA volume $r = 0.71$, indexed LV mass $r = 0.6$ and $p < 0.005$. Mean absolute error of M-LVEF versus AI-LVEF was 7.4 ± 6.6%. AI-LVEF, M-LVEF and other HFrEF biomarkers had a similar discrimination for HFrEF (AUC M-LVEF: 0.93 vs AI-LVEF: 0.88; 95% CI: -0.03 to 0.15; $p = 0.19$).

Discussion

In this project we validated a machine learning tool applied to ECG, previously diagnostic for HF and prognostic for related outcomes [5,6]. Second, we developed a pipeline for AI analysis of echocardiography to validate a method for obtaining LVEF more efficiently than manual methods [7]. Third, we integrated this information with next generation sequencing, metabolomics and volatilomics to reveal biological insights and identify novel diagnostic biomarkers (Figure 5). Lastly, we used a wrist worn wearable device and AECG to measure the effect of a VR-MS on a subset of HF patients and controls.

We showed that AECG, using logistic regression scores applied to conventional, spatial (vectorcardiography) and other ECG variables, has a diagnostic accuracy for detecting HF similar to NTproBNP. This result validates this method prospectively, which we have previously shown to have both diagnostic and prognostic value in the context of HFrEF [5,6]. Logistic regression and linear discriminant analysis, both forms of machine learning, applied to detailed ECG segmentation and highly curated databases, underpin the technology [12], which also demonstrates an

Figure 4. Photoplethysmography (top) and pulse wave tonometry (below). Healthy controls; heart rate (PPSG) (top left), heart failure; (top right). Example of Microsoft Aurora output (bottom).
PPSG: Photoplethysmography; VR: Virtual reality.

ability to track individual health status over time (Supplementary Figures 1 & 2). This therefore has the transparency and explainability that deep learning methods lack [3,4].

In this study we used AECG to investigate at baseline and during mental stress a number of ECG variables known to be associated with increased arrhythmic risk in HF patients. We showed that not only do patients with HF have increased spatial QRS-T angle at baseline, a biomarker associated with HF readmissions and mortality [6], but also increased alpha 2 RRV fractal dimension [25] and QT variability [26]. Both of these parameters are also strong predictors of mortality that moreover likely indicate, among other things, increased resting efferent cardiac sympathetic activity [25]. During VR-MS, alpha 2 also notably increased in healthy participants, but not in HF patients. The relatively blunted response in alpha 2 in HF patients during mental stress suggests that cardiac sympathetic activity might already be near maximum in such patients, in other words, 'reduced cardiac sympathetic reserve'. It should also be noted that whereas alpha 2 does not appear to be affected by 'physical' stress, for example, postural change [27], it was clearly increased by 'mental' stress in healthy subjects in this study. Since alpha 2 of RRV

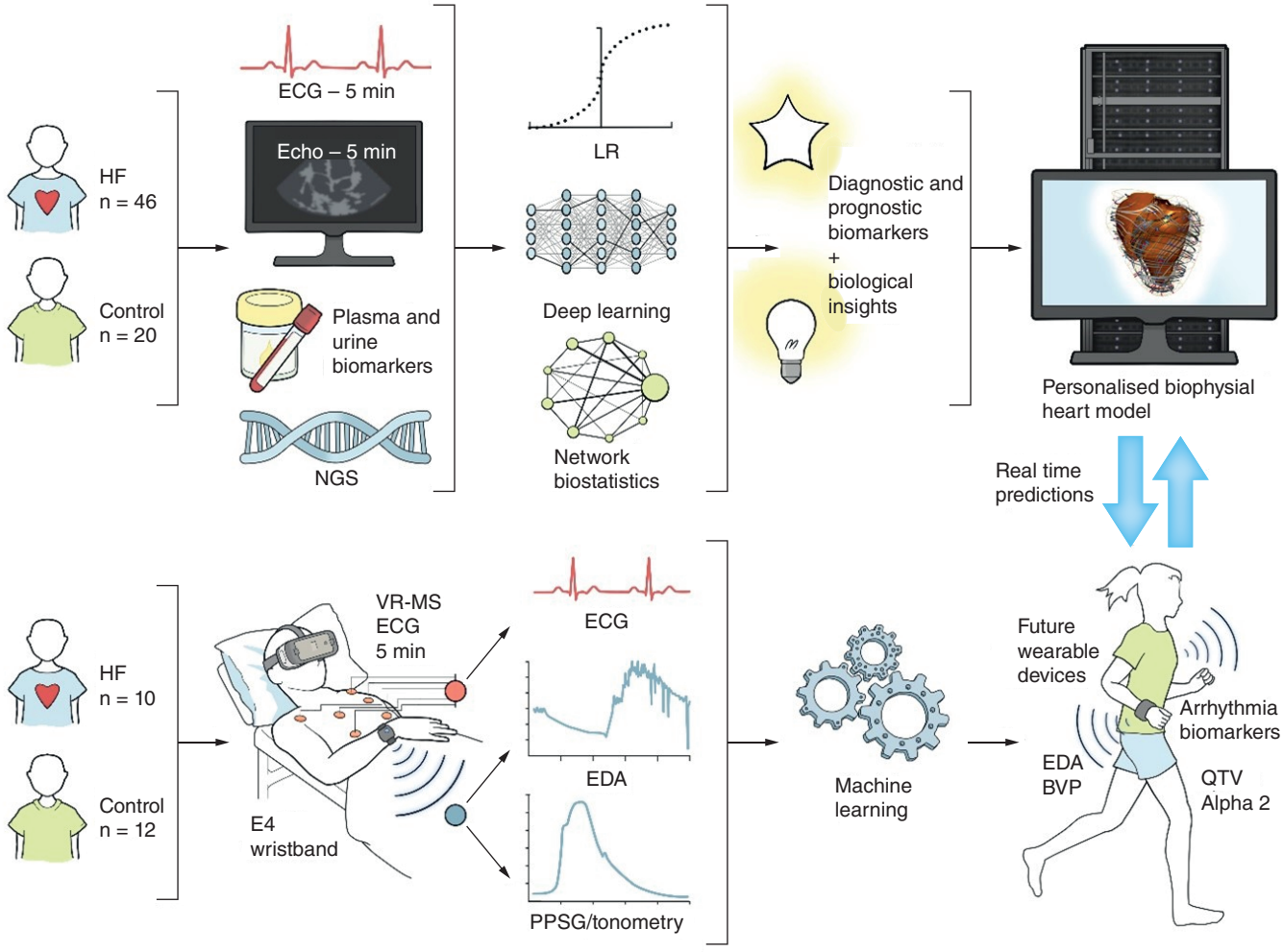

Figure 5. Study workflow, methods and results.

can be derived from single lead ECGs, this finding demonstrates potential promise for employing alpha 2 with wearable technologies to monitor mental stress in patients with and without HF.

We also identified at least two AECG variables, specifically the RNMSE_T and the root mean square of beat-to-beat QT interval variability in lead II, that were not only expectedly increased in HF patients at baseline, but also further relatively increased (worsened) by mental stress in HF patients compared with controls. Pending further validation, variables such as these might therefore hold promise for eventual use with wearable ECG technologies for monitoring arrhythmic propensity in real time.

Our study showed numerous metabolic changes associated with HF, which principally indicated abnormalities in mitochondrial metabolism, namely the citric acid cycle, ketone metabolism and kyneurinine pathway. One metabolite, acetone, was validated in the breath of patients with HF using a commerciallly available sensor. Our study, however, was underpowered to demonstrate any differences in the metabolite profiles of *TTN*tv carriers, particularly as this was confounded by their having a higher NTproBNP. Nor did we observe in *TTN*tv carriers an alteration in urinary N-terminal titin fragments previously shown to be a negative prognostic indicator in HF [28]. We were unable to demonstrate any statistically significant correlations between metabolomic biomarkers and AECG; however, metabolomics has previously shown that kynurenine pathway is associated with mental stress-induced LVSD and ketone bodies (acetate and beta hydroxybutyrate) with QTc in shift workers [29,30]. We were, however, able to show an ability to not only discriminate between healthy participants and HF patients using PPSG and EDA, but also identify the presence of mental stress with a high degree of accuracy. With this

knowledge it may be possible to develop wearable sensors, perhaps also monitoring metabolism [31], which will be capable of predicting HF exacerbations and short-term arrhythmic risk, influenced by mind–heart interactions in real time [32,33].

In our study we validated an Echo AI method provided by Zhang *et al.* [7] and showed that deep learning applied to a 5-min echocardiography protocol rapidly quantifies LVEF, equivalent to human interpretation. This method holds significant utility in the rapid identification of LVSD using POCUS in the ambulatory setting and opens up new opportunities for monitoring and titrating therapies in HF patients. We have previously shown the capability of AECG to identify both structural heart disease and LVSD, and allocate patients to POCUS screening versus full echocardiography [34,35]. Our intention going forward is to integrate all these sources of data into a virtual machine to apply biophysical electromechanical and circulatory computational modeling to better predict outcomes and response to therapies in HF patients [36].

Multiomics has been used fairly extensively in highly controlled cell-based and animal models of disease to identify novel biological pathways or casual genes [37,38]. Due to the high dimensionality and complexity of analysis multiomics is less often used in human studies; however, there is growing expertise in the field which demonstrates it is not only possible but a powerful tool in delivering personalized healthcare, tracking individual responses over time (Supplementary Figure 1). Multiomics has been used to deliver insights into human obesity and prediabetes [39,40]; however, to our knowledge it has not been extensively used in the diagnosis or stratification of human HF [41] or in combination with machine learning applied to echocardiography and ECG [42]. The use of clinical multiomics will be impeded by cost, time and complexity; however, machine learning is the logical tool to assimilate, predict and visualize results in a way which should disburden clinicians who are otherwise awash in data. To a simplistic degree we are working toward the implementation of some of the technologies outlined in this paper in a rapid cardiac screening clinic, using conventional blood tests, ECG and echocardiography 'omics delivered via a single platform' [19,20,34,35].

Limitations

This study was small and underpowered to identify metabolomic differences in specific subgroups, for example, *TTN*tv carriers. Multiple hypothesis testing increases the potential for Type I error; however, the discussion has been limited to points for which there is sufficient prior knowledge to make reasonable conclusions.

Conclusion

This study has demonstrated the feasibility of integrating multiple sources of 'omic clinical data and its potential clinical utility in the context of heart failure. This allowed the expansion of the clinical phenotype of HFrEF suggesting possible future directions for substratfiying patients and delivering personalised management strategies. Further work is needed to ensure the additional effort required to generate this data leads to a cost-effective improvement in patient outcomes.

Summary points

- Multiomics holds considerable promise for identifying biological pathways in heart failure (HF), which may have therapeutic or diagnostic ('theranostic') potential.
- Breath acetone and other metabolite biomarkers may be useful diagnostic or prognostic tools in human HF.
- Machine learning applied to echocardiography and electrocardiography could be used to expedite and enhance the sensitivity and specificity of these tools to both diagnose and risk stratify patients with HF.
- Deep phenotyping with wearable devices during external perturbation, such as mental stress testing, reveals novel insights into disease pathophysiology.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fca-2020-0225

Author contributions

PA Gladding contributed to ideation of the study, and is the main author. PA Gladding and M Jüllig contributed to statistics of the study. S Loader contributed to research co-ordination, patient enrolment and data collection. E Zarate, S Green and S Villas-

Boas contributed to gas chromatography–mass spectrometry metabolomics and analysis. K Smith, P Shepherd and P Kakadiya contributed to biobanking and next generation sequencing. W Hewitt contributed to echocardiography artificial intelligence coding and data analysis. E Thorstensen, C Keven and M Coe contributed to liquid chromatography–mass spectrometry metabolomics and analysis. B Nakisa, T Vuong and MN Rastgoo contributed to Empatica E4 machine learning analysis. M Jüllig contributed to figures and illustrations. V Starc and T Schlegel contributed to advanced ECG analysis. E Zarate, S Green, S Villas-Boas, B Nakisa, T Vuong, MN Rastgoo, V Starc and T Schlegel contributed to proofing.

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Ethical conduct of research

The NanoHF study (A Novel Nanosensor array for Heart Failure diagnosis) was approved by the Northern B Health and Disability Ethics Committee (16/NTB/115) (#16/680) and Waitematā District Health Board's IRB (#RM13458). In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Commentary

Future VIROLOGY

The role of artificial intelligence in tackling COVID-19

Neelima Arora*,[1], Amit K Banerjee[2] & Mangamoori L Narasu[1]

[1]Centre for Biotechnology, Institute of Science & Technology, JawaharLal Nehru Technological University, Hyderabad 500085, Telangana, India

[2]Biology Division, Indian Institute of Chemical Technology, Hyderabad 500007, Telangana, India

*Author for correspondence: Tel.: +91 961 825 4851; neelimaiict@gmail.com

> "AI can be harnessed for forecasting the spread of virus and developing early warning systems by extracting information from social media platforms, calls and news sites and provide useful information about the vulnerable regions and for prediction of morbidity and mortality"

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The past two decades were marked with the outbreaks of many viral diseases such as Chikungunya, Ebola, Zika, Nipah, H7N9 Bird flu, H1N1, SARS and MERS. The world woke up to this decade with a new disease outbreak. An outbreak of a novel Coronavirus emerged in Wuhan city in the Hubei province of China in December 2019. Most of the initially identified patients were traced back to the 'wet market' where live animals are slaughtered and sold. The market might have played a role as an amplification hotspot from where the virus spread to other parts of China and subsequently to 213 countries and territories in a very short time. The WHO named this disease 'COVID-19', which is an acronym of Coronavirus Disease 2019 on 11 February 2020. As of 17 August 2020, a total of 21.2 million confirmed cases and 761,000 deaths have been reported globally [1]. The worst outbreaks of COVID-19 are reported in the USA, India, Brazil and Russia where the number of cases has surpassed the confirmed cases in China. The WHO declared the current outbreak of COVID-19 a 'Public Health Emergency of International Concern' on 30 January 2020 and a 'pandemic' on 11 March 2020.

Although the fatality rate of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2; 2.9%) is much less compared with SARS-CoV (9.6%) and MERS-CoV (34.4%), the high infectivity rate of SARS-CoV-2 compared with other coronaviruses has become a global concern. Mortality and vulnerability to COVID-19 were found to be higher in males compared with females, which could be attributed to other gendered practices such as smoking [2]. The fatality rate of COVID-19 varied with an age gradient and it was also influenced by underlying co-morbidity, in other words, conditions such as diabetes, hypertension, cancer, cardiovascular diseases and chronic respiratory disease [3–5]. Vertical transmission of COVID-19 infection from mother to baby was not observed [6]. Children are vulnerable to COVID-19 but tend to show only mild symptoms [7].

SARS-CoV-2

The etiological agent was named as SARS-CoV-2 by the International Committee on Virus Taxonomy on 11 February 2020. SARS-CoV-2 is a beta coronavirus of zoonotic origin belonging to the subgenus *Sarbecovirus* in the *Orthocoronavirinae* subfamily of the family *Coronaviridae* transmitted to humans in a spillover event. Bats are thought to be the animal reservoir of SARS-CoV-2 but the other likely intermediate animal host is yet to be identified. The virus is a spherical particle of 70–90 nm [8], having spikes of glycoprotein projecting from its surface that bind to receptor angiotensin-converting enzyme 2 on the surface of the cell. These spikes give the virus a crown-like appearance.

The glycoprotein of SARS-CoV-2 has a furin polybasic cleavage site (PRRARS|V) located between the residues 682 and 685 at the boundary of two subunits S1/S2 that is catalyzed during biogenesis [9]. The presence of this

cleavage site in SARS-CoV-2 that is observed in avian influenza viruses but not related viruses like SARS-CoV and SARSr-CoVs makes it distinct and has an impact on entry, tropism, spread and pathogenicity of the virus [9,10]. Expression of furin proteases in the respiratory tract, brain, pancreas, liver, gastrointestinal tract and reproductive organs of the host enables the virus to infect different organs and also facilitates its release into the surrounding environment in many ways. At present, 249 protein structures and 255 whole-genome sequences belonging to SARS-CoV-2 are available in the public domain.

SARS-CoV-2 genome

A recent study suggested a single-source origin of SARS-CoV-2, as genomic sequences collected from different patients showed strikingly high identity and also indicated that SARS-CoV-2 is phylogenetically closer to bat-SL-CoVZC45 and bat-SL-CoVZXC21 [11]. Its genome size is approximately 30 kb [12]. A vast portion of the genome is occupied by two open-reading frames (ORF1a and ORF1b) that translate into pp1a and pp1ab polyproteins, which are then cleaved to 16 nonstructural proteins (nsp) like cysteine proteases, chymotrypsin-like, RNA-dependent RNA polymerase, helicase and so on. The rest of the genome encodes structural proteins like the spike(S), envelope (E), membrane (M) and nucleocapsid protein and 6–7 accessory proteins [13]. Genetic analysis revealed that SARS-CoV-2 has evolved in two lineages: ancestral S type and other more prevalent, aggressive and virulent L type derived from S type [14]. It is interesting to note that in the early stages of the epidemic, L type was more frequent, but its frequency decreased later and the frequency of S type increased, which can be attributed to differential selection pressure and epidemiological features [14].

Transmission

COVID-19 mainly spreads from human to human through direct contact by respiratory droplets during coughing or sneezing and through indirect contact route by fomites and regularly touched surfaces [15]. SARS-CoV-2 can remain viable on various surfaces for several hours to days [16]. Air-borne transmission is possible in a medical or hospital setting in processes that generate aerosols. Although fecal–oral transmission of COVID-19 has not been reported to date, it remains a potential route [17,18].

Clinical symptoms

Most patients experience mild flu-like symptoms including fever, cough, malaise, fatigue, sputum production and respiratory problems. Less common symptoms such as headache, hemoptysis and gastrointestinal symptoms with diarrhea and serious symptoms like pneumonia and bronchitis were also observed. Complications like Acute Respiratory Distress Syndrome, RNAaemia, acute cardiac injury, acute kidney injury and secondary infections [19] were reported in some patients. Other lab parameters associated with COVID-19 were low white blood cells and lymphocyte count, an increase in erythrocyte sedimentation rate, C-reactive protein, infiltrates and bilateral ground-glass opacity in lung CT scans.

Prevention & control

It is imperative to adopt control measures such as case isolation, contact tracing, quarantine to limit human-to-human COVID-19 transmission. Personal hygiene measures such as frequent hand washing, respiratory hygiene, social distancing, use of face masks/shields and disinfection of surfaces can help in reducing the transmission.

Screening & diagnosis

Discriminant clinical features like hyposmia (loss of smell) and hypogeusia (loss of taste) can be explored for preliminary diagnosis in telemedicine and mass screening [20]. Specimen samples collected from oropharyngeal and nasopharyngeal swabs or blood samples are used for diagnosis. Although routinely used for COVID-19 diagnosis in outbreak settings, sole reliance on CT scans can be misleading due to indistinguishable images with other viral pneumonia. Molecular test reverse transcriptase-PCR (RT-PCR) is recommended by WHO as the method of choice for detecting the SARS-CoV-2 nucleic acid for diagnosis of COVID-19. As the false-negative rate of RT-PCR is high, it is imperative to use CT scan of the chest as a supplementary diagnostic measure to confirm the diagnosis. Point-of-care immunodiagnostic assays that detect proteins from the COVID-19 virus or human antibodies generated against the virus in blood samples are also being used routinely to complement molecular tests due to low cost and fast results, but these methods suffer from poor sensitivity and are only qualitative [1]. Utility of these serological methods in public health settings for contact tracing and evaluating the success of

nonpharmaceutical interventions has been discussed elsewhere [21]. These serological methods have now received Emergency Use Authorization by the US FDA. CRISPR-Cas12-based assay that provides rapid results can be used in point-of-care testing in the future [22].

According to recent data from WHO, 13 candidate vaccines are being evaluated. An experimental vaccine developed by the University of Oxford/AstraZeneca has entered Phase III of clinical trials while vaccine candidates from CanSino Biological Inc./Beijing Institute of Biotechnology and Moderna/NIAID have reached Phase II trials and ten vaccine candidates have reached Phase I/II and Phase I stages. 129 other candidate vaccines are in the preclinical stage (WHO) and many are in pipeline [23,24].

Therapeutic agents

Some of the potential drugs against COVID-19 beingconsidered and evaluated are remdesivir (GS-5734), baricitinib, a combination drug ritonavir/lopinavir, Ribavirin®, umifenovir and IFN-β and other broad spectrum antiviral agents. Remdesivir was not found to be effective in treating COVID-19 patients in a placebo-controlled randomized trial of remdesivir [25]. In a recent development, the FDA has approved the use of remdesivir in confirmed and suspected cases of COVID-19. As of 25 June 2020, about 1235 clinical trials for various therapeutic agents against COVID-19 are being conducted across the globe [26].

Application of artificial intelligence in COVID-19 disease management

Unprecedented pace of efforts to address the COVID-19 pandemic situation is leveraged by big data and artificial intelligence (AI). Various offshoots of AI have been used in several disease outbreaks earlier. AI can play a vital role in the fight against COVID-19.

AI is being successfully used in the identification of disease clusters, monitoring of cases, prediction of the future outbreaks, mortality risk, diagnosis of COVID-19, disease management by resource allocation, facilitating training, record maintenance and pattern recognition for studying the disease trend. Several applications of AI that are garnering a lot of interest and raising hopes in the fight against COVID-19 are as follows:

AI in prediction & tracking

AI can be harnessed for forecasting the spread of virus and developing early warning systems by extracting information from social media platforms, calls and news sites and provide useful information about the vulnerable regions and for prediction of morbidity and mortality. Bluedot identified a cluster of pneumonia cases and predicted the outbreak and geographical location of the COVID-19 outbreak based on available data using machine learning. HealthMap collects the publicly available data on COVID-19 and makes it readily available to facilitate the effective tracking of its spread. Recently, the role of AI in identification and forecasting of COVID-19 outbreaks by employing multitudinal and multimodal data was emphasized [27].

AI in contact tracing

AI can augment mobile heath applications where smart devices like watches, mobile phones, cameras and range of wearable device can be employed for diagnosis, contact tracing and efficient monitoring in COVID-19 [28]. Applications like AI4COVID-19 that rely on audio recording samples of 2 s cough can be used in telemedicine [29].

AI in monitoring of COVID-19 cases

AI techniques are applied for monitoring patients in clinical settings and prediction of course of treatment. Based on the data derived from vital statistics and clinical parameters, AI may provide critical information for resource allocation and decision-making by prioritizing the need of ventilators and respiratory supports in the Intensive Care Unit [30]. AI can also be used for predicting the chances of recovery or mortality in COVID-19 and to provide daily updates, storage and trend analysis and charting the course of treatment.

AI in early diagnosis

AI was used for the detection and quantification of COVID-19 cases from chest x-ray and CT scan images [31–33]. Researchers have developed a deep learning model called COVID-19 detection neural network (COVNet), for differentiating between COVID-19 and community-acquired pneumonia based on visual 2D and 3D features extracted from volumetric chest CT scan [34]. Singh *et al.* developed a novel deep learning model using Multi-Objective Differential Evolution and convolutional neural networks for COVID-19 diagnosis using a chest CT

scan [35]. COVID-ResNet developed using automatic and discriminative learning rate and progressive image resizing performed better than COVID-Net in diagnosing COVID-19 [36]. Alom *et al.* developed a system called COVID_MTNet by applying improved Inception Recurrent Residual Neural Network and NABLA-3 network models for detection and localization of regions of interests from both x-ray images and chest CT scan [37]. Another study used AI-based classifiers for predicting the outcome of RT-PCR results of COVID-19 cases using 16 simple parameters derived from complete blood profile [38]. This may find application in reducing the number of RT-PCR tests in resource-poor settings.

AI in reducing the burden from medical practitioners & healthcare staff

AI-based triage systems can help in reducing the work burden of medical staff and healthcare workers by automating several processes such as imparting training to practitioners, determination of the mode of treatment and care by analyzing clinical data using pattern recognition approaches, digitalization of patient's reports and also by offering solutions that minimize their contact with the patients [39–41]. AI can be used for classification of patients based on the severity of symptoms, genetic disposition and clinical reports in different categories like mild, moderate and severe, so that different approaches can be adopted for handling the patients in the most effective manner. AI in telemedicine can also be used to eliminate the need of frequent and unnecessary hospital visits by distant monitoring of cases and recording of patient's data in asymptomatic cases or patients with mild symptoms. AI-based medical chatbots can also be used for consultations, thereby reducing the physical crowding of hospitals as well as the spread of infection and thus prevent weighing down of efficient operation of critical care services [42,43]. Chatbots like Clara from the Centre for Disease Control and Zini are providing much needed support to patients in remote settings [44]. A prognostic prediction algorithm predicted the mortality risk of patients by machine learning methods using extracted features derived from the data of other patients as training dataset [45]. A similar approach was used to predict the possibility of developing acute respiratory distress syndrome [46]. Service robots and anthropomorphic robots with AI core can be used for the delivery of essential services and routine tasks like cleaning, disinfecting and monitoring in hospital settings [47,48].

AI in protein structure prediction

AI can help in predicting the structure of important proteins crucial for virus entry and replication and provide useful insight that can pave way for drug development in a very short time. AlphaFold algorithm of Google Deep mind employed deep residual networks (DRN) called ResNets for predicting protein structures of membrane protein, protein 3a, nsp2, nsp4, nsp6 and papain-like C-terminal domain of SARS-CoV-2, which will give huge impetus to drug discovery programs [49]. DeepTracer, a program based on customized deep convolutional neural network, was used to derive protein complex structure of SARS-CoV-2 from high-resolution cryoelectron microscopy density maps and amino acid sequence [50].

AI in development of therapeutics

AI techniques can boost and complement traditional technologies by reducing the time required in bringing a drug from bench to bed by speeding up lead discovery, virtual screening and validation processes by a huge margin. AI can also accelerate the pace by deriving useful data for drug repurposing or drug repositioning by screening properties of already approved and validated drugs based on molecular descriptors and properties, which may not be possible for a human expert. BenevolentAI used machine learning methods to accelerate its drug discovery program and identified baricitinib as a potential drug against COVID-19 [51,52]. Insilico Medicine has identified several small molecules against COVID-19 using AI [53]. Another study combined virtual screening and supervised learning to identify potential drugs against COVID-19 [54]. Zhou *et al.* adopted an integrative network-based systems pharmacological methodology for finding potential drugs for SARS-CoV-2 from the already existing repertoire of drug molecules and drug combinations [53]. Several other AI-based endeavors including inclProject IDentif.AI (identifying infectious disease combination therapy with artificial intelligence) [55] and PolypharmDB [56] have been successful in identifying candidates against COVID-19. Many machine learning approaches and deep learning-based applications are also being used for expediting the drug discovery process [57–60].

AI in development of vaccines

Never before has mankind witnessed such a race for the development of a vaccine against a pathogen. The pace of the discovery can be accelerated manifold by harnessing the power of AI. Ong *et al.* predicted possible vaccine

candidates for COVID-19 using the Vaxign reverse vaccinology-machine learning platform that relied on supervised classification models [61].

AI in curbing spread of misinformation

Due to the avalanche of information, this pandemic has turned into an infodemic. Understanding knowledge, awareness and practices toward COVID-19 by tapping information from social media platforms like Twitter, Facebook etc. can help in devising the strategy to assemble and disseminate timely and correct information for mitigating the impact of COVID-19 [62,63]. Machine learning techniques can be used to identify trends and sentiment analysis and provide information regarding the origin of false information and help in curtailing the rumors and misinformation [64]. AI techniques can further be used for presenting a clear picture of recovery rates, accessibility and availability to healthcare and identification of the gaps. AI can provide the latest updates about the emerging evidence in diagnosis, treatment, spectrum of symptoms and therapeutic outcomes in this highly dynamic situation, which will help clinicians in real-world scenario and help public in overcoming fear and panic [65].

AI in genomics

Randhawa *et al.* devised a method for fast and accurate classification of available SARS-CoV-2 genomes by applying machine learning on identified genomic signatures [51]. Wang *et al.* used ontology-based side effect prediction framework and Artificial Neural Network to evaluate the side effects of Traditional Chinese Medicines for the treatment of SARS-CoV-2 [66].

Conclusion & future perspective

Adopting a three-pronged approach based on testing, isolation and contact tracing is warranted to combat COVID-19. It is necessary to exploit the available knowledge base to develop effective chemotherapeutic agents against COVID-19, taking cues from lessons learnt in the past during other such outbreaks.

As there is no silver bullet available to cure the disease, we need to hasten progress on all fronts ranging from surveillance and monitoring to prevention and treatment. As this is the third outbreak of a coronavirus in recent times and many coronaviruses are circulating in animal reservoirs, we must focus on deciphering the molecular mechanism of SARS-CoV-2 and other coronaviruses and increasing our preparedness by capacity building for preventing future outbreaks [67]. As the current scenario warrants the need for immediate delivery of solutions, response to this outbreak was hugely augmented by various digital technologies and AI [68]. AI was found to be on par with and even more accurate than human experts in COVID-19 diagnosis and drug discovery. We need bigger datasets for training AI models and a legal framework and ethical considerations for sharing data before AI takes the forefront in diagnosis and other areas. Several bottlenecks in harnessing AI to its full potential in the current scenario are availability and sharing of clinical and epidemiological data, computational resources, scalability, privacy and ethical concerns.

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Perspective

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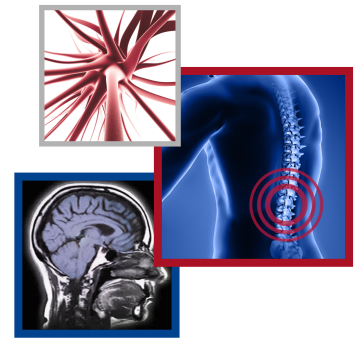

Immersive virtual reality to relieve exercise-induced pain caused by aerobic cycling

Carly LA Wender*[1,2]

[1]Center for Traumatic Brain Injury Research, Kessler Foundation, East Hanover, NJ 07936, USA

[2]Department of Physical Medicine & Rehabilitation, Rutgers-NJ Medical School, Newark, NJ 07102, USA

*Author for correspondence: Tel.: +1 973 324 8388; cwender@kesslerfoundation.org

Chronic pain affects 20% of the global population and is incredibly complex to treat. The burden of chronic pain is physical, emotional and financial, and prevalence rates continue to rise. Current treatments are ineffective long-term against pain and common comorbidities, including anxiety and depression, mood and sleep disorders, and social isolation. While a large body of evidence supports regular physical exercise as an effective long-term treatment for chronic pain and its comorbidities, exercise-induced pain and kinesiophobia are significant barriers to participation and adherence. Immersive virtual reality is a powerful short-term pain reliever, that, when combined with exercise, can help overcome these barriers. This perspective argues for the use of combined exercise and virtual reality treatment techniques to mitigate chronic pain.

Plain language summary: Chronic pain affects 20% of the global population and is incredibly difficult to treat. Chronic pain impacts physical and emotional health as well as one's financial independence. Current treatments are ineffective long-term against pain and common co-occurring symptoms, including anxiety and depression, mood and sleep disorders, and social isolation. While research supports regular physical exercise as an effective long-term treatment for chronic pain and its co-occurring symptoms, exercise-induced pain and kinesiophobia (i.e., fear of movement) are significant barriers to participation. Immersive virtual reality is a powerful short-term pain reliever, that, when combined with exercise, can help overcome these barriers. This perspective argues for the use of combined exercise and virtual reality treatment techniques to treat chronic pain.

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Keywords: chronic pain • exercise • exercise-induced pain • immersion • kinesiophobia • virtual reality

Chronic pain

Prevalence of chronic pain

After decades of ambiguity surrounding the diagnosis of chronic pain [1], the most recent International Classification of Diseases (ICD-11) defines chronic pain as pain that lasts or recurs for more than 3 months [2]. Due to the complex nature of this disease, there are two possible diagnoses: chronic primary pain, in which chronic pain is the disease itself, and chronic secondary pain, in which chronic pain is a symptom of another underlying condition, such as cancer or a neurological disorder [3,4]. Researchers and clinicians alike hope that with greater clarity and recognition of chronic pain as a disease, more efficacious treatments will emerge to combat this ever-growing pandemic.

Approximately 20% of adults reported having chronic pain in USA and Europe in the early 2000's, although experts believe this is likely an underestimate due to capturing rates of chronic primary pain exclusively [5]. A recently published paper from the Global Burden of Disease (GBD) study conducted in 2013 indicates that chronic pain may not only be the most impactful cause of morbidity and disability around the world right now, but may also pose the biggest health risk of the future [6].

Future Medicine

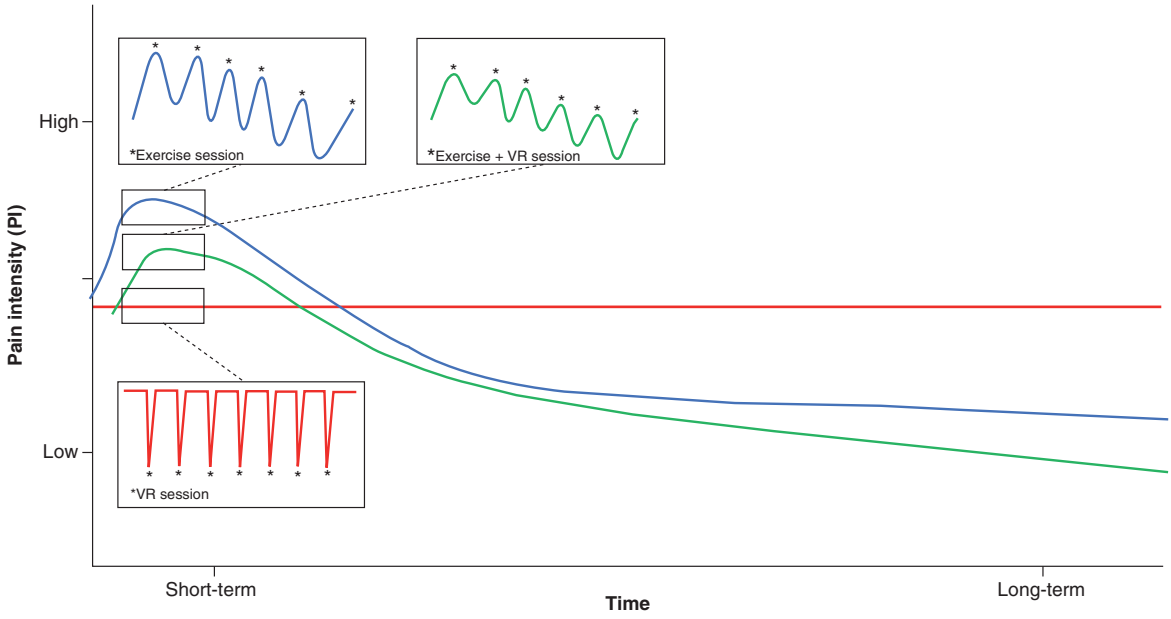

Figure 1. Virtual reality and exercise for chronic pain. This figure depicts the theoretical short-term and long-term effects of VR alone (red), exercise only (blue) and the combination of VR and exercise (green) on pain intensity for persons in chronic pain. In the short term, adding VR to exercise would lessen the initial increase in pain that accompanies exercise that commonly hinders adherence to training. This combination would ultimately allow for better long-term pain relief to exercise training alone.

Current treatment methods for chronic pain

Reliably effective treatments for chronic pain remain elusive, in large part, because of high inter-individual variability of many factors from symptom manifestation to biological and neurological consequences of chronic pain. For example, neurological studies have shown changes in motor and somatosensory processing in areas of the brain, commonly referred to as the pain matrix, due to sustained pain, but these changes differ based on location, intensity and duration of pain [7]. The high rate of comorbidities and subsequent behavioral changes associated with chronic pain conditions, including sleep disorders, anxiety or depression, changes in mood, social isolation and physical inactivity, have led many to believe that the most effective treatment for chronic pain will need to address several factors using a combination of techniques [8,9]. Pharmacologic treatments for chronic pain are perhaps the most commonly prescribed treatment, as it is by far the least complex and time intensive option for patients. Despite the ease of use, there is equivocal evidence for the effectiveness of pharmacologic pain-relievers and physiological side effects are commonly reported [10,11]. Opioids have emerged as the most popular pharmacologic remedy for chronic pain, but overuse and misuse have led to bigger problems of dependency, substance abuse, and a nation-wide opioid epidemic that has only compounded the already problematic burden of chronic pain [12].

Virtual reality & exercise for chronic pain

Immersive virtual reality (VR) and exercise, or regular physical activity (PA), are two potentially powerful treatment methods for chronic pain and its most common comorbidities. Evidence suggests that each treatment method on its own can improve pain. However, VR used alone in treatment only provides short-term pain relief, dissipating quickly once the VR exposure ends. While exercise does provide significant long-term pain relief, exercise can exacerbate pain in the short-term, which is a significant barrier to compliance and adherence. Therefore, we assert that by combining VR and exercise, we can utilize the powerful short-term pain relief of VR to overcome the immediate exercise-induced pain, thereby allowing persons with chronic pain to maintain exercise long enough to gain long-term benefits. This hypothesis is depicted in Figure 1.

A brief history of VR

While the popularity and availability of VR has increased exponentially in the last two decades, the technology has existed since the 1960′s, although the equipment was much bulkier and more restrictive than today's headsets.

Sutherland, the first well-known researcher of VR, defined the system as a "display connected to a digital computer [that] gives us a chance to gain familiarity with concepts not realizable in the physical world" [13]. He quickly realized how important complete immersion was to a person's experience and how quickly and easily people bought into being in a virtual environment (VE) [14]. Researchers in the 1990s strived to understand ways in which people's psychology and behavior changed while feeling present within a VE [15]. As early as 1999, the technology of VR was such that it became more accessible to the general public, prompting an exponential rise into research conducted exploring its potential value [16]. While the technology has greatly improved, and the possible applications of VR have expanded significantly [17], some basic questions remain unanswered. For example, what aspects of VR change human behavior the most and how does interpersonal variation moderate these effects? As research studies explore potential uses of VR, the possibilities and unanswered questions continue to outpace the rate at which research can answer those questions.

VR as a long-term pain-relieving technique for chronic pain

Evidence suggests that when VR is the sole treatment technique used to combat chronic pain, it is fairly ineffective. While VR can significantly reduce pain ratings due to chronic pain conditions during and shortly after exposure, the effects of VR are short-lived and do not mitigate long-term chronic pain. The success of VR for chronic pain is seen primarily in treatment methods where VR is used as an adjunct to other evidence-based practices. For example, VR combined with cognitive behavioral therapy has shown promise in helping people with chronic pain develop better coping skills, which may indirectly improve their pain by improving their quality of life [18,19]. While this area of research has become very popular in the last 10–15 years, researchers agree that more robust randomized controlled trials are required before clinical recommendations can be made about VR-based treatment techniques for people with chronic pain [20,21].

Exercise as a promising treatment for chronic pain

A large body of evidence suggests that regular PA and planned exercise are effective treatments for people with chronic primary or secondary pain due to a wide array of causes [22–24]. There are a number of hypothesized exercise-induced neurological and physiological changes that may explain reductions in chronic pain. Pain reduction due to exercise may stem in part from the endogenous opioid system that is activated by the body's interpretation of exercise as stress [25,26]. Other hypotheses suggest that regular PA or exercise helps regulate dysfunctional central pain inhibition and anti-inflammatory cytokines seen in those with chronic pain [25,27]. Finally, well-established improvements in mood, anxiety, depression, self-efficacy, and stress adaptation from exercise, potentially via change in the serotonergic system, may also contribute to the benefits of exercise on chronic pain by treating common comorbid symptoms [28–31].

Exercise-induced pain is a barrier to exercise in chronic pain

Despite knowing that regular PA is an effective treatment for chronic pain, activity levels in persons with chronic pain remain low, as they typically avoid activities that could exacerbate their pain [32]. Current pain and fear of future pain are common barriers to activity reported by those with chronic pain [33,34]. It is difficult to counteract this argument as exercise does cause pain in the short-term (i.e., exercise-induced pain).

Exercise-induced pain

Nociception is the biological process underlying the psychological construct of pain that people are familiar with. O'Connor and Cook defined nociception as "the reception of signals in the central nervous system (CNS) that are evoked by specialized sensory receptors (nociceptors) and that provide information about tissue damage or potential tissue damage [35]." There are four types of skeletal muscle afferent fibers that transmit signals from muscles to supraspinal brain. Type I and II afferents are not nociceptive and do not transmit pain-related signals. Type III and IV, also called A-delta and C fibers, respectively, are nociceptive afferents that respond to different painful stimuli. Type III fibers are mechanoreceptors that respond to high pressure stimuli while type IV fibers are chemoreceptors that respond to noxious chemicals. The mechanism of naturally occurring pain due to activated skeletal muscles during exercise, often described as 'dull-aching or cramping-type pain', is thought to involve both of these nociceptive fiber types [35].

When muscles contract above a certain intensity, relative to an individual's capacity, the resulting high pressure will stimulate type III afferents. For example, cycling below 50% of peak power output is not painful, while cycling

above 50% of peak power results in an intensity-dependent increase in quadriceps pain intensity (PI), which is thought to be caused, in part, by nociceptive signals to the brain via high pressure sensitive mechanoreceptors. This represents the initial, often described as 'dull or aching' pain people feel upon beginning exercise. With continued exercise and muscle contractions, a multitude of chemicals build up in the muscles and activate type IV nociceptive afferents. Anatomical studies in cats suggest there are about ten-times the amount of type IV nociceptive afferents in skeletal muscles than nociceptive type III afferents, suggesting a greater role of type IV afferents in pain perception during exercise [36]. Greater PI, often described as 'burning', is achieved by biomechanical activation of the larger quantity of type IV nociceptive afferents. Some of these chemicals work by directly activating the afferent fibers, such as bradykinin and potassium, while others work by sensitizing the afferent fibers, such as prostaglandins, leukotrienes and hydrogen ions. These endogenous algesics (pain causing agents) are all synthesized and/or released with tissue damage and/or high intensity muscle activity [35,37].

The most convincing evidence from human studies for the different roles of type III and IV nociceptive afferents in exercise-related pain comes from the 1997 seminal research study by Cook and colleagues. Every participant in this study experienced pain after cycling at 250 watts (W) for only 8 s. This is unlikely enough time for biochemicals to build up to a high concentration within muscles, indicating that a nociceptive response to the pressure of muscle contractions during exercise is enough for a person to perceive pain. Moreover, PI ratings were significantly higher during longer duration exercise at every power level above 100 W that was studied, including 250 W. While muscle biochemistry measures were not assessed in this study, the difference in PI ratings after significantly more time exercising lends support to the notion that a buildup of biochemicals in the muscle caused greater perceived pain [38]. Finally, lower ratings of PI during a short bout of cycling correspond to the dull aching or cramping feelings often felt with type III afferents as opposed to the dull, aching, and burning feelings reported that increase over time [35].

Cycling exercise

Cycling at any intensity above anaerobic threshold reliably causes quadriceps PI during exercise [39,40]. Muscle recruitment during cycling is a specific, systematic and coordinated effort that leads to direct force being applied to the crank to create the pedaling motion. Simply put, the hip and knee flexors lift to drive the pedal up and down. Research has shown the knee extensor muscle group, primarily the quadriceps muscles, to be the most important muscle group for cycling, as it provides the most force on the down stroke [41]. It logically follows that this muscle group is the primary source of pain during cycling and is the focus of most research on exercise-induced pain.

Cycling is pursued for multiple reasons, including transportation to work, recreation, fitness and sport. Bicycling is the sixth most common type of PA performed by adults in USA, with higher participation among men (6.3%) compared with women (3.3%) [42]. World-wide surveys of fitness professionals showed that indoor cycling (aka spinning) was especially popular from 2008 to 2012 [43]. High intensity interval training (HIIT), which typically involves indoor cycling, has been ranked between the first and third most popular fitness trend from 2014 to 2017 [44]. The popularity of HIIT stems, in part, from the time efficiency of short high intensity exercise bouts combined with evidence that cardiometabolic and physiological benefits of this exercise are comparable, if not superior, to longer, more moderate bouts [45]. However, higher intensity cycling leads to greater quadriceps muscle pain which may be a barrier to some.

Quadriceps pain during cycling may prevent people from adhering to cycling exercise training programs [46], although this has not been tested directly for cycling exercise in healthy adults. Pain exacerbation during movement has been shown to inhibit other types of exercise, such as breast pain reducing marathon running performance [47], and to be a barrier to exercise for groups with chronic pain conditions, such as in people with fibromyalgia [48] and osteoarthritis [49]. People with chronic pain conditions, or those going through physical rehabilitation for an injury or post-surgery, are often encouraged to cycle, as it is non-weight bearing and safe to use without supervision. However, people in chronic pain are less likely to commit to an activity like cycling that causes additional pain.

Virtual reality & exercise-induced pain

VR as a pain-relieving technique for acute pain

Several review papers published in the last decade have summarized work conducted on the success of VR on acute pain in both laboratory and real-world settings. One review of 11 high caliber randomized controlled trials (RCTs) reported a large mean effect size of VR on reducing pain (d = 0.94) caused by thermal stimuli or medical procedures, such as wound redressing in burn injured patients. This pain relief was seen only while participants

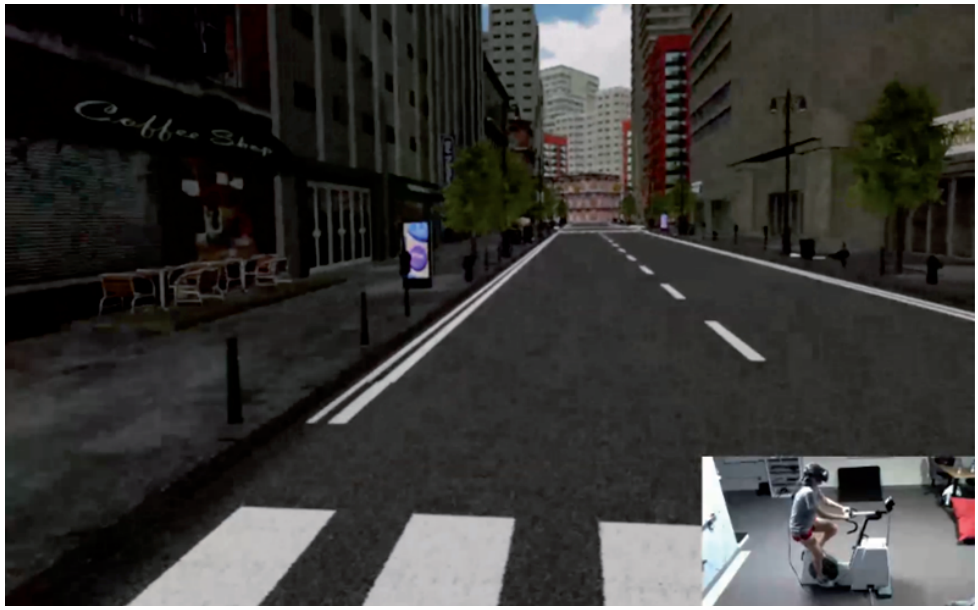

Figure 2. The virtual city scene that participants in the VR group interactively cycled through. The lower right shows a participant cycling while wearing the headset.

were using the VR and the greatest relief came from VR that was immersive, interactive, and of high technological quality [50]. A recent study echoes the significant effect of VR on experimentally induced pain from heat or pressure stimuli [51].

The precise mechanism by which VR has such a powerful effect on acute pain continues to elude researchers. One review article aimed to investigate psychological mediators of the analgesic effects of VR by evaluating 11 research studies that explored factors of immersion/presence, fun, and anxiety. Almost all studies that evaluated immersion/presence, or the feeling of being in the VE [52], showed positive correlations with pain thresholds or negative correlations with subjective PI ratings. All studies that measured fun showed strong positive correlations between fun ratings and pain relief. While no studies measuring anxiety examined it as a mediating factor, participants with lower baseline anxiety showed better pain reduction, indicating that anxiety may act as a moderating, rather than a mediating, factor [53]. Presence was higher in high-technology VR and fun ratings were higher in interactive VR, which may help explain why greater pain relief was previously reported in high-technology and interactive VR [50].

Virtual reality to decrease exercise-induced pain

Only three studies to date have investigated the effects of VR on acute, exercise-induced muscle pain. The first study elicited muscle pain with a continuous isometric biceps flexion of 20% of 1-repetition maximum (1RM) until exhaustion in 80 healthy young adults randomized to a VR or non-VR group [54]. Compared with the non-VR control group, mean biceps PI ratings for the VR group were lower after 1 and 2 min. Results showed enhanced exercise performance in the VR group, but the time to exhaustion exercise test is significantly less reliable than fixed time or distance trials [55].

The other two studies were conducted at the University of Georgia (UGA) and examined the effects of adding VR to cycling exercise in healthy college-aged adults. In the first study, immersive and interactive VR was added to Wingate sprints, or repeated 30-s sprint cycling trials at a high resistance (0.085 and 0.075 kilograms resistance to the flywheel per kilogram body weight for males and females, respectively) [56]. Ninety-four healthy young adults were randomized to cycle with one of two visual stimuli: the mental imagery group saw a static picture of a city scene inside the head-mounted display (HMD) and were told to imagine they were cycling through that city during the sprints, while the interactive VR group was immersed in a virtual city scene that they dynamically moved through at the speed at which they pedaled (Figure 2).

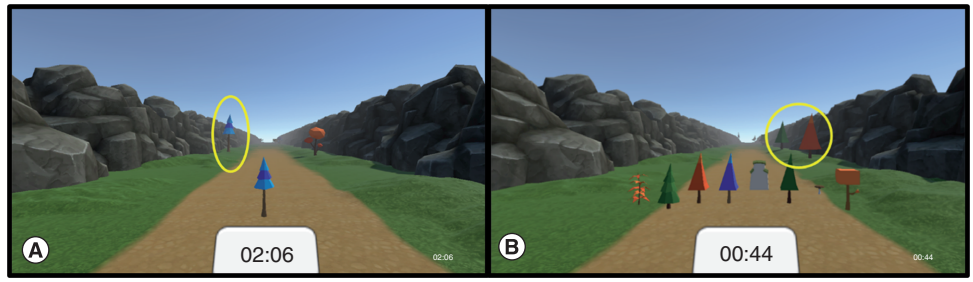

Figure 3. High and low perceptual load virtual environments. The virtual environment participants saw during the 10-min time trial in the **(A)** low perceptual load condition and **(B)** high perceptual load condition. The circle around items does not appear in the VR but is just to show that these are items participants should identify to as targets for the perceptual task.
Adapted from Wender *et al.*, unpublished data [under secondary review].

Immediately following each sprint, PI [38] and ratings of perceived exertion (RPE) were measured using 0–10 category scales with ratio properties [57]. There was a significant group × time interaction found for PI, such that in the VR condition, PI was 13.3% (M = 4.60 vs, 5.31, d = 0.28) and 11.8% (M = 5.68 vs 6.44, d = 0.27) lower at trials two and three, respectively. This experiment was the first to demonstrate that, without impacting cycling performance, there was a hypoalgesia (pain-reducing) effect of VR during brief, high intensity, painful, cycling exercise [56]. This finding was especially significant as other pain-reducing techniques have failed to reduce exercise-induced pain at such a high intensity [38,58–62]. This proof-of-concept study extended known effects of VR on acute pain to exercise-induced pain, but the mechanism by which this occurred was still a mystery.

Perceptual load (PL) is operationally defined as the number of unique objects present in a visual environment that may or may not be targets of interest. In the follow-up study at UGA, it was hypothesized that PL contributed greatly to the highly distracting nature of immersive VR as it is incomparably greater than the PL in other visual distractors, such as 2D videos [63]. According to the attentional load hypothesis, attention to one type of stimulus will decrease the processing of other concurrent perceptual information due to the sharing of limited resources [64]. It follows that cycling within a VE with a higher PL would limit the processing of muscle pain more than cycling within a VE with lower PL, where there is a greater capacity to process both the visual and muscle pain stimuli [65]. While this hypothesis is supported by previous studies where competing visual stimuli decreased the processing of noxious stimuli [66–68], no experiment had tested it using exercise-induced painful stimuli and VR. Using a within-subjects design, 43 healthy young adults completed a 10-min cycling time trial at a 'hard' intensity (RPE 15) under three conditions: no VR, low PL and high PL. The low and high PL conditions corresponded to the easier and harder perceptual task that participants were asked to do while cycling (Figure 3).

Unexpectedly, PI was significantly greater in the low PL (d = 0.472) and the high PL (d = 0.391) conditions than the no VR condition. Greater PI during the low PL condition was most likely explained by the significantly higher cycling performance. It was hypothesized that greater PI in the high PL condition was related to the significantly greater mental effort reported by participants, which related to less fun and more negative affect during exercise. The primary conclusion was that an engaging, but relatively easy, perceptual task in a VE with a low PL motivated participants to cycle harder despite reporting greater PI in their quadriceps muscles.

Conclusion

These three proof-of-concept studies support the pain-relieving effects of VR during exercise. Interactive and immersive VR reduced exercise-induced pain during bicep curls and leg cycling at moderate and high intensity. However, perceptual load, affect, and fun are important characteristics to attend to when designing a VE to exercise in. As negative affect/emotions are so closely linked with pain, a VE that stimulates negative emotion may exacerbate exercise-induced pain rather than alleviate it. These proof-of-concept studies in healthy individuals are a strong starting point, but more research is required before this approach can be integrated into a treatment technique for chronic pain.

Future perspective

While it is difficult to make conclusions from three very different research studies, it is clear that future research is warranted on the effects of VR on exercise-induced pain. For healthy individuals, attenuated pain during cycling could allow them to increase the intensity and/or duration of their exercise, thereby potentially increasing the health-related benefits of exercise or the ability to train for competition. For individuals with chronic pain, exercise may be perceived as too painful, and therefore avoided, despite the evidence-based benefits [69]. Techniques that allow for pain reduction during exercise, including VR, hold promise for breaking this fear-avoidance cycle. Specifically, such techniques could create an environment in which a patient in pain can exercise with attenuated or minimal pain and see clear mental and physical health benefits. This could also overcome the association of exercise with pain exacerbation, which could lead to greater exercise adoption and maintenance, and ultimately contribute to long-term physical and mental health benefits.

Employing VR in physical rehabilitation techniques for people with chronic pain has become very popular over the last decade, but the research studies have most often led to conclusions that it works as well as standard rehabilitation [70–72]. While outside the scope of this review, there may be promise in exergaming or VR-enhanced physical rehabilitation for people with chronic pain conditions. However, current studies have not employed exercise prescriptions previously shown to decrease pain, and have instead added VR to physical rehabilitation, which is distinct from exercise or PA. Researchers claim that exergaming or VR-enhanced exercise improves chronic pain outcomes because of decreased pain, greater enjoyment or higher motivation compared with standard home-based physical therapy, but these outcomes are rarely measured directly [73]. Moreover, the majority of these studies utilize non-immersive VR for fear of greater risk of simulator sickness, dizziness or nausea with immersive VR [74,75]. However, the experiments conducted combining exercise and cycling demonstrated that, with proper precautions, there is no reason to avoid using immersive VR with seated, cycling exercise.

Some studies have begun to explore and directly measure factors that might explain how VR-based treatments improve chronic pain, including a reduction in kinesiophobia or pain catastrophizing [76,77], increased motivation and enjoyment [78,79], or decreased perceived exertion during exercise [80]. More research is warranted to provide enough evidence that VR and exercise is a clinically safe and effective treatment method to combat all-cause chronic pain. This unique treatment modality could be incredibly impactful for the large population of people worldwide struggling with chronic pain conditions.

Executive summary

- The incidence rates of chronic pain globally continue to rise, meaning that effective, long-term treatment techniques still elude researchers and clinicians.
- The most common method of treatment today is pharmacological, which has shown equivocal results with dangerous side effects, including the long-time opioid epidemic.
- Aerobic exercise is an effective long-term treatment for chronic pain and co-occurring mental health issues, but common barriers to exercise in persons with chronic pain is exercise-induced pain and the fear that exercise will exacerbate current pain (i.e., kinesiophobia).
- Current exercise interventions do not target these aforementioned barriers, which leads to deconditioning, sedentary behavior, and a resurgence of chronic pain and subsequent negative consequences.
- Immersive virtual reality is a powerful tool for pain relief under acute pain stimuli, including exercise-induced pain, and should be utilized in exercise-based treatments to mitigate short-term barriers to exercise for people with chronic pain.
- Virtual reality has shown greatest success against chronic pain when combined with other effective, long-term treatment methods.

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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Research Article

Epigenomics

Salivary microRNAs identified by small RNA sequencing and machine learning as potential biomarkers of alcohol dependence

Andrew J Rosato[1], Xiaochun Chen[1], Yoshiaki Tanaka[2], Lindsay A Farrer[3,4,5,6,7], Henry R Kranzler[8], Yaira Z Nunez[1], David C Henderson[1], Joel Gelernter[2,9,10,11] & Huiping Zhang*[,1,3]

[1]Department of Psychiatry, Boston University School of Medicine, Boston, MA 02118, USA
[2]Department of Genetics, Yale University School of Medicine, New Haven, CT 06520, USA
[3]Department of Medicine (Biomedical Genetics), Boston University School of Medicine, Boston, MA 02118, USA
[4]Department of Neurology, Boston University School of Medicine, Boston, MA 02118, USA
[5]Department of Ophthalmology, Boston University School of Medicine, Boston, MA 02118, USA
[6]Department of Epidemiology & Boston University School of Public Health, Boston, MA 02118, USA
[7]Department of Biostatistics, Boston University School of Public Health, Boston, MA 02118, USA
[8]Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania & VISN4 MIRECC, Crescenz VAMC, Philadelphia, PA 19104, USA
[9]Department of Psychiatry, Yale University School of Medicine, New Haven, CT 06511, USA
[10]Department of Neuroscience, Yale University School of Medicine, New Haven, CT 06510, USA
[11]VA Connecticut Healthcare System, West Haven, CT 06516, USA
*Author for correspondence: Tel.: +1 617 358 3689; Fax: +1 617 414 1996; huipingz@bu.edu

Aim: Salivary miRNA can be easily accessible biomarkers of alcohol dependence (AD). **Materials & methods:** The miRNA transcriptome in the saliva of 56 African–Americans (AAs; 28 AD patients/28 controls) and 64 European–Americans (EAs; 32 AD patients/32 controls) was profiled using small RNA sequencing. Differentially expressed miRNAs were identified. Salivary miRNAs were used to predict the AD presence using machine learning with Random Forests. **Results:** Seven miRNAs were differentially expressed in AA AD patients, and five miRNAs were differentially expressed in EA AD patients. The AD prediction accuracy based on top five miRNAs (ranked by Gini index) was 79.1 and 72.2% in AAs and EAs, respectively. **Conclusion:** This study provided the first evidence that salivary miRNAs are AD biomarkers.

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Keywords: alcohol dependence • differential expression • machine learning • salivary microRNA • small RNA sequencing

Alcohol dependence (AD) is a common, complex and genetically influenced disorder. A reliable diagnostic tool for AD is needed to support efforts at prevention and treatment of the disorder. A current AD diagnosis depends primarily on self-reported symptoms, which are limited by inaccurate recall or reluctance of patients to give accurate information on their drinking behaviors or alcohol-related problems. Thus, there is considerable interest in the identification of biological measurements (or biomarkers) to assess a patient's current or past alcohol use.

Biochemical markers such as liver enzymes (e.g., γ-glutamyltransferase, aspartate aminotransferase and alanine aminotransferase) have been used to detect excessive ethanol consumption [1]. However, patients with liver diseases also have increased levels of these liver enzymes. Elevated erythrocyte macrocytic volume (MCV) is also common in AD patients [2], but the slow return of MCV to the reference value diminishes its potential as a relapse marker. Moreover, patients with macrocytic anemia also have an increased MCV [3]. Blood levels of alcohol and its byproducts (such as acetaldehyde, ethyl glucuronide and fatty acid ethyl ester) can reflect acute alcohol ingestion but not past drinking patterns or alcohol relapse [4]. Phosphatidylethanol (PEth), another byproduct of ethanol, can reflect drinking over the preceding weeks [5]. Most of the above biochemical markers (excluding PEth) are limited by their sensitivity and specificity in assessing alcohol abuse.

Future Medicine

Genetic and epigenetic factors are potential biomarkers of AD. Genome-wide association studies have identified AD-associated genetic variants, particularly those located in alcohol-metabolizing enzyme genes [6,7]. Nevertheless, the effect size of these genetic variants on AD risk is small [8]. So far, no genetic markers have been used as practical biomarkers for diagnosis of AD. Epigenetic markers, particularly miRNAs, are potential AD biomarkers. miRNAs are a class of small noncoding RNA molecules (containing about 22 nucleotides) that regulate gene expression via either translational repression or mRNA degradation at the post-transcriptional level [9–11]. As each miRNA can target multiple mRNAs and each mRNA can be regulated by multiple miRNAs, miRNAs play diverse roles in many cellular processes [12]. miRNAs have been implicated in a number of diseases, particularly cancer [13]. Evidence from rat [14] and human *post mortem* brain [15,16] studies suggest that adaptations to alcohol may be due in part to altered expression of a group of miRNAs and their target genes.

Although information on miRNA expression in postmortem brains of patients is critical for unraveling the epigenetic mechanisms of neuropsychiatric disorders including AD, it is of little use clinically because brain tissues are not easily accessible. Besides the existence of miRNAs in tissues and cells, miRNAs are also present in extracellular or body fluids including saliva. Extracellular miRNAs are highly stable and also RNase resistant because they are either contained in membranous vesicles [17,18] or bound to Argonaute proteins [19] or HDL [20]. There is evidence that extracellular miRNAs (such as salivary miRNAs) can serve as informative biomarkers for assessing the severity or presence of diseases [21,22]. However, no study is known to have examined miRNA expression alterations in the saliva of AD subjects.

In the present study, we investigated whether salivary miRNAs are potential biomarkers for detection of AD. We first used miRNA sequencing (miRNA-seq) technology to profile miRNA transcriptomes in the saliva of AD patients and healthy control subjects from both African–American (AA) and European–American (EA) populations. We then identified salivary miRNAs that are differentially expressed in AD patients, and used a machine learning approach to explore the utility of salivary miRNAs as biomarkers for identifying AD.

Materials & methods

Participants

56 African–Americans (AAs; 28 AD patients and 28 control subjects) and 64 European–Americans (EAs; 32 AD patients and 32 control subjects) participated in the present study. Participants were recruited from the community using advertisements such as posted bulletin board flyers and online advertising as well as word of mouth referrals. Participants were screened via telephone and scheduled for appointments if they met the inclusion/exclusion criteria. They were interviewed at the Yale University School of Medicine (APT Foundation; CT, USA). All subjects gave written informed consent to participate in the study. They were assessed using the Semi-Structured Assessment for Drug Dependence and Alcoholism [23,24] to derive diagnoses for lifetime substance use disorders including AD. All subjects were not affected with major psychotic disorders (schizophrenia and bipolar disorder). Among the 60 AD patients, 88.3% of them had one or more co-morbid other substance use disorders (such as cocaine, opioid, nicotine, marijuana, sedative and stimulant dependence). Control subjects were not affected with these substance use disorders. The participants were not seeking treatment, and abstinence from substances was not an inclusion criterion for either group. Demographic characteristics of the sample are summarized in Table 1.

Saliva collection & total RNA extraction

Saliva samples were collected from the above 120 subjects (56 AAs and 64 EAs). Subjects were asked to refrain from eating, drinking, smoking or chewing gum for 30 min before giving saliva samples. Whole saliva (about 2 ml) was collected using the Oragene•RNA (RE-100) for Expression Analysis Self-Collection Kit (DNA Genotek, Ottawa, Canada). Total RNA was extracted from the cell-free supernatant using the TRIzol LS Reagent (Life Technologies, CA, USA). Extracted RNA samples were further purified using the Agencourt RNAClean XP Kit (Beckman Coulter, MA, USA). RNA was quantified using a NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific, MA, USA). The size distribution and quality of extracted RNAs were assessed on an Agilent 2100 Bioanalyzer using an Agilent RNA 6000 Nano Chip. The RNA yield was about 150 ng/ml of saliva supernatant, and the mean RNA integrity number was 6.7.

miRNA-seq library preparation & sequencing

miRNA-seq was conducted at the Yale Center for Genome Analysis. Total RNAs (250 ng) extracted from saliva were used to construct small RNA sequencing libraries using the NEBNext® Multiplex Small RNA Library Prep

Table 1. Demographic information of the sample.

Demographic categories	African–Americans		χ^2/t-test	European–Americans		χ^2/t-test
	AD cases (n = 28)	Controls (n = 28)		AD cases (n = 32)	Controls (n = 32)	
Sex, males	12 (42.9%)	12 (42.9%)	χ^2 = 1.00, p = 1.000	16 (50.0%)	16 (50.0%)	χ^2 = 1.00, p = 1.000
Age, year (mean ± SD)	39 ± 13	39 ± 12	t = -0.11, p = 0.915	40 ± 12	46 ± 16	t = 0.99, p = 0.324
RIN (mean ± SD)	6.7 ± 1.7	6.3 ± 1.8	t = -0.86, p = 0.392	7.2 ± 1.2	6.7 ± 1.6	t = -1.34, p = 0.186
Co-morbid substance use disorders:						
– Cocaine dependence	11 (39.3%)	0 (0%)	–	23 (71.9%)	0 (0%)	–
– Opioid dependence	4 (14.3%)	0 (0%)	–	17 (53.1%)	0 (0%)	–
– Nicotine dependence	12 (42.9%)	0 (0%)	–	21 (65.6%)	0 (0%)	–
– Marijuana dependence	20 (71.4%)	0 (0%)	–	11 (34.4%)	0 (0%)	–
– Sedative dependence	1 (3.6%)	0 (0%)	–	8 (25.0%)	0 (0%)	–
– Stimulant dependence	1 (3.6%)	0 (0%)	–	4 (12.5%)	0 (0%)	–

AD: Alcohol dependence; RIN: RNA integrity number; SD: Standard deviation.

Set for Illumina® (Set 1; New England Biolabs, MA, USA) following the manufacturer's instruction manual. First, the 3′ SR adaptor was ligated to the 3′ end of RNAs, the reverse transcription primer was hybridized to the excess of the 3′ SR adaptor, and the 5′ SR adaptor was ligated to the 5′ end of the RNAs. Then, the ligation products were subjected to reverse transcription reactions to create single-stranded cDNAs. To enrich fragments with adapters on both ends selectively, cDNAs were amplified with 15 cycles of PCRs using a common primer and a primer containing an index tag (6 nt), which facilitated multiplexing and sequencing of different samples in a single lane of a flow cell. Size selection of miRNA-seq libraries (bands of 147 bp, corresponding to the size of adaptor-ligated miRNAs) was performed on a 6% polyacrylamide gel. The size, purity and concentration of miRNA-seq libraries were further assessed on an Agilent 2100 Bioanalyzer using a DNA 1000 chip. Finally, libraries generated from 12 salivary RNA samples were pooled and loaded in one lane of a flow cell for cluster formation. The colonized DNA served as the template for single-end 75-cycle sequencing using the HiSeq 2500 Sequencing System (Illumina, CA, USA).

miRNA-seq data processing

Raw sequence reads were processed by the Mapper module of miRDeep2 (v2.0.0.8) [25] to remove entries with noncanonical letters, clip the 3′ adaptor sequence AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC, discard sequence reads shorter than 18 nt and collapse identical sequence reads. Mapping sequence reads to the human genome (hg19) was performed by miRDeep2 mapper.pl script with the '-e -q -r 100 -s -h -n -m -j -l 18 -k AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC' options. The output files with sequence reads mapped to the human genome (hg19) were further processed with the miRDeep2.pl script to identify human miRNAs that were annotated in the miRBase database (v20) [26]. The total number of sequence reads for each miRNA was normalized to counts per million (CPM) by the total number of mapped sequence reads per sample. Three samples (one AA control sample and two EA control samples) were failed in miRNA-seq.

miRNA differential expression analysis

We performed miRNA differential expression analysis with the Bioconductor package edgeR [27,28]. miRNA expression levels (or counts of miRNAs per sample) were imported to edgeR and converted to CPM. The CPM were then normalized using the method TMM (trimmed means of M-values), which removed the miRNAs that were extremely low or high in expression and those that differed extensively across samples. The negative binomial distribution was used to model the variance of miRNA expression levels. A generalized linear model framework was used to compare miRNA expression differences between cases and controls, which included covariates sex, age,

and RNA integrity number (RIN). To identify common salivary miRNAs as biomarkers of AD, only those with CPM ≥100 in at least half of the subjects were retained in the AD prediction analysis.

miRNA target gene prediction & functional annotation

Genes (or mRNAs) potentially targeted by differentially expressed miRNAs were predicted using miRWalk (v3.0), the online Database on Predicted and Validated miRNA Targets [29,30]. Target genes with a prediction score of more than 0.8 and validated by a third party database such as TargetScan [31], miRDB [32] or miRTarBase [33] were subjected to gene annotation enrichment analysis, which was performed using the online Database for Annotation, Visualization and Integrated Discovery (DAVID; v6.8) [34].

Machine learning & AD prediction by salivary miRNAs

A Random Forest (RF) machine learning approach was applied to identify influential miRNAs for AD prediction using the randomForest R package [35]. An RF model was run using the AD status and miRNA expression levels of subjects in the dataset. The RF algorithm treated the expression level of each miRNA as a different variable in each decision tree and calculated each variable's importance to the model. Included in the importance calculation was each variable's Gini index [36], a measure of the importance of miRNAs in the RF model in predicting the disease status of subjects. In this study, each miRNA was assigned a Gini index based on its contribution to differentiate samples of AD and control subjects. The top ten, five or three miRNAs ranked by the Gini index were applied in AD prediction. The dataset was divided into training and test sets using random sampling for multiple train/test ratios: 50, 60, 70, 80 and 90%. An RF neural network model was created using the training dataset containing the case and control phenotype information. AD prediction was then performed using the RF neural network model on the test dataset. A confusion matrix was generated by comparing the predicted AD status of subjects to their actual status in the test dataset, which yielded the accuracy, sensitivity and specificity of the prediction analysis. The analysis for each ratio was performed ten-times, each time using a seed generated from a different random number. The mean of each of the above three statistics (accuracy, sensitivity and specificity) in ten permutations served as the final result.

Results

Differentially expressed salivary miRNAs in AD subjects

The average number of miRNA-seq reads per AA subject was 2,313,896 (±1,731,731), with 29.0% of the sequence reads mapped to the human genome and 0.2% mapped to human miRNA sequences. The average number of miRNA-seq reads per EA subject was 2,193,734 (±1,486,205), with 27.6% of the sequence reads mapped to the human genome and 0.2% mapped to human miRNA sequences. Among 2,588 different miRNAs detected in the saliva, 399 were expressed at a level of no less than 100 CPM in at least half of the subjects. Expression differences of these 399 miRNAs between cases and controls were analyzed by edgeR, and the results were visualized by volcano plots (Figure 1A & B). A list of miRNAs with $p < 0.050$ and FC (fold-change) >2 is provided in Table 2. Seven such miRNAs (miR-451a, miR-10a-5p, miR-100-5p, miR-3613-5p, miR-7704, miR-1290 and miR-4488) in AAs and five such miRNAs (miR-126-3p, miR-10a-5p, miR-1290, miR-4488 and miR-1273h-5p) in EAs were identified. Although the results did not withstand multiple testing correction (false discovery rate >0.05), expression changes of three miRNAs (miR-10a-5p, miR-1290 and miR-4488) were cross-validated in both AAs and EAs. They showed similar fold changes and the same direction of expression changes in both AA and EA AD subjects.

Pathways enriched in genes potentially targeted by differentially expressed miRNAs

Genes potentially targeted by the above three cross-validated miRNAs (miR-10a-5p, miR-1290 and miR-4488) were predicted by MiRWalk and validated by TargetScan, miRDB or miRTarBase. When the miRWalk prediction score was set at 0.8, 16 genes were predicted to be targets of miR-10a-5p, 104 genes were predicted to be targets of miR-1290, and 46 genes were predicted to be targets of miR-4488. Genes potentially targeted by these miRNAs were significantly overrepresented in gene ontology categories of DNA binding for miR-10a-5p ($P_{Bonferroni} = 0.019$), alternative splicing for miR-1290 ($P_{Bonferroni} = 0.021$), and calcium-dependent cell–cell adhesion for miR-4488 ($P_{Bonferroni} = 0.001$) (Supplementary Table 1).

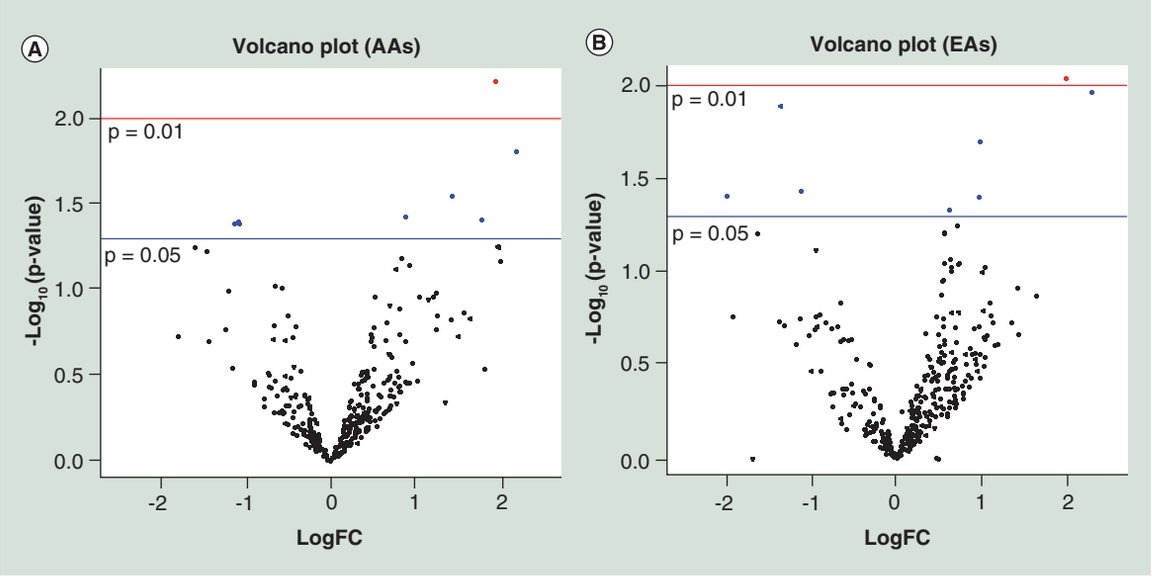

Figure 1. Volcano plot of miRNA expression changes. The volcano plot shows miRNA expression changes in the saliva of AAs **(A)** and EAs **(B)**. The y-axis corresponds to the $\log_{10}$(p-value), and the x-axis displays the $\log^2$ fold change value. The red dots represent differentially expressed miRNAs with $p < 0.01$; the blue dots represent differentially expressed miRNAs with $p < 0.05$; and black dots represent miRNAs whose expression levels did not reach statistical significance ($p > 0.05$).
AA: African–American; EA: European–American.

Table 2. Differentially expressed miRNAs in the saliva of subjects with alcohol dependence.

In AAs	logFC	logCPM	LR	p-value	FDR
hsa-miR-451a	1.93	10.75	7.49	0.006	>0.05
hsa-miR-10a-5p†	2.18	12.72	5.83	0.016	>0.05
hsa-miR-100-5p	1.43	12.49	4.79	0.029	>0.05
hsa-miR-3613-5p	1.77	10.71	4.22	0.040	>0.05
hsa-miR-7704	-1.07	11.93	4.21	0.040	>0.05
hsa-miR-1290†	-1.12	11.58	4.15	0.042	>0.05
hsa-miR-4488†	-1.06	12.64	4.15	0.042	>0.05
In EAs	logFC	logCPM	LR	p-value	FDR
hsa-miR-126-3p	2.00	12.90	6.77	0.009	>0.05
hsa-miR-10a-5p†	2.30	12.75	6.49	0.011	>0.05
hsa-miR-1290†	-1.35	11.40	6.17	0.013	>0.05
hsa-miR-4488†	-1.11	12.98	4.35	0.037	>0.05
hsa-miR-1273h-5p	-1.98	13.48	4.26	0.039	>0.05

†Consistent results in both AAs and EAs.
AA: African–American; EA: European–American; FDR: False discovery rate; logCPM: $\log_2$(counts per million); logFC: $\log_2$(fold change); LR: Likelihood ratio.

AD prediction by machine learning

The machine learning RF algorithm was used to define a cluster of miRNAs that predict AD status. Based on the expression levels of 399 common salivary miRNAs and the AD status of each subject, the RF algorithm constructed a multitude of decision trees at the training time and then output the mode of classes. Each miRNA was assigned a Gini index based on its contribution to differentiate samples of AD and control subjects. The Gini index of the top ten miRNAs in AAs and EAs are shown in Figure 2A & 2B, respectively. When the top five miRNAs (ranked by Gini index or their importance to AD prediction) were included in RF prediction analyses using a train/test sample ratio of 80/20, the AD prediction accuracy was 79.1 and 72.2% in AAs and EAs, respectively (Table 3). Inclusion of the top ten miRNAs (ranked by Gini index or their importance to AD prediction) in the RF prediction analyses

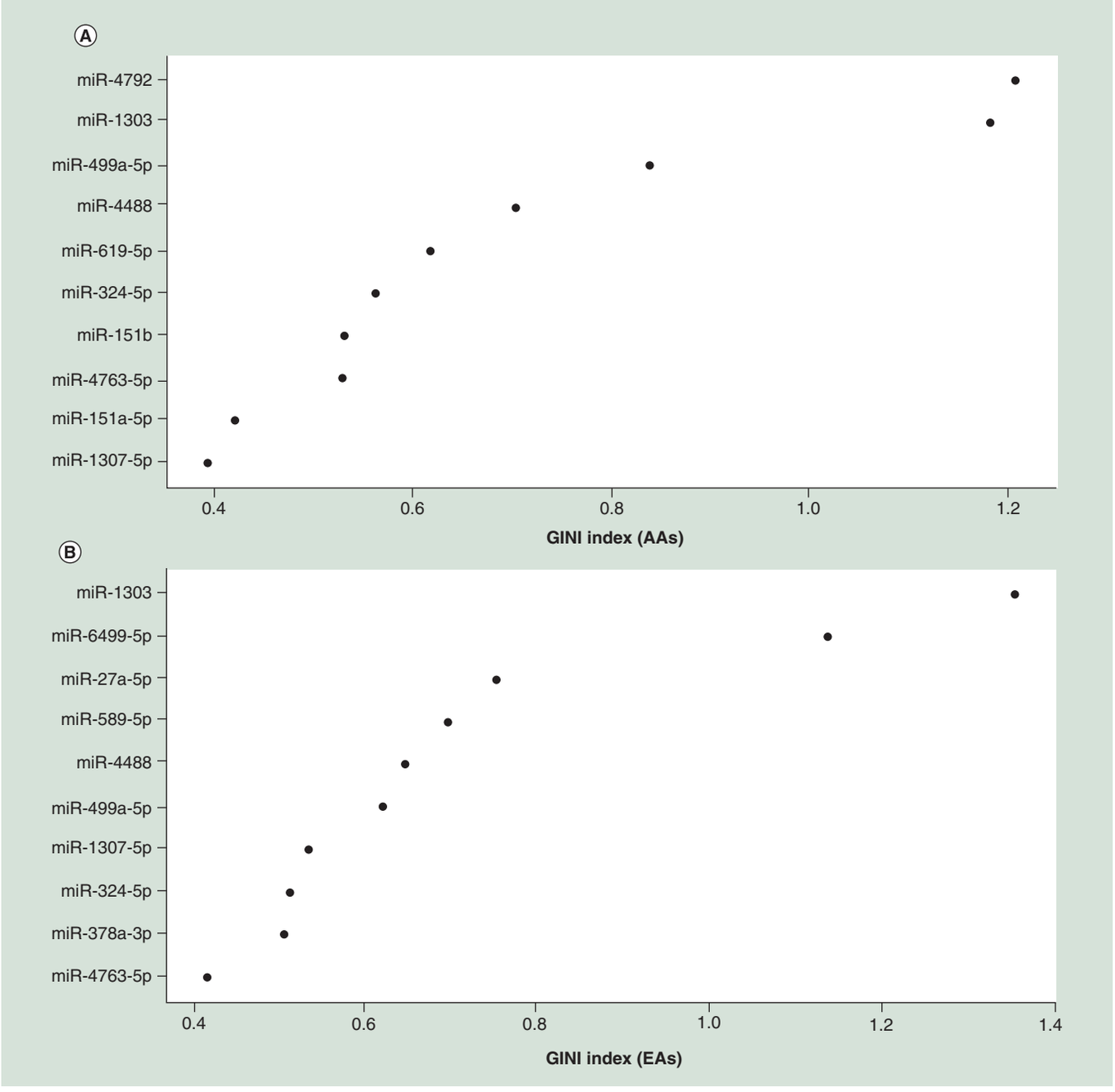

Figure 2. Machine learning-generated Gini indexes of the top ten miRNAs. The x-axis corresponds to Gini indexes generated by the Random Forest machine learning algorithm. The y-axis corresponds to the top ten miRNAs in AAs **(A)** and EAs **(B)**.
AA: African–American; EA: European–American.

decreased the AD prediction accuracy to 73.6% in AAs but slightly increased it to 75.4% in EAs. If the top three miRNAs (ranked by GINI index or their importance to AD prediction) were included in RF prediction analyses, the AD prediction accuracy was decreased in AAs (76.4%) and to a greater extent in EAs (64.6%).

Discussion

The identification of disease-specific biomarkers in easily accessible body fluids such as saliva can result in the early diagnosis and treatment of diseases. Given the important role of miRNAs in post-transcriptional regulation of gene expression and their stability and detectability in saliva, salivary miRNAs have been proposed as specific and reliable

Table 3. Random Forest prediction of alcohol dependence using miRNAs with the highest values of Gini index.

African–Americans				European–Americans			
Train/test	Accuracy (%)	Sensitivity (%)	Specificity (%)	Train/test	Accuracy (%)	Sensitivity (%)	Specificity (%)
Predicted by top ten miRNAs							
50/50	70.4	76.2	65.0	50/50	65.8	65.1	67.3
60/40	68.6	72.8	64.8	60/40	66.8	69.7	64.5
70/30	71.8	75.0	66.5	70/30	72.1	74.2	70.5
80/20	73.6	79.2	67.6	80/20	75.4	77.1	73.6
90/10	68.3	66.0	71.7	90/10	74.3	89.0	56.7
Predicted by top five miRNAs							
50/50	69.3	75.5	63.9	50/50	64.8	66.3	63.9
60/40	70.5	74.8	66.1	60/40	67.2	67.3	67.8
70/30	75.9	79.9	70.1	70/30	72.6	72.0	72.5
80/20	79.1	84.9	71.5	80/20	72.2	71.0	73.9
90/10	78.3	87.7	72.5	90/10	65.7	75.0	54.2
Predicted by top three miRNAs							
50/50	67.1	76.4	57.8	50/50	62.6	65.4	60.9
60/40	68.8	74.6	58.2	60/40	66.0	70.9	60.4
70/30	75.9	79.9	70.0	70/30	66.3	68.8	63.4
80/20	76.4	87.4	61.1	80/20	64.6	66.7	60.0
90/10	75.0	89.7	56.7	90/10	68.6	76.5	51.7

Top ten miRNAs in African–Americans (ordered by Gini indexes from high to low): miR-1303, miR-4792, miR-499a-5p, miR-4488, miR-619-5p, miR-324-5p, miR-151b, miR-4763-5p, miR-151a-5p, miR-1307-5p.
Top ten miRNAs in European–Americans (ordered by Gini indexes from high to low): miR-1303, miR-6499-5p, miR-27a-5p, miR-589-5p, miR-4488, miR-499a-5p, miR-1307-5p, miR-324-5p, miR-378a-3p, and miR-4763-5p.

biomarkers for the noninvasive diagnosis of diseases, including AD. The present study provided initial evidence that salivary miRNAs are potential biomarkers for AD prediction.

First, we identified differentially expressed miRNAs in the salvia of AD subjects by miRNA-seq. Three AD-associated miRNAs (miR-10a-5p, miR-1290 and miR-4488) were cross-validated in both AAs and EAs. In addition, four miRNAs (miR-451a, miR-100-5p, miR-3613-5p and miR-7704) were specific for AA AD subjects and two miRNAs (miR-126-3p and miR-1273h-5p) were specific for EA AD subjects (Table 2). The identification of population-specific miRNAs for AD is not surprising. It is known that gene expression can be population-specific [37], and subjects from different ethnic groups can have different vulnerabilities to AD [38]. Population-specific miRNAs for AD may confer susceptibility of subjects from a specific ethnic group to AD. Because the same set of genes may participate in reward pathways and addiction in subjects from different populations, a set of miRNAs that regulate the expression of these genes may show differential expression in AD subjects from different populations. Our miRNA differential expression analysis considered only common miRNAs (i.e., miRNAs with CPM no less than 100 in at least half of the subjects), even though miRNA-seq can detect miRNAs expressed at a level of a single copy. Because miRNAs present at low levels cannot be accurately quantified and low-expression miRNAs may be indistinguishable from sampling noise [39], low-expression miRNAs are inadequate as biomarkers for disease prediction. Therefore, we excluded low-expression miRNAs from our analyses in order to maximize the sensitivity of detecting differentially expressed miRNAs.

Second, our study demonstrated that salivary miRNAs could be used as biomarkers for AD prediction. A cluster of five miRNAs could achieve a prediction accuracy of over 70% for AD (Table 3). Although certain liver enzymes and alcohol metabolites have been evaluated as AD predictors, their limited specificity precludes them as biomarkers for the diagnosis of AD [1,4]. Specific and reliable biomarkers need to be developed to supplement or replace biochemical measurements in order to predict AD with high confidence. To our knowledge, no other published studies have explored the potential use of salivary miRNAs as biomarkers of AD. miRNAs have been incorporated in the diagnosis of several other diseases particularly cancers [40,41]. There is evidence that miRNA expression profiles can more accurately cluster poorly differentiated tumors than mRNA profiles [42]. In contrast to the relatively small differences in mRNA expression levels between cancer and normal cells, the expression levels of miRNAs can exhibit fold changes of tens to hundreds [43]. In the present study, we found that a cluster of salivary

miRNAs predicted AD with an accuracy of 79.1% in AAs and 72.2% in EAs (Table 3). In comparison to the predictive accuracy of miRNAs in physical diseases such as cancers (97.6% for lung cancer, 97.8% for hepatocellular carcinoma, and 95.0% for bladder carcinoma in a study with 41 lung cancer samples, 47 hepatocellular carcinoma samples and 20 bladder cancer samples as well as adjacent or normal tissues as controls) [44], the miRNA prediction accuracy of AD was relatively low. This may be due to the heterogeneity of AD, which is a complex genetic disorder in which there are substantial gene–environment interactions. In contrast, cancer biomarkers often are derived from somatic mutations [45], which are often more readily assessed and show greater variation than germline mutations such as those contributing to complex diseases like AD.

Third, our findings showed the utility of a machine learning approach in the development of an effective prediction system for AD. The traditional multivariate logistic regression model is commonly used to predict the outcome of a categorical dependent variable (e.g., a disease phenotype) from a set of predictors or independent variables. However, the success of logistic regression model (either forward or backward stepwise) for disease prediction depends on (1) choosing the correct predictor variables, (2) avoiding inclusion of highly correlated variables which can reduce model efficiency, and (3) not including too many predictor variables which can lead to model over-fitting. The major difference between the traditional approach and the machine learning approach for disease prediction is the number of predictor variables that can be considered initially. Our application of the RF-based machine learning prediction algorithm considered 399 common salivary miRNAs from which it extracted a small number of miRNAs for inclusion in the highest performing predictive model. Of interest, the prediction accuracy was not improved when using the top ten versus the top five miRNAs based on the Gini scores, suggesting the number of potentially useful miRNAs in AD prediction is limited. However, the prediction accuracy was reduced when using too few miRNAs (e.g., only the top three miRNAs based on the Gini scores) (Table 3). Including too many predictor variables can dilute the true association and lead to a large standard error with a wide and imprecise confidence interval. Conversely, including too few predictor variables can lower the predictive power of the model.

Fourth, our machine learning prediction analysis results indicated that it was more favorable to use Gini score-ranked top miRNAs than differentially expressed miRNAs in AD prediction. When the train/test ratio was 80/20 and differentially expressed miRNAs ($p < 0.05$ and $logFC > 1$) were included in the prediction analysis, the prediction accuracy was 63.6% in AAs and 56.2% in EAs (Supplementary Table 2). This suggests that a cluster of miRNAs generated by machine learning could be more powerful in predicting disease status than using differentially expressed miRNAs identified by statistical analysis, although the top miRNAs (e.g., miR-4488) generated by machine learning were also differentially expressed in AD subjects. We also noticed that the prediction accuracy was improved when the train/test ratio was increased (Table 3 & Supplementary Table 2). Previous studies have demonstrated that the training sample size is critical for training good classifiers [46]. In other words, more training data decrease the variance of the model, making it a more accurate general representation, and thus decreasing model overfitting.

Fifth, our study suggested that the integration of both machine learning and miRNA-seq approaches could yield a more precise prediction of AD. miRNA-seq is a type of RNA sequencing method (RNA-seq) for use in miRNA transcriptome profiling using a next-generation sequencing platform. It has higher detection sensitivity and specificity than gene expression microarray technology [47]. miRNA-seq can distinguish miRNAs with similar sequences and thus detect miRNA isoforms and novel miRNAs. When profiling miRNA transcriptomes in the saliva of human subjects by miRNA-seq, there is a concern that oral bacterial small RNAs can contaminate the sample. To avoid this, we aligned the sequences of 2588 miRNAs detected in the saliva of our AA and EA subjects against bacterial RNA sequences from the Human Oral Microbiome Database (www.homd.org). None of these salivary miRNA sequence reads were mapped to bacterial RNA sequences. Thus, miRNA-seq plus optimal sequence mapping algorithms facilitate accurate detection of human salivary miRNAs. The development of the disease prediction system using miRNA transcriptome profiling and machine learning is a novel approach with important potential clinical applications.

Conclusion & future perspective

Taken together, we described a method using miRNA-seq and RF-based machine learning to identify a cluster of salivary miRNAs as AD classifiers or predictors. We expect that this type of prediction system could be extended to other diseases based on miRNA expression changes that may also be present in the saliva of patients affected with them. To increase disease prediction accuracy, one could expand the machine learning component to include

additional types of biomarkers such as genetic variants and other epigenetic markers (DNA methylation and histone protein modifications), which regulate gene expression at the transcriptional level. This comprehensive approach could improve the diagnosis and treatment of a variety of diseases.

Summary points

- The current diagnosis of alcohol dependence (AD) depends primarily on self-reported symptoms. These may be augmented by alcohol-related biomarkers that reflect alcohol drinking patterns. The present study aimed to investigate whether salivary miRNAs are easily accessible AD biomarkers.
- We performed the first exploratory study on differential expression of salivary miRNAs in patients with AD in two populations (African–Americans [AAs] and European–Americans [EAs]) and using salivary miRNAs as easily accessible biomarkers to predict the presence of AD by the Random Forest machine learning method.
- Seven miRNAs were differentially expressed ($p < 0.05$ and $>$ two-fold change) in AA AD patients, and five miRNAs were differentially expressed ($p < 0.05$ and $>$ two-fold change) in EA AD patients. Three of the above salivary miRNAs (miR-10a-5p, miR-1290 and miR-4488) showed the same direction of expression changes in both AA and EA AD subjects. These three miRNAs potentially target genes involved in DNA binding, alternative splicing or calcium-dependent cell–cell adhesion.
- When the train/test sample ratio of 80/20 and the top five mRNAs (ranked by Gini index or their importance to AD prediction) were integrated in Random Forest prediction analyses, the AD prediction accuracy was 79.1 and 72.2% in AAs and EAs, respectively.
- These findings reflect miRNA expression changes in the saliva of AD patients, providing evidence that salivary miRNAs are potential biomarkers that identify the presence of AD. The findings need to be validated in a larger sample.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/epi-2018-0177

Author contributions

H Zhang and J Gelernter were responsible for the study concept and design. H Zhang and YZ Nunez performed the experiments. AJ Rosato, X Chen and H Zhang analyzed the data and wrote the manuscript. LA Farrer, HR Kranzler, YZ Nunez, DC Henderso and J Gelernter provided helpful comments on the manuscript. All authors critically reviewed content and approved the final version for publication.

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Financial & competing interests disclosure

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No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

All participants provided written informed consent approved by the Yale University Human Investigation Committee (HIC) (Protocol #: 0102012183).

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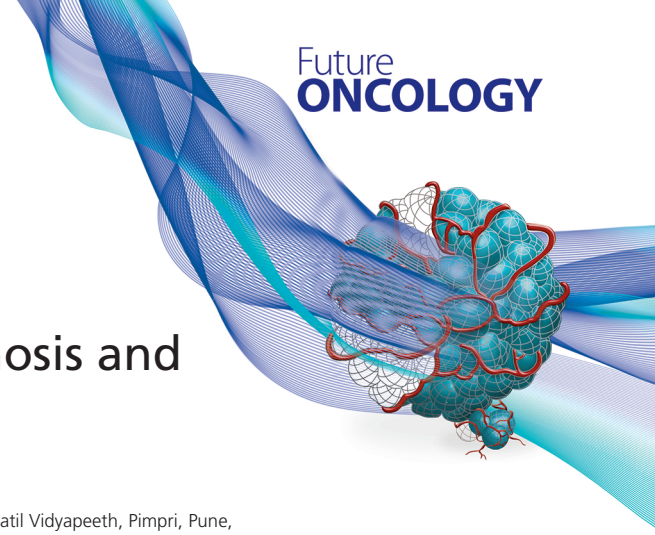

A critical appraisal on cancer prognosis and artificial intelligence

Sachin C Sarode*[,1], Nilesh Kumar Sharma[2] & Gargi Sarode[1]

[1]Department of Oral Pathology & Microbiology, Dr. D. Y. Patil Dental College & Hospital, Dr. D. Y. Patil Vidyapeeth, Pimpri, Pune, 411018, Maharashtra, India

[2]Cancer & Translational Research Laboratory, Dr. D.Y. Patil Biotechnology & Bioinformatics Institute, Dr. D.Y. Patil Vidyapeeth, Pune, 411033, Maharashtra, India

*Author for correspondence: Tel.: +91 992 249 1465; drsachinsarode@gmail.com

> "AI-based cancer prognostication has harnessed various factors such as patient characteristics (age, gender, habit, socio-economic status, etc.), tumor characteristics (type, site, stage, grade, etc.), environmental factors (diet, smoking, alcohol, etc.), imaging findings (computed tomography, MRI, nuclear imaging, etc.) and molecular marker expressions"

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Keywords: artificial intelligence • cancer • cancer biology • machine learning • microbial diversity • molecular carcinogenesis • prediction • prognosis • psychological stress

Artificial intelligence (AI) has been making tremendous strides in cancer research, especially in the domain of diagnosis, grading, management and prognosis [1]. AI models demonstrated superior predictive performance compared with traditional statistics in a majority of solid tumors [2]. AI-based cancer prognostication has harnessed various factors such as patient characteristics (age, gender, habit, socio-economic status, etc.), tumor characteristics (type, site, stage, grade, etc.), environmental factors (diet, smoking, alcohol, etc.), imaging findings (computed tomography, MRI, nuclear imaging, etc.) and molecular marker expressions [3]. Although the understanding of carcinogenesis is evolving day by day due to recent advances, it is still considered a highly complex event. Such complexities are responsible for intra-tumoral (within a tumor) and inter-tumoral (tumor by tumor) heterogeneities, which can impact the prognostication of cancer. Whether such complexities also impact AI-based prognostication is an important question and needs critical deliberation. With this view in mind, we appraised parameters that can impact AI decision-making in determining cancer prognosis. An understanding of these parameters would certainly enhance the perception of AI scientists and generate more research questions.

Molecular pathogenesis

In the human body, 100 trillion cells make up a network of cellular galaxies. Within a single cell, around 25,000 types of proteins and billions of their copy numbers create a part of these molecular galaxies that contribute to the complexity and heterogeneity of a single cell. Besides proteins, other molecular components such as water, ions and simple molecules add another level of molecular complexity. Moreover, 100 trillion cells interface with environmental factors, including diet, pollution, smoking, radiation, drugs and an environmental threat of electromagnetic radiation and silicon dioxide exposure from e-waste [4]. All together, normal cells in a healthy individual have enormous complexities that need to be well understood by AI scientists to further realize the complexities of carcinogenesis.

The molecular complexities further intensify when normal cells transform into malignant cells. Thousands of molecules and signaling pathways are altered to achieve survival, proliferation, invasion and migration goals [5]. Tumor-wise compositional variation in the tumor microenvironment further adds fuel to this complexity [6]. A great deal of intra-tumoral and inter-tumoral heterogeneity is attributed to the differential expression of signaling pathways and the tumor microenvironment and thus becomes an important predictor of biological behavior and the prognosis of a cancer patient. Although scientists are making efforts to quantify noncancerous cell populations

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using computational algorithms with different statistical frameworks and datasets [7], this important proposition has never been accounted for in AI-based predictive cancer research. After realizing this fact, AI scientists will need to incorporate molecular signatures along with tumor microenvironment status.

Psychological stress

It is a well-established fact that psychological stress and associated molecular events can initiate as well as promote carcinogenesis [8]. It can modulate the cancer-associated signaling pathways and leads to aggressive phenotypes in cancer. Invariably, psychological and emotional problems are an integral part of cancer patients' lives and have become an important determinant of prognosis [9]. It is well known that psychological interventions have a positive impact on the prognosis of a patient [10]. Despite this fact, psychological interventions in cancer patients are not routinely practiced in many low- and middle-income countries [11]. Incorporation of psychological status and intervention aspects into AI-based cancer prediction has not been attempted in the literature. In this regard, the only available study showed the highest prediction score of 81.2% by AI for psychological stress (elevated ACTH levels) in breast cancer patients [12]. However, ACTH levels are not the only true reflection of psychological stress and the prognostic aspect is not considered in the study. Hence, this research gap should be addressed in future studies with due consideration of all the psychological parameters (social, clinical and biochemical) of studying the predictive potential of AI in cancer patients. Perhaps a collaboration with psychiatric oncologists would be a pragmatic approach to addressing this issue.

Microbiome diversity

A great deal of microbial diversity exists in human bodies. A unique microbial signature has been reported in many solid tumors such as head and neck, colorectal and gastrointestinal malignancies. Due to protumorigenic and antitumorigenic potential of the human microbiome, the microbiome can modulate the cancer cell's biological behavior [13]. Thus, microbial composition and interaction have an indirect impact on determining the prognosis of the patient and can add complexity to AI-based cancer prognostication. In addition, we propose the need for awareness of the new classes of antibacterial and antiviral drugs that may alter the microbiome.

COVID-19 & cancer prognosis

Due to the growing global incidence of cancer, millions of cancer patients seem to be more susceptible to COVID-19 than the normal population. In this regard, numerous studies have been published regarding the prognosis of cancer patients who tested positive for COVID-19 [14]. A systematic review by ElGohary *et al.* [15] showed that the frequency of cancer among patients with confirmed COVID-19 was 2.1% (95% CI: 1.3–3.0) and mortality was 21.2% (95% CI: 14.7–27.6). Looking at these results, it is quite conceivable that pandemics such as COVID-19 have the potential to impact the prediction of cancer prognosis by AI, which can make the already established algorithms meaningless.

Other additional confounders

Immunocompromised comorbidities such as diabetes, AIDS, organ transplant and autoimmune disorders are some of the known validated prognosticators of increased recurrence and poor survival in cancer patients [16–18]. Various studies have shown significant differences in the survival of cancer patients with and without AIDS [17]. Although the survival gap between cancer patients with and without AIDS is reducing due to recent advancements in management, a small survival difference still exists ($\leq$ threefold) for cancers of the stomach, liver, anus, lung and brain and the most aggressive lymphoma subtype [18]. Certain inborn errors of metabolism are also known to result in predominantly immunologic phenotypes, manifesting in part as inborn errors of immunity [19,20]. These phenotypes are mostly caused by defects that affect the quality or quantity of essential structural building blocks (e.g., nucleic acids and amino acids), cellular energy economy (e.g., glucose metabolism), post-translational protein modification (e.g., glycosylation) or mitochondrial function. Presenting as multisystemic defects, they also affect innate or adaptive immunity, or both, and display various types of immune dysregulation [19,20]. These may add a layer of biological complexity to AI-based cancer prognostication. In low resource settings, especially in developing countries, such conditions are likely to go unnoticed in cancer patients. These confounding aspects need due consideration in future research on AI-based cancer prognosis.

Challenges & prospects

The growth and development of AI- and machine learning-based cancer prognostication will depend on the quality and quantity of data inputs that resolve the complexities discussed above. Hence, first and foremost is to make the large amount of data related to the aforementioned complexities in cancer available to AI scientists. One of the sources of raw data is associated with the supplementary materials in publications, which should be made available freely without any restrictions. AI scientists should be important stakeholders in the development of various databases on human cancer (genomic, proteomic, etc.) and the integration of databases with cancer research. Discoveries of cancer prognostic biomarkers by molecular biologists, pre-clinicians, and oncologists should be shared with AI scientists in vivid and easily understood ways, so that AI scientists will integrate these aspects into their programs catering to cancer prognosis.

Another major challenge for the AI scientist could be the validation of the data quality. The potential error during cancer prognosis could be accountable to the limitations in the accessibility of validated data. The reproducibility and robustness of the experimental results have become the parameter of quality in molecular research on cancer. Already, efforts have been initiated to address these problems in cancer biology, and possibly with the evolution of the same, AI would also be able to address this complexity [21].

There is a need for better infrastructure that can host both AI scientists and cancer biologists on one platform for better communication and understanding. As a measure of capacity building, we recommend the introduction of a basic course in AI for students and other stakeholders in the health science discipline. Similarly, AI students and scientists should also explore basic courses in medical oncology to further bridge the gap for such interdisciplinary research topics.

Conclusion

In conclusion, molecular signature, tumor microenvironment, patient's psychological status and the microbiome could influence the prognosis of the patient. Although these parameters are discussed briefly here, their complexities are far deeper and probably beyond the comprehension of today's AI scientists. For the development of an effective and reliable algorithm for cancer prognostication, there is a pressing need to integrate the aforementioned parameters into the system. The challenges of and remedies for the effective integration of such confounders have been discussed. Effective collaboration and capacity building among AI scientists and cancer biologists would make a difference in the future.

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The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending or royalties.

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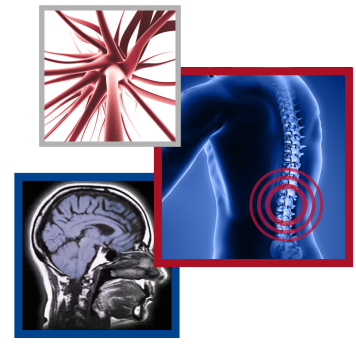

Use and efficacy of virtual, augmented, or mixed reality technology for chronic pain: a systematic review

Nadine S Matthie*[,1], Nicholas A Giordano[1], Coretta M Jenerette[2], Gayenell S Magwood[3], Sharon L Leslie[4], Emily E Northey[1], Caitlin I Webster[5] & Soumitri Sil[6]

[1]Nell Hodgson Woodruff School of Nursing, Emory University; Atlanta, GA 30322, USA
[2]College of Nursing, University of South Carolina; Columbia, SC 29208, USA
[3]College of Nursing, Medical University of South Carolina; Charleston, SC 29425, USA
[4]Woodruff Health Sciences Center Library, Emory University; Atlanta, GA 30322, USA
[5]Children's Healthcare of Atlanta; Atlanta, GA 30329, USA
[6]School of Medicine, Emory University; Aflac Cancer & Blood Disorders Center, Children's Healthcare of Atlanta; Atlanta, GA 30322, USA
*Author for correspondence: nadine.matthie@emory.edu

Aim: Characterize use and efficacy/effectiveness of virtual, augmented, or mixed reality (VR/AR/MR) technology as non-pharmacological therapy for chronic pain. **Methods:** Systematic search of 12 databases to identify empirical studies, of individuals who experience chronic pain or illness involving chronic pain, published between 1990 and 2021. JBI Critical Appraisal Checklists assessed study bias and a narrative synthesis was provided. **Results:** 46 studies, investigating a total of 1456 participants and including 19 randomized controlled trials (RCT), were reviewed. VR/AR/MR was associated with improved pain-related outcomes in 78% of the RCTs. **Conclusion:** While most studies showed effects immediately or up to one month post treatment, RCTs are needed to further evaluate VR/AR/MR, establish long-term benefits, and assess accessibility, especially among individuals who experience pain management disparities.

Plain language summary: Virtual, augmented and mixed reality (VR/AR/MR) are technologies that can be used to manage chronic pain. The use and effectiveness of VR/AR/MR were examined during a review of 46 research studies, which included 1456 participants and 19 randomized controlled trials (RCTs). In 78% of the RCTs, VR/AR/MR improved pain or pain-related outcomes. While most studies showed a benefit on pain immediately or up to 1 month after treatment, more research is needed to assess the long-term benefits of VR/AR/MR on pain and understand how these technologies provide pain relief in the body. Additionally, the accessibility and cost–effectiveness of VR/AR/MR must be evaluated. These areas for future research must consider individuals who experience disparities in the treatment of chronic pain.

Tweetable abstract: A systematic review of 46 studies, including 1456 participants and 19 RCTs, finds that virtual/augmented/mixed reality can have short-term benefits for individuals experiencing chronic pain. #VR/AR/MR #chronicpain

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Keywords: chronic conditions • chronic pain • disparities • pain management • technology • virtual reality

Chronic pain is a multidimensional health problem associated with reduced activity and productivity, disability, decreased quality of life, worsening chronic disease, psychological effects such as depression and anxiety and potential side effects and complications that may result from pain medications [1,2]. The International Association for the Study of Pain defines chronic pain as pain lasting or recurring for over 3 months [3]. In USA, approximately 50 million adults are affected by chronic pain and approximately 20 million experience high-impact chronic pain that often limits life or work activities [4]. The highest prevalence of chronic pain and high-impact chronic pain has been reported among women, individuals who live in rural areas, and older adults who were previously but not currently employed, experience financial instability and receive public health insurance [4]. Annually, chronic pain contributes to approximately US$560 to $635 billion in economic costs because of direct medical expenses, lost

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productivity and disability programs [5,6]. A multi-modal, multidisciplinary approach, such as the biopsychosocial care model, is required to manage chronic pain. This approach may include psychotherapy, complementary and integrative medicine, physical rehabilitation, interventional treatment and pharmacology [7]. Virtual reality (VR), augmented reality (AR) and mixed reality (MR) have emerged as promising, multi-modal, non-pharmacological approaches to pain management that are available to clinicians and individuals living with chronic pain.

The term 'virtual reality' was introduced in the late 1980's by computer scientist Jaron Lanier and it was popularized in the 1990's [8]. Virtual reality integrates computer graphics, body tracking and sensory input devices, visual displays, sounds and other sensations to create an immersive virtual environment [9]. People can engage with this computer-generated, simulated environment in several ways – such as by wearing a headset or head-mounted display (HMD), wearing goggles, or watching images projected onto a screen – and the degree of immersion varies with the type of equipment used to enter the environment. In the virtual environment, individuals can access various software programs (known as applications), including virtual gaming, exercise-based therapies, guided meditation and hypnosis. These applications can be operated via an increasing list of platforms, such as smartphones, computers, Microsoft's Xbox 360, Sony's PlayStation® VR and headsets, including Meta Quest's Oculus devices (such as the Oculus Quest) and HTC devices (such as the HTC VIVE) [10]. Augmented reality involves the real-time overlay of digital content on what a person sees in the real, physical world [11]. For example, a smartphone can be used on a city street to obtain information about buildings in one's field of vision [12] or individuals can play virtual games wherein they race toy cars on top of a table [13]. Augmented reality applications can be operated via smartphones, computers and projectors and AR glasses or headsets such as the Google Glass Enterprise Edition 2 and Oculus Quest 2. Mixed reality, a combination of VR and AR, allows individuals to see the real, physical world while also seeing virtual objects [11]. Applications for MR can be operated on similar platforms as VR and AR applications, and MR glasses such as the Microsoft HoloLens 2.

These technologies are hypothesized to work via various pathways to decrease chronic pain [14–16]. They promote distraction from chronic pain by diverting attention away from noxious stimuli and toward more pleasant or engaging stimuli [17]. They also provide a sense of control and can lead to possible cortical re-patterning, thereby producing analgesia [17,18]. In addition, VR/AR/MR-based approaches may serve to address factors that can exacerbate chronic pain by promoting behavioral skills for self-management and coping with pain. Because of these benefits, coupled with the creation of an immersive and engaging virtual environment, VR/AR/MR may be appealing, accessible, effective and scalable methods of implementing customized pain management for individuals at home, particularly for long-term chronic pain management.

Although several studies have demonstrated positive effects of VR/AR/MR on pain and pain-related outcomes, others have produced inconclusive evidence [19,20]. This systematic review was necessary because no comprehensive appraisal of the evidence has been published to date, and there are gaps in the literature regarding the use and efficacy/effectiveness of these technologies. A preliminary search of PROSPERO, MEDLINE, Cochrane Database of Systematic Reviews and *JBI Database of Systematic Reviews and Implementation Reports* revealed published reviews of VR effectiveness on musculoskeletal pain conditions, mental health and acute pain management. There are also ongoing reviews focused on VR effects in the context of rehabilitation programs (e.g., stroke, phantom limb pain and chronic pain), inpatient settings, cancer pain, burn injury and procedural pain. Yet, no current or in-progress systematic reviews specific to chronic pain across the pediatric and adult lifespan were identified. In addition, VR/AR/MR applications for pain have typically been used in clinic or hospital settings, but cost reductions and advances in the technology have created the potential for use at home [21]. A systematic review of the available VR/AR/MR studies for chronic pain will provide evidence for improving research and practice by informing the future development of VR/AR/MR-based interventions for chronic pain.

The overarching objective of this review is to evaluate the use and efficacy/effectiveness of VR/AR/MR technology, versus usual care or control (where possible), for chronic pain and pain-related outcomes. The following review questions were addressed among children, adolescents, and adults with chronic pain conditions:

- What are the types of VR/AR/MR applications or software that are used for pain management?
- What are the characteristics of VR/AR/MR applications or software that are used for pain management?
- How are VR/AR/MR applications or software used for pain management?
- What is the mechanism of action of VR/AR/MR interventions for reducing pain?
- Are VR/AR/MR interventions efficacious and cost-effective for pain management?

Methods

We conducted this systematic review by following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [22] and the Joanna Briggs Institute (JBI) methodology for systematic reviews of effectiveness evidence [23]. An *a priori* protocol was registered at PROSPERO 2019 (CRD42019117469).

Inclusion & exclusion criteria

This review considered experimental, quasi-experimental and non-experimental studies of children, adolescents, and adults of all ages and genders who experience a chronic condition or illness involving chronic pain, persistent pain, or recurrent pain that lasted for more than 3 months. Non-cancer chronic pain (primary) and chronic cancer pain (secondary) were included. Because of the focus on chronic pain, this review did not include studies wherein participants experienced acute, procedural, experimental, burn or postoperative pain. We considered studies that compared the intervention to usual care or a control condition, and evaluated VR, AR and/or MR technology for chronic pain and any pain-related outcomes. Pain-related outcomes include physical functionality, activities of daily living and quality of life. Among the methods of outcome measurement were validated instruments, observation and self-report.

Search strategy

A comprehensive literature search was undertaken to identify relevant, published studies. Search strategies were developed and conducted by an experienced medical librarian with input from the research team in accordance with the PRISMA guidelines [22] and were peer-reviewed by another medical librarian. Pre-identified sentinel articles were hand searched for keywords relating to the study objectives. The searches combined controlled vocabulary supplemented with keywords related to the concepts of chronic pain (e.g., intractable pain, persistent pain and recurrent pain), pain management (e.g., decreased pain, increased physical functioning and improved quality of life) and the intervention of VR (e.g., AR, virtual environment and immersive display). The search terms were then translated for each additional literature database and grey literature resource appropriate for the study topic. Searches were undertaken 3 October 2018, and rerun on 14 June 2021 and 23 November 2021. The searches were limited to English language and year of publication between 1 January 1990 and 31 December 2021. Prior to 1990, VR was used as a computer and gaming interface and its utilization in healthcare became popularized during the 1990's [8]. Reference lists in selected articles were also screened for additional studies.

12 bibliographic databases were searched: EBSCO's Business Source Complete, CINAHL, PsycInfo and Science and Technology Collection, Cochrane Database of Systematic Reviews, Embase.com, IEEE Xplore, JBI EBP Database, ProQuest Dissertations and Theses Global, MEDLINE via PubMed, Scopus.com and Web of Science Core Collection. The five grey literature sources searched were National Technical Reports Library, Open Grey, Papers First, Proceedings First, PROSPERO and REHABDATA. Clinical trials registries searched were Cochrane Central Register of Controlled Trials and ClinicalTrials.gov. The full electronic search strategies for all sources are provided in Supplementary Table 1. After the searches, all identified citations were collated and uploaded into EndNote X9 (Clarivate Analytics, PA, USA) and duplicates were manually removed.

Assessment of methodological quality

First, the primary reviewer screened the articles selected for retrieval. Eligible studies were then critically appraised independently by all clinical authors and non-author reviewers for methodological quality using JBI standardized critical appraisal instruments for randomized controlled trials (RCTs), quasi-experimental studies, analytical cross-sectional studies, case reports and case series [24]. The certainty of the evidence was subsequently assessed with the Grading of Recommendations, Assessment, Development, and Evaluation approach [25]. Lastly, the primary reviewer examined all the articles and critical appraisal instruments completed by the other reviewers. Any disagreements among the independent reviews were resolved by the decision of the primary reviewer.

Selected studies were included in the review if they met the minimum criteria: seven out of 13 items on the JBI Critical Appraisal Checklist for Randomized Controlled Trials, five out of nine items on the JBI Critical Appraisal Checklist for Quasi-Experimental Studies (non-randomized experimental studies), five out of eight items on the JBI Critical Appraisal Checklist for Analytical Cross-Sectional Studies, five out of eight items on the JBI Critical Appraisal Checklist for Case Reports and six out of 10 items on the JBI Critical Appraisal Checklist for Case Series [24]. Minimum criteria were checklist items identified as the most important methodological criteria based on each study design. For example, minimum criteria for RCTs included randomization, similarity of treatment

groups at baseline, similar treatment of groups except for the intervention of interest, intent-to-treat analysis, reliable measurement of outcomes, appropriate statistical analysis and appropriate trial design.

Data extraction

Data were independently extracted from included studies by all clinical authors and non-author reviewers using a researcher-developed tool that is provided in Supplementary Table 2. This tool, which expanded on the standardized JBI Data Extraction Form [26], was used to collect data specifically related to the review's purpose and objectives. Extracted data included specific details about the study populations, methods, interventions and outcomes of significance for the review objectives. To minimize errors after data extraction, the primary reviewer checked the data and clarified any discrepancies by reviewing the respective articles.

Data synthesis

A statistical meta-analysis of the data was not possible due to the heterogeneity of the study populations, interventions and comparators, outcome measurements and data analysis across the studies. Therefore, we utilized a vote-counting approach based on the direction of the effect reported in each RCT. A sign test was conducted, and a 95% confidence interval (CI) was computed for the RCTs included [27]. Statistical significance was $p < 0.05$. Additionally, characteristics of all included studies have been presented and discussed in narrative form, including tables (see Table 1) where appropriate.

Results

Study inclusion

A total of 1192 articles were identified through the searches. Duplicates (412) were excluded, leaving 780 articles to be screened in the initial title and abstract screening phase. The results were exported to an EndNote library and reviewed by the clinical authors. After excluding 707 articles based on the title and abstract because of unmet inclusion criteria or review objectives, 73 articles were eligible for full-text review and critical appraisal. An additional 14 articles were excluded during the full-text review phase, leaving 59 articles that met all the eligibility criteria for inclusion. After assessing the articles for methodological quality using the JBI standardized critical appraisal instruments [24], 46 were retained for inclusion in this review. Figure 1 shows the PRISMA flow diagram [71].

Characteristics of included studies

The 46 studies that were reviewed include 19 RCTs [19,20,28–44], 21 quasi-experimental studies [45–65] one analytical cross-sectional study [66] three case reports [67–69] and two pilot case series [14,70]. The total sample size for these studies was 1456 and the number of participants in the individual studies ranged from one [68,69] to 179 [30]. Characteristics of the studies are summarized in Table 1.

Of the studies, 42 included virtual reality, two included augmented reality and two included mixed reality. Among the 19 RCTs included in this review, the type of VR/AR/MR intervention, intervention duration and the control condition varied widely, including interventions without VR/AR/MR and treatment as usual. For example, four RCTs examined VR-based physical therapy approaches in comparison to in-person approaches [39–41,44], three compared virtual behavioral therapies to in-person therapies (e.g., cognitive behavioral therapy [CBT] and mirror therapy) [17,19,20,27,31,45] and one study compared the use of immersive VR gaming for distraction to self-mediated distraction interventions [32]. The follow-up period varied across the studies and ranged from 6 hours to 6 months. In 24 studies, there was no follow-up beyond the immediate post-intervention period.

All the studies, except one [60], involved adult participants who were ages 18 years and older. Chronic pain conditions were not mutually exclusive and were listed as: chronic back pain (n = 10), neuropathic pain (n = 8), chronic neck pain (n = 7), phantom limb pain (n = 6), complex regional pain syndrome (n = 5), fibromyalgia (n = 4), chronic pain (n = 2), various chronic pain conditions including headaches (n = 2), chronic pain syndrome (n = 1), rheumatoid arthritis and systemic lupus erythematosus (n = 1), chronic leg pain (n = 1), and upper body chronic pain post cancer surgery (n = 1). In the study involving adolescents, participants were ages 10–17 and they experienced chronic headache [60].

Of the participants in the included studies, 708 (48.6%) were females and 650 (44.6%) were males. It was unclear what genders were involved in four studies [34,38,53,65] because participants were either reported as females or males with no other gender categories specified. In another study, the gender for one participant was reported as

Table 1. Characteristics of included studies.

Study (year), country	Study design, study duration, and post-intervention follow-up	Sample size and population	Interventions (I) and control condition or comparator (C) included in the study	Outcomes reported	Ref.
Randomized controlled trials					
Austin *et al.* Australia	Randomized, cross-over trial; 1 day; no follow-up	16 adults (≥18 years old) with spinal cord injury and chronic neuropathic pain	I: 3D, head-mounted delivery of virtual environment C: 2D screen application of virtual environment	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[28]
Darnall *et al.* USA	Pilot RCT investigating feasibility and efficacy; 21 days; follow-up at 1 day post intervention	74 adults (ages 25–74 years old) with chronic back pain and fibromyalgia	I: 21-day, skills-based, self-management program based on principles of CBT, biofeedback, and mindfulness delivered via VR C: Audio delivery of 21-day, skills-based, self-management program	Pain: Yes Pain-related outcomes: Yes Mechanism of action: Yes Efficacy: Yes Cost–effectiveness: No	[29]
Garcia *et al.* USA	Randomized, placebo-controlled trial; 56 days; no follow-up	179 community-dwelling adults (ages 18–81 years old) with chronic low back pain	I: 8-week, 3D, immersive, VR pain self-management program that incorporates principles of CBT, mindfulness, and pain neuroscience education C: 8-week, non-immersive delivery of 2D nature footage and neutral music via Sham VR headset	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[30]
Garcia-Palacios *et al.* Spain	Pilot RCT investigating feasibility, acceptability, and preliminary efficacy; 3 weeks; follow-up at 3 weeks post intervention	61 adults (ages 23–70 years old) with fibromyalgia syndrome	I: Group CBT program with VR as an addition to activity pacing C: Treatment as usual (follow-up sessions with a rheumatologist for review of medication treatment)	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[19]
Jeon *et al.* Korea	Pilot pre-test and post-test study; 1 day; no follow-up	10 adults (ages 28–50 years old) with complex regional pain syndrome type I	I: Body swapping training video presented via VR, with mental rehearsal C: Body swapping training video presented via VR, without mental rehearsal	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[31]
Jin *et al.* Canada	Randomized, controlled crossover study; 1 day; no follow-up	20 adults (ages 30–75 years old) with chronic pain	I: Immersive VR game C: Self-mediated control with typical pain distraction activities used daily (e.g., reading, meditating, and playing a mobile game)	Pain: Yes Pain-related outcomes: No Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[32]
Lewis *et al.* United Kingdom	RCT; 6 weeks; follow-up at 2 weeks post intervention	45 adults (ages 18–78 years old) with complex regional pain syndrome and body perception disturbance	I: Visual illusions with digital manipulation of participants' hands using a mediated VR device C: Display of visual images, via a mediated VR device, without digital manipulation of participants' hands	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[33]
Matheve *et al.* Belgium	RCT; 1 day; no follow-up	48 adults (ages 18–65 years old) with chronic, nonspecific low back pain	I: Non-immersive VR games controlled by performing pelvic tilt exercises C: Performing pelvic tilt exercises, without VR games, according to a beep tone	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[34]
Nambi *et al.* Saudi Arabi	RCT; 4 weeks; follow-up at 6 months post intervention	60 adult university football players (ages 18–25 years old) with chronic low back pain	I #1: VR training (physical therapy using VR) with a VR game controlled by trunk movements I #2: Isokinetic training performed in an isokinetic dynamometer C: Conventional training of core muscles of the trunk, with stretching	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[35]
Nambi *et al.* Saudi Arabi	RCT; 4 weeks; follow-up at 6 months post intervention	54 adult university soccer players (ages 18–25 years old) with chronic low back pain	I #1: VR balance training, focused on core stability muscles, with a VR game controlled by trunk movements I #2: Combined physical rehabilitation using a Swiss ball for balance training of core stability muscles C: Conventional balance training (isotonic and isometric exercises) for core muscles, with stretching	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[36]

AR: Augmented reality; C: Control condition or comparator; CBT: Cognitive behavioral therapy; I: Interventions; RCT: Randomized controlled trials; SCSVR: Virtual reality.

Table 1. Characteristics of included studies (cont.).

Study (year), country	Study design, study duration, and post-intervention follow-up	Sample size and population	Interventions (I) and control condition or comparator (C) included in the study	Outcomes reported	Ref.
Nambi *et al.* Saudi Arabia	RCT; 4 weeks; follow-up at 8 weeks post intervention and 6 months post intervention	45 adult university football players (ages 18–45 years old) with chronic low back pain	I #1: VR balance training, focused on core stability muscles, with a VR game controlled by trunk movements I #2: Isokinetic training performed in an isokinetic dynamometer C: Conventional balance training (isotonic and isometric exercises) for core muscles, with stretching	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[37]
Nusser *et al.* Germany	RCT; 3 weeks; no follow-up	55 adults (≥18 years old) with non-traumatic chronic neck pain	I #1: Standard rehabilitation program (involving individual and group, general and neck-specific exercise therapy) and individual neck-specific sensorimotor training using a VR device I #2: Standard rehabilitation program and general sensorimotor training (skill exercises, balance exercises, and games) C: Standard rehabilitation program	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[38]
Rezaei *et al.* Iran	RCT; 4 weeks; follow-up at 5 weeks post intervention	42 adults (ages 22–46 years old) with non-specific chronic neck pain	I: VR video game, with increasing stages of difficulty, controlled by participants' head movements C: Conventional proprioceptive training (exercises included eye-follow, gaze stability, eye–head coordination and position sense, and movement sense)	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[39]
Rothgangel *et al.* The Netherlands	RCT; 10 weeks; follow-up at 6-months post intervention	75 adults (ages 44–74 years old) with a unilateral lower limb amputation who experience phantom limb pain	I #1: Traditional mirror therapy followed by tele-treatment at home with AR mirror therapy I #2: Traditional mirror therapy followed by self-delivered mirror therapy C: Sensorimotor exercises without mirror therapy followed by self-delivered sensorimotor exercises	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[20]
Sarig Bahat *et al.* Australia	RCT; 4 weeks; follow-up at 3 months post intervention	90 adults (≥18 years old) with chronic neck pain	I #1: VR kinematic training, with activity in the virtual environment controlled by participants' head movements I #2: Kinematic training using a head-mounted laser beam and wall poster C: Wait-list control	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[40]
Sarig Bahat *et al.* Australia	Pilot RCT; 5 weeks; follow-up at 3 months post intervention	32 adults (ages 26–55 years old) with chronic neck pain	I: Kinematic and VR training, with activity in the virtual environment controlled by participants' head movements C: Kinematic training using a head-mounted laser beam and wall poster	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[41]
Tejera *et al.* Spain	RCT; 4 weeks; Follow-up at 1 month post intervention and at 3 months post intervention	44 adults (ages 18–65 years old) with non-specific chronic neck pain	I: VR treatment, with activity in the virtual environment controlled by participants' neck movements C: Exercise treatment, with flexion, extension, rotation, and tilt exercises	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[42]
Venuturupalli *et al.* USA	Pilot, randomized, cross-over study investigating feasibility; 1 day; no follow-up	17 adults (≥18 years old) with physician-diagnosed autoimmune disorders and chronic pain	I: VR respiratory biofeedback environment C: VR guided mediation environment	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[43]
Yilmaz Yelvar *et al.* Turkey	RCT; 2 weeks; no follow-up	44 adults (ages 35–64 years old) with subacute and chronic, non-specific low back pain	I: Traditional physical therapy program (involving hot pack, TENS, deep heat with ultrasound, and therapeutic exercises) with integration of a 15-minute VR walking video C: Traditional physical therapy program	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[44]

AR: Augmented reality; C: Control condition or comparator; CBT: Cognitive behavioral therapy; I: Interventions; RCT: Randomized controlled trials; SCSVR: Virtual reality.

Table 1. Characteristics of included studies (cont.).

Study (year), country	Study design, study duration, and post-intervention follow-up	Sample size and population	Interventions (I) and control condition or comparator (C) included in the study	Outcomes reported	Ref.
Quasi-experimental studies					
Alemanno *et al.* Italy	Pre-test and post-test study; 4–6 weeks; no follow-up	20 adults (ages 19–72 years old) with chronic low back pain	I: VR-based sensorimotor rehabilitation using an avatar C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[45]
Botella *et al.* Spain	Pre-test and post-test study; 7 weeks; follow-up at 6 months post intervention	6 adults (47–65 years old) with fibromyalgia	I: Group CBT program with VR-based relaxation and mindfulness C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[46]
Fowler *et al.* USA	Implementation-effectiveness, pre-test and post-test study; 3 weeks; no follow-up	16 adult veterans (ages 28–63 years old) with chronic pain	I: VR distraction and exposure therapy, with increasing intensity of stimulation and movement C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[47]
Glavare *et al.* Sweden	Pre-test and post-test study; 6 weeks; no follow-up	12 adults (ages 18–65 years old) with chronic neck pain	I: Neck range of motion exercises using VR, with increasing levels of difficulty C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[48]
Hennessy *et al.* USA	Pilot study investigating content, usability, safety, and acceptance; 1 week; follow-up at 3–5 days post-intervention	12 adults (ages 43–60 years old) with chronic low back pain	I: VR walking modules with progressive movement exposure C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[49]
House *et al.* USA	Feasibility study; 8 weeks; follow-up at 8 weeks post intervention	6 adults (ages 22–78 years old), with upper body chronic pain post breast cancer surgery	I: Integrative VR rehabilitation games, with increasing stages of difficulty C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[50]
Igna *et al.* Romania	Pre-test and post-test study; 3 weeks; no follow-up	68 adults (ages 24–74 years old) with chronic back pain	I #1: Physiotherapy, medication, and mindfulness-based CBT I #2: Physiotherapy, medication, and VR-enhanced CBT C: Usual pharmacological and physiotherapy treatment	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[51]
Jones *et al.* USA	Pre-test and post-test study; 1 day; no follow-up	30 adults (ages 35–79 years old) with various chronic pain conditions	I: Immersive, 360-degree, VR fantasy landscape C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[52]
Liu *et al.* USA	Preliminary study investigating efficacy; 1 day; no follow-up	31 adults (ages 20–81 years old) with migraines, headaches, or other forms of chronic pain (not specified)	I: VR-guided meditation C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[53]
Matamala-Gomez *et al.* Spain	Pre-test and post-test study; 1 day; no follow-up	19 adults (ages 40–55 years old) with complex regional pain syndrome type 1 or type 2	I: Observation of virtual arm, with varying levels of transparency and size C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[54]
Mouraux *et al.* Belgium	Preliminary, pre-test and post-test study; 1 week; follow-up at 24 hours post intervention	22 adults (ages 18–75 years old) with chronic neuropathic pain	I: 3D, AR, mirror visual feedback therapy, with training exercises and virtual games of increasing levels of difficulty C: None	Pain: Yes Pain-related outcomes: No Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[55]
Ortiz-Catalan *et al.* Sweden and Slovenia	Pre-test and post-test study; 6 weeks; follow-up at 1 month post intervention, 3 months post intervention, and 6 months post intervention	14 adults (ages 26–74 years old) with chronic, intractable phantom limb pain	I: Phantom motor execution using myoelectric pattern recognition, AR, and VR, with virtual games controlled by phantom movements C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[56]

AR: Augmented reality; C: Control condition or comparator; CBT: Cognitive behavioral therapy; I: Interventions; RCT: Randomized controlled trials; SCSVR: Virtual reality.

Table 1. Characteristics of included studies (cont.).

Study (year), country	Study design, study duration, and post-intervention follow-up	Sample size and population	Interventions (I) and control condition or comparator (C) included in the study	Outcomes reported	Ref.
Putrino *et al.* USA	Pilot study; duration was not reported; no follow-up	8 adults (ages 44–71 years old) with neuropathic pain	I: Exposure to a scenic VR environment and a somatic VR environment (involving upper and lower extremity movements) C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[57]
Roosink *et al.* Canada	Proof-of-principle and feasibility study; 2 weeks; no follow-up	9 adults (ages 25–72 years old) with spinal cord injury and neuropathic pain	I: Interactive VR walking using an avatar, with virtual feedback C: Static presentation of a virtual scene	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[58]
Rutledge *et al.* USA	Feasibility study; duration was not reported; no follow-up	14 adult veterans (ages 37–76 years old) with an upper or lower extremity amputation, who experience phantom limb pain	I: Bicycling through a VR environment, as an avatar, using a bicycle pedaler and a customized pedal for prosthesis C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[59]
Shiri *et al.* Israel	Pre-test and post-test study; duration was not reported; follow-up at 1 month post intervention and 3 months post intervention	10 adolescents (ages 10–17 years old) with chronic headache	I: VR relaxation combined with biofeedback (tracking of galvanic skin response) C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[60]
Solcà *et al.* Switzerland	Pre-test and post-test, crossover study; 1 day; no follow-up	48 adults (ages 23–80 years old) with complex regional pain syndrome	I: Mirror therapy using synchronous heartbeat-enhanced VR (virtual hand flashing in synchrony with heartbeat) C: Mirror therapy using asynchronous heartbeat-enhanced VR	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[61]
Trost *et al.* USA	Pilot study investigating feasibility and preliminary efficacy; 2 weeks; follow-up at 7 days post intervention and at 2 weeks post intervention	27 adults (ages 23–70 years old) with complete paraplegia after spinal cord injury and neuropathic pain	I: Immersive, spatially tracked, VR walking (using an avatar), with virtual games C: View of avatar in 360-degree virtual scene with no control over virtual walking	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[62]
Villiger *et al.* Switzerland	Pre-test and post-test study; 4 weeks; follow-up at 12–16 weeks post intervention	14 adults (ages 28–71 years old) with neuropathic pain from chronic, incomplete spinal cord injury	I: VR-augmented neurorehabilitation, with VR tasks (of increasing stages of difficulty) for muscle training C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[63]
Won *et al.* USA	Pilot study investigating usability, acceptance, ease of use, and engagement; duration was not reported; follow-up at 1 month post intervention	9 adults (ages 19–60 years old) with complex regional pain syndrome	I: VR mirror visual feedback module, with avatar hands C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[64]
Zauderer *et al.* France	Pilot and feasibility study; 3 months; no follow-up	18 adults (≥18 years old) with non-specific chronic neck pain	I: Standardized, immersive, VR exercise therapy (including active cervical spine range of motion and eye-neck coordination exercises) and non-immersive VR exercise therapy (aerobic, mobility, and muscle strengthening exercises, and a personalized, home-based exercise program) C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[65]
Analytical cross-sectional study					
Solcà *et al.* USA	Cross-sectional, prospective, intervention study; 2 days; no follow-up	15 adults (ages 33–61 years old) with chronic leg pain	I #1: Personalized, visual, VR feedback of perceived SCS-induced paresthesia displayed on patient's virtual body I #2: Personalized, visual, VR feedback with rotation of the virtual body and spatial misalignment between visual VR feedback and SCS-induced paresthesia C: VR illumination of body with no SCS-induced paresthesia	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[66]

AR: Augmented reality; C: Control condition or comparator; CBT: Cognitive behavioral therapy; I: Interventions; RCT: Randomized controlled trials; SCSVR: Virtual reality.

Table 1. Characteristics of included studies (cont.).

Study (year), country	Study design, study duration, and post-intervention follow-up	Sample size and population	Interventions (I) and control condition or comparator (C) included in the study	Outcomes reported	Ref.
Case reports					
Ambron *et al.* USA	Pre-test and post-test study; 6 weeks; no follow-up	2 adults (specific ages were not provided) with unilateral transtibial amputation who experience phantom limb pain	I: VR games, of increasing levels of difficulty, using robot avatar legs controlled by participants' lower limb movements C: None	Pain: Yes Pain-related outcomes: No Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[67]
Oneal *et al.* USA	Pre-test and post-test study; 6 months; follow-up at 1 month post intervention	1 adult (age 36 years old) with chronic neuropathic pain from spinal cord injury	I: VR hypnosis and self-hypnosis at home between VR sessions C: Previous trial of standard hypnosis conducted with participant	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[68]
Ortiz-Catalan *et al.* Sweden	Pre-test and post-test study; 18 weeks; no follow-up	1 adult (age 72 years old) with an amputated limb who experiences phantom limb pain	I: AR, with the use of a virtual limb to play a game controlled by phantom motions C: None	Pain: Yes Pain-related outcomes: No Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[69]
Case series					
Garrett *et al.* Canada	Exploratory, mixed-methods, pre-test and post-test study; 4 weeks; follow-up at 6 hours post intervention and 24 hours post intervention	8 adults (ages 31–71 years old) with chronic pain	I: VR-based mindfulness and meditation, exposure to a VR fantasy landscape and a scenic VR environment, and virtual problem-solving games C: None	Pain: Yes Pain-related outcomes: Yes Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[14]
Sato *et al.* Japan	Pre-test and post-test study; duration was not reported; no follow-up	5 adults (ages 46–74 years old), with complex regional pain syndrome	I: Non-immersive, VR mirror visual feedback therapy, using an avatar hand, with hand exercises C: None	Pain: Yes Pain-related outcomes: No Mechanism of action: No Efficacy: Yes Cost–effectiveness: No	[70]

AR: Augmented reality; C: Control condition or comparator; CBT: Cognitive behavioral therapy; I: Interventions; RCT: Randomized controlled trials; SCSVR: Virtual reality.

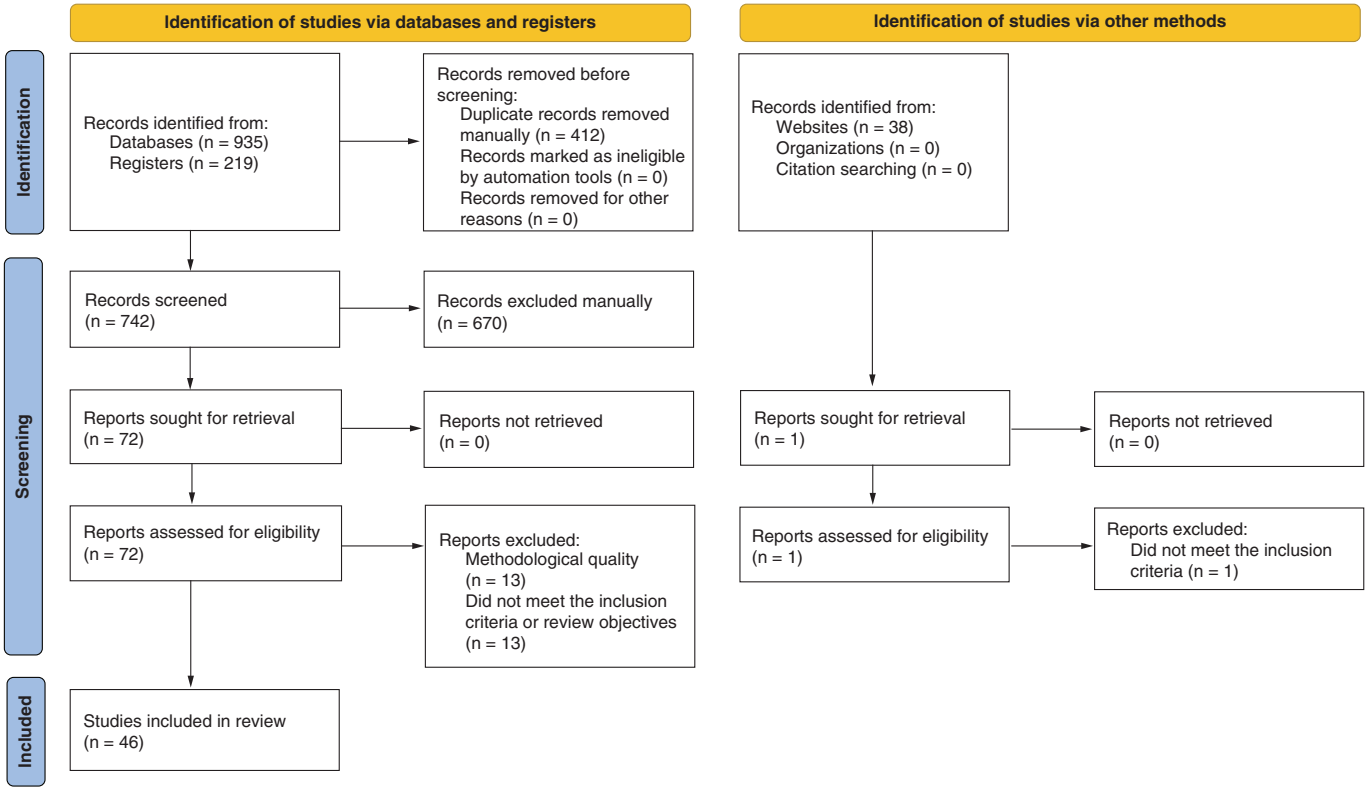

Figure 1. PRISMA flow diagram of study selection process.

'Other' [30]. Four studies had 100% females and six studies had 100% males. In two articles, data was not reported regarding gender [57,68].

Most of the articles (n = 32) did not provide data on race, ethnicity, or other sociodemographic factors (e.g., education, employment, and income). In eight studies, most of the participants were White [19,29,30,43,47,50,52,64] and in two studies, most of the participants were Black [49,62]. In one article, five participants were described as non-White and no data on race were reported for the remaining participants [59]. In another article, 96% of the participants were reported as White and no data were reported regarding the other participants' race [52].

Outcomes of interest and data collection instruments varied across the studies. All the studies included the reduction of pain and/or improvement of pain-related outcomes as study outcomes. Of the studies, 46 investigated the reduction of pain, 41 investigated the improvement of pain-related outcomes and 24 studies also evaluated the feasibility and/or acceptability of the technologies. Across the studies, pain-related outcomes included fear of movement, range of motion and kinematics, pain-related functional limitations or interference, emotional distress (such as depression), health status, daily functioning, functional disability, coping skills and quality of life. Outcomes related to feasibility and/or acceptability included acceptability of and satisfaction with VR, adverse effects or side effects and preferences in the type of VR experience.

Methodological quality

The overall quality of the RCTs was moderate, with a low risk of bias for most of the studies. Low risk of bias (or bias not serious) was related to having few study limitations such as the lack of blinding, a control group or follow-up. There was one RCT with a moderate risk of bias that was related to a lack of blinding of participants to treatment assignment, lack of blinding of those delivering treatment and lack of follow-up [44]. However, the authors reported that the participants and therapists were not blinded because of the nature of the intervention [44]. It was unclear whether at least one criterion was met in five RCTs because the information was not reported. These criteria included concealment of allocation to treatment groups, blinding of participants to treatment assignment, blinding of treatment assignment among those delivering treatment and blinding of outcome assessors to treatment assignment [29,31,32,39,43]. In two of these RCTs, it was unclear whether true randomization was used because the process of random assignment was not described [31,32]. Results of the critical appraisal assessments are provided in Supplementary Tables 3–7.

Results of the included studies consisted of both positive and negative findings; thus, publication bias was undetected. However, given the limitations of the included studies that were described above, the certainty of the evidence ranged from low to high with most of the studies demonstrating low certainty. This classification indicates that further research is highly likely to influence the confidence in the estimate of effect and is likely to change the estimate [25].

Review findings

The characteristics of the included studies are presented in Table 1 and a summary that addresses the sub-questions of this review are provided below.

Types of VR/AR/MR applications or software used for chronic pain management

The types of technology varied across the studies. Of the VR studies, 13 utilized immersive HMDs or headsets [28–31,42,43,47,48,53,57,59,61,65], 13 utilized immersive HMDs or headsets that were either tethered to computers and external cameras or required a computer to operate the software [14,32,38,40,41,49,52,54,59,62,64,66,67] and 14 utilized a desktop or laptop and displayed the non-immersive virtual environment on a desktop or laptop monitor, projector screen, or other screen [19,33–37,39,45,46,50,58,60,63,70]. One study utilized a device that was described as a VR helmet [68]; however, it was unclear whether the device was tethered or not. One study utilized a tethered, immersive HMD then transitioned to a portable VR headset and smartphone, when they became available, for participants' use at home if desired [59]. In one study, participants used video glasses to watch a virtual video on an iPod [44]. In another study, the VR device was not described [51].

Two studies used a non-immersive AR or MR system, consisting of a desktop computer and camera and presented the environment on a computer screen [55,56]. In one study, a VR and myoelectrically-controlled AR environment was presented on a computer screen [69]. Another study utilized a tablet with a built-in camera to display the AR environment [20].

Characteristics of VR/AR/MR applications or software used for chronic pain management

Several devices and systems were used in the studies. The most utilized hardware to deliver VR across studies was the HMD, predominantly the Oculus Rift® (n = 12). Other HMDs included the HTC VIVE and Samsung Oculus Gear VR, among others. These devices have built-in stereoscopic screens, which display separate images for each eye and sound and motion tracking sensors [72]. Other hardware used in the studies, such as the Wrap™1200VR and the Wrap920, are digital video eyewear products typically designed for AR applications [73]. Head-mounted displays and eyewear devices provide immersive video experiences for users. Systems included the Virtual Reality Rehabilitation System and the BrightArm Duo Rehabilitation System – an experimental robotic platform that modulates gravity loading on the upper extremities [50]. These types of systems are like the Microsoft Kinect and Nintendo Wii because they integrate haptics and projected images or avatars on screens so that users' motions are mimicked.

Various VR/AR/MR environments were used across the studies. In the context of this review, VR environment is a broad term that refers to a digital setting capable of arousing feelings of presence and immersion in VR/AR/MR users. Environments included VR or AR games, rehabilitation games or training exercises, VR programs or applications (such as a guided meditation application), VR experiences with and without gaming elements, software (such as a reality substitution software) and environments (such as a simulator for chronic pain treatment). While often designed for pleasure, VR games can also have therapeutic applications, such as distraction to mitigate painful experiences [74]. When VR/AR applications are used with sensors and haptics in rehabilitative settings to improve users' physical or cognitive functioning, they may be referred to as rehabilitation games, rehabilitation training, or exergaming [75]. As seen in the studies included in this review, the level of immersion in the VR environment can range from the projection of images on desktops or across screens in entire rooms to the use of avatars via HMDs.

Approaches for using VR/AR/MR applications or software for chronic pain management

The included studies applied a variety of approaches to using VR/AR/MR technology for chronic pain management. The approaches were not mutually exclusive and included: coping with chronic pain and/or associated psychosocial correlates (n = 14); rehabilitation therapy (physical or neuro rehabilitation) (n = 10); mirror therapy (n = 7); adjunct/enhancement to CBT (n = 4) or to replace guided imagery (n = 1) in the psychological treatment of pain; gaming (n = 3); virtual feedback or biofeedback (n = 3); prediction of motion intent (n = 2); visual feedback therapy or visual representation of spinal cord stimulation-induced paresthesia to enhance analgesia (n = 2); meditation and relaxation to reduce chronic pain and/or stress (n = 2); adjunct to activity management (n = 1) or an adjunct home therapy in chronic pain management (n = 1); graded exposure therapy for kinesiophobia (n = 2) and hypnosis (n = 1). Of the studies, 84.8% (n = 39) were conducted within a healthcare or research setting, such as a clinic or laboratory, while 15.2% (n = 7) were home-based. A group format was used to deliver the intervention in two studies [19,46].

Types of experiences that were provided by the VR/AR/MR applications or software were active (n = 25), passive (n = 14), or both (n = 7). Active experiences enabled participants to engage with interactive elements in the VR/AR/MR environment by completing specific tasks, such as shooting snowballs at targets. In contrast, passive or relaxing experiences allowed for immersion in the VR/AR/MR environment without active interaction, such as 'traveling' through the environment on a boat ride. The frequency or timing of VR/AR/MR delivery was two or more times in approximately 93.5% (n = 43) of the studies, with exposure to the VR/AR/MR environments, or dose, during each period of use ranging from one minute [33] to 2 hours [46]. The 2-hour experience was a group session in which a computer display, not an HMD, was used. In one study, participants were free to use the AR tele-treatment at home for their desired length and frequency [20]. However, participants used a tablet, not an HMD, to complete the tele-treatment. In another study, there was no set time limit for use of the technology, but the virtual environment was presented on a desktop monitor instead of an HMD [70]. Although the study duration was reported in three of the articles, the specific duration of VR/AR/MR use was not reported [42,56,58]. In five articles, the study duration was not reported (see Table 1). In another article, neither the study duration nor the specific duration of VR use was reported [64].

Mechanism of action of VR/AR/MR interventions for reducing chronic pain

Of the included studies, only one directly investigated the mechanism of action of VR/AR/MR for reducing chronic pain. In this study, the proposed mechanisms were mastery of behavioral skills for pain coping and enhanced self-

efficacy for pain self-management and treatment effects were attributed to the didactic and skills-based components of the immersive behavioral therapy [29]. In the remaining 45 articles, mechanisms of VR/AR/MR action were presented as the basis for the study or were discussed in support of study findings. These mechanisms were not mutually exclusive and included: cognitive and or/attentional distraction (n = 26); mechanisms of mirror therapy such as activation of the mirror neuron system, promotion of cortical reorganization, and provision of normalized visual feedback of movements to reduce pain perception (n = 7); activation of motor control mechanisms, function and movement execution, and/or coordination (n = 4); reversal of maladaptive changes in central neuroplasticity (n = 4); interactivity for motivation and enjoyment or training (n = 4); pain modulation mechanisms (n = 3); relaxation (n = 3); immersion (n = 2); cognitive-emotional mechanisms or emotional engagement (n = 2); modulation of the central body representation (n = 2); sensory feedback and activation of neurons to enhance motor activity (n = 2); promotion of self-efficacy for pain coping behaviors (n = 1); endorphin release (n = 1); alterations in the inflammatory process (n = 1); psychoneuromuscular theory (n = 1), activation of cortical and subcortical neuronal circuits to stimulate learning and recovery (n = 1) and visuotactile or visuomotor stimulations (n = 1).

Efficacy/effectiveness & cost-effectiveness of using VR/AR/MR interventions for chronic pain management

All 46 included studies investigated the efficacy/effectiveness of using VR/AR/MR for addressing pain and/or pain related outcomes as primary and/or secondary study objectives. However, the cost–effectiveness of using these technologies was not investigated. The efficacy/effectiveness findings provided here are not mutually exclusive.

There was a statistically significant reduction in pain intensity, phantom sensations, or pain unpleasantness in 29 (63%) of the 46 included studies. 19 of these 29 studies were RCTs, of which 78% (n = 15) demonstrated statistically significant benefits associated with the use of VR/AR/MR technology for pain (95% CI: 54%, 94%; p = 0.019) relative to the control group. Of these 15 RCTs, only one study utilized a sham VR headset as the control condition [30]. The remaining 14 RCTs utilized active control conditions without VR/AR/MR as the comparison, including an audio version of the content from the VR intervention program, mirror therapy, physical therapy, a rehabilitation program, and typical pain distraction activities. One of these studies also included a wait-list control as a second comparator [40]. In 82.7% (n = 24) of the 29 studies, effects on pain were found in the short-term (up to four weeks post-treatment) or immediate post-treatment period. Two studies found both short-term and long-term effects, with long-term effects at five weeks post intervention (n = 1) and 12–16 weeks post treatment (n = 1). Long-term effects were found in three studies, at 8 weeks post intervention (n = 1) and 6 months post intervention (n = 2). Although findings were not statistically significant in the remaining included studies (n = 17), some studies had clinically significant findings. For example, in one study, eight of 12 participants experienced an improvement in pain scores, with an average decrease of 7.8 points (SD = 5.1) [49]. In another study, VR conditions resulted in a 50% decrease in pain ratings [54].

In 52.2% (n = 24) of the included studies, there was a statistically significant improvement in various pain-related outcomes. These outcomes included: psychological correlates of pain such as affect, depression, anxiety, mood, or stress (n = 12); functional status, daily functioning, or mobility (n = 9); pain interference in activities of daily living and/or sleep (n = 6), kinesiophobia (fear of pain due to movement; n = 5), quality of life (n = 3), disability (n = 3), limb/joint range or strength (n = 2), cognitive functions (n = 2), coping skills (n = 1) and time spent thinking of pain (n = 1). In 75% (n = 18) of the 24 studies, effects on pain-related outcomes were found immediately post-treatment. Long-term effects were found in six of the studies, at 5 weeks post intervention (n = 1), 3 months post treatment (n = 1), 8 weeks and 6 months post intervention (n = 1) and 6 months post intervention (n = 3).

Other outcomes of interest

24 studies evaluated the feasibility and/or acceptability of using VR for pain and/or pain-related outcomes. In half of these studies, most participants reported satisfaction or high satisfaction with the VR experience or found VR to be an acceptable intervention for chronic pain. Participants described the experience as logical, useful, helpful and/or immersive [19,58,59]. They also reported high levels of enjoyment, motivation, attention [63] and engagement during the VR intervention [52]. In one study, two of 10 participants did not perceive the VR treatment as helpful [60]. However, there was an improvement in their pre-post treatment quality of life scores. In a few studies, some participants provided comments regarding limitations of the VR technology and practicality of its use as an adjunctive therapy. These participants reported frustration with using complex or cumbersome control systems, inability to use VR equipment during periods of severe pain and short-term duration of treatment effects [14];

an unpleasant weight of the study device – a helmet with an integrated HMD and sensors for head-movement tracking [38]; heaviness or bulkiness of the VR glasses or headset [43,48,65]; and discomfort in using corrective glasses with the headset [65].

Adverse effects or negative side effects were reported in 33.3% (n = 8) of these 24 studies. These effects included: nausea or motion sickness (4%, n = 4 to 24%, n = 6) [29,30,47]; mild nausea, rated at a level of 3 out of 10 (62.5%, n = 5 and 3.3%, n = 1) [14,52]; discomfort of device (5.9%, n = 1) [43]; dizziness in two of 98 study sessions [47]; transient musculoskeletal pain, physical fatigue and difficulties in maintaining attention (77.8%, n = 7) [58]; and 'slight' cybersickness (22.2%, n = 2) [64]. In one study, the presence or absence of adverse effects or negative side effects was not reported [47]. Some of these effects resolved with slowing the experience or taking a break from the device. Despite experiencing these effects, many participants either remained in the study because their ability to participate was not affected, expressed interest in using VR at home, and/or purchased a VR device to use at home.

Discussion

Effective pain management requires multifaceted interventions that employ pharmacological and non-pharmacological strategies. However, chronic pain management has posited a significant challenge for healthcare providers because a multidisciplinary treatment approach is lacking [19]. This systematic review of 46 studies suggests that VR/AR/MR can aid in providing patients with relief from chronic pain and improving pain-related outcomes.

Although several types of VR/AR/MR applications or software were utilized in several ways according to numerous mechanisms of action across the included studies, VR/AR/MR demonstrated statistically significant or potential clinical benefits for chronic pain and chronic pain-related outcomes. In the majority of the RCTs, the statistically significant benefits were demonstrated in comparison to active control conditions. The limited use of sham interventions and wait-listed control conditions inhibits our understanding of whether these findings, which were primarily short-term effects, are therapy-specific effects. For included studies in which the primary outcome measure was pain reduction, most of the studies reported high levels of pain reduction among study participants and benefits such as reduction of pain intensity, phantom sensations and pain unpleasantness. In studies that measured pain-related outcomes, the use of VR/AR/MR technology was also associated with substantial improvements. Benefits were demonstrated for outcomes such as pain interference, health status, fear of movement, functional capacity, perceived quality of life and coping strategies. In addition, some of the studies demonstrated the feasibility of VR/AR/MR use and high levels of acceptability among users and healthcare providers.

The VR/AR/MR interventions utilized among included studies were diverse, with VR being the most common technological approach employed. Few studies (n = 7) were home-based and only three of these studies included the option for use of a wireless device [20,30,59]. Additionally, participants in a few studies (n = 6) raised concerns regarding the convenience of the technology. These findings may help to improve the design, uptake and effectiveness of VR/AR/MR interventions; thus, they have important implications for long-term use of these technologies. There remain many barriers for patients seeking to access care at pain clinics or via integrative pain management clinicians, including costs and prohibitive distances to travel [76,77]. In addition, the coronavirus disease 2019 pandemic has further hastened the urgency to deliver effective nonpharmacological pain management interventions remotely to patients in the safety and comfort of their homes. The advancements in VR/AR/MR technology in recent years create the potential for increased accessibility and use of the technology in the patient's home environment as a part of their daily activities. Accordingly, utilizing VR/AR/MR modalities to manage chronic pain at home may be of interest to patients unable to travel or access in-person care [78]. Moreover, use of home-based interventions creates the opportunity for long-term evaluation of chronic pain and identification of patterns over time.

In studies that evaluated the acceptability and/or feasibility of VR/AR/MR, participants reported high satisfaction levels with the technology along with minimal, if any, adverse effects, or negative side effects. User satisfaction was specifically high in areas such as immersion, realism, helpfulness and usefulness of VR/AR/MR [19,58–60]. This underlines the fact that researchers must consider the nature of the virtual environments they design for VR/AR/MR interventions because the development of sophisticated VR technology may potentially be for naught if it does not appeal to the user [79]. The review finding reinforces the need for researchers to evaluate the level of immersion of their virtual environments and conduct analyses of how factors, such as immersion, affect pain and treatment outcomes [79].

Although this review focused on chronic pain management, our findings are consistent with current literature that has assessed the use of VR for various types of pain, including acute pain and found significant improvement in pain levels [79–81]. Most of the included studies did not directly address the mechanism of action for VR/AR/MR,

but over half of the studies cited the benefits of distraction in pain management and alluded to the benefits of pain reduction because of distraction. Changing the way that the brain physically registers pain through a complex combination of immersion, emotional engagement and cognitive distraction that is imbedded into the current experience draws attention away from the amount of pain being consciously experienced [32,74]. Stimulating the visual cortex while simultaneously engaging other senses, through features that allow users' minds to engage in an immersive experience, may have a substantial effect on moderating the processing of nociceptive stimuli and improving pain outcomes [17]. We infer that this process may be key in addressing and relieving chronic pain. Future research should characterize treatment mechanisms and duration of treatment effects across diverse patient populations living with chronic pain conditions. Addressing this gap will require investigations that capture both patient-reported outcomes and objective metrics, such as brain imaging, blood-based biomarkers and quantitative sensory testing.

Some of the included studies incorporated VR/AR/MR into evidence-based clinical interventions, such as hypnosis, biofeedback and physical therapy, resulting in significant improvements in pain and functional capacity [38,60,68]. Aligning VR/AR/MR with other modalities has become an emerging line of research, with some evidence that coupling of VR/AR/MR with methods such as hypnosis may be more effective for chronic pain management than either intervention alone [17]. One advantage of VR/AR/MR-based pain management interventions is the unique opportunity for managing chronic pain while also reducing biopsychosocial distress, anxiety and depression among patients [17,18,50,51,53]. Because pain-related outcomes can be triggered by psychosocial factors such as stress, the reduction of biopsychosocial stress may also include a potential effect of pain reduction [82].

We also aimed to assess the cost–effectiveness of VR/AR/MR interventions, but the included studies did not investigate cost-related outcomes. Interventions that involve VR/AR/MR could potentially be an affordable alternative for patients suffering from uncontrolled pain, especially as the cost of such technology, particularly VR, continues to decrease [21,81]. As the VR/AR/MR market continues to evolve, future studies are needed to assess the cost–effectiveness of such interventions for hospital, in-clinic and home use in addition to assessing feasibility of access to such interventions [81]. The combination of decreased technology costs, flexibility and customizability of immersive features and improvements in software and hardware design result in numerous potential applications for patients who are suffering from a wide array of acute and chronic pain conditions ranging from visceral to somatic pain [17]. These factors increase the potential and necessity for widespread dissemination of technology-based interventions throughout health systems [17,43], with the capability to continue treatment post-discharge. Therefore, VR/AR/MR technologies may be used to support individual and customized pain self-management, which can contribute to a decrease in healthcare expenses and expenditure of clinical resources.

Notably, most studies did not report data regarding race, ethnicity, or other sociodemographic factors. This may have been because most studies were conducted outside of the USA. While race is a socially constructed concept, it is paramount that future researchers assess and analyze socioeconomic and sociocultural contexts as well as the availability of resources and quality of infrastructure for persons with chronic pain. Addressing social determinants of health (SDOH) is at the forefront of achieving health equity. However, there was a paucity of attention to SDOH in the included studies, with demographics often limited to male/female gender, age, disease state, type of chronic pain and level of education. Attention to social-environmental-cultural context in future studies is particularly important given documented biases in healthcare. Such attention is also required when testing and refining intervention strategies for populations that have been historically marginalized because of race, ethnicity, or geographic location. Because pain is influenced by biological, psychological and social factors [83] and quality of life is a multidimensional concept often considered in investigations of pain, not examining social factors may contribute to further marginalization. Moreover, the acceptability and utility, access, mechanism of action, potential efficacy and customizability of VR/AR/MR technologies to individual needs may be affected by these factors [84].

Limitations of this review

There are some limitations to this systematic review. The specific inclusion criteria for this review may have limited the number of available studies. Despite conducting a comprehensive literature search, the final number of included studies may have been limited because the use of VR/AR/MR technology for chronic pain is still a developing area of research with few published studies. As a result, the number of RCTs and studies involving children and adolescents was also limited. Furthermore, this review only included studies published in the English language, potentially excluding studies otherwise eligible.

The heterogeneity of the study populations, interventions and comparators, outcome measurements and data analysis across the studies posed a challenge for synthesizing the results. Most of the studies included small sample sizes and in 45.6% (n = 21) of the studies, a comparison condition or comparator was not included. Because of these factors, the generalizability of the study results and the power of the findings are limited. Despite the heterogeneity in RCT outcomes, a count synthesis was conducted. However, a limitation of this approach is the inability to capture the magnitude of effect sizes. In addition, half of the studies did not include pain relief follow-up beyond the immediate post-intervention period. In a few of the studies that included a follow-up (n = 9), follow-up occurred within 1 month of treatment, resulting in insufficient data for determining VR's efficacy/effectiveness for long-term pain relief. Thus, there is a need for RCTs with larger sample sizes that are designed to provide high-quality evidence on the long-term efficacy of VR/AR/MR interventions. The RCTs included in this review tested a diverse set of VR/AR/MR interventions, of varying immersion and duration, with differing control groups, and were conducted on patients with a spectrum of chronic pain conditions, thus inhibiting our ability to inferentially ascertain the impact of these therapies. Nonetheless, there were significant findings that can be used to inform the future development of VR/AR/MR-based interventions for chronic pain. As the body of VR/AR/MR research grows, future systematic reviews may benefit from examining RCTs focused on comparing improvements in physical health functioning (e.g., physical therapy) and behavioral health functioning (e.g., CBT and mirror therapy) among patients with chronic pain conditions. Future studies examining the impact of VR/AR/MR compared with other pain management approaches would benefit from improved data reporting and interpretation as outlined by pain-focused international research groups, specifically when reporting group differences on patient-reported outcomes and pain medication utilization [85,86].

Conclusion

This review supports findings of current literature regarding the efficacy/effectiveness of VR/AR/MR in reducing pain and improving pain-related outcomes among patients living with chronic pain. The potential that innovative, non-pharmacological technologies, such as VR/AR/MR, offer individuals to cope with chronic pain is significant. While the efficacy/effectiveness of VR/AR/MR technology varied across studies, most studies showed short-term effects on reducing pain and improving pain-related outcomes. These pain-related outcomes included coping skills, daily functioning or functional capacity and perceived quality of life. Based on the findings of this review, there is no available evidence on the cost–effectiveness of using these technologies for home-based chronic pain management. However, the portability of VR/AR/MR enables use of these technologies in the delivery of home-based, pain self-management interventions to decrease chronic pain and its negative effects.

VR/AR/MR technologies can serve as efficacious methods of delivering non-pharmacological interventions for addressing treatment gaps in chronic pain management. Effective pain management must address psychosocial and behavioral factors while promoting self-management in conjunction with pharmacological and physical approaches [79,87]. VR/AR/MR technologies hold promise in addressing the various challenges that healthcare providers and patients have experienced in achieving effective pain management. As more rigorous research is conducted to evaluate the effectiveness of these technologies, data from such research can be used in support of their widespread dissemination throughout healthcare systems and in patients' homes.

Recommendations for practice

The following preliminary practice recommendations can be made:

- VR/AR/MR technologies can be effective methods for delivering interventions for chronic pain.
- VR/AR/MR-based interventions may be considered as a strategy to support home-based chronic pain management. This strategy may benefit historically marginalized individuals and those who live in locations where access to in-person interventions is limited.

Recommendations for research

The following recommendations can be made for future research:

- RCTs are required to evaluate VR/AR/MR technologies, particularly for home-based chronic pain management. There is a need for conducting more RCTs, with larger sample sizes, to generate data on a larger scale that can inform health systems in adopting VR/AR/MR interventions.

- Research should be conducted to evaluate the mechanisms of action of VR/AR/MR interventions for achieving pain relief.
  Further research is needed to identify and test specific mechanisms that result in pain relief from VR/AR/MR use and how specific factors, such as the type of equipment, intervention dose, along with the level of immersion and enjoyment of the VR/AR/MR environment, affect pain relief. This will require capturing patient-reported outcomes and objective pain-related measures (e.g., imaging, blood-based biomarkers, and quantitative sensory testing).
- Research should be conducted to explore the accessibility and cost-effectiveness of implementing VR/AR/MR-based interventions, especially in the home setting.
- Research of VR/AR/MR technologies should be conducted in partnership with members of historically marginalized groups, such as Black adults who experience chronic pain.
- Future VR/AR/MR programs should be tailored to the characteristics and needs of different patient groups.
  Although extensive research has demonstrated the effects of distraction for reducing pain, there is a need for further research that investigates tailored distraction techniques via VR/AR/MR in addressing different types and subtypes of pain that encompass individual, procedural, interventional, contextual, and social factors [88].
- Future studies should also assess the effects of combining VR/AR/MR with evidence-based pain management approaches such as CBT, mindfulness, and biofeedback.
- Future RCTs comparing VR/AR/MR with evidence-based pain management interventions should adhere to best data reporting and evaluation practices, including those outlined by the Initiative on Methods, Measurement, and Pain Assessment in Clinical Trials (IMMPACT).

Summary points

- Although the use of virtual, augmented, or mixed reality (VR/AR/MR) technology for chronic pain has increased, there is a dearth of literature regarding the use and efficacy/effectiveness of these technologies.
- This review of 46 empirical studies included 19 randomized controlled trials (RCTs) (n = 1011 participants), 21 quasi-experimental studies (n = 413), 1 analytical cross-sectional study (n = 15), three case reports (n = 4), and two pilot case series (n = 13), with a total of 1456 participants across all studies.
- Most of the included studies investigated VR, utilized immersive head-mounted displays, and did not include a follow-up beyond the immediate post-intervention period.
- In most studies, VR was utilized to cope with chronic pain and associated psychosocial correlates or was integrated into rehabilitation therapy.
- Efficacy/effectiveness outcomes included pain (46 studies) and pain-related outcomes (41 studies), such as functional status, psychological correlates of pain, and pain interference in activities of daily living.
- VR/AR/MR technology was associated with a statistically significant reduction in pain intensity, phantom sensations, or pain unpleasantness in 63% of the studies and a statistically significant improvement in various pain-related outcomes in 52.2% of the studies. Among these studies, 78% of the 19 RCTs had improved pain-related outcomes, with small to large effect sizes.
- In half of the 24 studies that evaluated the feasibility and/or acceptability of using VR for pain and/or pain-related outcomes, most participants reported satisfaction or high satisfaction with the VR experience or found VR to be an acceptable intervention for chronic pain.
- Adverse effects or negative side effects were reported in 33.3% of 24 studies and these effects were primarily mild.
- The overall quality of the studies was moderate, with a low risk of bias for most studies. Of the 19 RCTs, one study exhibited a moderate risk for bias, it was unclear if at least one criterion was met in 5 studies, and two studies did not utilize true randomization. In the RCTs, there was a wide range of results of high to low certainty, with overall low certainty reported.
- VR/AR/MR technology can be an effective method for delivering interventions for chronic pain.
- Clinical trials are needed to further evaluate VR/AR/MR technology for home-based chronic pain management, mechanisms of action of VR/AR/MR interventions for achieving pain relief, and accessibility and cost-effectiveness of implementing VR/AR/MR-based interventions, especially among members of historically marginalized groups.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/pmt-2022-0030

Author contributions

Conception and design of the work: N Matthie. Acquisition, analysis or interpretation of data for the work: All authors. Drafting the work or revising it critically for important intellectual content: All authors. Final approval of the version to be published: All authors. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: All authors.

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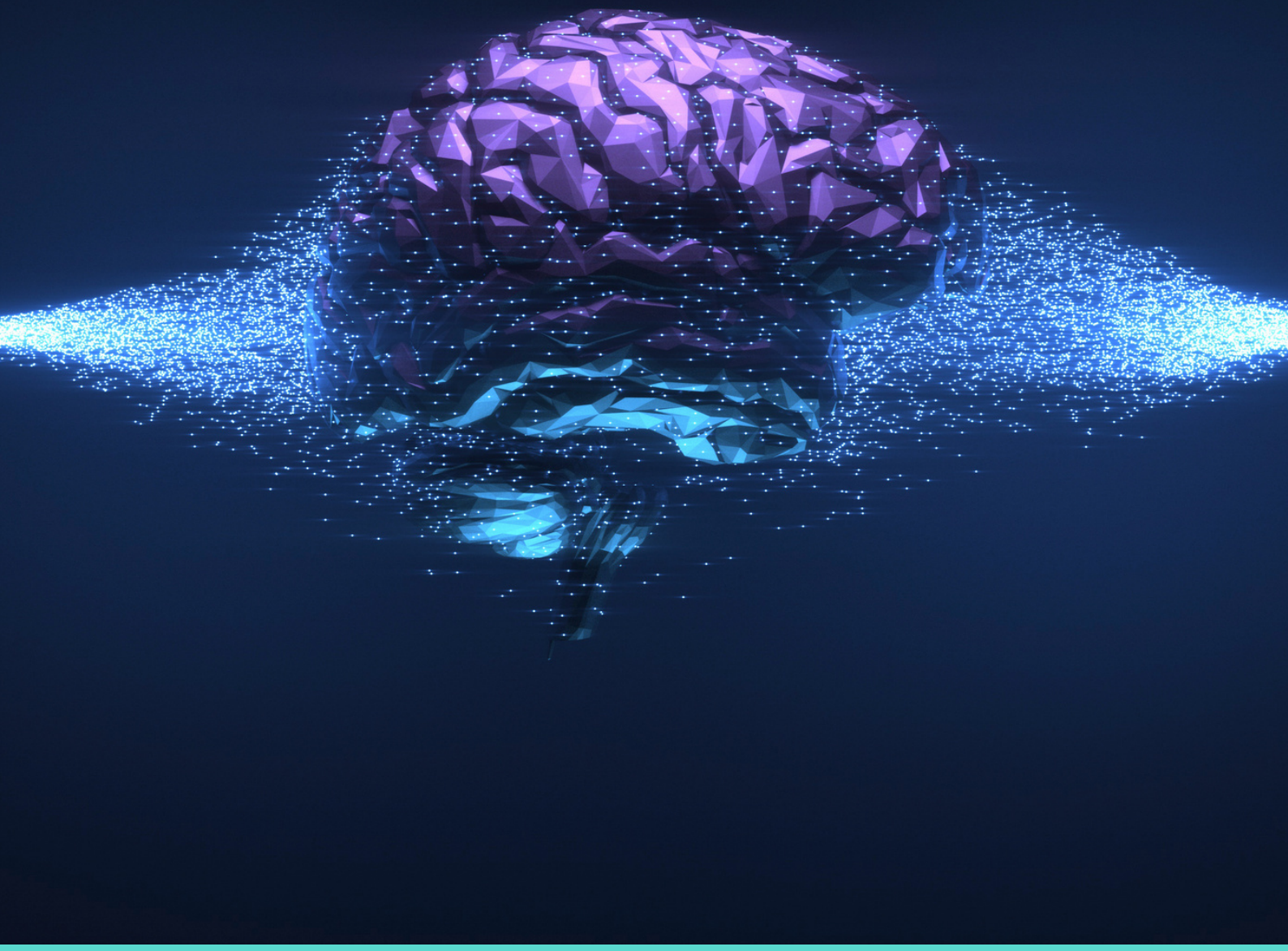

Contact us

Editorial Department

Digital Editor
Emma Hall
e.hall@future-science-group.com

Business Development and Support

Contact for Commercial Queries
sales@future-medicine-ai.com

@FutMedicineAI

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