SUPPLEMENT 3

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Research Dissemination Reports
醫療衞生研究基金
研究成果報告

Children and adolescent health
兒童和青少年健康

Infectious diseases
傳染病

Reproductive health
生殖健康

Pain
痛症

Mental health
精神健康

Stroke
中風

Cancer
癌症

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HONG KONG MEDICAL JOURNAL 香港醫學雜誌
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SUPPLEMENT 3

Health and Medical Research Fund

Research Dissemination Reports

STROKE

CANCER

Editorial

Dissemination reports are concise informative reports of health-related research supported by the Health and Medical Research Fund administered by the Health Bureau. In this edition, we present 12 dissemination reports of projects related to children and adolescent health, infectious diseases, reproductive health, pain, mental health, stroke, and cancer. In particular, research findings of three projects may provide insights to enhance clinical practices and help inform health policy formulation in Hong Kong.

The acquisition of antimicrobial resistance (AMR) is becoming a global public health concern, especially as international travel increases. The human gut microbiome is thought to play a major role in AMR acquisition and may provide a source of biomarkers for predicting AMR acquisition. As data in Asian populations is limited, Tun et al[1] aimed to establish the associations between gut microbiota, travel-associated risk factors, and AMR acquisition among 269 Hong Kong travellers. They found novel AMR genes among the microbiota profiles pre- and post-travel. Travel to low- and middle-income countries was linked with increased antibiotic resistance gene acquisition and richness. Eating raw seafood during travel was a risk factor of extended spectrum β-lactamase-producing Enterobacteriaceae acquisition.

Low back pain is a common disability worldwide and poses a considerable health and socioeconomic burden. Different combinations of pharmacological treatments have been tried over the years to treat severe symptoms, but the evidence base to support them is weak. Hung et al[2] conducted a multicentre, double-blind, randomised controlled trial in 296 Chinese adults who presented at participating emergency departments with acute non-specific low back pain. They were randomised to receive diclofenac plus placebo, diclofenac plus tramadol, or diclofenac plus tizanidine. The primary outcome was the 24-item Roland Morris Disability Questionnaire score, which assesses self-rated physical disability caused by low back pain. There was no significant difference in improvement of functional recovery, pain intensity, and return to work among the three arms at 7 days post-treatment. Therefore, the current findings do not support additional use of tizanidine or tramadol in addition to diclofenac in patients with acute low back pain.

Stroke self-management programmes can significantly improve health-related quality of life, self-efficacy, and functional independence among stroke survivors. Often, however, stroke survivors cannot access such programmes for a variety of reasons including functional impairment, lack of transportation, and unsuitable scheduling. Chau et al[3] conducted a randomised controlled trial aiming to develop and evaluate the effectiveness and cost-effectiveness of an innovative virtual multidisciplinary stroke care service among 274 Chinese community-dwelling stroke survivors. Participants of the programme had significantly improved health-related quality of life, self-efficacy, depressive level, and social participation. In addition, there were reduced emergency admissions and days of hospital stay. The flexible virtual setting of the programme addressed participants concerns on transportation limitations and scheduling conflicts. Although the virtual service is not more cost-effective than usual care, it is an effective model of service delivery for post-discharge community care and may provide reliable stroke-related support and information for stroke survivors, caregivers, and the public.

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Skin microbial signatures for eczema: a birth cohort study (abridged secondary publication)

TF Leung *, SKW Tsui, WH Tam, M Ip, MH Wang

KEY MESSAGES

1. Of 166 Chinese infants, 71 (43%) developed eczema during infancy; 33 (46.5%) of these infants had persistent eczema by 12 months of age.
2. Atopy with locally important allergens was associated with infantile eczema. Infants with more severe eczema were more likely to have persistent disease by 12 months of age.
3. Eczema was associated with higher transepidermal water loss over antecubital fossae in early life.
4. Infants with eczema had lower alpha diversity over the right antecubital fossa at birth and 6 months, and over the left popliteal fossa at 3 months and 12 months.
5. The compositions of bacterial communities over the left antecubital fossa and the left popliteal fossa were less clustered in infants with eczema at 12 months.

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Introduction

Eczema is the commonest chronic skin disease in childhood; it can cause substantial family stress, as well as social and financial burdens. Microbes inside the human body are associated with many complex diseases. The skin harbours hundreds of bacterial species, and their composition depends on the microenvironment (eg, humidity and temperature). The skin microbiome is involved in training the immune system, enhancing innate barrier immunity and homoeostasis, and triggering inflammatory cell recruitment and cytokine release.

The skin microbiome that modulates eczema susceptibility is poorly studied, mainly because of the lower microbial biomass present on the skin. Next-generation sequencing enables better understanding of the skin microbiome. Perturbations of the skin microbiome are associated with inflammatory dermatological conditions such as eczema. Eczema flares are generally associated with a low-diversity skin microbiome and *Staphylococcus aureus* dominance.

Most studies on this topic have been cross-sectional and thus could not support causal inferences. In Irish infants, skin microbiome composition and diversity shift over time; infants who developed eczema had significantly different bacterial communities on the antecubital fossa at 2 months of age.[1]

Additionally, skin dryness might predict eczema development in Caucasian infants. Transepidermal water loss (TEWL) before 6 months of age is a biomarker for eczema by 12 months of age.[2] However, the association between eczema and increased TEWL remains poorly defined. This study aimed to identify the skin microbial signatures in Chinese infants who develop eczema during infancy.

Methods

This study included (1) a discovery cohort of 166 healthy Chinese term singleton infants delivered vaginally who were free of any clinically evident dermatitis within 4 weeks after birth and whose mothers were free of any significant pregnancy-related diseases and peripartum sepsis, and (2) a replication cohort comprising two independent populations of preschool and school-age Chinese children from paediatric clinics with and without eczema, as well as participants in the SMART Baby birth cohort who were followed up for 2 years.[3]

All recruited infants were assessed for eczema through home visits at 1 and 3 months and clinic visits at 6 and 12 months. Eczema was diagnosed using the Hanifin and Rajka criteria, and disease severity was assessed by SCORAD. At each visit, clinical staff collected flocked skin swabs by 1 minute of firm rubbing over areas of 5×5 cm on the left and right antecubital fossae, left popliteal fossa, and anterior chest between the nipples. Within 2 hours, these swabs were transported on ice to the laboratory and stored at -80°C until sequencing. Skin biophysical parameters, including TEWL and skin hydration (SH), were measured over the volar forearm. At 12 months, all infants underwent skin

prick tests for six locally important allergens with positive (histamine 10 mg/ml) and negative (diluent) controls to determine atopy status. Participants were diagnosed as having transient eczema if it resolved at or before the 12-month visit or as having persistent eczema if it remained active at the 12-month visit.

Genomic DNA was extracted with a low biomass protocol and subjected to polymerase chain reaction amplification targeting the V1-V3 region of the 16S rRNA gene using primer pair 27F/534R (27F:5'-AGAGTTTGATCCTGGCTCAG-3'; 534R: 5'-ATTACCGCGGCTGCTGG-3'). Library preparation and 16S rRNA-based sequencing were then performed. Demultiplexed raw sequencing data were obtained in FASTQ format. Because the sizes of the original DNA fragments were shorter than two read lengths, paired-end reads were merged for improved genome assembly. The sequencing data were imported into QIIME2; DADA2 was used for denoising and removal of sequencing errors. Taxonomies of amplicon sequence variants were assigned using the *phyloseq* R package for microbiome analysis. Alpha diversity (Shannon and Simpson index) and beta diversity (unweighted UniFrac distance) were assessed.

Associations between clinical factors and eczema outcomes were analysed using the Mann-Whitney *U* test for continuous variables and the Pearson Chi-squared test for categorical variables; significant associations were then analysed by logistic regression. Longitudinal analyses were performed by generalised estimating equations (GEEs). The significance level was set to 5% for all analyses. Regarding the skin microbiome, single-timepoint microbial differences in alpha diversity indices among eczema groups were compared using the Kruskal-Wallis rank sum test, whereas longitudinal differences were analysed by GEEs. Associations between beta diversity and eczema outcomes over time were analysed by permutational multivariate analysis of variance with the *adonis* function. Taxa that were differentially abundant over time and among eczema groups were identified by the analysis of compositions of microbiomes with bias correction method.

For the replication cohort, the skin microbiome over the left antecubital fossae was assessed by whole-genome shotgun sequencing and by 16S rRNA sequencing. The predictivity of any skin microbial signature identified in the discovery cohort was compared with signatures identified in the two populations of the replication cohort.

Results

In total, 81 male and 85 female Chinese infants were recruited between October 2019 and November 2020; 44% had a maternal history of allergy and 44% had a paternal history of allergy. At 12 months, 151 (91%) infants remained in the study; 71 (47%) had developed eczema by 12 months of age, and 38 of these infants had transient eczema. Maternal smoking was associated with transient eczema (21.1% vs 7.4%, P=0.030).

Atopy was observed in 34 (28.6%) of 119 infants at 12 months and was associated with eczema ever (adjusted odds ratio=3.05, P=0.012). All infants with transient eczema had mild eczema. Compared with the transient eczema group, the persistent eczema group had a higher SCORAD at 6 months (9.8 vs 17.4, P=0.047).

Compared with infants with no eczema, infants with eczema ever had higher TEWL over the left antecubital fossae at 3 months (11.8 vs 8.2 g/m^2/hour, P=0.003) and over the right antecubital fossae at 12 months (10.2 vs 13.4 g/m^2/hour, P=0.005) [Table].

TABLE. Associations of transepidermal water loss (TEWL) with eczema outcomes

Measurement site and timepoint	Median (interquartile range) TEWL, g/m^2/hour				P value			
	No eczema (EN)	Eczema ever (EE)	Transient eczema (ET)	Persistent eczema (EP)	EN vs EE	EN vs ET	EN vs EP	ET vs EP
LAF at baseline	6.2 (5.2-7.7)	6.3 (5.0-8.7)	5.8 (4.9-8.3)	6.9 (5.0-9.1)	0.726	0.636	0.270	0.368
RAF at baseline	6.4 (5.3-8.5)	7.4 (5.3-8.2)	7.5 (5.0-8.0)	7.0 (5.6-9.7)	0.793	0.814	0.451	0.504
LAF at 1 month	8.9 (6.3-12.0)	8.7 (5.3-10.1)	9.2 (4.8-10.1)	7.5 (5.4-10.4)	0.265	0.512	0.253	0.650
RAF at 1 month	8.3 (5.8-11.6)	9.2 (5.1-12.2)	11.0 (8.3-13.4)	5.1 (3.8-10.5)	0.767	0.082	0.102	0.014*
LAF at 3 months	8.2 (6.5-10.7)	11.8 (8.6-17.6)	9.7 (7.5-17.6)	12.5 (10.5-17.4)	0.003	0.132	0.001	0.264*
RAF at 3 months	8.8 (6.1-15.2)	10.1 (7.2-15.6)	8.3 (6.6-15.3)	12.0 (8.7-15.6)	0.302	0.768	0.203	0.373*
LAF at 6 months	7.8 (5.7-11.4)	9.0 (6.4-12.7)	7.4 (6.0-11.7)	10.1 (7.5-14.4)	0.237	0.893	0.086	0.186
RAF at 6 months	9.1 (7.1-13.2)	9.5 (7.9-14.4)	9.2 (8.0-12.6)	10.8 (7.7-17.1)	0.261	0.647	0.183	0.332
LAF at 12 months	8.8 (7.4-11.8)	10.4 (7.5-15.4)	10.2 (6.9-14.7)	10.4 (7.7-16.3)	0.179	0.537	0.121	0.473
RAF at 12 months	10.2 (8.8-13.4)	13.4 (9.8-18.8)	15.8 (9.9-20.2)	11.8 (9.7-15.1)	0.005	0.006	0.076	0.249

Abbreviations: LAF = left antecubital fossa, RAF = right antecubital fossa

* Sample size ≤30

Compared with the persistent eczema group, the transient eczema group had higher TEWL over the right antecubital fossae at 1 month (5.1 vs 11.0 g/m^2/hour, P=0.014).

The effects of time on all eczema outcomes were significant (P<0.001). SH over both left antecubital fossae (10.8 vs 40.5 arbitrary units [AU], P<0.001) and right antecubital fossae (11.9 vs 42.2 AU, P<0.001) were lower at baseline and became stable at 1 month. Similar results were observed for TEWL over both the left antecubital fossae (7.1 vs 10.9 AU, P<0.001) and the right antecubital fossae (7.1 vs 12.7 AU, P<0.001), regardless of eczema outcomes. SH and TEWL trajectories did not differ significantly among eczema groups.

For skin microbiome analysis, 2345 skin swabs were collected from 166 participants across five timepoints. After quality control, a total of 42 268 249 reads from 2305 skin microbiome samples were analysed (15 908 reads per sample). We observed significant longitudinal changes in both alpha and beta diversity indices of the skin microbiome at all sampling sites from birth to 12 months of age (P<0.001, Fig). Regarding inter-group differences in diversity indices, we detected a significant association between eczema ever and skin microbiome measured over the left antecubital fossae; the association was stronger when participants were stratified into eczema groups (persistent eczema, transient eczema, and no eczema by 12 months). At 12 months, beta diversity over the left antecubital fossae differed between infants with and without eczema (P=0.004), as well as between infants with persistent eczema and infants with transient eczema (P=0.001). For biodiversity over other body sites, alpha diversity indices measured over the right antecubital fossae at birth differed between infants with and without eczema (P=0.026-0.032). Similar findings were observed for alpha diversity indices over the left popliteal fossa at 3 months (P=0.020) and 12 months (P=0.007-0.013). Beta diversity measured over the right antecubital fossae at 3 months differed between infants with and without eczema (P=0.026), as well as among infants with persistent eczema, transient eczema, and no eczema (P=0.012).

GEE analyses revealed that the skin microbiome over the right antecubital fossae had significant single-timepoint effects on eczema ever (Shannon: B= -0.139, 95% confidence interval [CI]= -0.263 to -0.016, P=0.027; Simpson: B= -0.051, 95% CI= -0.101 to -0.002, P=0.041) and persistent eczema (Shannon: B= -0.093, 95% CI -0.176 to -0.009, P=0.029). No such association was detected for skin microbiome samples obtained at other body sites (results not

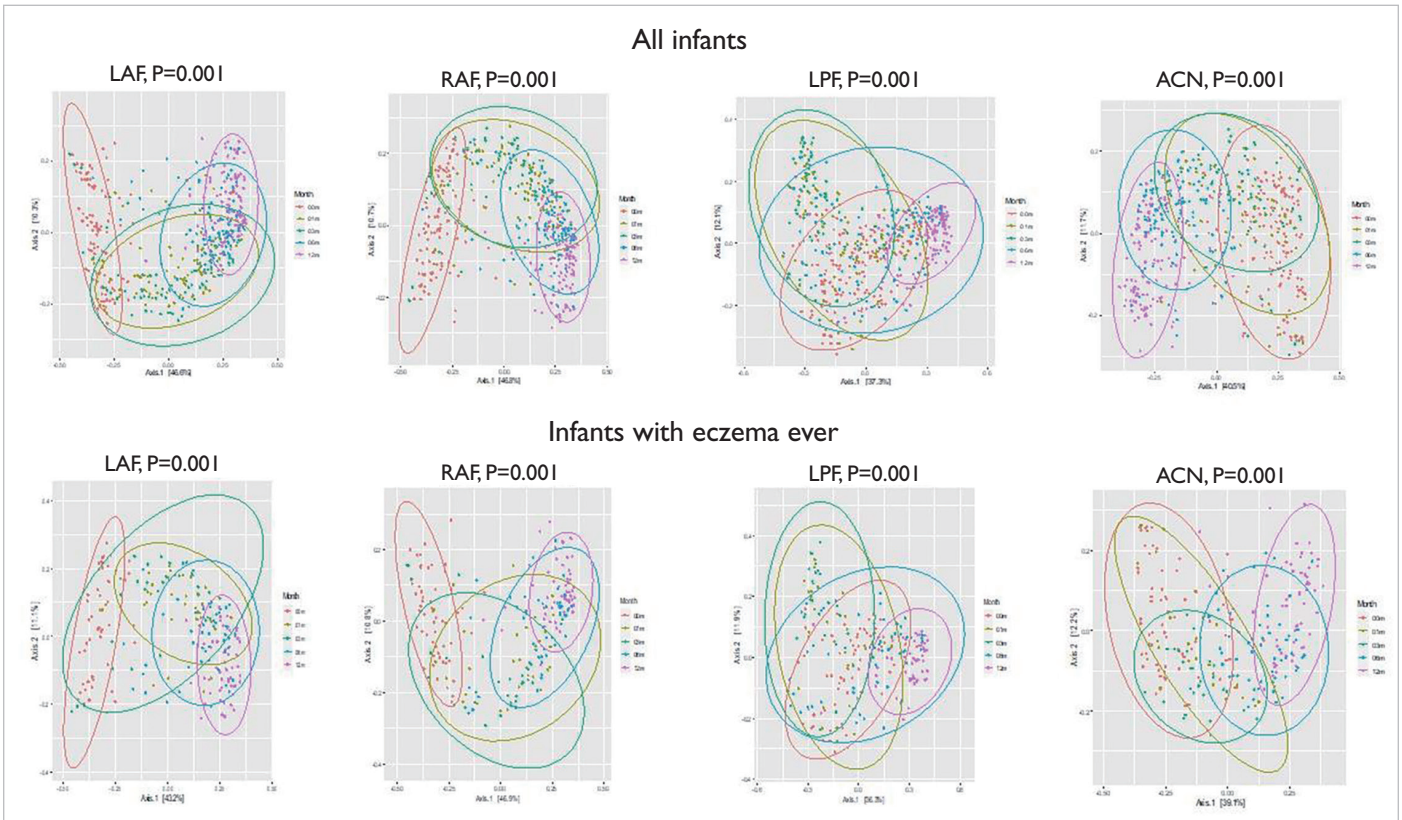

FIG. Principal coordinates analysis plots showing dynamic shifts in beta diversity indices over the left antecubital fossa (LAF), right antecubital fossa (RAF), left popliteal fossa (LPF), and anterior chest between the nipples (ACN) among all infants and infants with eczema.

shown). Additionally, GEE analyses did not show longitudinal effects of alpha diversity indices of the skin microbiome at any body site on the occurrence of eczema ever during infancy.

Multiple bacteria were associated with various eczema outcomes. For example, *Deinococcus* abundance over the right antecubital fossae at 1 month was significantly lower in infants with persistent eczema than in infants without eczema, whereas abundances of *Bergeyella* and *Chryseobacterium* over the right antecubital fossae at 3 months were lower in infants with persistent eczema than in infants with no eczema. However, these microbial signatures were not confirmed in the replication cohort. In the cross-sectional study, alpha diversity was lowest in lesional skin of infants with eczema (P<0.001) and lower in infants with atopic eczema at 12 months than in infants with non-atopic eczema (P<0.001).

Discussion

In the present study, eczema affected almost half of Chinese infants, although there was a high rate of remission by 12 months. Infantile eczema was associated with atopy only and not with other clinical, environmental, or biophysical factors. A family history of allergy was not reported to be a risk factor of eczema, probably owing to the small sample size.

The present study revealed postnatal alterations in biophysical parameters and suggested that the first month after birth is a vulnerable period owing to an immature skin barrier. Several studies from Japan and Ireland have supported our observation of an increase in TEWL within the first 1 to 2 months after birth. Patients with eczema exhibit dry and lichenified skin, and we found that TEWL at 3 months was associated with eczema. Our finding was consistent with that of other study.[4]

We found significantly lower alpha diversity among infants with eczema ever at several timepoints, suggestive of lower richness and evenness in the skin microbiome. This finding was consistent with that of a study of older children and adults.[5] Analyses of beta diversity suggested that bacterial community structures were altered in infantile eczema: infants with eczema ever exhibited significantly less clustered bacterial communities. This indicates that these infants harboured bacterial communities with greater phylogenetic distance among genera from different families.

Skin microbiome analyses in the replication cohort yielded discrepant findings among toddlers and preschool children with eczema. Alpha diversity was lower in older children with eczema but not in toddlers with eczema, compared with infants with no eczema. Furthermore, comparisons of the discovery and replication cohorts did not reveal any consistent microbial signature associated with eczema outcomes.

Conclusion

Atopy, biophysical parameters, and microbial diversity indices are predictive biomarkers for eczema outcomes in Chinese infants.

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Disclosure

The results of this research have been previously published in:

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Effects of long-term air pollutant exposure on respiratory health in Hong Kong primary school children: abridged secondary publication

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KEY MESSAGES

1. Chronic exposure to air pollutants (fine particulate matter and nitrogen dioxide) poses a threat to respiratory health among school children in Hong Kong.
2. Further studies are warranted regarding the health effects of long-term exposure to ozone.

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Introduction

Exposure to ambient air pollution increases mortality and morbidity. It is estimated that fine particulate matter ($PM_{2.5}$) alone contributed to 4 million deaths (7.5% of all deaths) worldwide in 2016.[1] Exposure to ozone (O_3) caused an additional 254 000 deaths and the loss of 4.1 million disability-adjusted life years (DALYs) secondary to chronic obstructive pulmonary disease in 2015.[2] However, the disease burden associated with nitrogen dioxide (NO_2) is not well-studied, despite its substantial adverse health effects.[2]

Compared with adults, children are more vulnerable and susceptible to the adverse effects of air pollutants. Hong Kong has various characteristics associated with air pollution such as a tropical climate, high population density, tall buildings surrounded by narrow roads with heavy traffic, and small cramped residential dwellings. Here, we aimed to investigate the effects of long-term exposure to a mixture of pollutants ($PM_{2.5}$, NO_2, and O_3) on respiratory health among Hong Kong primary school children.

Methods

This longitudinal prospective cohort study of school children across Hong Kong was conducted from 2012 to 2017. Details of the study have been reported elsewhere.[3] Briefly, we recruited 5573 children aged 6 to 16 years from 31 primary schools across Hong Kong. The students were followed up annually for 2 years. In the baseline and follow-up surveys, the parents or guardians of each student were asked to complete a self-administered questionnaire; anthropometric parameters and pulmonary function of each student were examined.

We developed a remote-sensing algorithm to estimate ground-level $PM_{2.5}$ concentrations with high-resolution (1 km) and good accuracy (average correlation up to 0.9). We also developed a space-time regression model to estimate ground NO_2 and O_3 concentrations. The annual average concentrations of $PM_{2.5}$, NO_2, and O_3 were assigned to students based on their home address and used as indicators of long-term air pollution exposure.

Outcome measures were lung function parameters including forced vital capacity, forced expiratory volume in 1 second, peak expiratory flow, and maximum mid-expiratory flow, as well as doctor-diagnosed respiratory diseases and self-declared respiratory symptoms. Linear mixed models with random slope effect terms were used to examine long-term associations between air pollution exposure and lung function parameters. Mixed effects logistic regression models with random effects of school and child levels were used to examine associations between long-term air pollution exposure and the occurrence of respiratory diseases or symptoms. A confidence level of 0.05 was assumed.

Results

This study included 5573 school children aged 6 to 16 years, with a total of 11 540 observation records (mean, 1.68; range, 1-3) [Table 1]. Of them, 4059 (72.8%) had undergone at least two follow-up surveys.

In single-pollutant models, a 5-μg/m^3 increase in $PM_{2.5}$ exposure was associated with the following

TABLE 1. Baseline characteristics of school children

Variable	All (n=5573)*	Boys (n=2841)*	Girls (n=2732)*	P value
Age, y	9.24±1.10	9.27±1.14	9.21±1.06	0.037
Body mass index, kg/m^2	17.46±3.25	17.92±3.48	16.99±2.93	<0.001
Air pollution exposures				
Fine particulate matter, μg/m^3	28.93±4.96	28.90±5.00	28.96±4.91	0.67
Nitrogen dioxide, μg/m^3	46.22±18.96	46.62±19.38	45.80±18.50	0.11
Ozone, μg/m^3	41.14±10.69	40.99±10.91	41.30±10.45	0.28
Lung function outcomes				
Forced expiratory volume in 1 second, mL	1670.12±343.18	1714.22±336.51	1622.85±344.04	<0.001
Forced vital capacity, mL	1924.49±392.35	1996.47±388.41	1847.34±381.83	<0.001
peak expiratory flow, mL/s	3775.69±824.19	3840.54±802.37	3706.19±841.59	<0.001
Maximum mid-expiratory flow, mL/s	1905.08±541.65	1894.23±528.33	1916.70±555.44	0.14
Passive smoking	1285 (23.56)	651 (23.39)	634 (23.73)	0.80
Asthma	192 (3.49)	121 (4.3)	71 (2.63)	0.001
Allergic rhinitis	2243 (40.76)	1306 (46.43)	937 (34.83)	<0.001
Sinusitis	162 (2.95)	109 (3.88)	53 (1.97)	<0.001
Bronchitis	700 (12.71)	389 (13.82)	311 (11.55)	0.013
Bronchiolitis	161 (2.92)	96 (3.41)	65 (2.41)	0.034
Pneumonia	61 (1.11)	27 (0.96)	34 (1.26)	0.35
Wheezing	548 (10.89)	320 (12.5)	228 (9.22)	<0.001
Dry cough	1478 (28.74)	816 (31.09)	662 (26.29)	<0.001
Phlegm	676 (13.17)	354 (13.53)	322 (12.81)	0.47

* Data are presented as mean±standard deviation or No. (%) of participants

magnitudes of change: -14.05 mL in forced expiratory volume in 1 second, -4.60 mL in forced vital capacity, 26.35 mL/s in peak expiratory flow, and -18.55 mL/s in maximum mid-expiratory flow (Table 2). Similarly, increased NO_2 exposure was associated with worse lung function; adjustment for $PM_{2.5}$ did not change the associations between NO_2 and lung function parameters. However, increased O_3 exposure was associated with better lung function; adjustment for $PM_{2.5}$ did not change the associations between O_3 and lung function parameters.

School children with a 5-μg/m^3 increase in $PM_{2.5}$ exposure was associated with an increased risk of sinusitis (odds ratios [OR]=1.14), whereas a 5-μg/m^3 increase in NO_2 exposure was associated with an increased risk of allergic rhinitis (OR=1.02) and a 5-μg/m3 increase in O_3 exposure was associated with an increased risk of asthma (OR=1.08).

Discussion

Among school children in Hong Kong, long-term exposures to $PM_{2.5}$ and NO_2 were associated with worse lung function. However, exposure to O_3 was associated with better lung function. Exposures to PM, NO_2, and O_3 resulted in higher odds of sinusitis, allergic rhinitis, and asthma, respectively.

O_3, a secondary pollutant, is often negatively correlated with PM and NO_x. The observed beneficial effects of O_3 on lung function might reflect decreases in PM and NO_x. Thus, further studies are warranted regarding the health effects of O_3 on respiratory health in children.

Inhaled particles from ambient surroundings can trigger interactions with pneumonocytes and may cause a cascade of inflammatory and systemic responses after entering the lung.[4] The biological effects of increased oxidative stress are concerning.[5] Evidence from animal studies has suggested that particulate matter can deplete antioxidants and related enzymes, produce free radicals, and trigger oxidative stress, inflammation, and pulmonary impairment.

Conclusion

Long-term exposure to ambient air pollutants ($PM_{2.5}$ and NO_2) is associated with potential harm to lung function, along with higher risks of respiratory diseases and symptoms.

TABLE 2. Associations between pollutants and lung functions

Pollutants and lung functions	Odds ratio (95% confidence interval) for every 5-μg/m³ increase in pollutant			P value
	All	Boys	Girls	
Fine particulate matter				
Forced expiratory volume in 1 second	-14.05 (-23.80 to -4.25)	-11.20 (-22.45 to 0.05)	-5.00 (-16.55 to 6.55)	<0.001
Forced vital capacity	-4.60 (-15.90 to 6.75)	-3.25 (-17.60 to 11.15)	-1.40 (-14.60 to 11.80)	<0.001
Peak expiratory flow	26.35 (-4.80 to 57.45)	28.05 (-7.45 to 63.50)	46.25 (5.45 to 87.10)	0.001
Maximum mid-expiratory flow	-18.55 (-37.10 to 0.00)	-9.15 (-28.55 to 10.20)	5.05 (-16.50 to 26.60)	<0.001
Nitrogen dioxide				
Forced expiratory volume in 1 second	-2.90 (-6.15 to 0.35)	-3.10 (-6.20 to 0)	-2.75 (-6.65 to 1.15)	<0.001
Forced vital capacity	-1.60 (-5.55 to 2.35)	-2.40 (-6.85 to 2.05)	-2.00 (-6.30 to 2.35)	<0.001
Peak expiratory flow	-7.45 (-20.55 to 5.65)	-8.35 (-21.50 to 4.8)	-3.00 (-18.60 to 12.60)	0.013
Maximum mid-expiratory flow	-3.05 (-11.00 to 4.9)	-3.65 (-8.75 to 1.4)	-1.15 (-8.40 to 6.15)	<0.001
Ozone				
Forced expiratory volume in 1 second	8.55 (3.35 to 13.7)	7.3 (2.1 to 12.5)	10.55 (4.4 to 16.7)	<0.001
Forced vital capacity	6.25 (0.2 to 12.35)	8.15 (0.85 to 15.5)	5.9 (-0.85 to 12.6)	<0.001
Peak expiratory flow	46.05 (25.4 to 66.75)	29.65 (7.4 to 51.85)	45.45 (20.4 to 70.5)	0.039
Maximum mid-expiratory flow	30.3 (17.75 to 42.8)	8.8 (0.2 to 17.45)	20.65 (8.3 to 33)	<0.001

Funding

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Disclosure

The results of this research have been previously published in:

1. Chen J, Zeng Y, Lau AK, et al. Chronic exposure to ambient $PM_{2.5}$/NO_2 and respiratory health in school children: a prospective cohort study in Hong Kong. Ecotoxicol Environ Saf 2023;252:114558.

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Statin safety among Chinese adolescents: a Mendelian randomisation study (abridged secondary publication)

SL Au Yeung *, HS Lam, YH Chan, CM Schooling

KEY MESSAGES

1. There is limited evidence on the long-term safety of statin use among adolescents.
2. As a form of genetic validation, this Mendelian randomisation study used randomly allocated variants within the HMGCR protein-encoding gene (leading to reductions in low-density lipoprotein cholesterol) to infer statin safety based on data from the Hong Kong 'Children of 1997' birth cohort.
3. Genetic evidence did not suggest substantial safety concerns about statins among Chinese adolescents.
4. This proof-of-concept study used a well-characterised birth cohort to infer the safety of drug targets among adolescents.

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Introduction

Three-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) inhibitors, collectively referred to as statins, are the first-line pharmacologic treatments for children with familial hypercholesterolaemia (FH); these inhibitors have demonstrated reductions in cardiovascular risk in adulthood. However, as obesity rates increase among adolescents, particularly in East Asian populations, statin prescriptions may be needed to mitigate the risk of atherosclerotic cardiovascular disease in non-FH adolescents who have elevated and uncontrolled lipid profiles. However, there has been a lack of safety studies regarding these medications in adolescents. It is in the public health interest to evaluate any adverse effects of statins in adolescents.

Real-world data, such as pharmaco-epidemiologic studies, are susceptible to confounding based on indications or immortal time bias. Mendelian randomisation studies have been used to investigate the adverse effects of medications, such as anti-hypercholesteremia and antihypertensive medications, in European adult populations. Here, we evaluated the association of genetically proxied inhibition of HMGCR with safety outcomes in a population-representative birth cohort in Hong Kong.

Methods

This Mendelian randomisation study had three instrumental variable assumptions. First, to ensure relevance, genetic variants within the *HMGCR* protein-encoding gene exhibiting associations with low-density lipoprotein cholesterol (LDL-C) were regarded as proxies for statin use. Second, the study assumed that genetic variants could not be confounded (eg, population stratification), ensuring independence. Third, the study assumed that genetic variants were independent of exposure-related outcomes, thereby addressing the exclusion restriction.[1]

The Hong Kong 'Children of 1997' birth cohort is a population-representative Chinese birth cohort that recruited 88% of all ethnic Chinese births (n=8327) between April and May 1997.[2] Recruitment was conducted at all maternal and children health centres in Hong Kong during the first postnatal visit for free preventive care and immunisations. Infant and family characteristics were recorded using a self-administered questionnaire. In 2005, record linkage was established to obtain routine information and clinical measurements (96% successful matching, n=7999). During the Biobank Clinical follow-up phase 1 in 2013 to 2016 and the supplementary Biobank Clinical follow-up phase 2 in 2017, 3460 and 158 participants, respectively, provided biospecimens (blood, saliva, urine, stool, hair, and nails) and completed comprehensive measurements (eg, anthropometrics).

Fasting blood samples and their derivatives (eg, buffy coat and plasma) were used for biochemical assays such as liver function tests. DNA was extracted from blood, buffy coat, or saliva samples; genotyping

was performed for 3582 participants. Phasing and genotyping imputation were conducted. For quality control, samples with a call rate <0.98, recorded sex not matching genetically inferred sex, second-degree relatedness or above, high heterozygosity (>3 standard deviations), or variants with a call rate <0.98 and imputation score <0.3 were excluded.

We identified genetic instruments to proxy the effects of HMGCR inhibitors using the Global Lipids Genetics Consortium, a genome-wide association study that included 188 577 middle-aged participants of predominantly European ancestry.[3] Six genetic variants (rs12916, rs17238484, rs5909, rs2303152, rs10066707, and rs2006760) are located within 100 kilobase pairs on either side of the *HMGCR* protein-encoding gene and associated with LDL-C at a genome-wide significance level ($P<5\times10^{-8}$).

To assess the validity of these variants in an East Asian population, we cross-verified the variant-LDL-C associations in the 'Children of 1997' birth cohort, with adjustments for age, sex, and the top six principal components of ancestry. We selected variants associated with LDL-C that displayed low linkage disequilibrium (clustering $r^2<0.3$, clustering window of 10 000 kilobases) in a reference panel of East Asian ancestry from the 1000 Genomes Project (Phase 3).

To maximise statistical power, an externally weighted genetic risk score for HMGCR was constructed for each participant. Specifically, the genetic risk score for HMGCR was constructed by summing the number of all LDL-C-lowering alleles for each variant in the *HMGCR* gene region, weighted according to the effect of each variant on LDL-C.[3] As in a previous study,[4] participants were first categorised using the median HMGCR score as proxies for statin use (participants with < median HMGCR score) and placebo (participants with ≥ median HMGCR score); they were subsequently categorised using the quartiles of HMGCR scores to assess potential dose-response relationships.

The outcomes included lipid profile (LDL-C, high-density lipoprotein cholesterol, and triglycerides), glycaemic traits (fasting glucose and glycated haemoglobin), liver function (alkaline phosphatase, alanine transaminase, albumin, and bilirubin) assessed at ~17.6 years during the Biobank clinical follow-up, and Tanner stage (breasts for girls and genitals for boys) and Tanner stage (public hair) at ~11 years.

The associations of HMGCR scores and potential confounders (sex, breastfeeding duration, highest parental education, housing type, and physical activity) were assessed using the Chi-squared test and analysis of variance. The associations of categorical HMGCR scores with outcomes were determined using multivariable linear and logistic regression, with adjustments for age, sex, and the top six principal components of ancestry. To preclude the possibility of false-positives due to inclusion of correlated variants, we also repeated the analysis using the index variant (rs12916) of *HMGCR* as a sensitivity analysis. The P value threshold after correction for multiple tests was set to 5×10^{-3} ($\alpha=0.05/10$).

Results

There were 1753 male and 1669 female participants with both valid genetic data and LDL-C measurements (Table). Two (rs12916 and rs17238484, LD $r^2=0.36$) of the six variants were selected to derive HMGCR scores, which were associated with LDL-C (-0.57 mmol/L, 95% confidence interval [CI]= -0.31 to -0.84). The HMGCR score was not associated with any of the potential confounders considered, other than parental education.

Compared with the reference group (participants with ≥ median HMGCR score), participants with < median HMGCR score had lower LDL-C (beta= -0.09 mmol/L, 95% CI= -0.13 to -0.04). In the dose-response analysis, lower HMGCR quartiles were associated with a stepwise decrease in LDL-C. In the sensitivity analysis, a T-allele increase in the HMGCR index variant rs12916 was associated with lower LDL-C (-0.07, 95% CI= -0.10 to -0.04). The HMGCR score was not associated with other lipid traits. For other outcomes, there were no associations of HMGCR scores below the median with glycaemic traits, liver function at age ~17.5 years, or Tanner stage (breasts for girls and genitals for boys) and Tanner stage (public hair) at age ~11 years. Sensitivity analyses yielded consistent findings, except that some HMGCR categories were nominally associated with lower glycated haemoglobin and lower albumin (Fig).

Discussion

The effect of HMGCR on LDL-C reduction was similar to the results of previous randomised controlled trials and Mendelian randomisation studies involving European and East Asian adults. A systematic review showed that short-term statin use (<48 weeks) among paediatric patients with FH was relatively safe.[5] Our study provides genetic evidence that long-term on-target effects of statins do not adversely affect glycaemic traits, liver function, or pubertal development in Chinese adolescents.

Consistent with previous studies, we did not find an association between genetic inhibition of HMGCR and liver function, although inadequate statistical power may explain the null findings. The rare statin-associated asymptomatic increase in transaminases (>3 times the upper limit of normal) and hepatotoxicity reported in clinical trials are likely due to idiosyncratic or immune

TABLE. Characteristics of participants in the 'Children of 1997' birth cohort according to 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) score

Variable	No. of participants	HMGCR score < median (n=1711)*	HMGCR score ≥ median (n=1711)*	P value
Sex	3422			0.43
Male		888/1711 (51.9)	865/1711 (50.6)	
Female		823/1711 (48.1)	846/1711 (49.4)	
Breastfeeding duration	3365			0.49
Never		882/1679 (52.5)	912/1686 (54.1)	
Partial or exclusive <3 months		664/1679 (39.6)	656/1686 (38.9)	
Exclusive ≥3 months		133/1679 (7.9)	118/1686 (7.0)	
Highest parental education level	3400			0.03
Grade 9 or below		483/1697 (28.5)	504/1703 (29.6)	
Grade 10-11		709/1697 (41.8)	762/1703 (44.8)	
Grade 12 or above		505/1697 (29.7)	437/1703 (25.7)	
Housing type at birth	3312			0.33
Home ownership		271/1643 (16.5)	269/1669 (16.1)	
Private flat		677/1643 (41.2)	652/1669 (39.1)	
Public/squatter/other		695/1643 (42.3)	748/1669 (44.8)	
Screen time, h	2324	4.87±2.67	4.84±2.47	0.62
Physical activity, h	1669	5.95±3.02	5.87±2.48	0.92
Low-density lipoprotein, mmol/L	3422	2.10±0.66	2.19±0.65	<0.001
High-density lipoprotein, mmol/L	3422	1.55±0.34	1.55±0.34	0.76
Triglycerides, log_{10}mmol/L	3422	-0.14±0.17	-0.13±0.18	0.63
Fasting glucose, mmol/L	3334	4.64±0.34	4.64±0.35	0.53
Glycated haemoglobin, %	3412	5.40±0.25	5.39±0.26	0.25
Albumin, g/L	3422	47.40±2.65	47.36±2.59	0.93
Bilirubin, log_{10}μmol/L	3180	1.06±0.20	1.05±0.20	0.39
Alkaline phosphatase, log_{10}IU/L	3422	1.89±0.14	1.89±0.14	0.65
Alanine transaminase, log_{10}IU/L	3099	1.24±0.19	1.24±0.19	0.49
Tanner stage (breasts/genitals)	918			0.76
1		181/468 (38.7)	174/450 (38.7)	
2		126/468 (26.9)	121/450 (26.9)	
3		124/468 (26.5)	116/450 (25.8)	
4		28/468 (6.0)	34/450 (7.6)	
5		9/468 (1.9)	5/450 (1.1)	
Tanner stage (pubic hair)	872			0.76
1		335/445 (75.3)	326/427 (76.4)	
2		77/445 (17.3)	64/427 (15.0)	
3		22/445 (4.9)	25/427 (5.9)	
4		11/445 (2.5)	11/427 (2.6)	
5		0/445 (0)	1/427 (0.2)	

* Data are presented as mean±standard deviation or No. (%) of participants

allergic reactions; in 2012, the US Food and Drug Administration recommended removal of routine periodic monitoring of liver enzymes among statin users.

This study had some limitations. First, the study was not able to assess pharmacological interventions administered at specific time points or doses, or the effects on individuals with particular indications (eg, FH), which is a common limitation of drug target Mendelian randomisation studies. Second, although

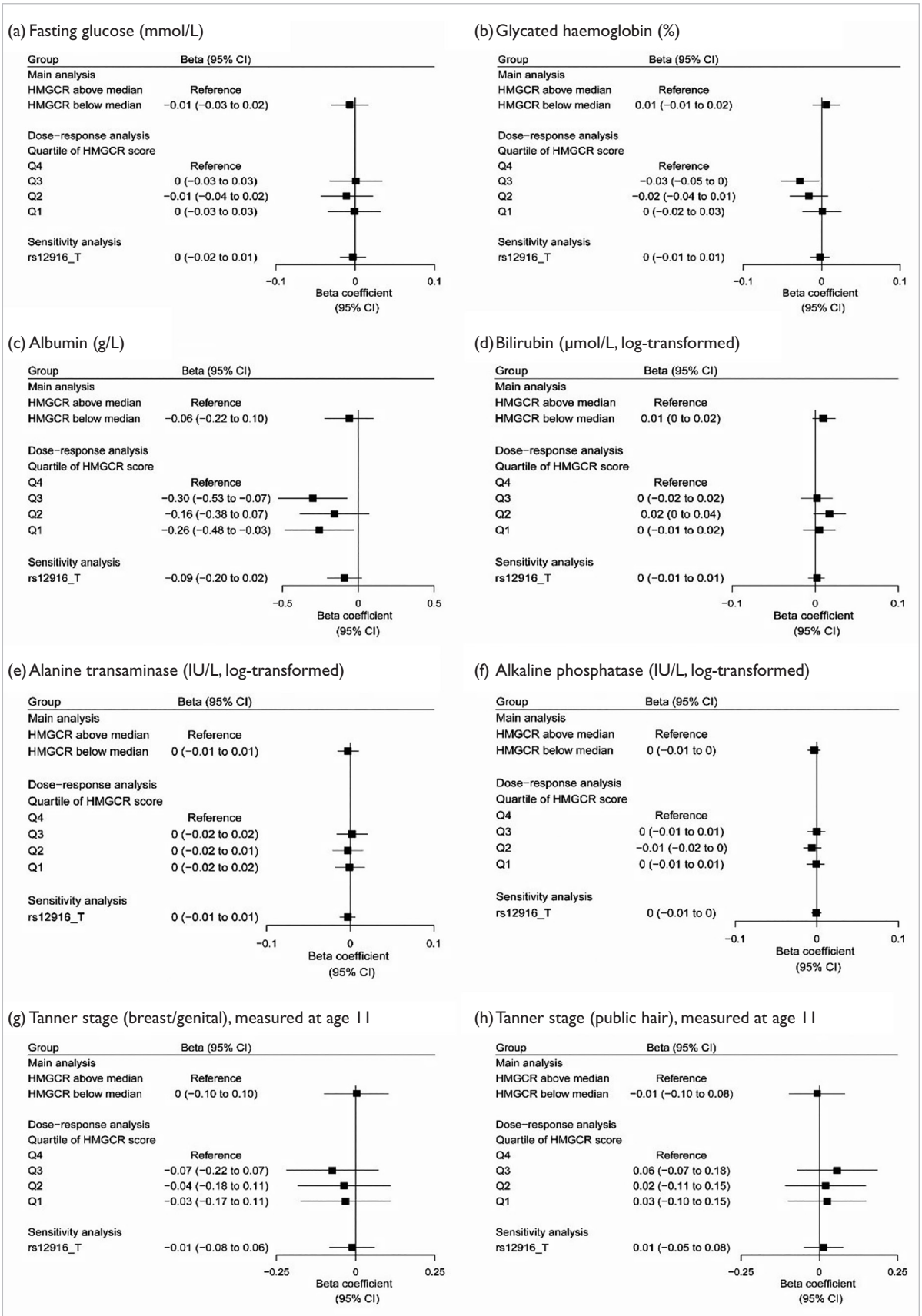

FIG. Association of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) low-density lipoprotein cholesterol score categories with (a) fasting glucose, (b) glycated haemoglobin, (c) albumin, (d) bilirubin, (e) alanine transaminase, (f) alkaline phosphatase, (g) Tanner stage (breasts/genitals), and (h) Tanner stage (pubic hair) in the 'Children of 1997' birth cohort.
Abbreviation: 95% CI = 95% confidence interval

the genetic variants for HMGCR inhibition were selected in people of European descent and validated in people of East Asian descent, future investigations should use ethnicity-specific genome-wide association studies to generate ethnicity-specific HMGCR variants. Third, because of sample size and statistical power constraints, we were unable to consider sex-specific associations and exclude the possibility of false-negatives, even though this is one of the largest studies in Chinese adolescents to date. Finally, we did not include all possible adverse effects such as muscle symptoms and related biomarkers. A broader spectrum of potential adverse effects should be assessed in larger studies that link electronic health records for clinical outcome with Biobank studies (eg, the All of Us study,[6] which recruited more than 40 000 participants aged 18 to 29 years).

Conclusion

This study did not yield evidence to suggest substantial concerns about long-term statin safety in Chinese adolescents. This proof-of-concept study showed the use of well-characterised birth cohorts with genetic and phenotypic data to facilitate assessments of drug target efficacy and safety among adolescents.

Funding

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Disclosure

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Acquisition of antimicrobial resistance after travel to resource-limited countries: a multi-layer metagenomic epidemiological study (abridged secondary publication)

HM Tun *, BJ Cowling, R Bruzzone, K Fukuda

KEY MESSAGES

1. Pre- and post-travel resistome and microbiota profiles showed separation in microbial communities and novel antimicrobial resistance genes.
2. Consumption of raw seafood, Actinobacteria richness, Erysipelotrichaceae UCG-003, *Blautia, Butyricicoccus, and Ruminiclostridium 9* were associated with the acquisition of extended spectrum β-lactamase-producing Enterobacteriaceae.
3 Travel to low and middle-income countries was associated with acquisition of antibiotic resistance genes.

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Introduction

Antimicrobial resistance (AMR) acquisition may occur through international travel. The colonisation of extended spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) has been reported among international travellers, regardless of infection status.[1] The human gut microbiota serve as AMR transporters and are associated with AMR acquisition during international travel and therefore are potential biomarkers to predict AMR acquisition. However, previous studies have mostly focused on Western populations.[2] Because ethnicity-associated lifestyle factors strongly influence gut microbiota,[3] evidence from Western populations may not be directly applicable to Asian populations. Therefore, we aimed to establish associations among gut microbiota, travel-associated risk factors, and AMR acquisition in Asian travellers. We also characterised the effects of control measures during the first wave of the COVID-19 pandemic on gut microbiota and resistome profiles in healthy individuals.

Methods

We recruited 269 Hong Kong travellers and asked them to submit two faecal samples and complete two questionnaires within 4 days before and after travel. Among the travellers, 35.7% participated during the first wave of COVID-19 in Hong Kong.

From faecal samples, ESBL-E was isolated and then subjected to antimicrobial susceptibility testing and taxonomic identification; DNA was extracted and aliquoted for real-time polymerase chain reaction quantification of AMR genes, 16S rRNA amplicon sequencing, and shotgun metagenomic sequencing. Sequencing reads for 16S and metagenomic data were analysed. Resistomes were quantified.

We developed a functional metagenomics platform to identify novel AMR genes in post-travel samples. Metagenomic DNA was pooled according to travel region and partially digested; digestion bands with sizes >700 bp were purified. The plasmid vector pZE21-MCS was digested and dephosphorylated. The random digests and plasmid were ligated and transformed into electrocompetent *Escherichia coli* cells. Cells were recovered in Luria-Broth medium and then spread onto Luria-Broth agar with kanamycin and various antibiotics for screening.

Comparisons of ESBL-E carriers and non-carriers were performed using permutational multivariate analysis of variance and principal coordinates analysis. Discriminant genera were identified using the linear discriminant analysis effect size pipeline. Predictors for acquisition of ESBL-E during travel were identified using three models that comprised different sets of predictors. The area under the receiver operating characteristic curve (AUC) was calculated for each model. Distributions of genus predictors were compared using the Wilcoxon rank-sum test.

Results

The pre- and post-travel resistome microbiota profiles showed similar separation in communities

and species richness (P=0.51, Fig 1). In total, 55 novel resistance genes were acquired after travel. Genes conferring resistance to bacitracin, macrolides, mupirocin, polymyxin, quinolones, and sulphonamides increased after travel, although these differences were not significant. The identification of new AMR genes contributed to expansion of antibiotic resistance gene (ARG) databases.

Overall ARG richness and diversity in travellers varied between those visiting low- or middle-income countries and those visiting high-income countries, although the differences were not significant. These findings were supported by the quantitative polymerase chain reaction results; the relative abundances of five common ARGs (*cfxA*, *tetM*, *tetQ*, *ermB*, and *aac(6')-aph(2")*) did not differ significantly between pre-and post-travel samples. To identify potential discriminant ARGs acquired during travel, we analysed changes in ARG abundances and identified 24 ARGs with significant differences (log^2 fold change >1 or <-1, P<0.05) before and after travel. Notably, 23 of these 24 ARGs were enriched after visits to low- or middle-income countries. In contrast, travellers visiting high-income countries exhibited depletion of resistance genes; six of the 24 ARGs were diminished. These findings suggest that travellers visiting high-income countries acquire fewer resistance genes.

Most ESBL-E isolates (n=55, 91.7%) from post-travel samples were resistant to multiple antibiotics (Fig 2). There was significant separation of post-travel microbial communities between ESBL-E-positive participants and ESBL-E-negative participants (n=34, P=0.038; weighted UniFrac distance, P=0.020). Moreover, we observed significantly lower Actinobacteria richness in ESBL-E-positive participants than in ESBL-E-negative participants (P=0.008).

We constructed a reference model based on 13 pre-travel gut microbial predictors, including Actinobacteria richness and 12 genera. To test whether the combination of travel-related risk factors

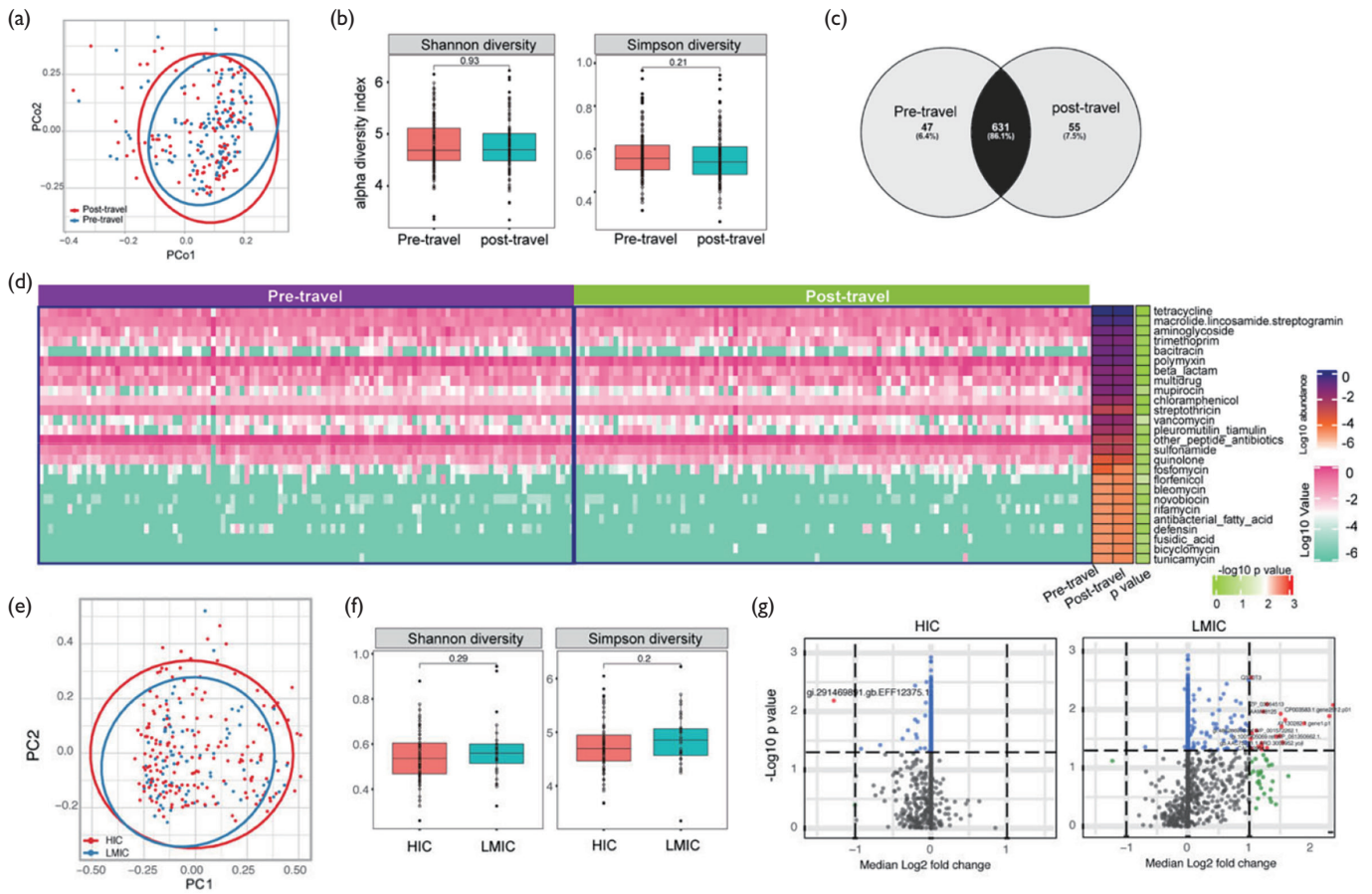

FIG 1. Metagenomic profile of changes in resistome between pre- and post-travel gut microbiota, and between samples collected after visits to high-income versus low- or middle-income countries: (a) principal coordinates analysis of weighted UniFrac distances between pre- and post-travel samples, (b) changes in alpha diversity between pre- and post-travel samples, (c) Venn plot showing the overlap of antibiotic resistance genes between pre- and post-travel samples, (d) heatmap comparing abundances of antibiotic resistance genes between pre- and post-travel samples, and (e, f, and g) changes in alpha diversity, beta diversity, and abundance in the resistomes of travellers who visited low- or middle-income countries versus high-income countries.

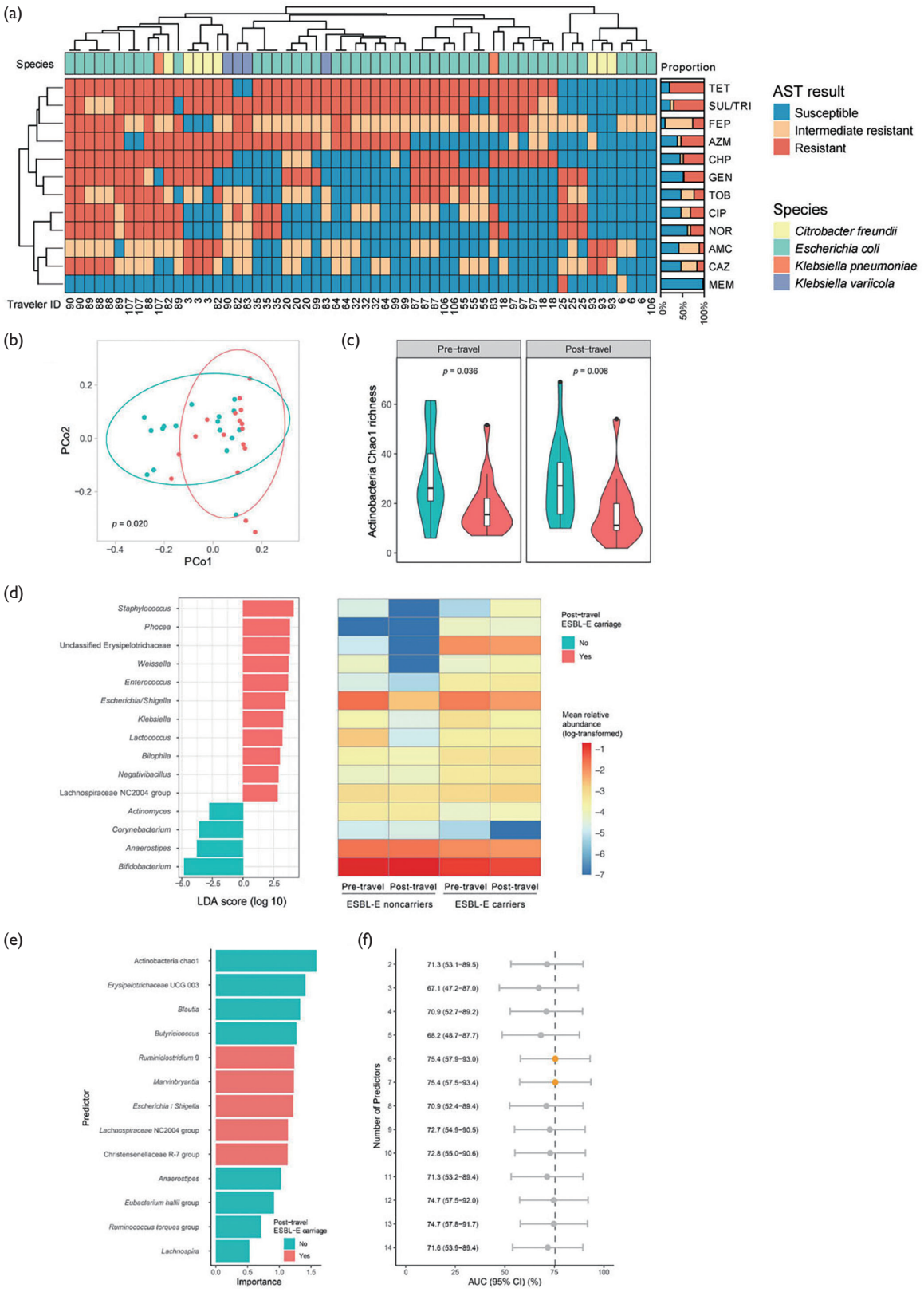

FIG 2. Travellers with extended spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) positivity after travel have distinct gut microbiota: (a) antimicrobial susceptibility testing (AST) of ESBL-E isolates in terms of amoxycillin (AMC), ceftazidime (CAZ), cefepime (FEP), meropenem (MEM), gentamycin (GEN), tobramycin (TOB), azithromycin (AZM), tetracycline (TET), ciprofloxacin (CIP), norfloxacin (NOR), trimethoprim-sulfamethoxazole (SUL/TRI), and chloramphenicol (CHP); (b) principal coordinates analysis of weighted UniFrac distances between ESBL-E-positive and -negative travellers; (c) Actinobacteria richness between ESBL-E-positive and -negative travellers; (d) linear discriminant analysis (LDA) score of genera between post-travel ESBL-E-positive and -negative travellers ($P<0.05$); (e) the twelve most important genus predictors among post-travel ESBL-E-positive and -negative travellers; and (f) areas under the curve (AUC) for combinations of raw seafood consumption and microbial predictors. Dashed line indicates the area under the curve of 75.4% for the reference model using Actinobacteria and 12 genera.
Abbreviation: 95% CI = 95% confidence interval

and microbial predictors could improve prediction, we established a series of models using combinations of raw seafood consumption and the stepwise addition of microbial predictors. A model with raw seafood consumption and five genus predictors (Actinobacteria richness, Erysipelotrichaceae UCG-003, *Blautia*, *Butyricicoccus*, and *Ruminiclostridium 9*) demonstrated an AUC of 75.4% (95% confidence interval=57.9%-93.0%).

Discussion

After travel, compared with ESBL-E-positive participants, ESBL-E-negative participants were associated with higher Actinobacteria richness and higher abundances of short-chain fatty acid producers, along with lower abundances of several opportunistic pathogenic genera. Potential gut acidity secondary to increased levels of short-chain fatty acids could inhibit ESBL-E colonisation.[4] Our findings likely reflect the association between compromised gut homeostasis and ESBL-E colonisation.

Raw seafood consumption during travel is a risk factor for ESBL-E acquisition, which could be attributed to seafood farming near areas of coastal run-off or the use of antibiotics in aquaculture. Thus, we suggest that travellers avoid raw seafood consumption during travel. The predictive power of Actinobacteria richness plus raw seafood consumption was higher than that of raw seafood consumption alone. Therefore, both travel-related risk factors and baseline gut microbiota are important for predicting ESBL-E acquisition during travel.

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Disclosure

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1. Peng Y, Liang S, Poonsuk K, et al. Role of gut microbiota in travel-related acquisition of extended spectrum β-lactamase-producing Enterobacteriaceae. J Travel Med 2021;28:taab022.
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Molecular epidemiology of hepatitis C virus transmission networks among men who have sex with men in Hong Kong

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KEY MESSAGES

1. Sexual transmission of hepatitis C virus (HCV) was common among men who have sex with men (MSM).
2. HCV infections among MSM living with HIV in Hong Kong formed a distinct closely knit cluster.
3. MSM had a shorter infection history at HCV diagnosis, compared with non-MSM.
4. The major driving force of the HCV/HIV syndemic was confined to local transmission clusters.

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Introduction

The major route of hepatitis C virus (HCV) transmission is blood contact including transfusion of contaminated blood products and needle-sharing among people who inject drugs. An increasing number of HCV infections has been reported among men who have sex with men (MSM) living with HIV in the Asia-Pacific region.[1] HCV infection and transmission are predominately associated with high-risk sexual activities involving mucosal trauma and recreational drug use for sex (chemsex) among both HIV-positive and -negative MSM because they share similar risk factors.[2]

In Hong Kong, HIV prevalence has been low among people who inject drugs, owing to the low-threshold methadone harm reduction programme. The disease burden of HIV/HCV co-infections is small among people who inject drugs, compared with MSM. This study investigated the molecular epidemiology of HCV infection among MSM living with HIV in Hong Kong to (1) delineate the transmission paths of HCV among MSM living with HIV, (2) evaluate factors associated with HCV transmission in the community, and (3) compare infection histories of intra-host variants with non-MSM.

Methods

We recruited MSM who had a diagnosis of HCV infection at the time of HIV diagnosis or experienced seroconversion during follow-up. We aimed to enrol 40 co-infected individuals, based on the estimated HCV infection prevalence of 4% among approximately 1000 HIV patients diagnosed between 2016 and 2019. A blood sample was collected from each MSM, and a survey about sexual behaviours was administered.

Two control groups were set up for comparative analysis. The first control group comprised MSM living with HIV without HCV infection at HIV diagnosis. The second control group included archived RNA specimens from non-MSM without HIV who had HCV infection and were blood donors and people who inject drugs. The total sample size was estimated to be 160.

Viral RNA was extracted. To construct near-complete genomes, 5 to 8 μL of viral RNA were reverse-transcribed and amplified. Primers were used to amplify near full-length genomes for both HIV-1 and HCV; the polymerase chain reaction products were purified.

Viral DNA libraries were prepared from amplicons to enable multiplex whole genome sequencing with MinION. MinKNOW was used to manage the sequencing experiments and to perform basecalling and demultiplexing. A custom bash command script was written to process the Nanopore reads for whole genome sequence reconstruction. To remove low-quality reads, all sequence reads were filtered. After quality checks, all reads were mapped to the reference sequence (HIV-1:HXB2 HCV: NC_004102). Variant and consensus calling were performed. The resultant consensus sequences were converted to the FASTA format.

For both HIV and HCV sequences, pairwise TN93+G+I distances were calculated to construct the respective networks with a 4% distance threshold. Undirected networks were analysed at the network level by measuring the average degree, diameter, clustering coefficient, and density. The networks were then joined to identify bridging nodes that

connected both the HIV and HCV networks. Node-level network parameters included degree, closeness centrality, and average shortest path length.

Whole genome sequences of HCV were subtyped. All sequences identified as subtype 3a were selected for analyses. The best-fit model was selected by likelihood scores, and priors were estimated. Bayesian coalescent skyline analysis was conducted to determine the time to the most recent ancestor (tMRCA) and its population dynamics. The 95% highest probability density interval of the age of the tree was calculated.

A custom bash command script was written for the bioinformatics pipeline to generate within-host variants. Quality-filtered reads were sampled to a size of 10000-read file to ensure computational feasibility while preserving the number of major variants identified. Sampled reads were mapped to the consensus sequence. The SAM files were converted to binary format, sorted, and indexed. After identifying minor variant sequences, clusters were defined as having at least 30 sequences.[3] To identify the infection history of an individual, individuals exhibiting at least 10 within-host variants were selected for phylodynamic analysis. Factors associated with the diversity of within-host variants and the infection history estimated by the tMRCA were determined using the Mann-Whitney *U* test and generalised linear models.

Results

In total, 132 and 115 HCV and HIV sequences were analysed, respectively. Of the HCV sequences, 67 (51%) were from MSM living with HIV and HCV, 44 (33%) were from people who inject drugs, and the remaining 21 (16%) were from blood donors. The most common HCV subtype was 3a (35%, n=46), followed by 6a (23%, n=31), 1b (20%, n=26), and 1a (16%, n=21). Five (4%) and three (2%) subtype 2a and 3b sequences were also identified. Most participants were men (89%, 116/131); the median age at sample collection was 39 years (interquartile range, 30-48 years). Most HIV sequences included were from men (96%, 107/112); the median age at data collection was 31 years (interquartile range, 27-43 years). The three major HIV subtypes were CRF01_AE (44%), CRF07_BC (24%), and B (24%). Other minority subtypes included C, CRF02_AG, CRF08_BC, CRF55_01B, and G.

Most HCV sequences (72%, 96/132) were connected in the network, with four major clusters: subtype 3a (n=42), 6a (n=24), 1a (n=6), and 1b (n=5) [Fig 1]. There were eight smaller components comprising two or three nodes. The subtype 3a cluster consisted of 44% connected nodes, forming a single dense cluster (density=0.997) with a network diameter of 2 and a clustering coefficient of 0.996. In contrast, the subtype 6a cluster was sparse, with a density of 0.279, a longer network diameter of 4, and a clustering coefficient of 0.67. Subtypes 1a and 1b clusters formed a clique-like structure with six and five nodes, respectively; there were three triads and five dyads. Almost all sequences in the subtype 3a network were from MSM living with HIV who formed a clique. Separately, the subtype 6a cluster was primarily composed of people who inject drugs.

Phylodynamic analysis of HCV subtype 3a in MSM was performed. Considering the high genetic similarity among the sequences predominated by MSM, the effective population size during the data collection period did not significantly differ over time, and the history of the tree was short (Fig 2).

Of the 132 HCV sequences, 85 (64%) had at least one within-host variant; 55 of these had ≥10 within-host variants and were analysed. The median of the mean tMRCA across all trees was 6.67 years (95% highest probability density=5.58-7.88 years). MSM had a significantly shorter infection history than non-MSM (1.77 vs 10.85 years, P=0.001). Although HCV subtype was not associated with infection history (P=0.51), subtype 6a had a more diverse within-host variant population (4 vs 15, P=0.0006). After adjustment according to subtype, MSM had a less diverse within-host variant population (P<0.001) and a shorter tMRCA (P=0.017).

Discussion

In Hong Kong, HCV transmission was phylogenetically densely connected among HIV co-infected MSM. At the 4% distance threshold, the HCV sequences in people living with HIV showed unique clustering patterns, which varied according

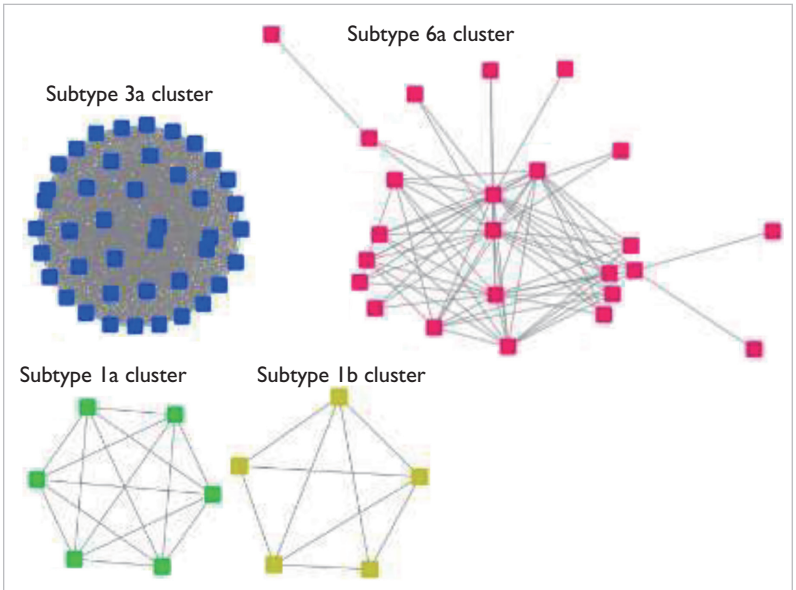

FIG 1. Transmission network of hepatitis C virus: each node represents an HCV sequence, and an edge connecting two nodes indicates a genetic distance of <4%.

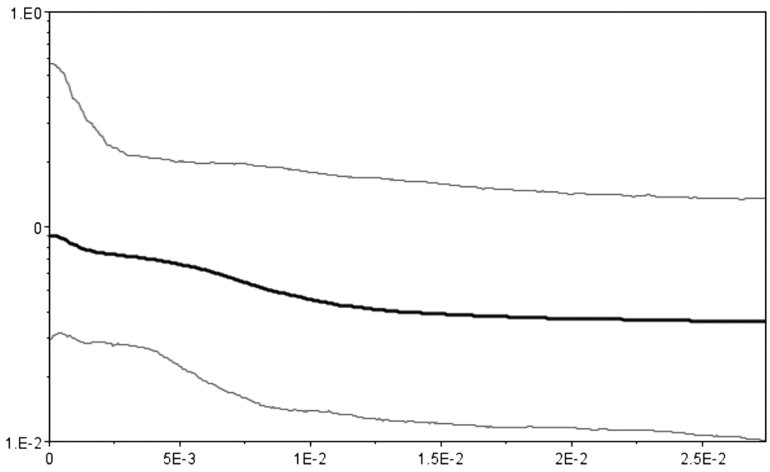

FIG 2. Temporal change in the effective population size of hepatitis C virus subtype 3a sequences: black line represents the median effective population size, and grey lines represent the 95% highest probability density. The x-axis represents time, and the y-axis represents the effective population size using a log10 scale.

to subtype. Most clusters were clique-like, reflecting a pattern of continuous local transmission of HCV in the community within a short period. Notably, the subtype 6a cluster was loosely connected with a low density and a low clustering coefficient. The transmission history of the entire cluster was assumed to be longer such that sufficient time was allowed for in vivo mutations before the virus was transmitted to another individual. Some non-local cases may have originated from neighbouring cities where HCV strains were similar, but less similar than those in the local core transmission cluster; these peripheral nodes formed a star network topology. This observation was confirmed by phylodynamic analysis, in which the estimated duration of infection history was <1 year.

The diversity of within-host variants can infer the infection history and the undiagnosed period; MSM had a shorter within-host variants tMRCA and a less diverse within-host variants population, compared with non-MSM. This indicates that non-MSM had a longer undiagnosed period, whereas MSM were diagnosed soon after HCV infection.[4] The lack of HCV screening, suboptimal access to treatment, and structural barriers may have contributed to the prolonged periods without diagnosis or treatment among non-MSM.[5] The HIV/HCV co-infected MSM were recruited from HIV specialist clinics; clinical management procedures could have contributed to the earlier diagnosis and treatment.

There were some limitations in this study. The transmission networks generated were based on pairwise genetic distances and did not imply direct transmission relationships. The sequences can never be complete because there could be undiagnosed individuals in the population as well as missing nodes owing to refusal to join the study; these missing nodes could have affected the network configuration. As most clusters formed a clique-like structure, the addition or removal of a node would not affect the overall configuration, except in rare instances where an entire cluster was not sampled.

Conclusion

The major driving force of the HCV/HIV syndemic was confined to local transmission clusters. Targeted intervention is warranted to prevent outbreaks in the MSM community. As routine HCV testing in HIV specialist clinics can shorten the diagnosis period, similar testing strategies should be offered to the wider MSM community, particularly individuals involve in chemsex and slamming, to pre-empt HCV transmission.

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Disclosure

The results of this research have been previously published in:

1. Kwan TH, Wong BCK, Wong KH, Lee SS. Hepatitis C co-infection in people living with HIV-epidemiologic differences between men who have sex with men MSM and non-MSM. Front Public Health 2022;10:925600.
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Sialyl-Lewis(x) interaction for sperm selection in assisted reproductive treatment: abridged secondary publication

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KEY MESSAGES

1. Capacitated human spermatozoa initiate fertilisation via binding to the zona pellucida (ZP). The sialyl-Lewis(x) (sLeX) sequence is the most abundant terminal sequence on the glycans of human ZP glycoproteins involved in spermatozoa-ZP binding. Compared with unbound spermatozoa, ZP- or sLeX-bound spermatozoa have better fertilisation potential and quality in terms of morphology, DNA integrity, chromatin integrity, protamination, and global methylation.
2. Four sLeX-binding proteins of capacitated spermatozoa were identified: chromosome 1 open reading frame 56 (C1orf56), ZP-binding protein 1, heat shock-related 70 kDa protein 2, and sperm acrosome membrane–associated protein 1.
3. Clorf56 translocated to the cell surface of the spermatozoa acrosomal region during capacitation. Treatment with anti-C1orf56 antibody inhibited spermatozoa-ZP binding and ZP-induced acrosomal reactions. Purified C1orf56 from capacitated spermatozoa were able to bind human ZP.
4. The in vitro fertilisation rate was not associated with the percentage of capacitated spermatozoa expressing C1orf56. However, the percentage of C1orf56-positive spermatozoa in the acrosome-reacted population was significantly lower in cycles with a fertilisation rate <60% than in cycles with a fertilisation rate ≥60%. These results suggest that C1orf56 has important roles after ZP-binding and acrosomal reactions.
5. Sperm quality can be significantly enhanced by selection methods involving ZP, sLeX, and annexin V microbeads.

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Introduction

Infertility affects approximately 15% of reproductive-aged couples worldwide. The live birth rate after use of assisted reproductive technology (ART) is approximately 30%. Sperm quality is a major factor influencing fertilisation success. Only fertilisation-competent spermatozoa survive natural selection mechanisms involving anatomical, biochemical, and physiological barriers in a highly specialised microenvironment.[1] In ART, motile and morphologically normal spermatozoa are routinely isolated by either swim-up or density gradient centrifugation. However, these two markers are unable to select spermatozoa with high fertilisation potential and genetic quality.

Human fertilisation begins when a capacitated spermatozoon binds to the zona pellucida (ZP) of an oocyte. The spermatozoa-ZP interaction serves as a natural sperm selection mechanism in vivo and can potentially be used for sperm evaluation and selection in clinical settings.[2] The sialyl-Lewis(x) (sLeX) sequence is the most abundant terminal sequence on the glycans of human ZP glycoproteins involved in spermatozoa-ZP binding.[3] This phenomenon suggests that the human ZP selectively interacts with spermatozoa exhibiting high fertilisation capacity and genetic integrity. This study aimed to determine the roles and clinical applications of human ZP and sLeX in selecting fertilisation-competent spermatozoa.

Methods

Semen samples were collected from normal men. Oocytes were collected from infertile women. The direct swim-up method was used to isolate motile and viable spermatozoa. Spermatozoa-ZP coincubation assays were performed. Ionophore- or ZP-induced acrosomal reactions were performed to prepare acrosome-reacted sperm. ZP- or sLeX-bound spermatozoa were collected. The fertilisation competence and quality of ZP- or sLeX-bound spermatozoa were determined in terms of

morphology, acrosome status, motility, viability, ZP-binding capability, DNA fragmentation rate, DNA damage, chromatin integrity, protamine deficiency, and methylation level.

Sperm plasma membrane proteins were extracted. Surface sLeX-binding protein expression patterns were investigated on uncapacitated, capacitated, and acrosome-reacted spermatozoa. The associations between surface sLeX-binding protein expression and spermatozoa fertilisation competence/quality were determined. Capacitated spermatozoa were pre-incubated in medium supplemented with functional blocking anti–chromosome 1 open reading frame 56 (C1orf56) antibody. Isotype-matched antibody was used as a control. Spermatozoa were washed before evaluation of their fertilisation competence. The effects of 1 mg/mL anti-C1orf56 antibody for 120 minutes on the sLeX binding capabilities of spermatozoa were determined by flow cytometry. C1orf56 was purified from human spermatozoa using immuno-affinity chromatography. Purified C1orf56 was labelled with Alexa Fluor-594. Matched hemizona were incubated with 1 mg/mL labelled C1orf56 with or without anti-C1orf56 neutralising antibody for 3 hours; binding was observed using a fluorescence microscope.

To investigate clinical applications of the sperm-sLeX interaction, couples attending the infertility clinic were recruited. The standard gonadotrophin-releasing hormone agonist long protocol was used. Conventional insemination was performed 4 hours after oocyte retrieval, and fertilisation was checked 16 to 18 hours later. Normal fertilisation was regarded as the appearance of two pronuclei. The fertilisation rate (FR) was defined as the number of two pronuclei zygotes observed divided by the total number of inseminated oocytes × 100. Men with in vitro FR ≥60% or <60% were compared. The surface expression of C1orf56 in spermatozoa was determined by flow cytometry. The potential application of sLeX for sperm selection during intracytoplasmic sperm injection (ICSI) was determined.

Results

Sperm-ZP binding is associated with two protein markers: heat shock 70 kDa protein 2 (HSPA 2) and sperm acrosome membrane–associated protein (SPACA) 3. Compared with unbound spermatozoa, ZP- or sLeX-bound spermatozoa had significantly higher expression levels of HSPA2 and SPACA 3, as well as significantly higher rates of normal morphology, DNA integrity, chromatin integrity, protamination, and global methylation.[4] Additionally, altered H3K9Me1 histone methylation and DNA methylation of small nuclear ribonucleoprotein polypeptide N were demonstrated in ZP- or sLeX-bound spermatozoa.[4]

We identified four sLeX-binding proteins in capacitated spermatozoa: C1orf56, ZP-binding protein 1, HSPA 2, and SPACA 1.[5] The acrosomal region of spermatozoa exhibited C1orf56 immunoreactive signals with intensities that increased after capacitation; this phenomenon indicated C1orf56 translocation to the cell surface during capacitation. Treatment with anti-C1orf56 antibody decreased the number of capacitated spermatozoa bound to the ZP, spermatozoa-sLeX binding, and ZP-induced acrosomal reactions of capacitated spermatozoa. Fluorescence-labelled C1orf56 also specifically bound to the ZP of human oocytes.[5] However, spermatozoa fertilisation competence/quality was not associated with surface C1orf56, clusterin, HSPA 2, SPACA 4, or ZP-binding protein 1 (data not shown).

The FR rate was not associated with the percentage of capacitated spermatozoa expressing C1orf56. However, the percentage of C1orf56-positive spermatozoa in the acrosome-reacted population was significantly lower in cycles with an FR <60% than in cycles with an FR ≥60%; these results suggest that C1orf56 has important roles after ZP-binding and acrosomal reactions.[5] The high- and low-motility groups did not significantly differ in terms of C1orf56 expression. Similarly, samples with normal morphology of >4% and ≤4% did not significantly differ in the capacitated and acrosome-reacted subpopulations.[5]

Compared with unselected controls, ZP-bound spermatozoa had a significantly higher rates of normal morphology, DNA integrity, protamination, and global methylation (Table). Similar enhancing effects were observed in sLeX-bound and annexin V microbead-selected sperm.

Discussion

In the present study, ZP-bound spermatozoa had significantly higher expression levels of HSPA 2 and SPACA 3, compared with unbound spermatozoa. Moreover, ZP-bound spermatozoa had significantly higher rates of normal morphology, DNA integrity, chromatin integrity, protamination, and global methylation, compared with unbound spermatozoa.[4] These findings confirmed the utility of the spermatozoa-ZP interaction in the selection of fertilisation-competent spermatozoa for ART and in provision of diagnostic information about the fertilisation potential and genetic qualities of spermatozoa. Methods involving ZP, sLeX, and annexin V microbeads are viable approaches for sperm selection.

The contribution of C1orf56 to the spermatozoa-ZP interaction was demonstrated by the binding of purified C1orf56 to the ZP, as well as the inhibitory effect of anti-C1orf56 antibody on the spermatozoa-ZP interaction.[5] There was

TABLE. Comparisons of fertilisation competence and quality among sperm samples (n=10) isolated by various selection methods

Sperm selection method	DNA fragmentation rate, %	Protamination deficiency, fluorescence intensity/sperm	Methylation, fluorescence intensity/sperm	Normal morphology, %
Unselected control	16.2±5.0	119189.4±33450.8	346.7±115.5	4.6±2.3
Zona pellucida	4.4±2.7*	31426.6±14122*	1212.6±428.1*	15.2±7.0*
Sialyl-Lewis(x) sequence	10.5±5.3*	98729.6±27056.3	691.0±383.4*	8.2±3.1*
Physiological intracytoplasmic sperm injection dish	11.9±4.6	90530.6±47736.8	540.6±399.3	7.1±3.3
Annexin V microbeads	8.7±4.1*	108768.8±63358.l	833.3±367.2*	8.7±3.8*

* P<0.05, compared with unselected controls

no significant difference in C1orf56 expression on capacitated spermatozoa between the high FR (≥60%) and low FR (<60%) groups. This lack of difference is likely due to the presence of multiple ZP receptors; a decrease in one such receptor can be compensated by others.[2] The C1orf56 level in the acrosome-reacted spermatozoa was positively associated with FR. This observation suggests that C1orf56 has important roles after ZP-binding and acrosomal reactions.

Conclusion

Our results support the notion that the development of a robust and reproducible selection method incorporating the ZP binding ability of spermatozoa can improve the overall workflow and pregnancy outcomes of ART.

Funding

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Disclosure

The results of this research have been previously published in:

1. Leung ETY, Lee BKM, Lee CL, et al. The role of spermatozoa-zona pellucida interaction in selecting fertilization-competent spermatozoa in humans. Front Endocrinol (Lausanne) 2023;14:1135973.
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No benefit of additional tramadol or tizanidine to diclofenac for acute low back pain: abridged secondary publication

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KEY MESSAGES

1. There was no significant difference in improvement of functional recovery, pain intensity, and return to work among the three groups of diclofenac plus tramadol, diclofenac plus tizanidine, and diclofenac plus placebo.
2. Our findings do not support the use of tramadol or tizanidine in addition to diclofenac in patients with acute low back pain.

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Introduction

The prevalence of low back pain (LBP) in Hong Kong was estimated to be 21% to 44.1%,[1,2] which is higher than that reported by international studies. LBP can result from over 60 different medical conditions. Apart from malignancy, infection, and fracture, the common causes of mechanical LBP include lumbar strain, herniated disc with radiculopathy, facet osteoarthritis, and lumbar spinal stenosis. It is recommended that nonsurgical management is an effective initial management for these LBP, and there is no need for radiographic or laboratory evaluation.

Emergency departments (ED) play a vital role in the management of patients with acute LBP and severe symptoms. In the United States, among patients with acute LBP, 48% reported functional impairment 3 months after discharge from ED; 42% reported moderate or severe pain; and 46% continued to use analgesic drugs.[3] This shows the lack of optimal treatment for patients with acute LBP in the emergency setting.

For patients with recognised risk factors for chronic LBP, international guidelines from 13 countries recommend management with patient education, early and gradual activation and avoidance of bed rest, the use of analgesic medications and manipulation therapy, and early and aggressive multimodal treatments.[4] Most guidelines recommend non-steroidal anti-inflammatory drugs (NSAID) as the first-line medication. However, for patients with more severe symptoms and functional impairments, the recommendation for second-line analgesic as an add-on of NSAID is less clear. Tramadol and skeletal muscle relaxants have been recommended, but no evidence suggests that they are preferable.

Tramadol is a weak opioid with a dual-model action. It is a selective agonist of mu-opioid receptors; it inhibits neuronal norepinephrine and serotonin reuptake. Tramadol is largely metabolised by the CYP 2D6 enzyme, for which approximately 10% of the Caucasian population (but <1% in the Chinese population) have genetic polymorphism. This suggests a more consistent predictable response

to tramadol in our population. Its common adverse effects include constipation, nausea, dizziness, headache, and drowsiness. The abuse potential is low. Continuous use of tramadol for up to 3 months is safe and effective for patients with chronic pain.

Tizanidine is a commonly used muscle relaxant for acute and non-specific LBP. Tizanidine is an alpha2-adrenargic agonist and an antispasticity and antispasmodic drug. Its onset is 1 to 2 hours, and the action duration is 3 to 6 hours. Its adverse effects are mild and include xerostomia (39%), somnolence (38%), asthenia (25%), and dizziness (12%). Tizanidine decreases the gastrointestinal adverse effects of NSAID (diclofenac); this suggests combined use of diclofenac and tizanidine for patients with more severe symptoms.

Methods

We conducted a multicentre, double-blind, randomised controlled trial.[5] Adult patients with acute non-specific LBP who attended the ED of the Prince of Wales Hospital, Pamela Youde Nethersole Eastern Hospital, and United Christian Hospital were randomly assigned to receive diclofenac plus tramadol, diclofenac plus tizanidine, or diclofenac plus placebo in a 1:1:1 ratio. Patients with a direct injury or fall or with signs of major pathologies were excluded. Patients were followed up for 4 weeks. Outcome measures include the Roland-Morris Disability Questionnaire (RMDQ), numeric rating scale (NRS), adverse effect profile, drug compliance, sick leave period, and return to work capacity (including light duty).

Results

A total of 291 patients were randomly assigned to receive diclofenac plus tramadol (n=93), diclofenac plus tizanidine (n=99), or diclofenac plus placebo (n=99). Of the patients, 90.4% had lower back pain and 9.6% had sciatica. All patients had severe pain and disability, with RMDQ scores ranging from 15.3 to 16.3, NRS (rest) ranging from 4.1 to 4.6, and NRS (activity) ranging from 7.3 to 7.9. However, only 23.7% of patients fully complied with the regimen of at least three of four recommended doses; 53.6% of participants were non-compliant with intake of less than one of the four recommended doses.

Using the intention-to-treat analysis, there were no significant differences between groups in terms of changes in RMDQ score, NRS (at rest), and NRS (activity) on day 7.

Significantly more patients in the tizanidine group and tramadol group than the placebo group reported adverse events including sleepiness and dizziness. There were also more nausea and vomiting in the tramadol group than the placebo group.

Discussion

Adding tramadol or tizanidine to diclofenac did not improve functional recovery of patients with acute LBP. Therefore, the use of additional tramadol or tizanidine in the ED setting is not supported. However, the low compliance drove the effect estimate towards the null hypothesis (ie, no difference). Further studies should take into account of the low compliance.

In a systematic review of muscle relaxants for non-specific LBP, non-benzodiazepine antispasmodics are suggested to increase the risk of an adverse event. Furthermore, in patients taking tramadol, adverse events of dizziness, sleepiness, nausea, and vomiting are reported as causes for discontinuation of treatment. The high incidence of adverse events may have resulted in the low compliance in our study. Thus, physicians should be cautious when prescribing tramadol or tizanidine to patients with acute LBP.

Despite the poor compliance, patients in all three groups had improvements in functional outcomes (RMDQ score) and pain relief (NRS) at days 7 and 28. This is consistent with the results from the United States population that functional impairment improved in most patients from 1 week to 3 months. However, 31% to 45% of our patients could not return to their work capacity at day 28.

Funding

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Disclosure

The results of this research have been previously published in:

1. Hung KKC, Lam RPK, Lee HKH, et al. Comparison of diclofenac with tramadol, tizanidine or placebo in the treatment of acute low back pain and sciatica: multi-center randomized controlled trial. Postgrad Med J 2024:qgae052.

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5. Hung KKC, Lam RPK, Lee HKH, et al. Comparison of diclofenac with tramadol, tizanidine or placebo in the treatment of acute low back pain and sciatica: multi-center randomized controlled trial. Postgrad Med J 2024:qgae052.

Improving workplace mental health literacy in Hong Kong: abridged secondary publication

LT Lam *, P Wong, MK Lam, P Reddy

KEY MESSAGES

1. An intervention comprising a workplace environmental scan and a psychoeducation training course for workplace issues and mental health literacy effectively improved mental health literacy and mental well-being among workers.
2. This intervention should be integrated into the Occupational and Health Safety legislation as compulsory workplace training. In proportion to the workforce size, trained officers should be available to provide support when workers require mental health assistance.

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Introduction

Full-time workers in Organisation for Economic Co-operation and Development countries are estimated to spend approximately 37% of their time working on a normal day.[1] Workplace mental health is a major health concern. The workplace is an important venue for preventing mental health problems and promoting mental wellness.[2] In its Mental Health Action Plan 2013-2020, the World Health Organization states that the workplace should be the main focal environment for mental health promotion.[3] Community-based prevention and early intervention programmes (including those in the workplace) for employees may contribute to early identification and intervention of mental health problems. There are two main types of intervention programmes: person-directed and organisation-directed.[4] The effects of person-directed intervention programmes tend to be short (≤6 months); in contrast, the effects of organisation-directed programmes can last for ≥12 months. Programmes targeting both the organisation and the individual should be developed.[4] This study aimed to assess the effects of a workplace mental well-being intervention programme for organisations and individuals on improving mental health literacy, work-related burnout and stress, and health-related quality of life among workers.

Methods

This phase III waitlist cluster randomised controlled trial used different work sites or branch offices as the primary units of randomisation. The intervention programme comprised an organisation-directed component and an individual-directed component. For the former, a senior social worker with expertise in workplace issues conducted workplace environment scans using the Moos Work Environment Scale.[5] De-identified and aggregated assessment data were provided to the management, along with a professional interpretation of the findings. Possible strategies were offered to resolve the identified issues. Site management was encouraged to use this information to improve the work environment. For the latter, the Workplace Mental Health First Aid training course, which comprises a series of self-paced online e-Learning modules on stress reduction and burnout prevention, was provided to workers. After completion of the course, a face-to-face group session was conducted to allow participants to clarify any queries with the trainer and to gain hands-on experience through communication skills practice.

The primary outcome measure was mental health literacy among workers, which was assessed using the Australian National Mental Health Literacy and Stigma Survey.[6] Its Chinese version has been validated by the Mental Health Association of Hong Kong. Considering the local context, only relevant components were used: correct recognition of a mental problem, help-seeking intention, stigmatisation, and social distancing. Secondary outcomes included burnout, stress, and health-related quality of life. Burnout was evaluated using the Maslach Burnout Inventory.[7] Stress was assessed using the Anxiety subscale of the Depression, Anxiety, and Stress Scale, which has good reliability and validity.[8,9] Health-related quality of life was measured using the European Quality of Life-5 Dimensions.[10,11] Participants were assessed at

baseline, after completion of the intervention, and at a 3-month follow-up. Intention-to-treat analyses were performed.

TABLE 1. Baseline characteristics of participants (n=456)

Characteristic	Control (n=227)*	Intervention (n=229)*	P value
Age, y	40.5±1.1	40.9±1.4	0.575
Male sex	97 (42.7)	118 (51.5)	0.104
University or above education level	173 (76.2)	190 (83.0)	0.109
Married	135 (59.5)	136 (59.4)	0.988
Full-time employment	227 (100)	227 (99.1)	0.407
Flexible hours	82 (36.1)	82 (35.8)	0.926
Regular exercise	178 (78.4)	166 (72.5)	0.198
Smoker	8 (3.5)	9 (3.9)	0.864
Drinker (moderate/heavy)	4 (1.8)	4 (1.7)	0.981
Intended to resign	99 (43.6)	107 (46.7)	0.203
Burnout			
Emotional exhaustion	20.1±0.8	20.9±0.8	0.611
Depersonalisation	6.5±0.4	6.6±0.4	0.897
Professional accomplishment	28.0±1.0	28.0±0.8	0.999
Stress	7.1±0.3	7.4±0.3	0.541
Quality of life (self-rating)	80.7±1.4	70.3±1.0	0.568
Mental health literacy			
Correct recognition of a mental problem	3.3±0.05	3.3±0.03	0.567
Help-seeking intention	12.1±0.2	12.2±0.3	0.611
Stigmatisation	24.5±0.5	24.9±0.5	0.213
Social distancing	12.1±0.3	12.2±0.4	0.709

* Data are presented as mean±standard deviation or No. (%) of participants

TABLE 2. Outcome measures after the intervention programme (n=456)

Outcome measures	Control (n=227)*	Intervention (n=229)*	P value (adjusted)
Burnout			
Emotional exhaustion	20.5±1.0	20.9±0.6	0.872
Depersonalisation	6.9±0.6	7.4±0.3	0.689
Professional accomplishment	27.7±1.2	28.2±0.8	0.035
Stress	7.5±0.5	6.7±0.4	0.015
Quality of life (self-rating)	80.5±1.6	81.5±0.8	0.375
Mental health literacy			
Correct recognition of a mental problem	3.2±0.1	3.4±0.1	0.003
Help-seeking intention	11.9±0.2	12.9±0.3	<0.001
Stigmatisation	24.5±0.6	26.3±0.5	<0.001
Social distancing	12.3±0.6	11.9±0.5	0.160

* Data are presented as mean±standard deviation

Results

Of 456 participants recruited from five corporations, 229 (50.2%) were randomly allocated to receive the intervention programme first. All participants completed the online modules and the face-to-face session. The intervention and control groups were comparable in terms of baseline characteristics (Table 1).

After the intervention, compared with the control group, the intervention group had improved mental health literacy scores in terms of correct recognition of a mental problem (3.4 vs 3.2, P=0.003), help-seeking intention (12.9 vs 11.9, P<0.001), and stigmatisation (26.3 vs 24.5, P<0.001); improved stress score (6.7 vs 7.5, P=0.015); and improved burnout scores in terms of professional accomplishment (28.2 vs 27.7, P=0.035) [Table 2].

Of the 456 participants, 273 (60.0%) responded to the 3-month follow-up survey. Improvement in the mental health literacy scores persisted in terms of help-seeking intention (P=0.014), stigmatisation (P<0.001), and social distancing (P<0.001) [Table 3].

Discussion

Our findings support the efficacy of the workplace mental health intervention programme on improving mental health literacy and alleviating stress and burnout, which are precursors to more severe mental health illnesses. Improved mental health literacy is protective against mental health problems. Work is a key component of daily life; workers' mental states affect the work environment, and the work environment affects workers. Thus, worker and work environment variables should be addressed concurrently to improve the overall well-being of workers. The COVID-19 pandemic has accelerated the use of digital health solutions for healthcare provision, health education, and health promotion.

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Disclosure

The results of this research have been previously published in:

1. Lam LT, Wong P, Lam MK. Protocol for a phase III wait-listed cluster randomised controlled trial of an intervention for mental well-being through enhancing mental health literacy and improving work friendliness in Hong Kong. Trials 2019;20:672.
2. Lam LT, Lam MK, Reddy P, Wong P. Factors

TABLE 3. Outcome measures at baseline, post-intervention, and 3-month follow-up

Outcome measures	Baseline (n=456)*	Post-intervention (n=456)*	3-month follow-up (n=236)*	P value
Burnout				
Emotional exhaustion	20.5±11.0	20.5±11.7	20.9±11.1	0.175
Depersonalisation	6.5±5.7	7.3±6.0	8.0±6.2	0.543
Professional accomplishment	27.5±8.8	27.4±9.3	27.8±8.9	0.332
Stress	7.2±4.1	6.9±4.2	7.3±4.1	0.813
Quality of life (self-rating)	80.3±11.5	81.0±10.7	81.3±12.0	0.084
Mental health literacy				
Correct recognition of a mental problem	3.2±0.6	3.4±0.7	3.3±0.7	0.195
Help-seeking intention	12.4±1.9	12.7±2.3	12.7±2.1	0.014
Stigmatisation	24.7±3.6	25.8±4.1	25.8±3.9	<0.001
Social distancing	12.5±3.2	12.1±3.3	11.9±3.5	<0.001

* Data are presented as mean±standard deviation

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Virtual multidisciplinary stroke care clinic for community-dwelling stroke survivors: a randomised controlled trial (abridged secondary publication)

JPC Chau *, SHS Lo †, AYL Lau, VWY Lee, KC Choi, EWC Shum, SS Hung, VCT Mok, EKC Siow, JYL Ching, SKY Lam

KEY MESSAGES

1. A virtual multidisciplinary stroke care clinic (VMSCC) service significantly improved participants' self-efficacy, depressive level, and social participation, while reducing emergency admissions and lengths of hospital stay during readmission.
2. Common challenges for stroke survivors accessing community rehabilitation services such as transportation limitations and scheduling conflicts were addressed by the flexible virtual delivery of the VMSCC service.
3. The VMSCC service can serve as a model for post-discharge community care that offers a high level of support and reliable information to aid stroke survivors by promoting their recovery and adjustment to their altered roles and functions.

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Introduction

Stroke self-management programmes can significantly improve self-efficacy and functional independence among stroke survivors,[1,2] but timely support for stroke survivors is often delayed because of functional impairments, disruptions to social life, lack of transportation to rehabilitation centres, and inadequate scheduling. We aimed to evaluate the effectiveness and cost-effectiveness of a virtual multidisciplinary stroke care clinic (VMSCC) service for community-dwelling stroke survivors.

Methods

We developed an online platform to promote stroke recovery and offer support to survivors and caregivers. The platform contains a total of 89 videos developed by a multidisciplinary team of healthcare professionals.

Ten pairs of stroke survivors and caregivers were invited to engage in two rounds of face-to-face semi-structured interviews. The VMSCC service was developed based on their feedback, expectations, and perceived facilitators and barriers to using the service.

In total, 205 male and 130 female stroke survivors aged 24 to 88 (mean, 62) years from 10 public hospitals were randomly allocated at a 1:1 ratio to receive either VMSCC service or usual care. Stroke survivors' self-efficacy in self-management, satisfaction in performance, depression, social participation, emergency admission to hospital, and length of hospital stay were assessed at baseline (T0) as well as 3 months (T1) and 6 months (T2) after the study began. A generalised estimating equation model was used to compare differential changes in outcomes at T1 and T2 relative to T0 between the control and intervention groups.

Total costs incurred for both the VMSCC service and usual care were calculated, as were incremental cost-effectiveness ratios expressed as the incremental cost per one-time decrease in emergency admission to hospital and 1-day decrease in length of hospital stay during the 6-month study period.

Qualitative feedback regarding the service was elicited from a random sample of 12 participants in the intervention group. The interview data were transcribed verbatim and analysed thematically.

Results

Of the 335 stroke survivors enrolled, 274 (81.8%) completed the study. Most (n=288, 86.0%) were

recovering from the first-ever stroke, whereas 47 (14.0%) were recovering from a recurrent stroke. Most (n=286, 90.2%) strokes were ischaemic.

Compared with the control group, the intervention group had greater improvement at 6 months after study commencement in terms of self-efficacy (mean difference=4.61, 95% confidence interval [CI]=0.17-9.05, P=0.042), depression (mean difference= -2.34, 95% CI= -4.06 to -0.61, P<0.01), and social participation (mean difference=5.10, 95% CI=0.64-9.56, P=0.025). However, the two groups were comparable in terms of the change in the Stroke Self-Management Behaviours Performance Scale at T1 and T2.

The VMSCC service was more effective but more expensive than usual care in reducing the number of emergency admissions to hospital (65% probability) and the length of hospital stay (47% probability).

Twelve stroke survivors in the intervention group were interviewed. Two themes were identified: (1) acceptance of the VMSCC service as a means of accessing healthcare services, and (2) provision of remote stroke self-management support. Participants perceived that the VMSCC service is an alternative means of accessing healthcare services and is more convenient, less time-consuming, and safer. Additionally, they found that the online platform helped to increase their confidence in managing post-stroke challenges.

Discussion

Participants perceived that the VMSCC service enhanced participants' knowledge of stroke by providing access to comprehensive information about stroke and self-care through videos. The video content was considered more helpful than other resources in Hong Kong. By incorporating an online video chat and a Skype-based hotline, the VMSCC eliminated transportation challenges, enabled better use of time, and provided practical guidance on daily self-care for stroke survivors between medical follow-up appointments. Participants were satisfied with the VMSCC service. Regular online video calls could help to detect care-related problems, symptoms of recurrent stroke, and other healthcare issues. Survivors felt reassured that they were cared for, monitored, and followed up even after discharge from the hospital.

The VMSCC intervention was based on the self-efficacy theory, which identifies perceptions of one's own capabilities as a key variable affecting stroke outcomes.[3] Self-efficacy is negatively correlated with depression.[4] The VMSCC service had significant group-by-time interaction effects on self-efficacy and depression, supporting the development of a self-management programme based on the self-efficacy construct.[5] Moreover, the VMSCC service resulted in greater reductions in the number of emergency admissions to hospital and the length of hospital stay, compared with usual care, but its cost was slightly higher (US$74).

One limitation of this study was the exclusion of stroke survivors with severe cognitive and communication impairments that could hinder Internet use and communication by phone.

Funding

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Disclosure

The results of this research have been previously published in:

1. Lo SHS, Chau JPC, Lau AYL, et al. A virtual multidisciplinary stroke care clinic for community-dwelling stroke survivors: a randomized controlled trial. Stroke 2023;54:2482-90.
2. Lam SKY, Chau JPC, Lo SHS, et al. User engagement in the development of a home-based virtual multidisciplinary stroke care clinic for stroke survivors and caregivers: a qualitative descriptive study. Disabil Rehabil 2022;44:5983-9.
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ImmuneMirror for evaluation of the genomic and transcriptomic features of resistance to immunotherapy for gastrointestinal tract cancer: abridged secondary publication

W Dai *, ML Lung, KO Lam, CL Chiang, H Zheng

KEY MESSAGES

1. We developed an integrative ImmuneMirror pipeline to evaluate tumour mutation burden, microsatellite instability status, human leukocyte antigen type, predicted neoantigen load, top-ranked neoantigens with T cell immunogenicity, and expression of innate anti-PD-1 resistance signatures.
2. We established a web server incorporating a machine learning model for neoantigen prediction and prioritisation.
3. In gastrointestinal tract cancers (including colorectal cancer, oesophageal squamous cell carcinoma, and hepatocellular carcinoma), an elevated neoantigen load was associated with good clinical outcomes in patients with oesophageal squamous cell carcinoma and poor clinical outcomes in patients with hepatocellular carcinoma.
4. A subset of microsatellite instability–high colorectal cancers with lower neoantigen load was shown to exhibit an advanced T stage.
5. The neopeptide YMCNSSCMGV derived from the TP53 hotpot mutation G245V restricted by HLA-A02 was identified in a patient with oesophageal squamous cell carcinoma. Experimental validation revealed high binding affinity between HLA-A02 and TP53G245V (YMCNSSCMGV).

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Introduction

Gastrointestinal tract (GIT) cancers have become the leading cause of cancer mortality in Hong Kong, responsible for 29.6% of cancer-relevant deaths. In particular, colorectal cancer (CRC), oesophageal squamous cell carcinoma (OSCC), and hepatocellular carcinoma (HCC) are among the top 10 major causes of cancer-related death. Clinical outcomes for patients with advanced-stage cancer metastasis remain poor.

The Food and Drug Administration has approved the clinical use of programmed death-1 (PD-1)/programmed death-ligand 1 (PDL-1) inhibitors (pembrolizumab and nivolumab) for metastatic CRC and gastric cancer. Nonetheless, response rates to these immunotherapies remain low in unselected patients; most patients only achieve a partial response. Thus, it is important to understand the mechanisms of resistance and identify biomarkers that predict treatment outcomes, thereby facilitating patient stratification.[1]

Multiple mechanisms are involved in resistance to cancer immunotherapy.[2] Although key genomic and transcriptomic features (mismatch repair deficiency; mutations of specific genes such as *BRCA2*, *B2M*, *JAK1/JAK2*, *PTEN*, *AKT1*, and *EGFR*; and enrichment of innate anti-PD-1 resistance signatures) have been associated with treatment efficacy,[2,3] the use of multiple standalone and web-based tools to extract these features complicated next-generation sequencing data analysis. Moreover, a lack of user-friendly integrative tools hinders the translation of these valuable findings into relevant clinical trials. Therefore, we established an all-in-one computational framework (ie, ImmuneMirror) that incorporates benchmark tools to characterise key genomic features and gene expression signatures associated with responses to PD-1/PDL-1 inhibitors.

Methods

In total, 805 normal-tumour paired GIT cancer samples were analysed. Whole-exome sequencing data and RNASeq data were obtained from various archives.

The ImmuneMirror web server was implemented using the Shiny web framework.

Various R packages were used to design the interactive web interface. The R package *emayili* was used to send automatic emails from the server to users.

To build a prediction model for neoantigen identification, we first gathered neopeptides (from 19 studies) with experimentally confirmed T cell responses as the training data. The class distribution of the dataset was extremely unbalanced due to the low proportion of T cell-positive neoantigens. Consequently, conventional classification algorithms were expected to bias towards the minority class, resulting in poor prediction performance. Therefore, we used random forest learning algorithms, which were evaluated using the area under the receiver operating characteristic curve (AUC). We evaluated synthetic minority over-sampling and balanced sampling techniques for the random forest method; the estimated AUCs for the test set were 0.8294 and 0.8679, respectively. The balanced random forest method outperformed the other method, indicating that it was the optimal model for neoantigen prediction; thus, it was implemented in our computational platform.

The accuracy of identifying human leukocyte antigen (HLA) types at 4-digit resolution was assessed using the benchmark dataset from the 1000 Genomes Project, which included 271 Asian samples with known HLA typing results. The accuracies of HLA type identification were 98.3% for class I alleles and 85.3% for class II alleles. We evaluated the accuracy of microsatellite instability subtype prediction in ImmuneMirror, compared with The Cancer Genome Atlas database for CRC patients. The sensitivity, specificity, and accuracy were 97.37%, 99.56%, and 99.25%, respectively.

Results

We developed the ImmuneMirror pipeline to evaluate tumour mutation burden, microsatellite instability status, HLA type, predicted neoantigen load, top-ranked neoantigens with T cell immunogenicity, and the expression of selected gene signatures (Fig 1). The pipeline can support Linux, Mac, and Windows operating systems. We implemented the machine learning model to prioritise neoantigens for HLA class I. Final outputs of the pipeline included germline and somatic estimated tumour mutation burdens, microsatellite instability status, HLA type, predicted neoantigen load, top-ranked putative neoantigens with T cell immunogenicity, and the expression of selected gene signatures. The ImmuneMirror pipeline can be obtained from the GitHub repository (https://github.com/weidai2/ImmuneMirror/).

We also developed the ImmuneMirror web server to identify potential neoantigens restricted by major histocompatibility complex (MHC) classes I and II molecules. Users can upload a VCF file, specify a set of alleles for both MHC classes I and II, and select peptide lengths via the interface. A URL link to download the results is automatically emailed to the user. The web server is freely available at http://immunemirror.hku.hk/App/.

We tested the pipeline on a Linux operating system (Ubuntu 20.04). One pair of samples with 13 threads required 24 hours of processing time. Moreover, we used the pipeline to process multiple pairs of samples with different types of cancers. Users can use the pipeline to process a list of samples; the actual processing time is dependent on the computation speed and resources of the user's device. For successful pipeline implementation, we recommend using a device with at least 64 GB of

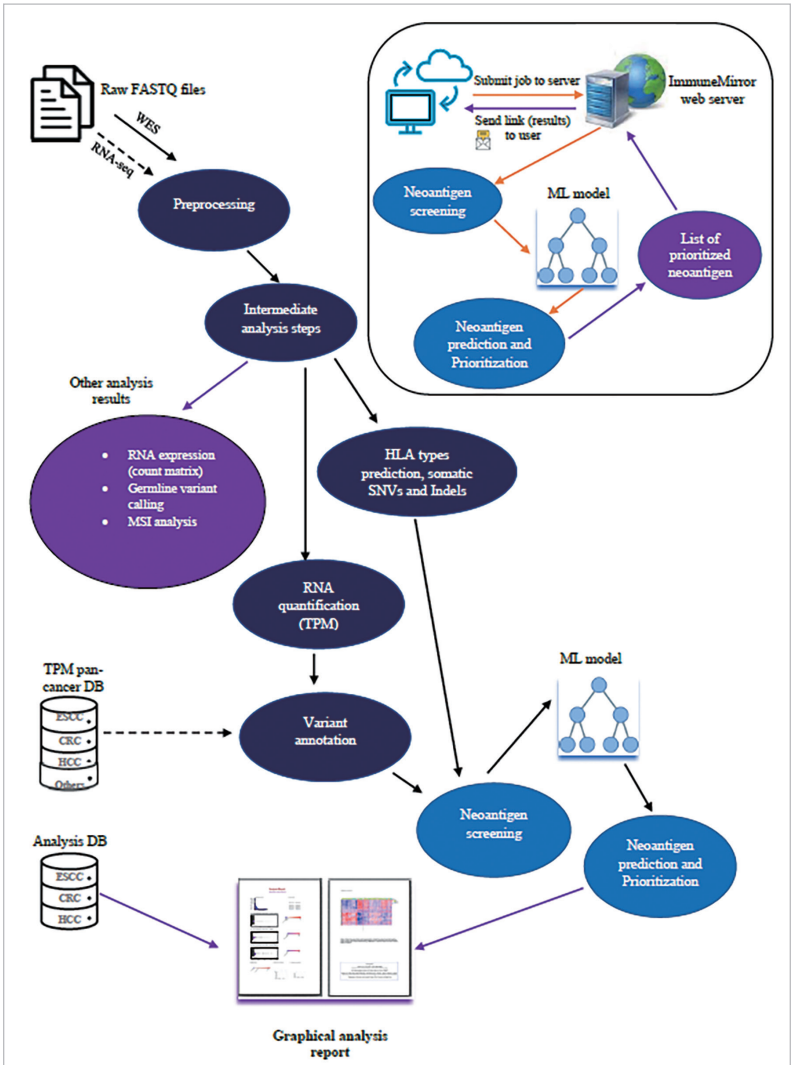

FIG 1. ImmuneMirror workflow: raw FASTQ files are processed for human leukocyte antigen (HLA) subtype prediction, single-nucleotide variant and Indel detection, variant annotation, and neoantigen prediction and prioritisation. A graphical analysis report is generated for each patient. The web server accepts VCF files as input, and a web link to the analysis results (list of prioritised neoantigens) is emailed to the user.

Abbreviations: CRC = colorectal cancer, HCC = hepatocellular carcinoma, and ESCC = oesophageal squamous cell carcinoma

TABLE. Comparison of bioinformatics tools currently available for neoantigen prediction

Index	Software	Input	Raw data type	Source	Method for prioritisation	Docker image	Web server/app	Class I prediction	Class II prediction	Multiple prediction methods
1	ImmuneMirror (current study)	FASTQ, VCF	Whole-exome sequencing, RNASeq	Mutation	✓	✓	✓	✓	✓	✓
2	NeoPredPipe	VCF	-	Mutation				✓	✓	
3	MuPeXI	VCF	-	Mutation			✓	✓		
4	TSNAD	FASTQ	RNASeq	Gene fusion		✓		✓		
5	pVAC-Seq	VCF	-	Mutation	✓	✓		✓	✓	✓
6	CloudNeo	VCF, BAM	RNASeq	Mutation			✓	✓		
7	TIminer	VCF, FASTQ	RNASeq	Mutation	✓			✓		
8	INTEGRATE-Neo	FASTQ	Whole-genome sequencing, RNASeq	Gene fusion				✓		
9	Neopepsee	FASTQ, VCF	RNASeq	Mutation	✓			✓		
10	Vaxrank	VCF, BAM	RNASeq	Mutation				✓		
11	OpenVax	FASTQ	Whole-exome sequencing, RNASeq	Mutation	✓	✓		✓	✓	✓
12	TruNeo	FASTQ	Whole-exome sequencing, RNASeq	Mutation, gene fusion	✓			✓		✓
13	ScanNeo	BAM	RNASeq	Indels				✓		✓
14	NeoFuse	FASTQ	RNASeq	Gene fusion				✓		

RAM and sufficient storage for the pipeline (80 GB), its supporting files (483 GB), and analysis results directory (45 GB for one pair of samples). Input/output file formats and detailed instructions are provided on the website.

We compared the bioinformatics tools currently available for neoantigen prediction (Table). Only ImmuneMirror has all six features (prioritisation method, docker image, web server, MHC class I prediction, MHC class II prediction, and multiple prediction algorithms). ImmuneMirror accepts inputs of raw fastq files from both RNASeq (tumour) and whole-exome sequencing (matched normal-tumour pairs) data. Similar to pVAC-Seq, ImmuneMirror can be used for neoantigen prediction restricted by MHC classes I and II, whereas pVAC-Seq accepts VCF files only. ImmuneMirror accepts VCF files that contain somatic mutations detected by MuTect2 for identification of potential neoantigens from both MHC classes I and II molecules.

We collected a total of 805 samples of GIT cancers. After quality checking, we analysed 691 samples with valid data, including 316 CRCs, 290 OSCCs, and 85 HCCs. On average, ImmuneMirror identified 17 (range, 0-316), 5 (range, 0-76), and 6 (range, 0-64) neoantigens per patient with CRC, ESCC, and HCC, respectively. Importantly, the neoantigen load was associated with favourable clinical outcomes and longer overall survival in patients with OSCC, whereas it was associated with poor clinical outcomes and shorter overall survival in patients with HCC ($P<0.05$). In patients with CRC, although the neoantigen load was not associated with overall survival, we identified a subgroup of patients with mismatch repair deficiency who had much lower neoantigen loads for both MHC classes I and II (Fig 2); these patients exhibited an advanced T stage (T4 vs others, 30.8% vs 0%, $P=0.011$).

We compared neoepitopes in 10 cancer-related genes with hotspot mutations and identified 12 putative neoepitopes derived from TP53, STAT3, and RAB35 that demonstrated high affinity with HLA-A*02:01, HLA-A*11:01, HLA-A*33:03, HLA-A*33:01, HLA-A*03:01, and HLA-A*02:06 HLA alleles. The neoepitope $TP53^{G245V}$ (YMCNSSCMGV), which was restricted by HLA-A*02, was identified in a patient with OSCC (Fig 2). This mutation affected the binding of p53 to DNA and interfered with the protein's transcriptional activity. RNASeq data indicated that this mutant was widely expressed in tumour tissue.

Conclusion

ImmuneMirror is reliable and effective for identification of genomic and transcriptomic features

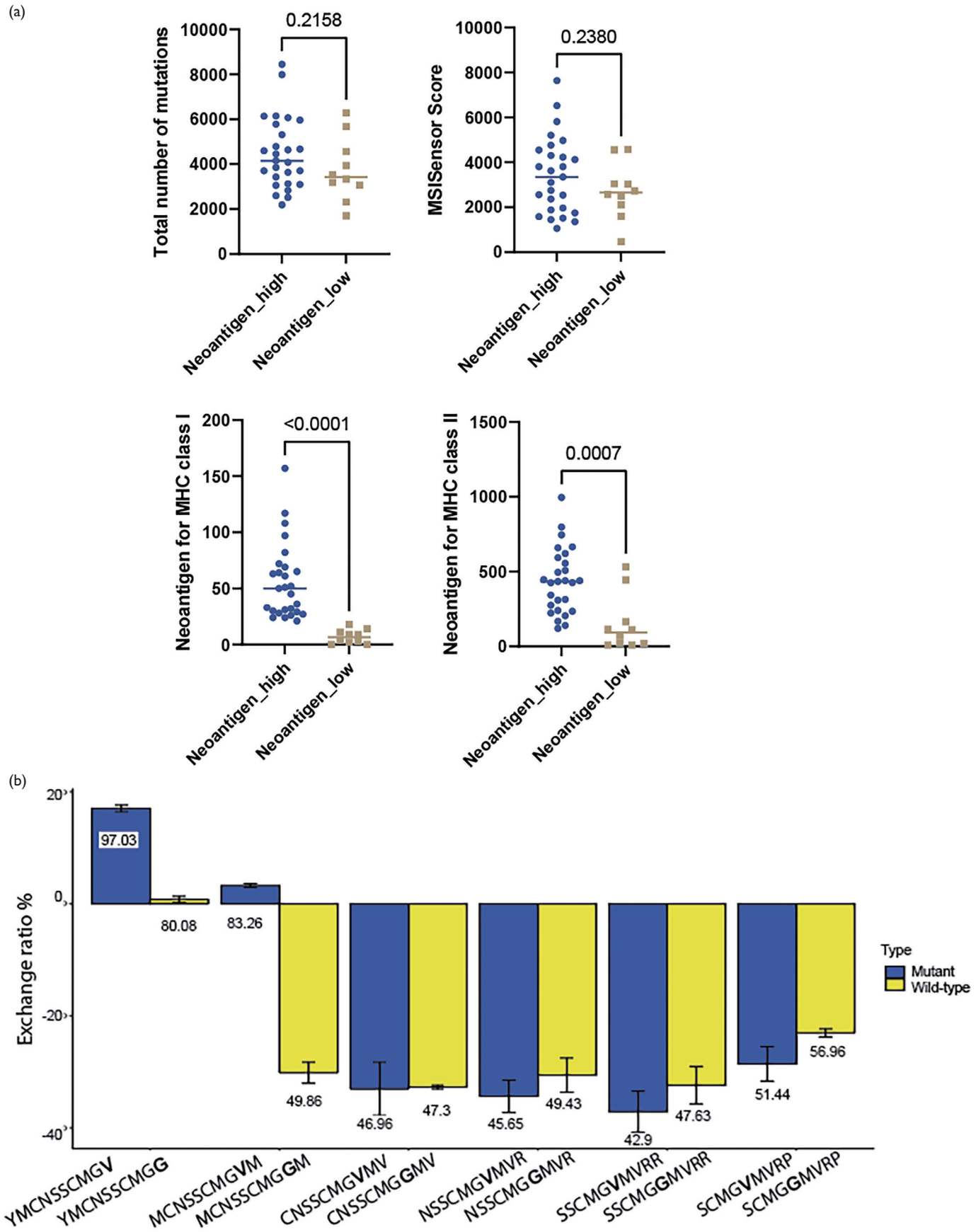

FIG 2. (a) A subgroup of patients with colorectal cancer who have mismatch repair deficiency and low neoantigen loads for major histocompatibility complex (MHC) classes I and II. (b) Validation of binding affinity between human leukocyte antigen–A02 and neoepitopes derived from TP53 mutations. Exchange ratios of $TP53^{G245V}$ mutants to wild-type peptide are shown (>80% is the cut-off for positive values).

associated with responses to immunotherapy. We developed a machine learning model to predict putative neoantigens and identify neoantigens derived from hotspot mutations that can serve as actionable targets in cancers.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#07182016). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Chuwdhury GS, Guo Y, Chiang CL, et al. ImmuneMirror: a machine learning-based integrative pipeline and web server for neoantigen prediction. Brief Bioinform 2024;25:bbae024.

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Community health worker–led multimedia intervention to increase cervical cancer screening uptake among South Asians: a randomised controlled trial (abridged secondary publication)

CL Wong *, WKW So, DNS Chan

KEY MESSAGES

1. The rate of cervical cancer screening uptake among South Asian women are significantly lower than that among the general population of Hong Kong.
2. A community health worker–led multimedia intervention is effective in improving cervical cancer screening uptake and readiness to undergo screening, while mitigating perceived barriers to cervical cancer screening, among South Asian women in Hong Kong.

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Introduction

Cervical cancer screening enables early detection and treatment of pre-malignant lesions, thereby improving survival rates and reducing medical costs. In Hong Kong, >50% of South Asian women have never been screened,[1] owing to various barriers such as health illiteracy, insufficient awareness, misunderstandings about cancer and screening, difficulty accessing to medical services, and language barriers.[1,2]

Multimedia interventions led by community health workers (CHWs) may increase the use of cervical screening services among ethnic minority women.[3-5] The CHWs receive training and help connect community members with healthcare providers.[4] The involvement of CHWs in intervention delivery enhances cultural appropriateness and healthy behaviors.[5] We developed a CHW-led multimedia intervention guided by the Health Belief Model and the PRECEDE-PROCEED framework. The effectiveness of this intervention was evaluated in a cluster randomised wait-list controlled trial. We aimed to evaluate the effects of a CHW-led multimedia intervention on cervical cancer screening uptake among South Asian women in Hong Kong, and to assess the effects of the intervention on readiness to undergo screening and the beliefs regarding cervical cancer screening among these women. The cost of implementing the intervention was also evaluated.

Methods

This study was conducted between September 2018 and January 2020. Six female CHWs (two Indians, two Nepali, and two Pakistani) were recruited from six different non-governmental organisations. They were aged 32 to 47 years, married, had completed at least secondary education, and had resided in Hong Kong for >10 years. They received 14 hours of training in seven sessions that covered information about cervical cancer and screening, barriers to screening services uptake, and strategies to overcome these barriers.

The six non-governmental organisations were randomised to either the intervention group (n=3) or the wait-list control group (n=3). Each CHW was allocated to either group. The CHW–led intervention included multimedia education, monthly telephone follow-up, and navigation assistance. South Asian women aged ≥25 years who were sexually active, had no history of cancer, and had not taken a Pap test in the past 5 years were invited to attend a multimedia education session that involved two half-hour health talks related to cervical cancer and screening. They then watched a video to reinforce the information they received; they were also given a booklet that included information provided in the health talk. In the 3 months after multimedia education, CHWs conducted monthly telephone follow-up to reinforce the women's knowledge and motivate them to undergo screening. The CHWs also provided assistance including arranging appointments for screening and accompanying women for screening.

Data were collected at three time points: baseline (T0), immediately after the intervention (T1), and 3 months after the intervention (T2). The primary outcome was cervical cancer screening uptake at T1 and T2; the secondary outcomes were readiness to undergo screening, as well as perceived susceptibility, perceived severity, perceived benefits,

TABLE 1. Baseline characteristics of participants (n=402)

Characteristics	Intervention (n=201)*	Control (n=201)*	P value
Age, y	41.6±8.5	40.1±8.6	0.066
Duration living in Hong Kong, y	17.3±10.2	15.3±8.8	0.051
Ethnicity			1.000
Pakistani	67 (33.3)	67 (33.3)	
Nepali	67 (33.3)	67 (33.3)	
Indian	67 (33.3)	67 (33.3)	
Education			0.600
Primary school or below	65 (32.3)	55 (27.4)	
Secondary school	58 (28.9)	59 (29.4)	
College	43 (21.4)	43 (21.4)	
University or above	35 (17.4)	44 (21.9)	
Employment status			0.287
Full-time	52 (25.9)	42 (20.9)	
Part-time	14 (7.0)	21 (10.4)	
Housewife	135 (67.2)	138 (68.7)	
Monthly family income, HK$			0.169
<10 000	36 (17.9)	23 (11.4)	
10 000-19 999	72 (35.8)	62 (30.8)	
20 000-29 999	30 (14.9)	41 (20.4)	
≥30 000	13 (6.5)	17 (8.5)	
Do not know	50 (24.9)	58 (28.9)	
Marital status			0.487
Single	0	1 (0.5)	
Married/cohabiting	189 (94.0)	191 (95.0)	
Separated/divorced/widowed	12 (6.0)	9 (4.5)	
No. of children	2.30±1.18	2.37±1.23	0.580
Religion			0.062
Hinduism	94 (46.8)	71 (35.3)	
Islam	61 (30.3)	71 (35.3)	
Buddhism, Sikhism, Christianity or no religion	46 (22.9)	59 (29.4)	
Family history of cervical cancer			
No	169 (84.1)	168 (83.6)	0.441
Yes	9 (4.5)	5 (2.5)	
Do not know	23 (11.4)	28 (13.9)	
Had any cervical disease before			
No	198 (98.5)	197 (98.0)	0.703
Yes	3 (1.5)	4 (2.0)	
Had any cervical cancer screening before (>5 y)			
No	130 (64.7)	122 (60.7)	0.409
Yes	71 (35.3)	79 (39.3)	
Know the clinics/centres providing cervical cancer screening			
No	140 (69.7)	139 (69.2)	0.914
Yes	61 (30.3)	62 (30.8)	
Annual physical check-up			
No	150 (74.6)	159 (79.1)	0.287
Yes	51 (25.4)	42 (20.9)	
Have any health insurance			0.059
No	167 (83.1)	180 (83.6)	
Yes	34 (16.9)	21 (16.4)	
Any doctor suggests the participant undergoing Pap test			0.324
No	168 (83.6)	175 (87.1)	
Yes	33 (16.4)	26 (12.9)	
Friends suggest the participant undergoing Pap test			0.615
No	86 (42.8)	91 (45.3)	
Yes	115 (57.2)	110 (54.7)	
Family suggests the participant undergoing Pap test			0.117
No	153 (76.1)	139 (69.2)	
Yes	48 (23.9)	62 (30.8)	
Ever received a reminder letter from doctor or healthcare organisation for cervix examination			0.162
No	187 (93.0)	179 (89.1)	
Yes	14 (7.0)	22 (10.9)	

* Data are presented as mean±standard deviation or No. (%) of participants

perceived barriers, and self-efficacy of undergoing cervical cancer screening. The cost of the intervention, including training and implementation of the intervention, was assessed at T2. The effectiveness of the CHW-led intervention was evaluated by comparing the proportions of women who reported cervical cancer screening uptake and indicated readiness to undergo screening. Changes in primary and secondary outcomes between the two groups were compared using generalised estimating equations.

Results

Of 402 South Asian women, 195 in the intervention group and 192 in the control group completed the study. The baseline characteristics of the two groups were comparable (Table 1). Compared with controls, a higher proportion of participants in the intervention group reported having undergone a Pap test in T2 (P=0.005), readiness to undergo screening at T1 (P<0.001) and T2 (P<0.001), and greater reduction in perceived barriers at T1 (P=0.047) and T2 (P=0.041) [Table 2]. The cost of CHW training was HK$13 500, and the cost of implementation was HK$164 580.6. The total cost of intervention was HK$178 080.6. The average cost of intervention per participant for each 1% increase in the Pap test uptake rate was HK$20.2 (178 080.6/195-0/192)/(97.9%-52.6%).

TABLE 2. Cervical cancer screening uptake, readiness to undergo screening, and beliefs regarding cervical cancer screening between groups

Outcomes	Control (n=192)*	Intervention (n=195)*	Odds ratio (95% confidence interval)	P value
Cervical cancer screening uptake				
T1	73 (38.0)	110 (56.4)	2.09 (0.34-12.82)	0.424
T2	101 (52.6)	191 (97.9)	42.73 (3.09-591.82)	0.005
Readiness to undergo screening				
T0	52 (27.1)	30 (15.4)	0.49 (0.18-1.35)	0.169
T1	161 (83.9)	194 (99.5)	37.36 (6.78-205.82)	<0.001
T2	161 (83.9)	194 (99.5)	37.36 (6.78-205.82)	<0.001
			Mean difference (95% confidence interval)	
Perceived susceptibility				
T0	2.20±0.97	2.12±0.94	Reference	
T1	2.14±0.95	2.01±0.91	-0.06 (-0.42 to 0.30)	0.749
T2	2.39±1.66	2.13±0.93	-0.19 (-1.80 to 1.42)	0.818
Perceived severity				
T0	3.10±0.84	3.16±0.90	Reference	
T1	3.45±0.84	3.27±0.94	-0.24 (-1.41 to 0.93)	0.684
T2	3.34±1.04	2.67±0.92	-0.74 (-2.17 to 0.70)	0.315
Perceived benefits				
T0	3.75±0.62	3.73±0.65	Reference	
T1	3.95±0.57	4.03±0.51	0.10 (-0.28 to 0.48)	0.605
T2	4.01±0.68	4.20±0.38	0.21 (-0.25 to 0.67)	0.367
Perceived barriers				
T0	2.58±0.59	2.90±0.69	Reference	
T1	2.50±0.61	2.14±0.46	-0.68 (-1.35 to -0.01)	0.047
T2	2.66±0.87	2.11±0.46	-0.86 (-1.69 to -0.04)	0.041
Self-efficacy				
T0	3.36±0.91	2.68±1.15	Reference	
T1	3.62±0.89	3.94±0.87	0.99 (-0.45 to 2.43)	0.177
T2	3.64±0.97	4.15±0.89	1.19 (-0.58 to 2.96)	0.188

* Data are presented as mean±standard deviation or No. (%) of participants

Discussion

The CHW-led multimedia intervention was able to increase cervical cancer screening uptake and readiness to undergo screening, while reducing perceived barriers to cervical cancer screening, among South Asian women in Hong Kong. The rate of cervical cancer screening uptake increased from 56.4% upon completion of the intervention to 97% at 3 months after the intervention.

Despite the significant effects of the intervention on reducing perceived barriers to screening, no significant effects were observed concerning other screening-related beliefs. This finding implies that efforts to reduce perceived barriers to screening are more effective for promoting screening uptake, compared with efforts to enhance perceived susceptibility, severity, benefits, and self-efficacy of undergoing cervical cancer screening. Perceived barriers constituted the only factor that contributed to the low rate of cervical cancer screening uptake among South Asians in Hong Kong.

There were some limitations in this study, including potential selection bias related to the small number of available clusters, the absence of comparison groups for individual components to determine their effects, the small sample size in each cluster, the short follow-up period, the inability to include all South Asian women who do not undergo a Pap test every 3 years, and the limited generalisability to the South Asian populations in other countries. Future studies should assess the long-term effects of the intervention on outcomes and explore the application of CHW-led interventions for other ethnic groups.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#01170958). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

Disclosure

The results of this research have been previously published in:

1. Wong CL, Choi KC, Chen J, Law BMH, Chan DNS, So WKW. A community health worker-led multicomponent program to promote cervical cancer screening in South Asian women: a cluster RCT. Am J Prev Med 2021;61:136-45.
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Family-based multimedia intervention to increase colorectal cancer screening uptake among South Asians in Hong Kong: a randomised control trial (abridged secondary publication)

WKW So *, CWH Chan, KC Choi, DNS Chan, M Krishnasamy

KEY MESSAGES

1. A family-based multimedia intervention was effective in increasing colorectal cancer (CRC) screening uptake among South Asians aged 56 to 75 years. A greater proportion of participants in the intervention group attended a medical consultation with a family doctor for faecal immunohistochemical testing and submitted a stool sample, compared with the control group (P<0.001).
2. Of the 29 community centres and non-governmental organisations we approached, 25 (86.2%) promoted the intervention to South Asians in Hong Kong. Participants were highly satisfied with the intervention.
3. The family-based multimedia intervention should be incorporated into routine health promotion activities for South Asians in Hong Kong.

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Introduction

Colorectal cancer (CRC) screening, such as faecal immunohistochemical testing (FIT) and colonoscopy, is effective in the early detection and prevention of CRC. Despite the availability of CRC screening in Hong Kong, its uptake remains low, especially among South Asians.[1] Common barriers to CRC screening utilisation among South Asians include language barriers and limited awareness of CRC screening,[2] especially among older adults. Interventions to enhance their knowledge about and access to CRC screening are needed. Multimedia interventions are acceptable to South Asians and effective in the dissemination of health knowledge.[3] We hypothesised that participation by younger South Asians would increase their awareness of CRC screening and empower them to encourage their older family members to undergo CRC screening. This study aimed to evaluate the effectiveness of a family-based, culturally sensitive, and linguistically appropriate multimedia intervention regarding CRC screening among South Asians aged 56 to 75 years, based on the reach-effectiveness-adoption-implementation-maintenance framework.

Methods

This was a cluster-randomised controlled trial with waitlist control groups. Based on the Health Belief Model, a family-based multimedia intervention was developed. An advisory panel consisting of five members from South Asian communities in Hong Kong provided recommendations on strategies to enhance the cultural relevance of the intervention, which included a PowerPoint presentation, health information booklets, and a video clip. The PowerPoint presentation covered topics such as CRC risk factors and symptoms, myths and misconceptions about CRC, prevention and early detection of CRC, FIT procedures, the current CRC screening programme in Hong Kong, and the implications of a positive FIT result. The health information booklets were intended to reinforce the knowledge gained. The video clip emphasised the importance of family support in modifying CRC screening behaviours. Cultural relevance was enhanced by using South Asian actors and actresses.

Participants were recruited from six districts as dyads of older family members aged 56 to 75 years and their younger family members aged 18 to 55 years. The participants were from India, Pakistan or Nepal who had no personal history of CRC, had not undergone CRC screening in the previous year, and had never participated in a cancer screening intervention. Cluster randomisation was conducted at the district level. Only the outcome assessors were blinded to group allocation.

The intervention comprised a 90-minute health

talk using the PowerPoint presentation, during which the video clip was presented and the stool collection procedures for FIT were demonstrated. The health information booklets were distributed after the talk. Each dyad was scheduled to attend a medical appointment with a family doctor for an FIT consultation within 1 month. A site coordinator of South Asian origin accompanied the dyads to these appointments. Control dyads received the intervention within 2 months of completing the outcome assessments.

The primary outcome was the effectiveness of the intervention in promoting CRC screening uptake among the older family members, measured as the percentage of older family members who visited a family doctor for an FIT consultation and returned their stool sample for FIT. We also assessed the effectiveness of the intervention in promoting the willingness of younger family members to encourage their older family members to undergo FIT, as well as their readiness to assist older family members with FIT. Satisfaction among the intervention dyads was assessed. Data were collected upon recruitment, immediately after intervention, at the medical visit within 1 month of intervention, and upon return of the stool sample 2 months later.

Results

In total, 320 dyads were recruited from six districts and then randomly assigned according to district level in a 1:1 ratio to either the intervention or control group. Of these, 117 (73.1%) intervention dyads and 146 (91.3%) control dyads completed the study. Table 1 shows the baseline characteristics of the participants.

A greater proportion of older family members in the intervention group visited a family doctor for FIT immediately after the intervention, compared with the control group (71.8% vs 6.8%, P<0.001, Table 2). Among the older family members who returned their stool sample for FIT 2 months later, all in the control group returned the sample with aid from their younger family members. In contrast, 62.2% of older family members in the intervention group returned the sample by themselves.

Immediately after the intervention, the proportions of young family members who expressed willingness to encourage and readiness to assist their older family members to undergo FIT decreased by 16.6% and 22.5%, respectively, among participants in the control group, whereas the proportions remained unchanged among participants in the intervention group. This difference suggests that the intervention was able to maintain the younger family members' willingness to encourage and readiness to assist their older family members to undergo FIT.

Of the 29 partner organisations, 25 (86.2%) engaged in our intervention by promoting it to community peers, whereas 20 (69.0%) supported implementation of the intervention by providing a venue. Of 42 family doctors, 27 (64.3%) agreed to conduct FIT for the older family members. Approximately 72% of participants in the intervention dyads attended the medical appointment with a family doctor after the intervention. Eighteen partner organisations were willing to continue implementing the intervention at their centres, and 97% of participants in the intervention group were satisfied with the intervention.

Discussion

The family-based intervention was effective in enhancing FIT uptake among South Asians aged 56 to 75 years: the proportion of older family members who returned their stool sample for FIT was 10-fold higher in the intervention group than in the control group. The higher FIT uptake may be attributed to increased knowledge about FIT and its importance, which increased participants' willingness to undergo FIT. Notably, compared with the control group, a greater proportion of the older family members in the intervention group returned the stool sample for FIT by themselves, rather than relying on their younger family members. This difference suggests that the intervention enhanced self-efficacy among older family members in the intervention group. Moreover, the efficacy of the intervention was demonstrated by the greater proportions of younger family members who were willing to encourage their older family members to undergo FIT and were ready to assist with stool sample collection.

Conclusion

A family-based multimedia intervention was effective in enhancing CRC screening uptake among South Asians aged 56 to 75 years in Hong Kong. The intervention was well received by participants; it should be incorporated into health promotion programmes offered at non-governmental organisations that serve South Asians. The government should allocate more training resources to enable these staff members to act as programme instructors for implementation of the intervention.

Funding

This study was supported by the Health and Medical Research Fund, Health Bureau, Hong Kong SAR Government (#01170848). The full report is available from the Health and Medical Research Fund website (https://rfs2.healthbureau.gov.hk).

TABLE 1. Baseline characteristics of participants

Characteristic	Older family members (n=320)		P value	Younger family members (n=256)		P value
	Control (n=160)*	Intervention (n=160)*		Control (n=129)*	Intervention (n=127)*	
Age, y	65.1±6.1	65.0±6.2	0.849	36.4±8.1	35.4±8.7	0.355
Sex						
Male	64 (40.0)	61 (38.1)	0.731	62 (48.1)	36 (28.3)	0.001
Female	96 (60.0)	99 (61.9)		67 (51.9)	91 (71.7)	
Country of origin			0.108			0.131
Pakistan	21 (13.1)	35 (21.9)		17 (13.2)	28 (22.0)	
India	31 (19.4)	25 (15.6)		25 (19.4)	18 (14.2)	
Nepal	108 (67.5)	100 (62.5)		87 (67.4)	81 (63.8)	
No. of household members			0.274			0.175
1-2	22 (13.8)	12 (7.5)		16 (12.4)	7 (5.5)	
3-4	48 (30.0)	58 (36.3)		45 (34.9)	49 (38.6)	
5-6	63 (39.4)	63 (39.4)		46 (35.7)	54 (42.5)	
≥7	27 (16.9)	27 (16.9)		22 (17.1)	17 (13.4)	
Monthly household income, HK$			0.003			0.020
<10 000	50 (31.3)	36 (22.5)		25 (19.4)	20 (15.7)	
10 000-19 999	35 (21.9)	22 (13.8)		26 (20.2)	23 (18.1)	
20 000-29 999	21 (13.1)	17 (10.6)		35 (27.1)	26 (20.5)	
≥30 000	10 (6.3)	7 (4.4)		15 (11.6)	7 (5.5)	
Don't know	44 (27.5)	78 (48.8)		28 (21.7)	51 (40.2)	
Education level			0.891			0.298
Primary or below	96 (60.0)	93 (58.1)		7 (5.4)	10 (7.9)	
Did not complete secondary	26 (16.3)	28 (17.5)		13 (10.1)	20 (15.7)	
Completed secondary	15 (9.4)	19 (11.9)		46 (35.7)	51 (40.2)	
Matriculation	10 (6.3)	7 (4.4)		31 (24.0)	22 (17.3)	
Tertiary (non-degree) or degree or above	13 (8.1)	13 (8.1)		32 (24.8)	24 (18.9)	
Marital status			0.070			0.097
Single/divorced/widowed	12 (7.5)	22 (13.8)		19 (14.7)	29 (22.8)	
Married	148 (92.5)	138 (86.3)		110 (85.3)	98 (77.2)	
Have a part-time/full-time job			0.150			0.024
No	103 (64.4)	115 (71.9)		46 (35.7)	63 (49.6)	
Yes	57 (35.6)	45 (28.1)		83 (64.3)	64 (50.4)	
Hong Kong permanent resident			0.356			0.748
No	12 (7.5)	8 (5.0)		4 (3.1)	5 (3.9)	
Yes	148 (92.5)	152 (95.0)		125 (96.9)	122 (96.1)	
Family history of cancer			0.110			0.290
No	136 (85.0)	141 (88.1)		110 (85.3)	116 (91.3)	
Yes	16 (10.0)	7 (4.4)		12 (9.3)	6 (4.7)	
Unsure	8 (5.0)	12 (7.5)		7 (5.4)	5 (3.9)	
Have health insurance			0.999			0.999
No	155 (96.9)	155 (96.9)		124 (96.1)	123 (96.9)	
Yes	5 (3.1)	5 (3.1)		5 (3.9)	4 (3.1)	
Acculturation score	1.46±0.75	1.48±0.73	0.811	2.39±0.76	2.28±0.92	0.294
Acculturated (score ≥3)			0.479			0.919
No	144 (90.0)	140 (87.5)		78 (60.5)	76 (59.8)	
Yes	16 (10.0)	20 (12.5)		51 (39.5)	51 (40.2)	
Preference for doctor's spoken language			0.015			0.406
No preference	0	4 (2.5)		1 (0.8)	3 (2.4)	
English only	7 (4.4)	6 (3.8)		7 (5.4)	11 (8.7)	
Mother tongue or familiar language but not English	128 (80.0)	139 (86.8)		89 (69.0)	89 (70.1)	
English or other familiar languages	25 (15.6)	11 (6.9)		32 (24.8)	24 (18.9)	

* Data are presented as mean±standard deviation or No. (%) of participants

TABLE 2. Outcome measures for older and younger family members

Outcome	Control*	Intervention *	P value
Older family members (n=263)	n=146	n=117	
Visited a family doctor for faecal immunohistochemical testing (FIT) within 1 month of intervention	10 (6.8)	84 (71.8)	<0.001
Returned a stool sample 2 months later	n=10	n=82	
Participant himself/herself	0	51 (62.2)	<0.001
Family member	10 (100)	19 (23.2)	
Site coordinator	0	12 (14.6)	
Result of FIT			0.999
Negative	10 (100)	79 (96.3)	
Positive	0	3 (3.7)	
Younger family members			
At baseline (n=256)	n=129	n=127	
Willing to encourage their older family members to undergo FIT	125 (96.9)	120 (94.5)	0.342
Ready to assist older family members with stool sample collection	126 (97.7)	122 (96.1)	0.498
At post-intervention (n=208)	n=117	n=91	
Willing to encourage their older family members to undergo FIT	94 (80.3)	89 (97.8)	<0.001
Ready to assist older family members with stool sample collection	88 (75.2)	87 (95.6)	<0.001

* Data are presented as No. (%) of participants

Disclosure

The results of this research have been previously published in:

1. So WKW, Chan DNS, Law BMH, Choi KC, Krishnasamy M, Chan CWH. A family-based multimedia intervention: a potential strategy to promoting colorectal cancer screening utilisation among South Asian ethnic minorities. Accessed 17 October 2022. Available from: https://www.isncc.org/Blog/13011110.
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AUTHOR INDEX

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