Understanding the molecular determinant of rhinoviruses in functional group by the virus-genotype to disease-phenotypes association

PUN, CS[1]; WANG, HC[1]; TAO, KP[1,2]; TSUN, GS[1]; YU, WY[1]; CHEN, XY[1]; WANG, MH[4]; CHAN, PKS[2,3]; LEUNG, TF[1,2]; CHAN, RWY[1,2]

[1]Department of Paediatrics, Faculty of Medicine, The Chinese University of Hong Kong, HKSAR
[2]CUHK-UMCU Joint Research Laboratory of Respiratory Virus & Immunobiology, Department of Paediatrics, Faculty of Medicine, The Chinese University of Hong Kong, HKSAR
[3]Department of Microbiology, Faculty of Medicine, The Chinese University of Hong Kong, HKSAR
[4]Division of Biostatistics, The Jockey Club School of Public Health and Primary Care, Faculty of Medicine, The Chinese University of Hong Kong, HKSAR

Introduction

Rhinovirus (RV) infection happens all the time, which is one of the causes of common cold. A more severe disease spectrum has been found to be associated with infants and children, since the discovery of the new RV species C in 2010. RV was also found to be strongly associated with the development of asthma with early life exposure.

There are 168 genotypes of RV, which are classified into three species: RV-A, -B and -C. We hypothesise that the genotypes may induce different biological pathways, resulting in various disease phenotypes, including acute bronchiolitis, asthma exacerbation, pneumonia, and upper respiratory tract infection. In this study, we would like to identify the "functional group" of the RV genotypes by evaluating its virus-genotype to a disease-phenotype association.

Objective

To identify the functional group of RV and understand their pathogenesis for better disease management.

Hypothesis

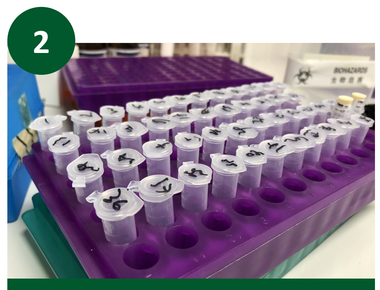
The certain groups of genotypes in RV-A, RV-B and RV-C triggers a similar biological mechanism which can classify into different functional groupings, in which causes different diseases in humans. For instance, highly inflammatory and tissue remodeling.

Methodology

1,209 frozen archive of the nasopharyngeal aspirate (NPA) specimens, collected from hospitalised children aged 1-17, tested positive with enterovirus in two regional tertiary hospitals, from January 2015 to December 2016, were examined. The VP4/VP2 viral protein region were Sanger sequenced and identified the genotype by matching against the RV-prototype database by BLAST+. The symptoms of diagnosis in the discharge notes of the patients were reviewed, and the cases were categorised into acute bronchiolitis, asthma exacerbations, pneumonia, upper respiratory tract infection (URTI) and febrile convulsions. The association of the virus-genotype to disease-phenotype were evaluated by the Fisher's Exact tests.

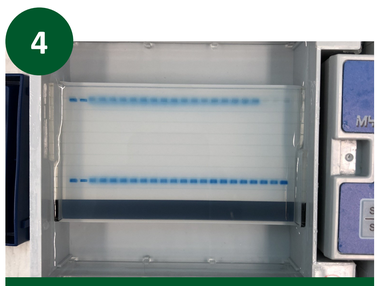
1

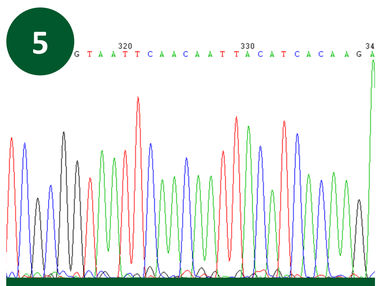
Collection of NPA

2

RNA extraction

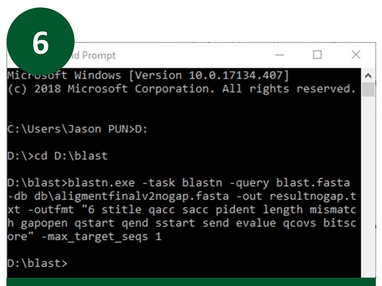
3

Reverse transcription & PCR

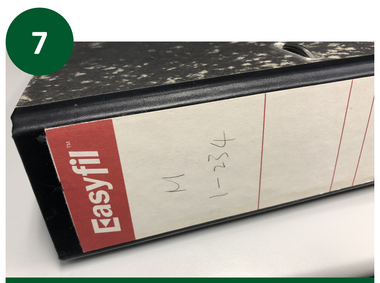
4

Gel electrophoresis

5


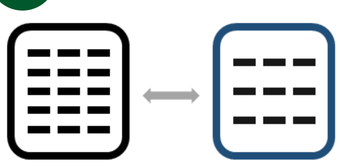
Sanger sequencing

6


Genotype identification

7


Case review

8

Data matching

9

Analysis

香港中文大學
The Chinese University of Hong Kong

香港中文大學醫學院
Faculty of Medicine
The Chinese University of Hong Kong

DEPARTMENT OF PAEDIATRICS
Faculty of Medicine
The Chinese University of Hong Kong

Supervisor: Prof. Renee W. Y. Chan
Student Name: PUN Chun Sang
Programme: PhD in Medical Sciences
Date of publish: 1st June 2019

BLAST+ and Rhinovirus Database

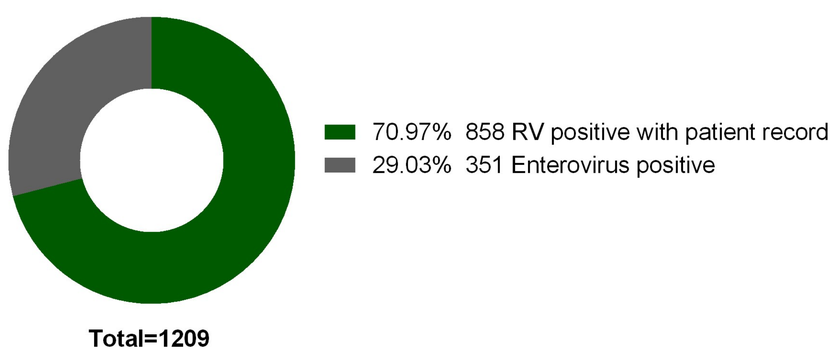
The sequence were searched against the BLAST+ proprietary, customised Rhinovirus database constructed in-house. Only the sequences that met the strict criteria below would be accepted to obtain a high quality identification.

Similarity	e-value	Alignment Length
RV-A >= 89.5% RV-B >= 90.5% RV-C >= 89.5%	<= 0.001	>= 355 bp (>= 90% coverage of the prototype)

1. The criteria in similarity and alignment length are gold standards in the field of rhinovirus research (McIntyre et al., 2013)
2. The e-value is number of hits that can be found by chance when searching a database of a particular size. The closer it is to zero, the more significant the match is.

Result

As of 1st June, 2019, 1,209 cases were investigated. 70.97% of the cases were successfully genotyped, while 29.03% are identified to be Enterovirus positive.



The virus-genotype to disease-phenotype association was conducted using Fisher's Exact test by SciPy in Python. The tables below demonstrated summary of the associations.

Specific RV genotype detected vs other RV genotypes detected

Patients that had	Interested symptom diagnosed	Interested symptom not diagnosed
Specific RV genotype infected	a	b
Other specific RV genotype infected	c	d

Genotype	a	b	c	d	p-value	Odds ratio	Symptom diagnosed
C35	16	8	156	678	6.04E-07	8.692	Asthma exacerbation
C51	7	10	85	756	0.001	6.226	Pneumonia
A10	4	2	88	764	0.0016	17.364	Pneumonia
A45	3	6	35	814	0.0056	11.629	Febrile convulsions
C12	9	10	163	676	0.0062	3.733	Asthma exacerbation
C15	11	14	161	672	0.0084	3.28	Asthma exacerbation
C40	3	1	112	742	0.0085	19.875	Bronchiolitis
C37	5	3	167	683	0.0102	6.816	Asthma exacerbation
C42	4	5	88	761	0.0102	6.918	Pneumonia
A55	2	2	36	818	0.0108	22.722	Febrile convulsions
B27	4	2	168	684	0.0167	8.143	Asthma exacerbation
A19	8	8	193	649	0.0176	3.363	URTI
C2	7	15	108	728	0.0196	3.146	Bronchiolitis
A16	0	20	172	666	0.0201	0	Asthma exacerbation
C38	5	3	196	654	0.0202	5.561	URTI
A49	6	12	109	731	0.0242	3.353	Bronchiolitis
C45	0	16	201	641	0.0309	0	URTI

Future work

The RV genotypes in the functional groups with a strong association to the diseases-phenotype will be further analysed by complete viral genome sequencing. Unique molecular determinants that are related to the clinical outcomes might be identified by aligning individual virus strains. Further research on the biological implication of these determinants would be useful for drug development in curing diseases with high severity.