ISSN 2186-3644 Online ISSN 2186-361X

IRDR

Intractable & Rare Diseases Research

Volume 11, Number 3
August, 2022

www.irdrjournal.com

IRDR
Intractable & Rare Diseases Research

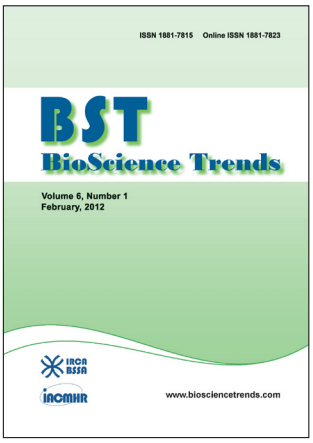
ISSN: 2186-3644
Online ISSN: 2186-361X
CODEN: IRDRA3
Issues/Year: 4
Language: English
Publisher: IACMHR Co., Ltd.

Intractable & Rare Diseases Research is one of a series of peer-reviewed journals of the International Research and Cooperation Association for Bio & Socio-Sciences Advancement (IRCA-BSSA) Group and is published quarterly by the International Advancement Center for Medicine & Health Research Co., Ltd. (IACMHR Co., Ltd.) and supported by the IRCA-BSSA.

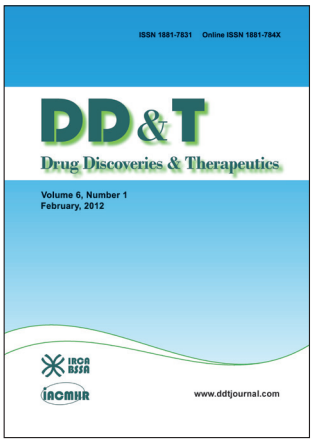
Intractable & Rare Diseases Research devotes to publishing the latest and most significant research in intractable and rare diseases. Articles cover all aspects of intractable and rare diseases research such as molecular biology, genetics, clinical diagnosis, prevention and treatment, epidemiology, health economics, health management, medical care system, and social science in order to encourage cooperation and exchange among scientists and clinical researchers.

Intractable & Rare Diseases Research publishes Original Articles, Brief Reports, Reviews, Policy Forum articles, Case Reports, Communications, Editorials, News, and Letters on all aspects of the field of intractable and rare diseases research. All contributions should seek to promote international collaboration.

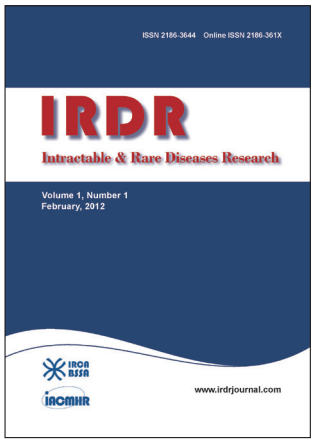
IRCA-BSSA Group Journals



ISSN: 1881-7815
Online ISSN: 1881-7823
CODEN: BTIRCZ
Issues/Year: 6
Language: English
Publisher: IACMHR Co., Ltd.
www.biosciencetrends.com



ISSN: 1881-7831
Online ISSN: 1881-784X
CODEN: DDTRBX
Issues/Year: 6
Language: English
Publisher: IACMHR Co., Ltd.
www.ddtjournal.com



ISSN: 2186-3644
Online ISSN: 2186-361X
CODEN: IRDRA3
Issues/Year: 4
Language: English
Publisher: IACMHR Co., Ltd.
www.irdrjournal.com

Intractable & Rare Diseases Research

Editorial and Head Office

Pearl City Koishikawa 603, 2-4-5 Kasuga, Bunkyo-ku,
Tokyo 112-0003, Japan

E-mail: office@irdrjournal.com
URL: www.irdrjournal.com

Editorial Board

CONTENTS

Volume 11, Number 3, 2022

Review

Original Article

Brief Report

Communication

Communication

Review

DOI: 10.5582/irdr.2022.01093

Incidence and prevalence of 121 rare diseases in China: Current status and challenges: 2022 revision

Yanqin Lu[1,2,*], Qingxia Gao[3], Xiuzhi Ren[4], Junfeng Li[2], Dan Yang[2], Zijian Zhang[2], Jinxiang Han[1,2]

[1] Department of Endocrinology and Metabology, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Ji'nan, Shandong, China;
[2] Key Laboratory for Biotech Drugs of the National Health Commission, Key Laboratory for Rare & Uncommon Diseases of Shandong Province, Biomedical Sciences College & Shandong Medicinal Biotechnology Centre, Shandong First Medical University & Shandong Academy of Medical Sciences, Ji'nan, Shandong, China;
[3] Neck-Shoulder and Lumbocrural Pain Hospital, Shandong First Medical University, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong, China;
[4] Orthopedic Surgery, The People's Hospital of Wuqing District, Tianjin, China.

SUMMARY The current study updated data on the incidence and prevalence of 121 rare diseases listed in *China's First List of Rare Diseases* to provide rationales and references for the development and promotion of rare-disease-related policies. The National Health Commission of the People's Republic of China issued the *Rare Disease Diagnosis and Treatment Guide* (2019) (denoted here as *China's Rare Disease Diagnosis and Treatment Guide*). Then 121 diseases were registered with the national rare disease diagnosis and treatment network. The incidence/prevalence of 121 rare diseases varied from country to country. Data are available for a total of 76 rare diseases (76 of 121 rare diseases, 62.81%) in China, including data on the incidence of 23 rare diseases (19.01%) and data on the prevalence of 66 (54.55%). There are data on the incidence/prevalence of 112 rare diseases (112 of 121 rare diseases, 92.56%) at the global level, including data on the incidence of 86 rare diseases (71.07%) and data on the prevalence of 91 (75.21%). On average, the incidence of progressive muscular dystrophies, hyperphenylalaninemia, citrullinemia, and methylmalonic acidemia is over 1/10,000 in China. The prevalence of coronary artery ectasia, congenital scoliosis, retinitis pigmentosa, severe congenital neutropenia, congenital hyperinsulinemic hypoglycemia, and osteogenesis imperfecta is over 1/10,000 in China. All of these figures are beyond the cut-off of 1/10,000 according to the 2021 definition of rare diseases in China. As registration and investigation of rare diseases continues, the spectrum of rare diseases in some provinces is expanding. Diseases such as idiopathic pulmonary arterial hypertension, hepatolenticular degeneration, hemophilia, amyotrophic lateral sclerosis, idiopathic pulmonary fibrosis, and multiple sclerosis are relatively prevalent in some regions and cities of China. Registration efforts promote the correction of incidence/prevalence data, development of orphan drugs, coverage by medical insurance, and development of clinical and diagnostic pathways.

Keywords rare disease, incidence, prevalence, definition, *China's Rare Disease Diagnosis and Treatment Guide*

1. Introduction

Rare diseases are defined by the World Health Organization (WHO) as diseases with a prevalence between 0.65-1‰ (*1*). Different definitions of rare diseases have been adopted in some countries. The European Union defines a rare disease as a life-threatening or chronically debilitating disease with a prevalence of 5/10,000; in the US, a rare disease is defined as a disease with a prevalence of less than 7.5 per 10,000 and affecting fewer than 200,000 patients (*2*). In 2021, a rare disease was defined as a disease with an incidence of less than 1/10,000 in newborns, a prevalence of less than 1/10,000, or a condition that affects fewer than 200,000 people in China (*3*). Currently, there are roughly 7,000 different rare diseases affecting 3.5–5.9% of individuals worldwide, which amounts to 263–446 million individuals (*4*).

Rare diseases are hard to assess due to the large number and diversity of conditions; this is especially true

in China, which has a large population. Epidemiological data on rare diseases are limited, and the incidence or prevalence of most major rare diseases is still unclear worldwide. Over the last few years, the Chinese Government has announced a series of policies to support diagnosis and treatment of rare diseases, including fast-tracking orphan drugs, coverage by medical insurance, and disease registration. In 2018, five bodies including the National Health Commission of the People's Republic of China and the National Medical Products Administration issued *China's First List of Rare Diseases* in May 2018 (denoted here as *China's Rare Disease List*) (*5,6*).

The release of *China's Rare Disease List* has greatly promoted the diagnosis and treatment of rare diseases. At present, more than 40 types of drugs for rare diseases have been included in the national medical insurance drug catalogue. Some policies and laws have been drafted to facilitate scientific research, diagnosis and treatment, drug access, and medical care for rare diseases.

The current study updated the incidence and prevalence of 121 rare diseases with recent registration numbers for these rare diseases based on the 2019 version (*3*). The OMIM database was searched for genetic information on 121 rare diseases in *China's Rare Disease List*. Incidence and prevalence data and case numbers in *China's Rare Disease List* were collected from the *China's Rare Disease Diagnosis and Treatment Guide*, the *Research Report on the definition of rare diseases in China,* Taiwan's National Health Insurance Research Database, Orphanet (www.orpha.net), MalaCards (https://www.malacards.org/) and literature in CNKI, Wanfang, and PubMed. Here, diseases in the list were mapped to a standard ICD 10 (International Classification of Diseases), disease classification and code (National Clinical Version 1.1), and ORPHA code.

2. Current incidence/prevalence of 121 rare diseases in China

One hundred and twenty-one rare diseases in 10 different categories and their genetic information are listed in Supplementary Table S1 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=114*). Most of these rare diseases are genetic in origin. The use of high-throughout sequencing technology in rare diseases has accelerated the discovery of the genes causing these diseases. Genotypic and phenotypic variation contributes to the complexity of rare diseases. Clinical symptoms of rare diseases overlap those of common diseases, leading to the misdiagnosis of rare diseases. The codes for 121 rare diseases are listed in Supplementary Table S2 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=115*).

Based on a comprehensive analysis of the literature, official websites, and *China's Rare Disease Diagnosis and Treatment Guide,* data on the incidence/prevalence of 76 rare diseases (62.81%) were available for 121 rare diseases in China. Data on the incidence of 23 rare diseases (19.01%) was reported, along with data on the prevalence of 66 (54.55%). The incidence/prevalence of 21 rare diseases (17.36%) was cited from *China's Rare Disease Diagnosis and Treatment Guide.* Data on 73 diseases (60.33%) were retrieved from articles, literature databases, and official websites. Global data on the incidence/prevalence of 112 rare diseases (112 of 121 rare diseases, 92.56%) were available, including data on the incidence of 86 rare diseases (71.07%) and data on the prevalence of 91 (75.21%). The incidence/prevalence of 64 of 121 rare diseases (52.89%) has been reported in Europe, including the incidence of 30 rare diseases (24.79%) and the prevalence of 46 (38.02%). The incidence/prevalence of 37 of 121 rare diseases (30.58%) has been reported in the US, including the incidence of 18 rare diseases (14.88%) and the prevalence of 25 (20.66%). Details are shown in Table 1 and Supplementary Table S3 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=116*) (*3,7-24*).

The cut-off of 1/10,000 in newborns was adopted as a unique criterion for the 2021 definition of rare diseases in China. In the past, the newborn incidence of 8 different rare diseases was available in China and 31 rare diseases (0.26%) were cited in *China's Rare Disease Diagnosis and Treatment Guide* (Table 1).

Incidence/prevalence data in China is mainly from Taiwan and Shanghai. Data on the incidence of 10 (8.26%) of 121 rare diseases and the prevalence of 53 (43.80%) were available from Taiwan, compared to data on the incidence of 19 rare diseases (15.70%) and the prevalence of 36 (29.75%) from the Chinese mainland. Regional trends are evident in some rare diseases, *e.g.*, the incidence of citrullinemia is 1.87/100,000 in South China and 0.03/100,000 in North China. A high incidence of 1/100,000 was reported for methylmalonic acidemia (MMA) in North China and 1.27/100,000 in Yacheng, Jiangsu Province. The prevalence of Larson syndrome is 4.86/100,000 in Shanghai and 0.021/100,000 in Taiwan (Table 1).

3. Rare diseases with a high incidence/prevalence in the first list of 121 rare diseases

In the first list of 121 rare diseases, some diseases have a relatively high incidence or prevalence in China. Hyperphenylalaninemia, phenylketonuria, albinism, 21-hydroxylase deficiency, progressive muscular dystrophies, citrullinemia, and methylmalonic acidemia are relatively prevalent. The incidence of progressive muscular dystrophies is 2.53/10,000 (Table 1), which is close to the worldwide incidence of 2.19. Duchenne muscular dystrophy (DMD) is reported to have an incidence of 2/10,000 and Becker muscular dystrophy (BMD) is reported to have an incidence

Table 1. Newborn incidence, incidence, and prevalence of 121 rare diseases in *China's First List of Rare Disease*

No.	Disease	Newborn incidence /100,000 persons	Incidence /100,000 persons	Prevalence /100,000 persons
1	21-Hydroxylase deficiency	5-10 (*10*)	3.08 (Dongguan, Guangzhou) (*18*) 3.03 (Liuzhou, Guangxi) (*19*)	/
2	Albinism	/	5.56[b]	/
3	Alport syndrome	/	/	/
4	Amyotrophic lateral sclerosis	/	0.6 (Hong Kong)[b] 0.51 (Taiwan) (*42*)	3.1 (Hong Kong)[b] 1-9 (Taiwan)[a] 3.33 (Taiwan) (*9*)
5	Angelman syndrome	/	/	0.29 (Taiwan) (*9*)
6	Arginase deficiency	0.1-0.33 (*10*)	/	0.43 (Zhejiang) (*43*)
7	Asphyxiating thoracic dystrophy (Jeune syndrome)	0.77-1 (*10*)	/	/
8	Atypical hemolytic uremic syndrome	/	/	0.03 (Shanghai) (*40*) 0.068 (Taiwan) (*9*)
9	Autoimmune encephalitis	/	/	/
10	Autoimmune hypophysitis	/	/	/
11	Autoimmune insulin receptopathy (Type B insulin resistance)	/	/	/
12	β-ketothiolase deficiency	/	0.10 (*10*)	/
13	Biotinidase deficiency	/	/	0.025 (Taiwan) (*9*)
14	Cardiac ion channelopathies	2.5 (*10*)	/	/
15	Carnitine deficiency	/	1.27 (Yancheng, Jiangsu) (*44*)	2.4 (Shanghai)[b] 3.1 (Zhejiang)[b] 1.1 (Hongkong)[b] 0.8 (China, Taiwan)[b] 2.5 (*21*) 0.60 (Taiwan) (*9*)
16	Castleman disease	/	/	/
17	Charcot-Marie-Tooth disease	/	/	1.44 (Taiwan) (*9*)
18	Citrullinemia	/	10.87 (South China)[b] 0.03 (North China)[b] 8.66 (Taiwan) (*43*)	/
19	Congenital adrenal hypoplasia	/	/	/
20	Congenital hyperinsulinemic hypoglycemia	/	/	23.2 (Shanghai) (*40*) 0.29 (Taiwan) (*9*)
21	Congenital myasthenic syndrome	/	/	/
22	Congenital myotonia syndrome (Non-dystrophic myotonia)	/	/	/
23	Congenital scoliosis	/	/	202.43 (Luohe He'nan) (*45*) 295.98 (Females, Luohe, He'nan) (*45*) 110.63 (Males, Luohe, He'nan) (*45*)
24	Coronary artery ectasia	/	/	656 (Beijing) (*3*)
25	Diamond-Blackfan anemia	/	/	0.19 (Shanghai) (*40*)
26	Erdheim-Chester disease	/	/	/
27	Fabry disease	0.91-2.5 (*10*)	/	0.12 (Shanghai) (*40*) 1.34 (Taiwan) (*9*)
28	Familial Mediterranean fever	/	/	/
29	Fanconi anemia	/	/	0.11 (Shanghai) (*3*)
30	Galactosemia	2.08 (*10*) 0.53 (China) (*10*)	0.25 (Taiwan) (*21*)	0.53 (Zhejiang)[b] 0.11 (Taiwan) (*9*)
31	Gaucher's disease	1.25 (China) (*10*)	1.24 (Shanghai)[b]	0.22 (Shanghai) (*40*) 0.15 (Taiwan) (*9*)
32	General myasthenia gravis	/	0.68 (*11, 13*) 0.89 (Taiwan) (*11, 13*)	/
33	Gitelman syndrome	/	/	/
34	Glutaric acidemia type I	/ /	1.67[b] 0.77 (Zhejiang) (*21*)	/
35	Glycogen storage disease (Type I or II)	/	2 (Taiwan)[b] 1 (Taiwan) (*21*)	1.51 (Shanghai) (*40*)

Note: [a]data from MalaCards; [b]data from *China's Rare Disease Diagnosis and Treatment Guide (2019)*.

of 0.5/10,000 (Supplementary Table S3, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=116*). The global incidence of familial Mediterranean fever, idiopathic pulmonary fibrosis, hyperornithinemia-hyperammoniemia-homocitrullinuria syndrome, congenital scoliosis, multiple sclerosis, cardiac ion channelopathies, Charcot-Marie-Tooth disease, pulmonary cystic fibrosis, Marfan syndrome, retinitis pigmentosa, hemophilia, and Fabry disease is relatively high (greater than 2/10,000), but there are no data on these diseases from China (Supplementary Table S3, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=116*).

Some diseases have a relatively high prevalence, such as coronary artery ectasia, congenital scoliosis, retinitis pigmentosa, severe congenital neutropenia, congenital

Table 1. Newborn incidence, incidence, and prevalence of 121 rare diseases in *China's First List of Rare Disease* (continued)

No.	Disease	Newborn incidence /100,000 persons	Incidence /100,000 persons	Prevalence /100,000 persons
36	Hemophilia	/	/	2.73[b] 2.7 (Chinese mainland) (*46*) 6.46 (Shanghai) (*40*) 5.5 (Males, Chinese mainland) (*47*) 2.0 (Blood group A, 8 Chinese provinces) (*48*) 9.1 (Males, Taiwan) (*49*) 6.4 (Hong Kong) (*50*)
37	Hepatolenticular degeneration (Wilson's disease)	/	/	2.85 (8 Chinese provinces) (*48*) 3.69 (Shanghai) (*40*)
38	Hereditary angioedema	/	/	0.034 (Taiwan) (*9*)
39	Hereditary epidermolysis bullosa	/	/	0.30 (Taiwan) (*9*)
40	Hereditary fructose intolerance	5 (*10*)	/	/
41	Hereditary hypomagnesemia	/	/	/
42	Hereditary multi-infarct dementia (Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy)	/	/	/
43	Hereditary spastic paraplegia	/	/	0.48 (Taiwan) (*9*)
44	Holocarboxylase synthetase deficiency	/	/	/
45	Homocystinuria	0.33-0.5 (*10*)	/	/
46	Homozygous hypercholesterolemia	/	/	0.20 (Taiwan) (*9*)
47	Huntington's disease	/	/	1.23 (Taiwan) (*9*)
48	Hyperornithinemia-hyperammoniemia-homocitrullinuria syndrome	/	/	/
49	Hyperphenylalaninemia	9.62 (China) (*10*)	9.62 (China, 1985-2011)[b] 12.67 (Yancheng, Jiangsu) (*44*)	/
50	Hypophosphatasia	1 (*10*)	/	0.02 (Shanghai) (*40*) 0.017 (Taiwan) (*9*)
51	Hypophosphatemic rickets	/	/	0.52 (Taiwan) (*9*)
52	Idiopathic cardiomyopathy	/	/	/
53	Idiopathic hypogonadotropic hypogonadism	/	/	/
54	Idiopathic pulmonary arterial hypertension	/	/	1.56 (Taiwan) (*9*) 6.25 (Shanghai) (*40*)
55	Idiopathic pulmonary fibrosis	/	/	/
56	IgG4-related disease	/	/	/
57	Inborn errors of bile acid synthesis	/	/	0.013 (Taiwan) (*9*)
58	Isovaleric acidemia	0.63 (China) (*10*)	0.63[b] 0.063 (Shanghai) (*21*) 0.027 (Taiwan) (*21*)	0.047 (Taiwan) (*9*)
59	Kallmann syndrome	/	/	0.20 (Taiwan) (*9*)
60	Langerhans cell histiocytosis	/	/	/
61	Laron syndrome	/	0.021 (Taiwan) *(9)*	4.86 (Shanghai) (*40*) 0.021 (Taiwan) (*9*)
62	Leber hereditary optic neuropathy	/	/	1.092 (Xingtai)[b]
63	Long chain 3-hydroxyacyl coA dehydrogenase deficiency	/	0.4[b]	/
64	Lymphangioleiomyomatosis	10-16.67 (TSC) (*10*)	/	/
65	Lysine urinary protein intolerance	1-1.75 (*10*)	/	/
66	Lysosomal acid lipase deficiency	/	/	0.0042 (Taiwan) (*9*)
67	Maple syrup urine disease	0.2-0.59 (*10*) 0.72 (China) (*10*) 1 (Taiwan) (*10*)	0.72 (Shanghai) (*21*)	0.72[b] 1 (Taiwan)[b] 0.1-0.9 (Taiwan)[a] 0.12 (Taiwan) (*9*)
68	Marfan syndrome	/	/	0.75 (8 Chinese provinces) (*48*)
69	McCune-Albright syndrome	/	0.1-0.9 (Taiwan)[a]	0.089 (Taiwan) (*9*)
70	Medium chain Acyl coA dehydrogenase deficiency	1.9-9.4 (*10*) 0.66 (China) (*10*)	0.67 (Chinese mainland) (*43*) 0.28 (Zhejiang) (*21*) 1.43 (Shanghai) (*51*) 2.13 (Shandong) (*51*) 0.38 (Taiwan) (*43*)	0.034 (Taiwan) (*9*) 0.74 (Shanghai) (*21*)

Note: [a]data from MalaCards; [b]data from *China's Rare Disease Diagnosis and Treatment Guide (2019)*.

Table 1. Newborn incidence, incidence, and prevalence of 121 rare diseases in *China's First List of Rare Disease* (continued)

No.	Disease	Newborn incidence /100,000 persons	Incidence /100,000 persons	Prevalence /100,000 persons
71	Methylmalonic acidemia	0.59-2 (*10*) 3.57-10 (China) (*10*) 1.16 (Taiwan) (*10*)	10 (North China)[b] 1.27 (Yancheng, Jiangsu) (*44*)	1.16 (Taiwan)[b] 3.57 (Chinese mainland)[b] 3 (Shanghai) (*21*) 1.5 (Zhejiang) (*21*) 0.22 (Taiwan) (*9*)
72	Mitochondrial encephalomyopathy	/	/	/
73	Mucopolysaccharidosis	/	/	0.46 (Taiwan) (*9*)
74	Multi-focal motor neuropathy	/	/	/
75	Multiple Acyl coA dehydrogenase deficiency	/	/	/
76	Multiple sclerosis	/	/	3.4 (Males, 8 Chinese provinces) (*48*) 6.3 (Females, 8 Chinese provinces) (*48*) 7.02 (Taiwan) (*9*) 4.8 (Hong Kong) (*52*)
77	Multiple system atrophy	/	/	0.65 (8 Chinese provinces) (*48*)
78	Myotonic dystrophy	/	/	0.70 (Taiwan) (*9*)
79	N acetylglutamate synthase deficiency	/	/	/
80	Neonatal diabetes mellitus	/	/	0.0042 (Taiwan) (*9*)
81	Neuromyelitis optica	/	0.278 (*7*)	/
82	Niemann-Pick disease	/	/	0.12 (Shanghai) (*40*) 0.059 (Taiwan) (*9*)
83	Non-syndromic deafness	186 (*10*)	/	/
84	Noonan syndrome	40-100 (*10*)	/	/
85	Ornithine transcarbamylase deficiency	7.14 (*10*)	/	0.089 (Taiwan) (*9*)
86	Osteogenesis imperfecta (Brittle bone disease)	5-6.67 (*10*)	/	11.3 (8 Chinese provinces) (*48*) 1.45 (Taiwan) (*9*) 0.38 (Shanghai) (*40*)
87	Parkinson's disease (Young-onset, Early-onset)	/	/	7.39 (Taiwan) (*3*)
88	Paroxysmal nocturnal hemoglobinuria	/	1[b] 2.7(Mudanjiang, Heilongjiang)[b]	0.8 (8 Chinese provinces) (*48*) 1.419 (6 Chinese provinces) (*20*) 0.45 (Taiwan) (*9*)
89	Peutz-Jeghers syndrome	/	/	/
90	Phenylketonuria	/	8.48[b] 1.82 (Taiwan) (*21*)	1.17 (Taiwan) (*9*) 0.10 (Shanghai) (*40*)
91	POEMS syndrome	/	/	/
92	Porphyria	/	/	0.44 (Taiwan) (*9*)
93	Prader-Willi syndrome	/	/	1.22 (Taiwan) (*3*) 0.23 (Shanghai) (*40*)
94	Primary combined immune deficiency	1-1.33 (*10*)	/	/
95	Primary hereditary dystonia	/	/	/
96	Primary light chain amyloidosis	/	/	/
97	Progressive familial intrahepatic cholestasis	/	/	0.059 (Taiwan) (*9*)
98	Progressive muscular dystrophies	16.67-27.78 (*10*)	25.30[b]	/
99	Propionic acidemia	/	/	0.6-0.7[b] 0.03 (Shanghai) (*40*) 0.055 (Taiwan) (*9*)
100	Pulmonary alveolar proteinosis	/	/	/
101	Pulmonary cystic fibrosis	4-55.56 (*10*)	/	0.064 (Taiwan) (*9*)
102	Retinitis pigmentosa	/	/	26.43[b] 23.38 (Rural areas around Beijing) (*53*)
103	Retinoblastoma	5-6.67 (*10*)	/	1-9 (Taiwan)[c]
104	Severe congenital neutropenia	0.4 (*10*)	/	25.7 (Shanghai) (*40*)
105	Severe myoclonic epilepsy in infancy (Dravet syndrome)	/	/	0.25 (Taiwan) (*9*)
106	Sickle cell disease	/	/	/
107	Silver-Russell syndrome	1-3.33 (*10*)	/	/
108	Sitosterolemia	/	/	0.017 (Taiwan) (*9*)
109	Spinal bulbar muscular atrophy (Kennedy disease)	/	/	/
110	Spinal muscular atrophy	/	/	1.71 (Taiwan) (*9*)
111	Spinocerebellar ataxia	/	/	4.41 (Taiwan) (*9*)

Note: [a]data from MalaCards; [b]data from *China's Rare Disease Diagnosis and Treatment Guide (2019)*.

Table 1. Newborn incidence, incidence, and prevalence of 121 rare diseases in *China's First List of Rare Disease* (continued)

No.	Disease	Newborn incidence /100,000 persons	Incidence /100,000 persons	Prevalence /100,000 persons
112	Systemic sclerosis	/	/	/
113	Tetrahydrobiopterin deficiency	/	/	0.021 (Taiwan) (*9*)
114	Tuberous sclerosis complex	10-16.67 (*10*)	/	1-9 (Taiwan)[a] 2.16 (Taiwan) (*9*)
115	Tyrosinemia	/	/	0.042 (Taiwan) (*9*)
116	Very long chain acyl coA dehydrogenase deficiency	/	/	/
117	Williams syndrome	4.26 (Hong Kong) (*10*)	/	1.07 (Taiwan) (*9*)
118	Wiskott-Aldrich syndrome	0.4-1 (*10*)	/	0.03 (Shanghai) (*40*) 0.064 (Taiwan) (*9*)
119	X-Linked agammaglobulinemia	/	/	/
120	X-linked adrenoleukodystrophy	0.26 (*10*)	/	/
121	X-linked lymphoproliferative disease	/	/	0.03 (Shanghai) (*40*)

Note: [a]data from MalaCards; [b]data from *China's Rare Disease Diagnosis and Treatment Guide (2019)*.

hyperinsulinemic hypoglycemia, Laron syndrome, spinocerebellar ataxia, multiple sclerosis, amyotrophic lateral sclerosis, hepatolenticular degeneration (Wilson's disease), hemophilia, osteogenesis imperfecta, and tuberous sclerosis complex. The prevalence of coronary artery ectasia, congenital scoliosis, retinitis pigmentosa, severe congenital neutropenia, congenital hyperinsulinemic hypoglycemia, and osteogenesis imperfecta is higher than 1/10,000, according to the new 2021 definition of rare diseases in China, though some data are limited by region (Table 1). The prevalence of congenital hyperinsulinemic hypoglycemia is 2.32/10,000 in Shanghai and 0.029/10,000 in Taiwan. Like the incidence, the global prevalence of rare diseases differs greatly from that in China (Table 1, Supplementary Table S3, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=116*).

4. The development and promotion of the rare disease list and registration: Data collection and information sharing

Before the release of *China's Rare Disease List,* the Shanghai Municipal Health Commission published the "*Major Rare Diseases in Shanghai (2016 Edition)*" that included 56 rare diseases (Supplementary Table S4, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=117*) (*25*). After excluding infectious diseases, tumors, poisoning, and traumatic diseases in the Orphanet catalogue, searching the literature and websites, and comparing diseases to the Chinese version of ICD-10, a catalogue of 4,299 rare diseases and 1,049 ICD codes was created in 2016 by the Shandong Association for the Prevention and Treatment of Rare Diseases. On March 1, 2017, the Shandong Provincial Health Commission began registering cases of 68 rare diseases according to their prevalence and drug availability (Supplementary Table S4, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=117*) (*26*). Provincial Grade-III Class-A hospitals (according to the Chinese Hospital Ranking System) including maternal and child health hospitals, children's hospitals, and general hospitals were required to register these 68 diseases. This was later expanded to 121 rare diseases under the national rare disease diagnosis and treatment network. Thus far, more than 29,000 cases have been registered (unpublished data). The Chinese Organization for Rare Disorders (CORD), the largest non-official rare disease organization, has advocated for a list of 147 rare diseases. The criteria used for inclusion of these disorders included the following: (a) the global incidence of rare diseases; (b) rare diseases that have been treated with drugs at home and abroad; (c) diseases recognized by domestic organizations for rare diseases; (d) the list of rare diseases in Taiwan; (e) the rate of clinical detection by domestic genetic testing facilities; (f) the list of major rare diseases in Shanghai; and (g) rare diseases of great social concern (Supplementary Table S2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=115*) (*27*).

Despite the lack of epidemiological data on and registration of rare diseases, rare diseases have been specifically defined in China, and this has greatly affected their diagnosis and treatment. In February 2019, the National Health Commission issued the *Rare Disease Diagnosis and Treatment Guide* (2019), which cited data on the incidence/prevalence of rare diseases based on *China's Rare Disease List* (*28*). The national rare disease diagnosis and treatment network was created at the same time, and it included 1 leading national hospital (Beijing Union Medical College Hospital), 32 leading provincial hospitals, and 291 member hospitals in the network (*29*). In October 2019, member hospitals in the network were encouraged to start registering 121 rare diseases on November 1, 2019. Registration was dated back to the beginning of 2015 (*30*). Nearly 500,000 cases have been registered according to a notice from the National Health Commission (*31*). The first national rare disease

registry (the National Rare Diseases Registry System of China; NRDRS) was created in 2016; it includes a total of 62,590 cases of 166 diseases or types of diseases (*32*).

5. The relationship between the rare disease list and the 2021 definition

There is no unified standard for the definition and classification of rare diseases. The definition is dynamic, and it changes from nation to nation. Initially, rare diseases were defined despite the lack of epidemiological data in China. Then, they were defined based on prevalence and the number of people affected or the incidence in newborns (*33*). A rare disease is a disease with an incidence less than 1/10,000, as recognized by the Rare Disease and Drug Review Committee, and it is documented by a relevant department in Taiwan. The definition benefited from the rare disease reporting system that was established in 2000, which covers disease incidence, treatment fees, and treatment outcomes (*34*). As national registration continues, more reports of rare diseases will be collected. The emphasis will be on rare diseases with a relatively high incidence/prevalence, drug availability, and greater recognition. The 2021 definition will definitely increase the understanding of more rare diseases as more epidemiological data becomes available. Moreover, it will guide medical insurance coverage, orphan drug R&D, and designations.

China is a country with a high prevalence of birth defects, with an estimated prevalence of 5.6% (*35*) . Birth defects keep rising with the high growth rate. The number of children with birth defects was 977,000 in 1996, but that figure jumped to 1.53 million in 2011, with a growth rate as high as 70.9% (*35*). Newborn screening is an important policy in China, and it started in 1980. In 2016, the Department of Maternal and Child Health Care and Community Health of the National Health Commission and the Foundation for Foundation for Intervention in and Relief of Birth Defects in China jointly launched a relief project for birth defects (genetic metabolic diseases); the project included 78 diseases, 43 of which were listed in *China's First List of Rare Diseases (36)* . The incidence in newborns was included in the definition of rare diseases in China. Epidemiological data on rare diseases will help to tally the number of patients with rare diseases. This will facilitate interventions in and help to prevent birth defects and improving the health of the population.

6. Rare diseases beyond the 121 diseases in the list and their spectrum

As part of a "Project to Study and Attempt to Control Rare Diseases in China" (no. 2013BAI07B00) under the "Twelfth Five-year Plan" National Program to Support Science and Technology, the Shandong Association for the Prevention and Treatment of Rare Diseases conducted an epidemiology study of rare diseases in nearly 100 tertiary hospitals in China in collaboration with research institutes in Shandong and 6 other provinces. A total of 40,5589 patients with 952 rare diseases (2.27% of all hospitalized patients) were recorded by 93 hospitals in the 7 provinces and at least half of the rare diseases were congenital diseases (*37*). A survey and literature review revealed that 5,749 cases of 323 rare diseases were identified in Shandong Province (*38*). The Rare Disease Branch of the Beijing Medical Association has conducted studies of rare diseases since 2013; using the rare diseases listed on European websites related to rare diseases as a template, the Rare Disease Branch has collected and analyzed 404,312 cases from tertiary hospitals in Beijing. As a result, the Rare Disease Branch identified 1,423 rare diseases (*37*). Preliminary research by the Rare Disease Branch yielded information on 121 diseases in *China's First List of Rare Diseases*, including the number of inpatients, the disease distribution by province/municipality, affected age groups, and the rate of repeated hospitalization at 96 level A tertiary hospitals. Although national epidemiological data are lacking, data on diseases in the database have been mined, which is also an effective approach for an epidemiological study (*39*).

Out of over 15 million hospitalized patients, a total of 54,468 patients with 102 rare diseases were identified. Sixty-nine-point-seven-two percent of those cases involved the ten leading rare diseases, including idiopathic pulmonary arterial hypertension, Langerhans cell histiocytosis, amyotrophic lateral sclerosis, idiopathic pulmonary fibrosis, systemic sclerosis, hepatolenticular degeneration, retinitis pigmentosa, Marfan syndrome, homozygous familial hypercholesterolemia, and congenital scoliosis (*39*). In the NRDRS, hemophilia, idiopathic pulmonary arterial hypertension, spinocerebellar ataxia, Alport syndrome, myasthenia gravis, phenylketonuria, methylmalonic acidemia, multiple sclerosis, osteogenesis imperfecta, and spinal muscular atrophy were the ten leading rare diseases, accounting for 71.19% of total cases (*32*) . From the Shanghai list of 33 rare diseases, 16,933 cases were identified from 2013 to 2016. The disease spectrum in terms of age, gender, and yearly changes was described. The proportion of inpatients and outpatients and the burden of hospitalization were analyzed. Severe congenital neutropenia, congenital hyperinsulinemic hypoglycemia, hemophilia, hepatolenticular degeneration, idiopathic pulmonary arterial hypertension, and congenital adrenal hyperplasia were identified as the most prevalent rare diseases in Shanghai (*40*). In December 2021, Tongji Hospital, a leading provincial hospital and member of the network, released an *Investigation on rare diseases in Hubei Province* that included 109 rare diseases; amyotrophic lateral sclerosis, multiple sclerosis, hemophilia, multiple system atrophy, autoimmune encephalitis, Marfan syndrome, Castleman

disease, idiopathic pulmonary fibrosis, coronary artery ectasia, and optical neuromyelitis were the most prevalent rare diseases (*41*). Taking this spectrum in different regions and cities into consideration, idiopathic pulmonary arterial hypertension, hepatolenticular degeneration, hemophilia, amyotrophic lateral sclerosis, idiopathic pulmonary fibrosis, and multiple sclerosis are relatively prevalent.

7. Conclusion

In light of the drafting of *China's First List of Rare Diseases* and the promotion of national or regional registration of rare diseases, the number of registered cases has increased massively, and especially for 121 rare diseases. More cases will be assembled and disease and epidemiological data will be collected as registration continues, though there are limited data on the incidence and prevalence of 121 rare diseases. Since there are more than 7,000 rare diseases, the rare disease list should be continuously updated. With the 2021 definition of rare diseases in China, the number of registered rare diseases will increase. The national rare disease diagnosis and treatment network is expected to expand as more hospitals are enrolled and expert knowledge on rare diseases spreads. Hence, different types of rare diseases will be identified and registered. Based on updated registration data, highly prevalent diseases should be removed from the list of 121 rare diseases. Registration could promote the development of orphan drugs, improve medical insurance coverage, and enhance diagnosis and treatment. In addition to registration, the conducting of follow-ups and collection of biosamples should be enhanced.

Funding: This work was supported by a grant from the Project to Promote Academics of Shandong First Medical University (no. 2019LJ001).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Melnikova I. Rare diseases and orphan drugs. Nature Reviews Drug Discovery. 2012; 11:267-268.
2. Nguengang Wakap S, Lambert DM, Olry A, Rodwell C, Gueydan C, Lanneau V, Murphy D, Le Cam Y, Rath A. Estimating cumulative point prevalence of rare diseases: Analysis of the Orphanet database. Eur J Hum Genet. 2020; 28:165-173.
3. He J, Tang M, Zhang X, Chen D, Kang Q, Yang Y, Hu J, Jin C, Song P. Incidence and prevalence of 121 rare diseases in China: Current status and challenges. Intractable Rare Dis Res. 2019; 8:89-97.
4. Shourick J, Wack M, Jannot AS. Assessing rare diseases prevalence using literature quantification. Orphanet J Rare Dis. 2021; 16:139.
5. He J, Kang Q, Hu J, Song P, Jin C. China has officially released its first national list of rare diseases. Intractable Rare Dis Res. 2018; 7:145-147.
6. Bureau of Medical Administration, National Health Commission. Notice on issuance of the first list of rare diseases. *http://www.nhc.gov.cn/yzygj/s7659/201806/393a9a37f39c4b458d6e830f40a4bb99.shtml* (accessed March 10, 2022). (in Chinese)
7. Yuan P, Li Z, Xia T, Li H. Population investigation of albinism in China for 25 years- Review and future. Chin J Birth Health & Heredity. 2006; 4-6.
8. Loirat C, Fremeaux-Bacchi V. Atypical hemolytic uremic syndrome. Orphanet J Rare Dis. 2011; 6:60.
9. Taiwan Health Promotion Administration. Statistical Table of Reported Cases of Rare Diseases. *https://www.hpa.gov.tw/Pages/Detail.aspx?nodeid=1559&pid=10254* (accessed March 10, 2019). (in Chinese)
10. Ding J, Wang L. 121 Rare Diseases Handbook. China Medical Science and Technology Press. Beijing, 2019.
11. Zhan S, Siu J, Wang Z, Yu H, Bezabeh T, Deng Y, Du W, Fei P. Focal point of Fanconi anemia signaling. Int J Mol Sci. 2021; 22.
12. Berry GT. Classic galactosemia and clinical variant galactosemia. In: GeneReviews (Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, eds.). Seattle (WA), 1993.
13. Pillai NR, Stroup BM, Poliner A, Rossetti L, Rawls B, Shayota BJ, Soler-Alfonso C, Tunuguntala HP, Goss J, Craigen W, Scaglia F, Sutton VR, Himes RW, Burrage LC. Liver transplantation in propionic and methylmalonic acidemia: A single center study with literature review. Mol Genet Metab. 2019; 128:431-443.
14. Li Q, Yang C, Feng L, Zhao Y, Su Y, Liu H, Men H, Huang Y, Korner H, Wang X. Glutaric acidemia, pathogenesis and nutritional therapy. Front Nutr. 2021; 8:704-984.
15. Racis L, Tessa A, Di Fabio R, Storti E, Agnetti V, Casali C, Santorelli FM, Pugliatti M. The high prevalence of hereditary spastic paraplegia in Sardinia, insular Italy. J Neurol. 2014; 261:52-59.
16. The Subspecialty Group of Endocrinology H, and Metabolic Diseases, Society of Pediatrics, Chinese Medical Association. Common sense in diagnosis and treatment of hyperphenylalaninemia. Chin J Ped. 2014; 420-425.
17. Tournis S, Yavropoulou MP, Polyzos SA, Doulgeraki A. Hypophosphatasia. J Clin Med. 2021; 10.
18. Ye L, Yuan H, Cai X. Analysis of screening results for 21-hydroxylase deficiency in newborns in the Dongguan area. Contemporary Medicine Forum. 2014; 164-165.
19. Pan L, Zheng M, Xie L, Cai R, Tan J, Yang J, Huang L. Screening for 21-hydroxylase deficiency in newborns in Liuzhou. Chin J Birth Health & Heredity. 2013; 68-69.
20. Wang C. A retrospective study of rare diseases in China Biology Medicine Database. Master's Thesis, Jinan University. 2015.
21. Chen J. Treatable Rare Diseases (Chen J, eds.). Shanghai Jiao Tong University Press, Shanghai, China. 2017; 38-136.
22. Roberts AE. Noonan syndrome. In: GeneReviews (Adam MP, Everman DB, Mirzaa GM, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Amemiya A, eds.). University of Washington, Seattle (WA), 1993.
23. Zhang SY. Compendium of China's First List of Rare Disease. People's Medical Publishing House. Beijing, 2018.

24. Hosahalli Vasanna S, Pereda MA, Dalal J. Clinical features, cancer biology, transplant approach and other integrated management strategies for Wiskott-Aldrich syndrome. J Multidiscip Healthc. 2021; 14:3497-3512.
25. Shanghai Municipal Health Commission. Notice on printing and distribution of the *List of Major Rare Diseases in Shanghai* (2016 Edition). *http://wsjkw.sh.gov.cn/fybj2/20180815/0012-59645.html* (accessed March 10, 2022). (in Chinese)
26. Liu X. Descriptive epidemiology investigation and analysis of the rare diseases in eight provinces, China. China Academic Journal Electronic Publishing House. 2016.
27. Chinese Organization for Rare Disorders. Reference list of rare diseases in China. https://www.sohu.com/a/114982149_119250 (accessed March 10, 2022). (in Chinese)
28. General Office, National Health Commission. Notice on publication of the Rare Diseases Diagnosis and Treatment Guide (2019). *http://www.nhc.gov.cn/yzygj/s7659/201902/61d06b4916c348e0810ce1fceb844333.shtml* (accessed March 10, 2022). (in Chinese)
29. Bureau of Medical Administration, National Health Commission. Notice on the establishment of a national cooperative network for the diagnosis and treatment of rare diseases. *http://www.nhc.gov.cn/yzygj/s7659/201910/be9343380e414adb8c8d641ae8967492.shtml* (accessed March 10, 2022). (in Chinese)
30. Bureau of Medical Administration, National Health Commission. Notice on the registration of information on the diagnosis and treatment of rare diseases. *http://www.nhc.gov.cn/yzygj/s7659/201910/be9343380e414adb8c8d641ae8967492.shtml* (accessed March 10, 2022). (in Chinese)
31. Department of Publicity, National Health Commission. Transcript of the State Council Information Office's regular policy briefing on July 8, 2021. *http://www.nhc.gov.cn/xcs/s3574/202107/8c7bce96b85c48498df15fb1bd0434eb.shtml* (accessed March 10, 2022). (in Chinese)
32. Guo J, Liu P, Chen L, Lv H, Li J, Yu W, Xu K, Zhu Y, Wu Z, Tian Z, Jin Y, Yang R, Gu W, Zhang S. National Rare Diseases Registry System (NRDRS): China's first nationwide rare diseases demographic analyses. Orphanet J Rare Dis. 2021; 16:515.
33. Lu Y, Han J. The definition of rare disease in China and its prospects. Intractable Rare Dis Res. 2022; 11:29-30.
34. Xiao J, Wang C. Support system for rare disease prevention and protection: Experience in Taiwan and insights. Social Security Studies. 2018; 2:92-105.
35. Ministry of Health of the People's Republic of China. Report on the control of birth defects in China 2012. *http://www.gov.cn/gzdt/att/att/site1/20120912/1c6f6506c7f811bacf9301.pdf* (accessed March 10, 2022). (in Chinese)
36. Foundation for Intervention in and Relief of Birth Defects in China. Birth defect (genetic metabolic disease) relief project. *http://www.csqx.org.cn/list.aspx?id=877919995095&prjid=48###* (accessed March 10, 2022). (in Chinese)
37. Ding J WL. Report of rare diseases in China (2018) (Ding J, Wang L, eds). China Medical Science Press, Beijing, 2018; 9.
38. Zhao H, Cui Y, Zhou X, Pang J, Zhang X, Xu S, Han J. Study and analysis of the state of rare disease research in Shandong Province, China. Intractable Rare Dis Res. 2012; 1:161-166.
39. Shi XM LH, Wang L, Wang ZX, Dong CY, Wang YF, Yao C, Zhan SY, Ding J, Li Y. Study on the current situation of China's First List of Rare Diseases based on 15 million hospitalizations. Natl Med J China 2018; 98:3274-3278.
40. Cai X, Genchev GZ, He P, Lu H, Yu G. Demographics, in-hospital analysis, and prevalence of 33 rare diseases with effective treatment in Shanghai. Orphanet J Rare Dis. 2021; 16:262.
41. Hubei Daily. "Survey of rare diseases in Hubei Province" released. *http://www.shandong.gov.cn/art/2017/3/2/art_97564_280621.html?from=singlemessage&isappinstalled=0* (accessed March 10, 2022). (in Chinese)
42. Tsai CP, Wang KC, Hwang CS, Lee IT, Lee CT. Incidence, prevalence, and medical expenditures of classical amyotrophic lateral sclerosis in Taiwan, 1999-2008. J Formos Med Assoc. 2015; 114:612-619.
43. Zhang SY. Compendium of China's First List of Rare Disease (Zhang SY, eds.). People's Medical Publishing House, Beijing, 2018; 86-503.
44. Lu H, Zheng J, Yao Y, Yang M, Ya S, Shi N, Lu J. Retrospective analysis of screening results using TMS to identify inherited metabolic diseases in newborns in Yancheng. Chin J Birth Health & Heredity. 2016; 83-85.
45. Li Y, Cui W, Ya X, Wang H. Epidemiology of congenital scoliosis in Luohe. Chin J Ped Surg. 2017; 221-224.
46. Qu Y, Nie X, Yang Z, Zhan S. Meta-analysis of the prevalence of hemophilia in mainland China. Chin J Hematol. 2014; 65-68.
47. Zhao Z. Advances in screening newborns for inherited metabolic diseases. Chin J Pract Ped. 2014; 586-589.
48. Liu X. Descriptive epidemiology investigation and analysis of the rare disease in eight provinces, China. Master's Thesis, Pharmacy, Jinan University. 2016.
49. Tu TC, Liou WS, Chou TY, Lin TK, Lee CF, Chen JD, Cham TM, Chung MI. Prevalence, incidence, and factor concentrate usage trends of hemophiliacs in Taiwan. Yonsei Med J. 2013; 54:71-80.
50. Ling S, Chan G, Shing M, Yue H, Lee A, Chan C, Lee C, Kwong K, Li C. Children and adolescents with haemophilia in Hong Kong: An epidemiological and clinical review. Hong Kong J Paed. 2006; 13-19.
51. Zhou H, Li Y, Tian L. Progress in diagnosis and treatment of medium-chain acyl-coenzyme A dehydrogenase deficiency. Chin J Pract Ped. 2019; 22-25.
52. Lau KK, Wong WW, Sheng B, Yu IT, Fung BH, Li HL, Ma KF, Wong LK, Li PC. The clinical course of multiple sclerosis patients in Hong Kong. J Neurol Sci. 2008; 268:78-82.
53. You QS, Xu L, Wang YX, Liang QF, Cui TT, Yang XH, Wang S, Yang H, Jonas JB. Prevalence of retinitis pigmentosa in North China: The Beijing Eye Public Health Care Project. Acta Ophthalmol. 2013; 91:e499-500.

Received July 21, 2022; Revised August 18, 2022; Accepted August 22, 2022.

**Address correspondence to:*
Yanqin Lu, The First Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, No. 16766 Jingshi Road, Ji'nan, Shandong 250013, China.
E-mail: yqlu@sdfmu.edu.cn

Released online in J-STAGE as advance publication August 25, 2022.

Review

DOI: 10.5582/irdr.2022.01080

Anterior cervical discectomy and fusion without plate (ACDFWP) versus anterior cervical disc arthroplasty (ACDA) for cervical spondylosis: A meta-analysis and literature review

Jiajie Peng[1,§], Sihan Li[2,3,§], Xiangying Lin[3], Degui Zhong[4], Rong Zheng[3], Minghan Huang[3], Pengfei Li[5], Hongmei Song[3,*], Tetsuya Asakawa[6,*]

[1] Department of Orthopedics, Zhongshan Jishuitan Orthopedic Hospital, Zhongshan, Guangdong, China;
[2] School of Basic Medical Sciences, Guangzhou University of Chinese Medicine, Guangzhou, Guangdong, China;
[3] Department of Gastroenterology, The Second People's Hospital Affiliated to Fujian University of Traditional Chinese Medicine, Fuzhou, Fujian, China;
[4] Department of Orthopedics, Foshan Hospital of Traditional Chinese Medicine, Foshan, Guangdong, China;
[5] Department of Nephrology, The People's Hospital Affiliated to Fujian University of Traditional Chinese Medicine, Fuzhou, Fujian, China;
[6] Institute of Neurology, The Third People's Hospital of Shenzhen, Shenzhen, Guangdong, China.

SUMMARY This meta-analysis compared the clinical outcomes between two alternative surgeries for patients with cervical spondylosis, namely anterior cervical discectomy and fusion (ACDF) without plate (ACDFWP) vs. anterior cervical disc arthroplasty (ACDA). We searched databases, including PubMed, EMBASE, Cochrane Library, Google Scholar, and Web of Science (firstly available-2019). A standard meta-analysis was performed with the included studies. A Risk of Bias in Non-randomized Studies of Interventions (ROBINS-I) tool was used for the evaluation of the study quality of nonrandomized-controlled trials (nRCTs), while a Risk of Bias (RoB) battery was used for randomized controlled trials (RCTs). Eight studies involving 640 patients were included. No significant difference was found in the indices of Neck Disability Index (NDI) score, Visual Analog Score (VAS), Japanese Orthopaedic Association (JOA) score, operative time, blood loss, Swallowing Quality of Life Score (SWAL-QL), and complications. Cervical alignment was significantly better in the ACDFWP than in ACDA (mean difference (MD) = −0.67, 95% confidence interval (CI) [−1.11, −0.23], $P = 0.003$, $I^2 = 20\%$). Although the alternative ACDFWP was slightly superior in terms of the index of cervical alignment, the limited research on this subject present insufficient evidence. Further well-designed studies are warranted in the future.

Keywords cervical spondylosis, anterior cervical discectomy and fusion without plate, anterior cervical disc arthroplasty, cervical alignment, meta-analysis

1. Introduction

Cervical spondylosis is a common disease characterized by progressive degeneration in the cervical spine and is regarded as a natural process of aging. The main symptoms of cervical spondylosis are neck pain and neck stiffness. Recently, an increasing number of young people are experiencing cervical spondylosis. It has been a public health concern related to remarkable disease burden. Since the 1950s, anterior cervical discectomy and fusion (ACDF) with plate has been regarded as the gold standard of therapy for cervical spondylosis (*1-3*). Although it has been well documented regarding its satisfactory efficacy, some complications, such as dysphagia (*4*), hoarseness (*2*), and adjacent segment disease (ASD) (*3*) are also reported. Some authors believe that these complications are caused by the influence of anterior vertebral plate fixation (*4*). Kepler *et al*. found that the fusion itself may contribute to more pressure between the adjacent segmental discs (*5*). Moreover, ASD might also result from the progression of cervical degeneration (*6,7*). Thus, the conventional ACDF with plate has limitations, and the adverse events cannot be ignored. To prevent these adverse events, alternative surgeries, such as ACDF without plate (ACDFWP) and anterior cervical disc arthroplasty (ACDA) were developed against cervical spondylosis (Figure 1).

Currently, many studies have reported a comparison of the clinical outcomes of conventional ACDF (with

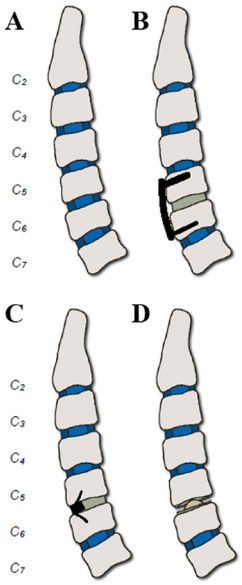


Figure 1. Schematic diagram of the surgeries for cervical spondylosis. A. Schematic diagram of the cervical spine. **B.** Conventional anterior cervical discectomy and fusion (ACDF) with plate. **C.** Anterior cervical discectomy and fusion without plate (ACDFWP). **D.** Anterior cervical disc arthroplasty (ACDA). Most of previous studies compared ACDF with plate and ACDA (B *vs.*D), in the present study we compared ACDFWP *vs*. ACDA (C *vs*. D).

plate) and alternative ACDFWP. Recently, Cheung *et al.* found that ACDFWP provides better clinical outcomes than conventional ACDF (with plate) (*8*). Other studies have also compared the clinical outcomes between conventional ACDF (with plate) and ACDA. Latka *et al*. reported that compared to conventional ACDF (with plate), ACDA significantly lowered the probability of ASD development at the 60-month follow-up. (*9*). And Maharaj *et al*. compared ACDA and ACDF (including with and without plate). They found that ACDA has superiority in terms of reoperation rate and reduction in neurological deficits (*10*). However, to our knowledge, no meta-analyses have compared the clinical outcomes between alternative surgeries, ACDFWP and ACDA. Thus, in order to provide a complete chain of evidence to enable the selection of an appropriate surgical technique for cervical spondylosis treatment by the clinicians, we designed this study to compare the clinical outcomes of the alternative ACDFWP and ACDA (Figure 1), strictly as per the guidelines of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (*11*).

2. Materials and Methods

2.1. Literature search strategy

English-language search of databases, including PubMed, EMBASE, Cochrane Library, Google Scholar, and Web of Science was performed with the keywords of "cervical spondylosis" AND "anterior cervical" AND "arthroplasty" AND "discectomy and fusion" OR "replacement". Studies from firstly available-2021 were included.

As per the inclusion criteria, (*i*) studies that compared the clinical outcomes between ACDA and ACDFWP in patients with cervical spondylosis, (*ii*) studies where complete data were available, (*iii*) randomized controlled trials (RCTs) and nonrandomized-controlled trials (nRCTs), and (*iv*) studies wherein at least one of the following assessments for clinical outcomes were reported: Neck Disability Index (NDI) score, Visual Analog Score (VAS), Japanese Orthopaedic Association (JOA) score, cervical alignment (The overall sagittal alignment of the cervical spine was calculated by the Cobb angle between the C2 and C7 vertebrae on the lateral radiograph), operative time, blood loss, complications, and Swallowing Quality of Life Score (SWAL-QL), were included. Furthermore, (*i*) non-English studies, (*ii*) review papers, (*iii*) meta-analyses, (*iv*) case reports and serious case reports, as well as (*v*) letters were excluded.

2.2. Data extraction

Three authors (JP, SL, XL) performed the literature search, read the title and abstract, and then screened the studies as per the inclusion/exclusion criteria. Subsequently, a senior scientist (TA) cross-checked and confirmed the quality of the included literature. Three authors (JP, SL, HS) extracted information (information of enrolled patients, therapy, experimental design, and outcome assessments) from the final selection of studies. We classified the assessments into two groups. One group contained indices that directly evaluated the clinical outcomes, including the NDI, VAS, JOA and cervical alignment; anther group contained the other factors associated with the surgeries, including operative time, blood loss, complications, and SWAL-QL. We discussed the data every day to reach consensus. Finally, all the data were checked by third-party authors (DZ, RZ, MH, PL) before submission for the meta-analysis.

2.3. Statistical analyses

We designed and performed this study strictly as per the PRISMA guidelines (*11*). We employed a RevMan 5.3 software (The Nordic Cochrane Center, The Cochrane Collaboration, Copenhagen, Denmark) for the meta-analysis. The odds ratio (OR) and the corresponding 95% confidence interval (CI) were calculated for the dichotomous outcomes. The mean difference (MD), and the 95% CI were calculated for the continuous data. During the homogeneity test, when $p \geq 0.1$ and $I^2 \leq 50\%$, the studies were considered to be homogeneous, which were analyzed with a fixed-effect model; when

$p < 0.1$ and $I^2 > 50\%$, the studies were regarded to be heterogeneous, which were analyzed with a random-effects model. Subgroups analyses were performed for considering the subgroups in some groups, such as VAS (neck and arm), cervical alignment (whole and local) and complications (ASD is regarded as the most important adverse event).

2.4. Assessment of study quality of the included studies

Three authors (SL, JP and RX) performed quality assessment for the study. A Risk of Bias (RoB) in Non-randomized Studies of Interventions (ROBINS-I) tool (*12*) was employed to assess the study quality of nRCTs. A RoB battery developed by the Cochrane Collaboration was used for the RCTs (*13*).

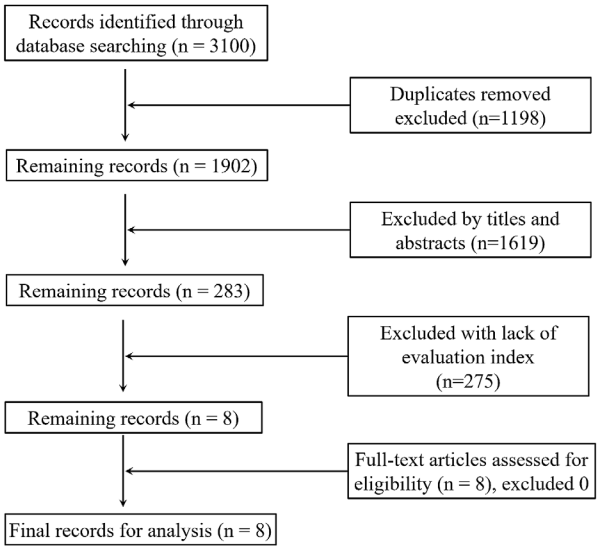


Figure 2. Flow chart of the searching strategy and selection of literature reports.

3. Results

3.1. Literature searching results

Total 3100 studies were identified in the first search. First, duplicate studies (1,198) and those with an inappropriate title and abstract (1,619) were excluded. Thereafter, the remaining 283 studies were read and selected within which 275 items were excluded. Finally, 8 studies were included and submitted for the meta-analysis (Figure 2). The characteristics of the included studies are listed in Table 1.

3.2. Assessments of the study quality

Figure 3 shows the results of the assessments of study quality using a RoB tool (for RCTs) and a ROBINS-I tool (for nRCTs). Overall, three RCTs had low RoB. Only one study (Qizhi *et al*. 2016) had a high risk of blinding, and one study a had high risk of other bias (Donk *et al*. 2017b). The study quality of the included RCTs was satisfactory (Figure 3A). However, the study quality of the included nRCTs was weaker. The study by Park *et al*. 2008 had a serious RoB and that by Vorsic *et al*. 2010 had a critical RoB. Confounding bias was involved in 2 studies (*14,15*). One study had missing data (*14*). Overall, two studies were of low quality (*14,15*), and other 3 studies were identified as having moderate study quality (Figure 3B). We could not evaluate the publication bias because only 3 RCTs and 5 nRCTs were included in this study.

3.3. Assessments of the postoperative clinical efficacy achieved with the ACDFWP and ACDA.

Figure 3 shows the results of the assessments of study quality using a RoB tool (for RCTs) and a ROBINS-I

Table 1. Characteristics of the included studies

Included Trials	Study Design	Sample size	Average age ($\bar{y}$)	Gender (M/F)	Level	Implants
Park *et al*. 2008	R	ACDA: 21	45	11/10	1	Mobi-C
		ACDFWP: 32	47	20/12		Solis
Vorsic *et al*. 2010	P	ACDA: 40	48.1	13/27	1/2	ProDisc-C
		ACDFWP: 40	51.3	12/28		ChronOS
Park *et al*. 2012	R	ACDA: 22	39.9	19/3	1	ProDisc-C
		ACDFWP: 21	44.3	11/10		Solis
Qizhi *et a*l. 2016	RCT	ACDA: 14	46.79	9/5	2	Discover
		ACDFWP: 16	48.13	11/5		Zore-P
Shi *et al*. 2016a	R	ACDA: 60	46.5	24/36	1	Discover
		ACDFWP: 68	47.4	33/35		Zore-P
Shi *et al.* 2016b	P	ACDA: 55	48.9 ± 7.0	30/25	1	Discover
		ACDFWP: 57	50.6 ± 7.2	37/20		Zore-P
Donk *et al*. 2017a	RCT	ACDA: 50	44.1 ± 6.4	24/26	1	Bryan
		ACDFWP: 47	43.1 ± 7.5	25/22		Brantigan
Donk *et al*. 2017b	RCT	ACDA: 50	53.6 ± 6.9	24/26	1	Bryan
		ACDFWP: 47	52.2 ± 8.1	25/22		Brantigan

ACDA: anterior cervical disc arthroplasty; ACDFWP: anterior cervical discectomy and fusion without plate; R: Retrospective; P: Prospective; RCT: Randomized controlled study.

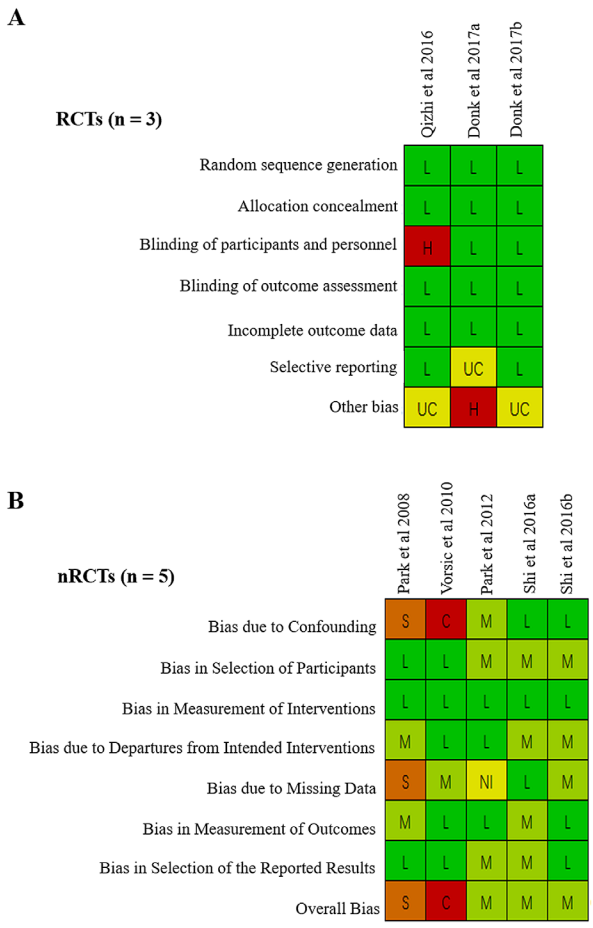


Figure 3. Quality assessment of the involved literatures with a RoB tool for RCTs (A) and a ROBINS-I tool for nRCTs (B). L = Low, UC = Unclear, H = High, M = Moderate, S = Serious, C = Critical, NI = No information.

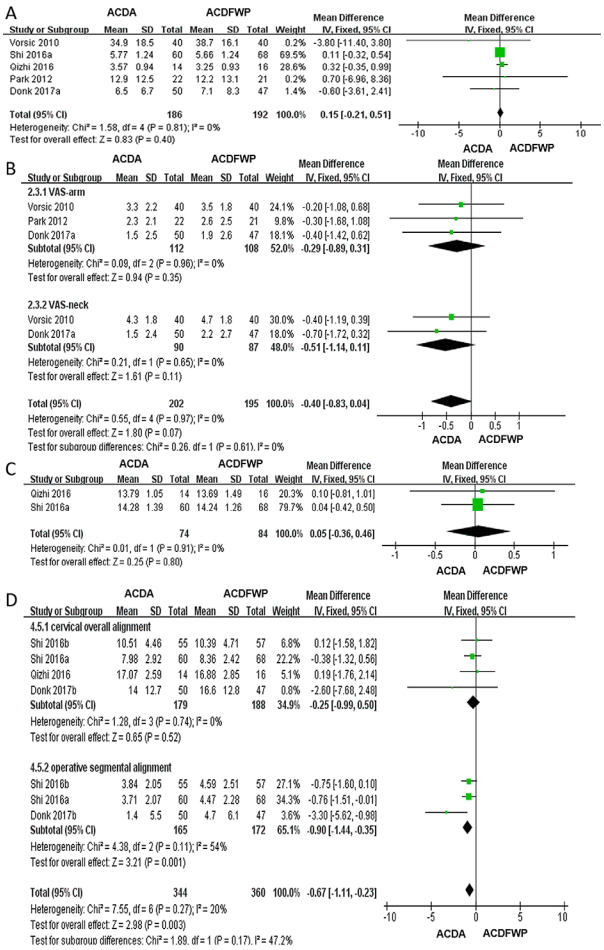


Figure 4. Meta-analysis for the clinical assessments of ACDA and ACDFWP. A. Forest plot for the scores of NDI. **B.** Forest plot for the scores of VAS. **C.** Forest plot for the scores of JOA. **D.** Forest plot for the scores of cervical alignment.

tool (for nRCTs). Overall, three RCTs had low RoB. Only one study (Qizhi *et al*. 2016) had a high risk of blinding, and one study a had high risk of other bias (Donk *et al*. 2017b). The study quality of the included RCTs was satisfactory (Figure 3A). However, the study quality of the included nRCTs was weaker. The study by Park *et al*. 2008 had a serious RoB and that by Vorsic *et al*. 2010 had a critical RoB. Confounding bias was involved in 2 studies (*14,15*). One study had missing data (*14*). Overall, two studies were of low quality (*14,15*), and other 3 studies were identified as having moderate study quality (Figure 3B). We could not evaluate the publication bias because only 3 RCTs and 5 nRCTs were included in this study.

3.3.1. NDI

From among the studies that have reported postoperative NDI scores, one study (*14*) was excluded owing to lack of data on standard deviation of NDI. The remaining five studies (*15-19*) reported the postoperative NDI for 378 patients. No significant difference was observed in the NDI scores of the ACDFWP and ACDA (MD = 0.15, 95% CI [−0.21, 0.51], $P = 0.40$, $I^2 = 0\%$) (Figure 4A).

3.3.2. VAS

Four studies (*14-16,19*) reported the postoperative VAS scores, within one study (*14*) was withdrawn for analysis because they did not report the standard deviation. The remaining three studies suggested non-significant differences in the VAS scores of the two groups (MD = −0.40, 95% CI [−0.83, 0.04], $P = 0.07$, $I^2 = 0\%$). We also introduced subgroups analysis and found no difference between the subgroups (Figure 4B).

3.3.3. JOA

Two studies reported the postoperative JOA scores (*17,18*), with 158 patients being enrolled. The pooled analysis showed no significant difference in the postoperative JOA scores of the ACDFWP and ACDA (MD = 0.05, 95% CI [−0.36, 0.46], P = 0.80, $I^2 = 0\%$) (Figure 4C).

3.3.4. Cervical alignment

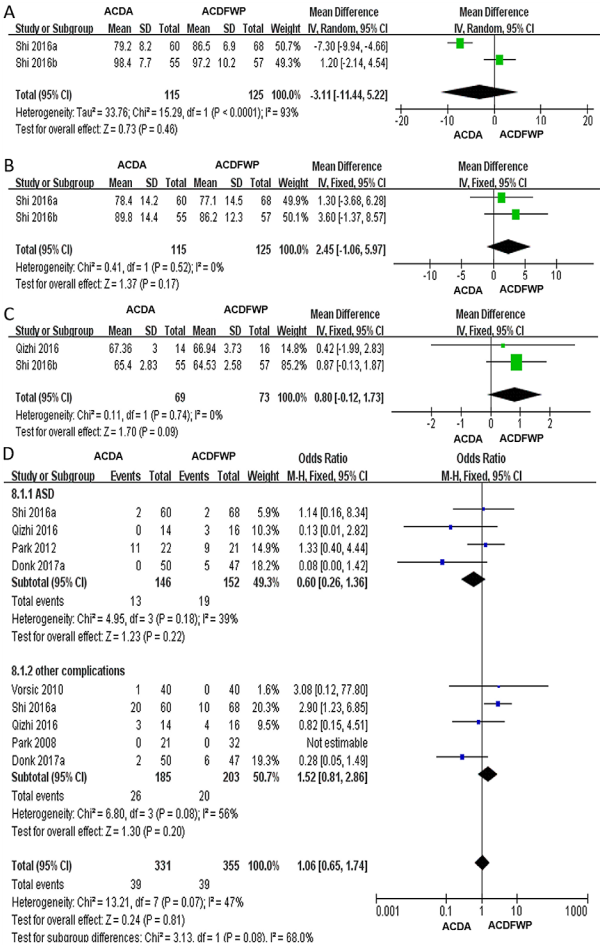


Figure 5. Meta-analyses of the other outcome indices of ACDA and ACDFWP. A. Forest plot for the operative time. **B.** Forest plot for the scores of blood loss. **C.** Forest plot for the scores of SWAL-QL. **D.** Forest plot for the scores of complications.

Six studies (*14,16-18,20,21*) reported the cervical alignment, while two (*14,16*) were excluded because they did not report the standard deviation. We conducted subgroups analyses to decrease the potential heterogeneity, and no significant differences were observed between the subgroups. Overall, the remaining four studies indicated that the cervical alignment was better in the ACDFWP (*vs.* ACDA, MD = −0.67, 95% CI [−1.11, −0.23], $P = 0.003$, $I^2 = 20\%$) (Figure 4D).

The assessment of postoperative clinical efficacy showed that the ACDFWP exhibited better efficacy only in the cervical alignment. No other significant difference was found in the other 3 indices.

3.4. Assessments of other clinical factors

3.4.1. Operation time

Four studies reported the operation time; two studies (*14,16*) were excluded owing to lack of data on standard deviation. Analysis of the remaining two studies (*18,20*) that included 240 patients found no difference between the ACDFWP and ACDA (MD = −3.11, 95% CI (−11.44, 5.22), $P = 0.46$, $I^2 = 93\%$) (Figure 5A).

3.4.2. Blood loss

Two studies reported blood loss (*18,20*). Total 240 patients were enrolled. However, no significant difference was identified between the ACDFWP and ACDA (MD = 2.45, 95% CI [−1.06, 5.97], $P = 0.17$, $I^2 = 0\%$) (Figure 5B).

3.4.3. Swallowing Quality of Life Score

SWAL-QL was reported in two studies (*17,20*) that involved 142 patients enrolled. No significant difference was found in the SWAL-QL of the two groups (MD = 0.80, 95% CI [−0.12, 1.73], P = 0.09, $I^2 = 0\%$) (Figure 5C).

3.4.4. Complications

Complications were reported in 6 studies (*14-19*) that involved 431 patients enrolled. No significant difference was observed in the complications between the two groups (MD = 1.06, 95% CI [0.65, 1.74], $P = 0.81$, $I^2 = 47\%$) (Figure 5D). Particularly, with regard to the frequency of occurrence of ASD, there were 19 cases in the ACDFWP and 13 cases in the ACDA, no significant difference was found between two groups (Z =1.23, $P = 0.22$) (Figure 5D).

No significant difference was found in the other clinical-related indices.

4. Discussion

The present study compared the efficacy and other factors associated with the clinical outcome of ACDFWP and ACDA. To our knowledge, this is the first meta-analysis to compare these two alternative surgeries. We found that the ACDFWP seems had better efficacy in terms of cervical alignment than that ACDA. No significant difference was found in the other indices. Our results suggest a slight superiority of ACDFWP. These findings might contribute to the selection of surgery type in patients with cervical spondylosis. However, the relatively small number of included studies lowered the evidence level.

In the present study, 8 studies (3 RCTs and 5 nRCTs, 640 patients enrolled) were evaluated. Although 3 RCTs were included, the quality of the included RCTs were satisfactory, and the quality of the included nRCTs was moderate (Figure 3). Evidence-based comparison of the studies has limited worthiness.

With respect to the comparisons of clinical efficacy, the indices of NDI (MD = 0.15, 95% CI [−0.21, 0.51], $P = 0.40$, $I^2 = 0\%$), VAS (MD = −0.40, 95% CI [−0.83, 0.04], $P = 0.07$, $I^2 = 0\%$), and JOA (MD = 0.05, 95% CI [−0.36, 0.46], $P = 0.80$, $I^2 = 0\%$) showed the same

tendency. The homogeneity of the involved studies was high. We did not find any difference between the two surgeries. However, the results of the cervical alignment (MD = −0.67, 95% CI [−1.11, −0.23], P = 0.003, I^2 = 20%) indicated better efficacy of ACDFWP (Figure 4). Under the support of cage, ACDF could achieve good operative alignment recovery.

With regard to the indices of other factors, we found no difference between the operation time [MD = −3.11, 95% CI (−11.44, 5.22), P = 0.46, I^2 = 93%], blood loss [MD = 2.45, 95% CI (−1.06, 5.97), P = 0.17, I^2 = 0%], SWAL-QL [MD = 0.80, 95% CI (−0.12, 1.73), P = 0.09, I^2 = 0%], and complications [MD = 1.06, 95% CI (0.65, 1.74), P = 0.81, I^2 = 47%]. There was no difference in the operation time and blood loss, postoperative QOL, and complications. ASD is the most important complication that must be considered while selecting the surgery. The aim of developing these alternative surgeries was to reduce the risk of complications, particularly, the onset of ASD because placing the anterior plate during the conventional ACDF is risky (*22,23*). It was reported that the incidence of ASD in ACDFWP was significantly lower than that in conventional ACDF (*24*). ACDA is another alternative surgery that is believed to lower the incidence of ASD onset. Previous evidence shows that ACDA contributes to maintain the motion at the involved segments and had a lower reoperation rate than those in conventional ACDF (*1,25-27*); a current study proved the long-term efficacy of ACDA in reducing the risk of ASD (*9*). However, our study found no difference between the ACDFWP and ACDA (X^2 = 4.95, P = 0.18, I^2 = 39%) (Figure 5).

However, conventional ACDF (with plate), alternative ACDFWP, and ACDA, all the existing surgeries have their specific advantages and disadvantages. *i*) Conventional ACDF *vs*. alternative ACDF: Although alternative ACDF achieved low onset of ASD, the conventional ACDF (with plate), as the gold standard therapy for cervical spondylosis, also has its merits. Current two studies indicated that conventional ACDF is good for ensuring greater restoration of alignment in the operative segment (*21,28*). A long-term follow-up study found that the operative segmental alignment gradually decreased, and the overall alignment increased with time (*21*). *ii*) Convention ACDF *vs*. ACDA: In addition to the low risk of ASD, ACDA has good efficacy. According to the results of several meta-analyses (*29,30*), the efficacy and safety of ACDA were superior to those of conventional ACDF. After a long-term follow-up, Ma *et al*. reported that ACDA was superior to conventional ACDF in terms of VAS and the overall success rate (*30*). Zhao *et al*. found that ACDA achieved a higher SF-36, a larger range of movement, a higher rate of neurological improvement, a lower VAS, a lower NDI, and a lower reoperation rate than conventional ACDF (*29*). Other studies have also indicated that ACDA was slightly better than the conventional ACDF in terms of the VAS score (*15,16,19*) and SWAL-QL (*17,20*). However, ACDA also has its disadvantages. Tian *et al*. reported that ACDA might contribute to promote the progression of heterotopic ossification, especially for patients who had been preoperatively developed (*31*). Latka *et al*. reported that ACDA involved a longer operation time and more blood loss than the conventional ACDF. A longer traction on the structures of the soft neck is a major disadvantage of ACDA (*9*). *iii*) Alternative ACDF *vs*. ACDA (the present study): We found that only the alternative ACDF exhibited a slight benefit in the cervical alignment as compared to ACDA. No significant difference was found in the other indices between alternative ACDF and ACDA. In sum, both the conventional and alternative surgeries have their strengths and weaknesses. Based on the evidence available today, they cannot replace each other. The appropriate surgery should be seriously selected according to the individual pathophysiological state of each patient.

There are certain limitations of this study, as follows: *i*) The small number of included studies; only 3 RCTs were included; only studies published in English were included. Two studies by the same authors were included, namely Shi 2016a (*20*) and Shi 2016b (*18*), and Donk 2017a (*19*) and Donk 2017b (*21*). Although we have investigated the studies and excluded the possibility of overlap of patients, but the data sources were too narrow, which lowered the reliability of the evidence. *ii*) The study quality of some the included studies was low. *iii*) The heterogeneity of some items (operation time, complications) was high, lowering the level of evidence. *iv*) Long-term observation was not performed in all included literatures, which is required on the study of ASD. More rigorous studies with long-term follow-up are expected to compare the alternative ACDF and ACDA, particularly for the onset of ASD.

5. Conclusions

In this study, we compared the clinical outcomes between the alternative ACDFWP and ACDA. We found that the ACDFWP exhibited slightly better cervical alignment than ACDA. No significant difference was found in the other items, including NDI, VAS, JOA, operation time, blood loss, SWAL-QL, and complications. These findings may contribute to the selection of the appropriate surgery for patients with cervical spondylosis. However, limited studies exist; thus, the evidence level is low. Larger, better-planned studies are required in the future.

Funding: This work was supported by Fujian Natural Science Foundation (2016J01560), grant from the Youth Program of the Health Commission of Fujian Province (2016-ZQN-73).

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Loumeau TP, Darden BV, Kesman TJ, Odum SM, Van Doren BA, Laxer EB, Murrey DB. A RCT comparing 7-year clinical outcomes of one level symptomatic cervical disc disease (SCDD) following ProDisc-C total disc arthroplasty (TDA) versus anterior cervical discectomy and fusion (ACDF). Eur Spine J. 2016; 25:2263-2270.
2. Nanda A, Sharma M, Sonig A, Ambekar S, Bollam P. Surgical complications of anterior cervical diskectomy and fusion for cervical degenerative disk disease: a single surgeon's experience of 1,576 patients. World Neurosurg. 2014; 82:1380-1387.
3. Laxer EB, Brigham CD, Darden BV, Bradley Segebarth P, Alden Milam R, Rhyne AL, Odum SM, Spector LR. Adjacent segment degeneration following ProDisc-C total disc replacement (TDR) and anterior cervical discectomy and fusion (ACDF): does surgeon bias effect radiographic interpretation? Eur Spine J. 2017; 26:1199-1204.
4. Hofstetter CP, Kesavabhotla K, Boockvar JA. Zero-profile Anchored Spacer Reduces Rate of Dysphagia Compared With ACDF With Anterior Plating. J Spinal Disord Tech. 2015; 28:E284-290.
5. Kepler CK, Hilibrand AS. Management of adjacent segment disease after cervical spinal fusion. Orthop Clin North Am. 2012; 43:53-62, viii.
6. Hilibrand AS, Carlson GD, Palumbo MA, Jones PK, Bohlman HH. Radiculopathy and myelopathy at segments adjacent to the site of a previous anterior cervical arthrodesis. J Bone Joint Surg Am. 1999; 81:519-528.
7. Helgeson MD, Bevevino AJ, Hilibrand AS. Update on the evidence for adjacent segment degeneration and disease. Spine J. 2013; 13:342-351.
8. Cheung ZB, Gidumal S, White S, Shin J, Phan K, Osman N, Bronheim R, Vargas L, Kim JS, Cho SK. Comparison of Anterior Cervical Discectomy and Fusion With a Stand-Alone Interbody Cage Versus a Conventional Cage-Plate Technique: A Systematic Review and Meta-Analysis. Global Spine J. 2019; 9:446-455.
9. Latka D, Kozlowska K, Miekisiak G, Latka K, Chowaniec J, Olbrycht T, Latka M. Safety and efficacy of cervical disc arthroplasty in preventing the adjacent segment disease: a meta-analysis of mid- to long-term outcomes in prospective, randomized, controlled multicenter studies. Ther Clin Risk Manag. 2019; 15:531-539.
10. Maharaj MM, Mobbs RJ, Hogan J, Zhao DF, Rao PJ, Phan K. Anterior cervical disc arthroplasty (ACDA) versus anterior cervical discectomy and fusion (ACDF): a systematic review and meta-analysis. J Spine Surg. 2015; 1:72-85.
11. Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, Clarke M, Devereaux PJ, Kleijnen J, Moher D. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ. 2009; 339:b2700.
12. Sterne JA, Hernan MA, Reeves BC, *et al*. ROBINS-I: a tool for assessing risk of bias in non-randomised studies of interventions. BMJ. 2016; 355:i4919.
13. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JA, Cochrane Bias Methods G, Cochrane Statistical Methods G. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. BMJ. 2011; 343:d5928.
14. Park JH, Roh KH, Cho JY, Ra YS, Rhim SC, Noh SW. Comparative analysis of cervical arthroplasty using Mobi-c® and anterior cervical discectomy and fusion using the Solis® -cage. J Korean Neurosurg Soc. 2008; 44:217-221.
15. Vorsic M, Bunc G. ProDisc-C versus fusion with Cervios chronOS prosthesis in cervical degenerative disc disease: Is there a difference at 12 months? Evid Based Spine Care J. 2010; 1:51-56.
16. Park JY, Kim KH, Kuh SU, Chin DK, Kim KS, Cho YE. What are the associative factors of adjacent segment degeneration after anterior cervical spine surgery? Comparative study between anterior cervical fusion and arthroplasty with 5-year follow-up MRI and CT. Eur Spine J. 2012; 22:1078-1089.
17. Qizhi S, Lei S, Peijia L, Hanping Z, Hongwei H, Junsheng C, Jianmin L. A Comparison of Zero-Profile Devices and Artificial Cervical Disks in Patients With 2 Noncontiguous Levels of Cervical Spondylosis. Clin Spine Surg. 2016; 29:E61-66.
18. Shi S, Zheng S, Li XF, Yang LL, Liu ZD, Yuan W. Comparison of 2 Zero-Profile Implants in the Treatment of Single-Level Cervical Spondylotic Myelopathy: A Preliminary Clinical Study of Cervical Disc Arthroplasty versus Fusion. PloS One. 2016; 11:e0159761.
19. Donk R, Verbeek A, Verhagen W, Groenewoud H, Hosman A, Bartels R. What's the best surgical treatment for patients with cervical radiculopathy due to single-level degenerative disease? A randomized controlled trial. PloS One. 2017; 12:e0183603.
20. Shi S, Li XF, Zhao QT, Yang LL, Liu ZD, Yuan W. Risk Factors for Dysphagia After Single-Level Anterior Cervical Decompression with Arthroplasty or Fusion: A Prospective Study Comparing 2 Zero-Profile Implants. World Neurosurg. 2016b; 95:148-155.
21. Donk RD, Arnts H, Verhagen WIM, Groenewoud H, Verbeek A, Bartels RHMA. Cervical sagittal alignment after different anterior discectomy procedures for single-level cervical degenerative disc disease: randomized controlled trial. Acta Neurochir (Wien). 2017; 159:2359-2365.
22. Burkhardt BW, Simgen A, Wagenpfeil G, Reith W, Oertel JM. Adjacent Segment Degeneration After Anterior Cervical Discectomy and Fusion With an Autologous Iliac Crest Graft: A Magnetic Resonance Imaging Study of 59 Patients With a Mean Follow-up of 27 Years. Neurosurgery. 2018; 82:799-807.
23. Alhashash M, Shousha M, Boehm H. Adjacent Segment Disease After Cervical Spine Fusion Evaluation of a 70 Patient Long-Term Follow-Up. Spine. 2018; 43:605-609.
24. Lu Y, Bao W, Wang Z, Zhou F, Zou J, Jiang W, Yang H, Zhang Z, Zhu X. Comparison of the clinical effects of zero-profile anchored spacer (ROI-C) and conventional cage-plate construct for the treatment of noncontiguous bilevel of cervical degenerative disc disease (CDDD): A minimum 2-year follow-up. Medicine (Baltimore). 2018; 97:e9808.
25. Pisano A, Helgeson M. Cervical disc replacement surgery: biomechanical properties, postoperative motion, and postoperative activity levels. Curr Rev Musculoskelet Med. 2017; 10:177-181.
26. Hisey MS, Bae HW, Davis RJ, Gaede S, Hoffman G, Kim KD, Nunley PD, Peterson D, Rashbaum RF, Stokes J, Ohnmeiss DD. Prospective, Randomized Comparison of Cervical Total Disk Replacement Versus Anterior Cervical Fusion: Results at 48 Months Follow-up. J Spinal Disord

Tech. 2015; 28:E237-243.
27. Yang X, Janssen T, Arts MP, Peul WC, Vleggeert-Lankamp CLA. Radiological follow-up after implanting cervical disc prosthesis in anterior discectomy: a systematic review. Spine J. 2018.
28. Di Martino A, Papalia R, Albo E, Cortesi L, Denaro L, Denaro V. Cervical spine alignment in disc arthroplasty: should we change our perspective? Eur Spine J. 2015; 24 Suppl 7:810-825.
29. Zhao GS, Zhang Q, Quan ZX. Mid-term efficacy and safety of cervical disc arthroplasty versus fusion in cervical spondylosis: A systematic review and meta-analysis. Biomed Rep. 2017; 6:159-166.
30. Ma Z, Ma X, Yang HL, Guan XM, Li X. Anterior cervical discectomy and fusion versus cervical arthroplasty for the management of cervical spondylosis: A meta-analysis. Eur Spine J. 2017; 26:998-1008.
31. Tian W, Han X, Liu B, He D, Lv Y, Yue J. Generation and Development of Paravertebral Ossification in Cervical Artificial Disk Replacement. Clin Spine Surg. 2017; 30:E179-E188.

Received July 26, 2022; Revised August 9, 2022; Accepted August 10, 2022.

[§]These authors contributed equally to this work.

**Address correspondence to:*
Hongmei Song, Division of Science and Education, The Second People's Hospital Affiliated to Fujian University of Traditional Chinese Medicine, No. 282 Wusibei Road, Fuzhou 353003, China.
E-mail: 13305912709@163.com

Tetsuya Asakawa, Institute of Neurology, The Third People's Hospital of Shenzhen, 29 Buji Bulan Road, Shenzhen, Guangdong 518112, China.
E-mail: asakawat1971@gmail.com

Released online in J-STAGE as advance publication August 12, 2022.

Mini-Review

DOI: 10.5582/irdr.2022.01060

Smooth muscle motility disorder phenotypes: A systematic review of cases associated with seven pathogenic genes (*ACTG2*, *MYH11*, *FLNA*, *MYLK*, *RAD21*, *MYL9* and *LMOD1*)

Ninon Fournier[1], Alexandre Fabre[1,2,*]

[1] APHM, Timone Enfant, Pediatric Multidisciplinary Department, Marseille, France;
[2] Aix-Marseille Université, INSERM, GMGF, Marseille, France.

SUMMARY Smooth muscle disorders affecting both the intestine and the bladder have been known for a decade. However, the recent discovery of genes associated with these dysfunctions has led to the description of several clinical phenotypes. We performed a systematic review of all published cases involving seven genes with pathogenic variants, *ACTG2*, *MYH11*, *FLNA*, *MYLK*, *RAD21*, *MYL9* and *LMOD1*, and included 28 articles describing 112 patients and 5 pregnancies terminated before birth. The most commonly described mutations involved *ACTG2* (75/112, 67% of patients), *MYH11* (14%) and *FLNA* (13%). Twenty-seven patients (28%) died at a median age of 14.5 months. Among the 76 patients for whom this information was available, 10 (13%) had isolated chronic intestinal pseudo-obstruction (CIPO), 17 (22%) had isolated megacystis, and 48 (63%) had combined CIPO and megacystis. The respective proportions of these phenotypes were 9%, 20% and 71% among the 56 patients with *ACTG2* mutations, 20%, 20% and 60% among the 10 patients with *MYH11* mutations and 50%, 50% and 0% among the 7 patients with *FLNA* mutations.

Keywords smooth muscle motility disorders, chronic intestinal pseudo-obstruction, megacystis, ACTG2, MYH11, FLNA, MYLK, RAD21, MYL9, LMOD1, mutations

1. Introduction

Smooth muscle motility disorders are a set of congenital diseases often associated with chronic intestinal pseudo obstruction (CIPO), hypoperistalsis, megacystis and/or microcolon (*1-3*). There are no pathognomonic signs and until recently, these disorders could only be diagnosed clinically, often at birth but sometimes prenatally (*4,5*). The characteristic symptom is abdominal distension, suggestive of mechanical obstruction but without radiologically or surgically detectable mechanical obstruction or any organic, systemic or metabolic disease (*6,7*). The rarity of these conditions means that epidemiological data are scarce and only small case series have been reported (*8*). In the last few years however, several genes with pathogenic variants that encode proteins involved in smooth muscle contraction have been associated with these phenotypes: *ACTG2* (*9-24*), *MYH11* (*24-28*), LMOD1 (*29*), *MYLK* (*30*), MYL9 (*31*), *FLNA* (*32-34*) and *RAD21* (*35*). Little is known about the different phenotypes or their evolution. We therefore performed a systematic review of all published cases of smooth muscle motility disorders associated with mutations in these seven genes to summarize current genetic knowledge and provide an overview of the clinical phenotypes, treatment methods and outcomes of these congenital diseases in terms of the associated mutations.

2. Literature search strategies and analysis methods

2.1. Search strategies

The literature review was conducted according to PRISMA guidelines. A MEDLINE (PubMed) search was performed on 2 October 2020 using the following terms: "ACTG2 CIPO", "ACTG2 hypoperistalsis", "ACTG2 smooth muscle motility disorders", "MYH11 CIPO", "MYH11 hypoperistalsis", "MYH11 smooth muscle motility disorders", "FLNA hypoperistalsis", "FLNA CIPO", "FLNA smooth muscle motility disorders", "MYLK CIPO", "MYLK hypoperistalsis", "MYLK smooth muscle motility disorders", "RAD21 CIPO", "RAD21 hypoperistalsis", "RAD21 smooth muscle motility disorders", "MYL9 hypoperistalsis",

"MYL9 CIPO", "MYL9 smooth muscle motility disorders", "LMOD1 CIPO", "LMOD1 hypoperistalsis", and "LMOD1 smooth muscle motility disorders".

Another search was performed on March 2020 in Google Scholar for articles cited by Halim *et al.* 2017 (*29*), Moreno *et al.* 2018 (*31*), Gauthier *et al.* 2015, Bonora *et al.* 2015 (*33*), Oda *et al.* 2016 (*34*), and Wangler *et al.* 2014 (*10*).The resulting articles were included if they contained a clinical description of patients with mutations in one of the seven considered genes. No ethics approval was required under French law as the study only involved data analysis. Database data were used in accordance with the corresponding data use agreements.

2.2. Selection and description of cases

Cases were included if they were associated with mutations in either *ACTG2*, *MYH11*, *FLNA*, *MYLK*, *RAD21*, *MYL9* or *LMOD1* and if gastrointestinal or urologic symptoms were described. The data collected were the patients' sex, anthropometric data at birth and at last follow-up, prenatal manifestations (megacystis, hydronephrosis, dilated bowel), and digestive and urinary manifestations (signs of CIPO, presence of megacystis, description of colon). The mode of inheritance of the mutation, use and duration of parenteral nutrition, need for ostomy, bladder catheterization or vesicostomy, and mortality and cause of death were also recorded.

2.3. Statistical analysis

Statistical analyses were performed using biostatgv (*https://biostatgv.sentiweb.fr/*). Variables were expressed as number, percentage and median. Birth weight percentiles were calculated using Audipog (*https://www.audipog.net/Courbes-morpho*). Weight Z-scores were calculated using Peditool (*https://www.peditools.org/*). P-values < 0.05 were considered statistically significant. Protein–protein interaction networks were built using the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins; *https://string-db.org/*) database, using defaults settings with the highest confidence score (0.9) and without text mining.

3. Results

3.1. Population

Sixty-one articles were retrieved using the search terms, of which 28 satisfied the inclusion criteria (Supplemental Figure S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=119*). Among the 117 cases described (61 male, 53 female, 3 of unknown sex), 5 (2 male, 2 female and 1 of unknown sex) were only included in the analysis of prenatal signs because the corresponding pregnancies were terminated before birth. The patients' clinical characteristics are listed by gene mutation in Table 1. Follow-up information was available for 83 patients. The median age at last follow-up was 16.5 years (198 months; range, 0.2–882 months). The mortality rate was 28% (the information of the outcome was known for 95 patients, among them 27 patients have died), with a median age at death of 14.5 months (range, 0.2–414 months).

3.2. Mortality

The outcome of 17 patients (15%) was unknown. The mortality rate was 8.8 per 1,000 patients-years. Information on the cause of death was available in 16/27 cases: four patients each died of sepsis, multisystem organ failure, and single organ failure (liver failure, pancreatitis, cardiac arrest, and respiratory failure), two patients died after treatment was discontinued, one died of surgical complications and for one there was no obvious cause of the death. The Kaplan–Meier survival curve is shown in Supplemental Figure S2 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=119*).

3.3. Prenatal signs

Prenatal signs were described for 58/117 patients (50%), including 44/77 (57%) of those with an *ACTG2* mutation, 5/18 (28%) of those with an *MYH11* mutation, 4/14 (29%) of those with a *FLNA* mutation and all 5 patients with a *MYLK*, *MYL9* or *LMOD1* mutation (Supplemental Table S2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=119*). The prenatal signs were mainly in the urinary tract: megacystis (55 patients), hydronephrosis (14 patients), polyhydramnios (13 patients) and oligohydramnios (11 patients). Prenatal gastrointestinal signs such as a dilated or echogenic bowel were reported in six patients. For the 14 patients with available data the median birth term was 38 weeks and the median birthweight Z-score was 0.175 (Supplemental Table S3, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=119*).

3.4. Pattern of inheritance

The inheritance pattern of the *ACTG2* mutations was in most cases (54/77) autosomal dominant but five cases involved a family history of the mutation. The inheritance of the *MYH11*, *MYLK*, *MYL9* and *RAD21* mutations was autosomal recessive in 20/36 cases. X-linked recessive inheritance was described for one *FLNA* mutation.

3.5. Phenotypes

Information on the patients' clinical symptoms, including bladder and/or intestinal involvement, was available for 76 patients (Supplementary

Table 1. Patient clinical characteristics according to gene mutation

Gene	*n*	Male/Female	Alive/Dead	Age at last follow-up (months)	Age at death (months)	Death rate per 1,000 patients-year[a]	Prenatal signs[b], yes/no	CIPO yes/no	MEGACYSTIS yes/no	Colon micro/normal/mega
ACTG2	75 (67%)	33/40 (44%)	45/20	138 (0.2-882, *n* = 54)	21.5 (0.2-414, *n* = 12)	13.51	44/6	56/11	59/5	21/24/2
MYH11	16 (14.3%)	10/6 (63%)	14/2	378 (0.5-846, *n* = 16)	9.25 (0.5-18, *n* = 2)	3.52	5/0	14/2	8/2	3/-/-
FLNA	14 (12.5%)	13/1 (93%)	7/0	240 (72-618, *n* = 7)	-	0	4/0	7/7	3/2	-
MYLK	2 (1.8%)	1/1 (50%)	0/2	0.5 (*n* = 1)	0.5 (*n* = 1)	-	3/0	2/0	1/0	1/-/-
RAD21	3 (2.7%)	2/1 (67%)	2/1	354 (318-366, *n* = 3)	354 (*n* = 1)	11.6	-	3/0	-	-
MYL9	1 (0.9%)	0/1	0/1	20 (*n* = 1)	20 (*n* = 1)	-	1/0	1/0	1/0	1/0/0
LMOD1	1 (0.9%)	0/1	0/1	0.2 (*n* = 1)	0.2 (*n* = 1)	-	1/0	0/1	1/0	1/0/0
TOTAL	112	59/51 (54%)	68/27 (72%)	198 (0.2-882, *n* = 83)	14.5 (0.2-414, *n* = 18)	8.8/1000 patients per year	58/6 (91%)	83/21 (80%)	73/9 (89%)	27/24/2 (51%)

Data are expressed as number (percentage) or median (range, number of observations). *calculated only if more than 2 patients.CIPO, chronic intestinal pseudo-obstruction.

Table 1. Patient clinical characteristics according to gene mutation (continued)

Gene	Parenteral nutrition yes/no	Age at start of parenteral nutrition (months)	Parenteral nutrition weaning yes/no	Age at parenteral nutrition weaning (months)	Stomy yes/no	Ileostomy yes/no	Bladder catheterization or vesicostomy yes/no
ACTG2	39/9	0 (0-42, *n* = 19)	2/25	90 (*n* = 1)	16/42	29/28	36/3
MYH11	3/6	0 (0-0, *n* = 2)	0/2	-	1/7	1/7	1/0
FLNA	4/0	0 (0-0, *n* = 2)	1/1	9 (*n* = 1)	2/5	0/2	3/2
MYLK	-	-	-	-	0/1	1/0	1/0
RAD21	1/0	-	0/1	-	0/2	0/2	-/-
MYL9	1/0	0 (*n* = 1)	0/1	-	1/0	1/0	1/0
LMOD1	1/0	0 (*n* = 1)	0/1	-	0/1	0/1	1/0
TOTAL	49/15 (77%)	0 (0-42, *n* = 25)	3/31 (9 %)	49.5 (9-90, *n* = 2)	20/58 (26%)	32/40 (44%)	43/5 (90%)

Data are expressed as number (percentage) or median (range, number of observations). *calculated only if more than 2 patients.CIPO, chronic intestinal pseudo-obstruction.

Table S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=119*). Figure 1 shows a Venn diagram of the most common clinical phenotypes, along with separate diagrams for the three most commonly associated genes, *ACTG2*, *MYH11* and *FLNA*. The most common phenotype was CIPO associated with megacystis (in 68% of patients whose symptoms were described). A few patients with *FLNA* and *LMOD1* mutations had isolated CIPO and 4/10 patients with a *MHY11* mutation had either isolated CIPO (n = 2) or isolated megacystis (n = 2). Descriptions of the bladder (normal or enlarged) were lacking for all three patients with *RAD21* mutations; none of these patients had CIPO (Supplemental Table S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=119*).

The colon was described in 47/75 patients with an *ACTG2* mutation, among which 21 had microcolon, but only in 3/16 patients with a *MYH11* mutation, all three of whom had microcolon. Descriptions of the colon were given for all two patients with either a *MYL9* or *LMOD1*, and it was a microcolon for each. Only one description of the colon (microcolon) over the two patients for *MYLK* mutation. The colon was not described for any of the 17 patients with *FLNA* (n = 14) or *RAD21* (n = 3) mutations.

3.6. Managements

Total parenteral nutrition (TPN) was known for 64/112 patients and required in 49/64 patients (77%), 39 of whom had an *ACTG2* mutation. TPN was typically started soon after birth (median age at start, 0 months; range, 0.2–42 months, n = 25) and weaning, when mentioned (n = 34), was only achieved in 3 patients (9%). The age at weaning was only described for two patients, who were respectively weaned at 9 and 90 months. Intermittent catheterization or vesicostomy to ensure bladder decompression and prevent renal scarring or failure was mentioned for 48 patients, and performed for 43 of these (90%). Surgery was mentioned in 84 cases and performed for 71 of these patients (85%).

3.7. Protein interactions

Predicted protein-protein interactions using the STRING database indicate that five of the seven analyzed genes (*ACTG2*, *MYH11*, *MYLK*, *MYL9* and *LMOD1*) are functionally associated as part of a larger unit (Supplementary Figure 3, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=119*). The networks built separately for each protein in turn show that LMOD1 interacts with all four other proteins, while the remaining four (ACTG2, MYH11, MYL9 and MYLK) only interact with three of the other four. This analysis also confirms that RAD21 and FLNA do not interact with the other proteins. Note that three proteins appear in several networks: ACTA2 and TPM2 interact with ACTG2, MYH11, MYL9 and LMOD1, and TPM1 interacts with ACTG2, MYL9 and LMOD1.

4. Discussion

In this study, we reviewed all 117 published cases of smooth muscle motility disorder linked to seven genes with pathogenic mutations: *ACTG2*, *MYH11*, *FLNA*, *MYLK*, *RAD21*, *MYL9* and *LMOD1*. The main finding is that the most commonly described phenotype is combined CIPO and megacystis, similar to what has been described for megacystis microcolon intestinal hypoperistalsis syndrome (MMIHS) (*1,2,4*) — although microcolon was not systematically reported in the cases reviewed here — and to pediatric intestinal pseudo-obstruction (PIPO) (*36*).

We identified five major genes implicated in these cases, *ACTG2*, *MYH11*, *MYLK*, *MYL9* and *LMOD1*, as noticed previously by Ambartsumyan *et al.* (*6*). We started our analysis with the seven genes associated in the literature with smooth muscle motility disorder, and then classified the phenotypes of the corresponding patients, rather than starting with known phenotypes (such as MMIHS, CIPO, multisystemic smooth muscle dysfunction syndrome, hollow visceral myopathy, prune belly syndrome) to identify genes as done in previous studies. This is a major strength of this study, one of the largest reviews of published cases of smooth muscle motility disorder. Our results confirm that there is high phenotype variability for all the considered gene mutations, which clearly limits the possibility of associating distinct forms of smooth muscle motility disorder with particular genes. Note that other phenotypes, such as isolated megacystis and isolated CIPO, are also possible.

In a systematic review of patient outcomes in MMIHS (*37*), Gosemann and Puri found that 80% of children with MMIHS died before adulthood (the oldest MMIHS patents alive were reported to be 19 and 24 years old). The mortality rate in the cases reviewed here was 28%, with 84% survival at five years and 80% at ten years. The major causes of death were sepsis and multisystemic organ failure, probably as a result of improved management of these diseases. Soh *et al.* (*38*) found likewise in their 2015 review of cases in Japan, with 42% of children diagnosed with MMIHS dying of enteritis or sepsis and five and ten-year survival rates of 63% and 57%. Further studies to explore the outcomes and clinical effectiveness of total parenteral nutrition and with multiorgan transplantation with multidisciplinary care are required to evaluate current survival rates, even if no specialized treatment is yet available. Our review confirms that smooth muscle motility disorders are severe diseases, with total parenteral nutrition required in many cases and weaning

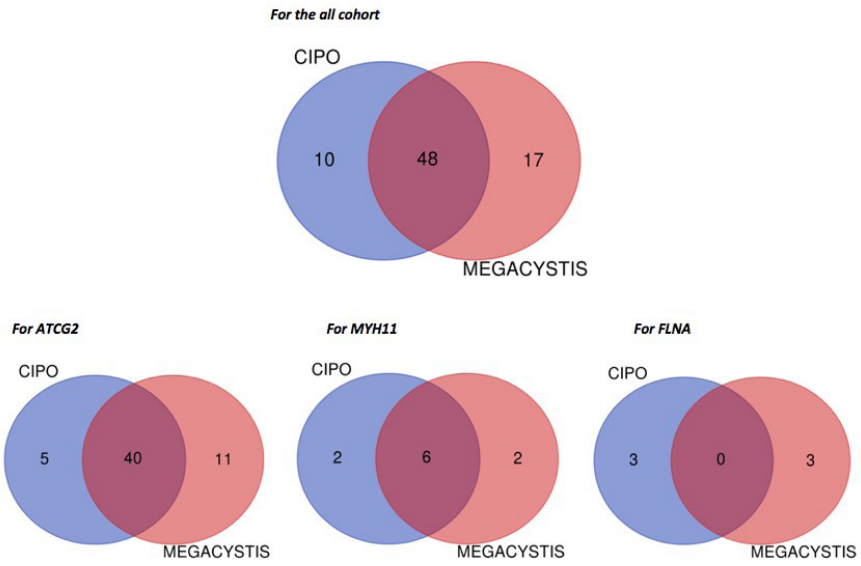


Figure 1. Venn diagrams of the most common clinical phenotypes among 112 patients with a pathogenic variant of *ACTG2*, *MYH11*, *FLNA*, *MYLK*, *RAD21*, *MYL9* or *LMOD1*, and separate diagrams for the three most commonly associated genes.

very rare (in only 9% of cases this series). It is unclear whether the better outcomes identified in this study are due to gene analyses or to improved care in the past few years.

The limitations of this study include different levels of missing patient data in the included articles, and the fact that cases were selected based on gastrointestinal and/or urological symptoms only, meaning, for example, that patients with an *FLNA* mutation but only neurological symptoms were not included. Our results may also be biased by the initial distribution of the genes with 67% of cases associated with an *ACTG2* mutation, the main gene encoding the smooth muscle actin found in enteric tissues, 14% associated with *MYH11* mutations, and 13% with *FLNA*, while only 6% of cases involved *MYLK*, *MYL9*, *RAD21* or *LMOD1* mutations.

Our analysis based on the STRING database suggests the five main genes (*ACTG2*, *MYH11*, *MYLK*, *MYL9* and *LMOD1*) are related as part of a protein interaction network, the same five genes identified by Ambartsumyan *et al.* (*6*) in cases of MMIHS. These proteins also share interaction partners, namely ACTA2, TPM1, TPM2. An *ACTA2* mutation has been described in a case of multisystemic smooth muscle dysfunction (*24*), a severe phenotype with visceral myopathy. Heterozygous variants of *ACTA2* were first described in individuals with familial thoracic aortic aneurysm (*39*), a phenotype also described for pathogenic *MYH11* variants. It would be interesting to see if the two other shared interaction partners, TPM1 and TPM2, are also implicated in other forms of motility disorders or might be predicted to be if no case has yet been described in the literature.

In conclusion, smooth muscle motility disorders are rare, often unrecognized, severe diseases, due to impaired smooth muscle function, whose complications are disabling and can be life-threatening in short to medium term. Our review highlights the variability of clinical phenotypes for each gene mutation, preventing any simple gene–phenotype association. Multigene panel testing of *ACTG2*, *MYH11*, *MYLK*, *MYL9* and *LMOD1* should be considered in patients with hypoperistalsis, signs of CIPO and/or enlarged bladder, or with prenatal signs such as megacystis. The variability of symptoms makes this group of smooth muscle motility disorders a diagnostic challenge but information on associated genetic variants should facilitate diagnosis and classification.

Acknowledgements

We thank Paul Guerry (Green Grow Scientific) for editing the article.

Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Berdon WE, Baker DH, Blanc WA, Gay B, Santulli TV, Donovan C. Megacystis-microcolon-intestinal hypoperistalsis syndrome: A new cause of intestinal obstruction in the newborn. Report of radiologic findings in five newborn girls. AJR Am J Roentgenol. 1976; 126:957-964
2. Puri P, Shinkai M. Megacystis microcolon intestinal hypoperistalsis syndrome. Semin Pediatr Surg. 2005;

14:58-63.
3. Thapar N, Saliakellis E, Benninga MA, Borrelli O, Curry J, Faure C, De Giorgio R, Gupte G, Knowles CH, Staiano A, Vandenplas Y, Di Lorenzo C. Paediatric intestinal pseudo-obstruction: Evidence and consensus-based recommendations from an ESPGHAN-led expert group. J Pediatr Gastroenterol Nutr. 2018; 66:991-1019.
4. Lashley DB, Masliah E, Kaplan GW, McAleer IM. Megacystis microcolon intestinal hypoperistalsis syndrome: bladder distension and pyelectasis in the fetus without anatomic ooutflow obstruction. Urology. 2000; 55:774.
5. Tuzovic L, Anyane-Yeboa K, Mills A, Glassberg K, Miller R. Megacystis-microcolon-intestinal hypoperistalsis syndrome: Case report and review of prenatal ultrasonographic findings. Fetal Diagn Ther. 2014; 36:74-80.
6. Ambartsumyan L. Megacystis-Microcolon-Intestinal Hypoperistalsis Syndrome Overview. 2019. In: Adam MP, Ardinger HH, Pagon RA, *et al.*, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2021.
7. El-Chammas K, Sood MR. Chronic intestinal pseudo-obstruction. Clin Colon Rectal Surg. 2018; 31:99-107.
8. Wymer KM, Anderson BB, Wilkens AA, Gundeti MS. Megacystis microcolon intestinal hypoperistalsis syndrome: Case series and updated review of the literature with an emphasis on urologic management. J Pediatr Surg. 2016; 51:1565-1573.
9. Thorson W, Diaz-Horta O, Foster J 2nd, Spiliopoulos M, Quintero R, Farooq A, Blanton S, Tekin M. De novo ACTG2 mutations cause congenital distended bladder, microcolon, and intestinal hypoperistalsis. Hum Genet. 2014; 133:737-742.
10. Wangler MF, Gonzaga-Jauregui C, Gambin T, *et al.* Heterozygous de novo and inherited mutations in the smooth muscle actin (ACTG2) gene underlie megacystis-microcolon-intestinal hypoperistalsis syndrome. PLoS Genet. 2014; 10:e1004258.
11. Klar J, Raykova D, Gustafson E, Tóthová I, Ameur A, Wanders A, Dahl N. Phenotypic expansion of visceral myopathy associated with ACTG2 tandem base substitution. Eur J Hum Genet. 2015; 23:1679-1683.
12. Tuzovic L, Tang S, Miller RS, *et al.* New insights into the genetics of fetal megacystis: ACTG2 mutations, encoding γ-2 smooth muscle actin in megacystis microcolon intestinal hypoperistalsis syndrome (Berdon Syndrome). Fetal Diagn Ther. 2015; 38:296-306.
13. Wangler MF, Beaudet AL. ACTG2-Related Disorders. 2015. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993–2020.
14. Halim D, Hofstra RM, Signorile L, *et al.* ACTG2 variants impair actin polymerization in sporadic megacystis microcolon intestinal hypoperistalsis syndrome. Hum Mol Genet. 2016; 25:571-583.
15. Lu W, Xiao Y, Huang J, Tao Y, Yan W, Lu L, Cao Y, Cai W. Mutation in actin γ-2 Responsible for Megacystis Microcolon Intestinal Hypoperistalsis Syndrome in 4 Chinese Patients. J Pediatr Gastroenterol Nutr. 2016; 63:624-626.
16. Matera I, Rusmini M, Guo Y, *et al.* Variants of the ACTG2 gene correlate with degree of severity and presence of megacystis in chronic intestinal pseudo-obstruction. Eur J Hum Genet. 2016; 24:1211-5.
17. Ravenscroft G, Pannell S, O'Grady G, *et al.* Variants in ACTG2 underlie a substantial number of Australasian patients with primary chronic intestinal pseudo-obstruction. Neurogastroenterol Motil. 2018; 30:e13371.
18. Whittington JR, Poole AT, Dutta EH, Munn MB. A Novel mutation in ACTG2 gene in mother with chronic intestinal pseudoobstruction and fetus with megacystis microcolon intestinal hypoperistalsis syndrome. Case Rep Genet. 2017; 2017:9146507.
19. Collins RRJ, Barth B, Megison S, Pfeifer CM, Rice LM, Harris S, Timmons CF, Rakheja D. ACTG2-associated visceral myopathy with chronic intestinal pseudoobstruction, intestinal malrotation, hypertrophic pyloric stenosis, choledochal cyst, and a novel missense mutation. Int J Surg Pathol. 2019; 27:77-83.
20. Milunsky A, Baldwin C, Zhang X, Primack D, Curnow A, Milunsky J. Diagnosis of chronic intestinal pseudo-obstruction and megacystis by sequencing the ACTG2 Gene. J Pediatr Gastroenterol Nutr. 2017; 65:384-387.
21. Milunsky A, Lazier J, Baldwin C, Young C, Primack D, Milunsky JM. Prenatal diagnosis of chronic intestinal pseudo-obstruction and paternal somatic mosaicism for the ACTG2 pathogenic variant. Prenat Diagn. 2017; 37:1254-1256.
22. Korğalı EÜ, Yavuz A, Şimşek CEÇ, Güney C, Kurtulgan HK, Başer B, Atalar MH, Özer H, Eğilmez HR. Megacystis microcolon intestinal hypoperistalsis syndrome in which a different de novo Actg2 gene mutation was detected: A case report. Fetal Pediatr Pathol. 2018; 37:109-116.
23. Assia Batzir N, Kishor Bhagwat P, Larson A, *et al.* Recurrent arginine substitutions in the *ACTG2* gene are the primary driver of disease burden and severity in visceral myopathy. Hum Mutat. 2020; 41:641-654.
24. Moreno CA, Metze K, Lomazi EA, Bertola DR, Barbosa RH, Cosentino V, Sobreira N, Cavalcanti DP. Visceral myopathy: Clinical and molecular survey of a cohort of seven new patients and state of the art of overlapping phenotypes. Am J Med Genet A. 2016; 170:2965-2974.
25. Dong W, Baldwin C, Choi J, Milunsky JM, Zhang J, Bilguvar K, Lifton RP, Milunsky A. Identification of a dominant MYH11 causal variant in chronic intestinal pseudo-obstruction: Results of whole-exome sequencing. Clin Genet. 2019; 96:473-477.
26. Kloth K, Renner S, Burmester G, Steinemann D, Pabst B, Lorenz B, Simon R, Kolbe V, Hempel M, Rosenberger G. 16p13.11 microdeletion uncovers loss-of-function of a MYH11 missense variant in a patient with megacystis-microcolon- intestinal-hypoperistalsis syndrome. Clin Genet. 2019; 96:85-90.
27. Wang Q, Zhang J, Wang H, Feng Q, Luo F, Xie J. Compound heterozygous variants in MYH11 underlie autosomal recessive megacystis-microcolon-intestinal hypoperistalsis syndrome in a Chinese family. J Hum Genet. 2019; 64:1067-1073.
28. Gilbert MA, Schultz-Rogers L, Rajagopalan R, Grochowski CM, Wilkins BJ, Biswas S, Conlin LK, Fiorino KN, Dhamija R, Pack MA, Klee EW, Piccoli DA, Spinner NB. Protein-elongating mutations in MYH11 are implicated in a dominantly inherited smooth muscle dysmotility syndrome with severe esophageal, gastric, and intestinal disease. Hum Mutat. 2020; 41:973-982.
29. Halim D, Wilson MP, Oliver D, *et al.* Loss of LMOD1 impairs smooth muscle cytocontractility and causes

megacystis microcolon intestinal hypoperistalsis syndrome in humans and mice. Proc Natl Acad Sci U S A. 2017; 114: E2739-E2747.
30. Halim D, Brosens E, Muller F, *et al.* Loss-of-Function Variants in MYLK Cause Recessive Megacystis Microcolon Intestinal Hypoperistalsis Syndrome. Am J Hum Genet. 2017; 101:123-129.
31. Moreno CA, Sobreira N, Pugh E, Zhang P, Steel G, Torres FR, Cavalcanti DP. Homozygous deletion in MYL9 expands the molecular basis of megacystis-microcolon-intestinal hypoperistalsis syndrome. Eur J Hum Genet. 2018; 26:669-675.
32. Jenkins ZA, Macharg A, Chang CY, *et al.* Differential regulation of two FLNA transcripts explains some of the phenotypic heterogeneity in the loss-of-function filaminopathies. Hum Mutat. 2018; 39:103-113.
33. Iqbal NS, Jascur TA, Harrison SM, Edwards AB, Smith LT, Choi ES, Arevalo MK, Chen C, Zhang S, Kern AJ, Scheuerle AE, Sanchez EJ, Xing C, Baker LA. Prune belly syndrome in surviving males can be caused by Hemizygous missense mutations in the X-linked Filamin A gene. BMC Med Genet. 2020; 21:38.
34. Oda H, Sato T, Kunishima S, *et al.* Exon skipping causes atypical phenotypes associated with a loss-of-function mutation in FLNA by restoring its protein function. Eur J Hum Genet. 2016; 24:408-414.
35. Bonora E, Bianco F, Cordeddu L, *et al.* Mutations in RAD21 disrupt regulation of APOB in patients with chronic intestinal pseudo-obstruction. Gastroenterology. 2015; 148:771-782.e11.
36. Gamboa HE, Sood M. Pediatric intestinal pseudo-obstruction in the era of genetic sequencing. Curr Gastroenterol Rep. 2019; 21:70.
37. Gosemann JH, Puri P. Megacystis microcolon intestinal hypoperistalsis syndrome: systematic review of outcome. Pediatr Surg Int. 2011; 27:1041-1046.
38. Soh H, Fukuzawa M, Kubota A, Kawahara H, Ueno T, Taguchi T. Megacystis microcolon intestinal hypoperistalsis syndrome: A report of a nationwide survey in Japan. J Pediatr Surg. 2015; 50:2048-2050.
39. Guo DC, Pannu H, Tran-Fadulu V, *et al.* Mutations in smooth muscle α-actin (ACTA2) lead to thoracic aortic aneurysms and dissections. Nat Genet. 2007; 39:1488-1493.

Received May 25, 2022; Revised August 15, 2022; Accepted August 25, 2022.

Address correspondence to:
Alexandre Fabre, Pediatric Multidisciplinary Department, Timone Enfant Hospital, APHM, Aix-Marseille University, 264 Rue Saint Pierre 13005 Marseille, France.
E-mail: alexandre.fabre@ap-hm.fr

Released online in J-STAGE as advance publication August 29, 2022.

Original Article

DOI: 10.5582/irdr.2022.01067

Need for revision of the ACMG/AMP guidelines for interpretation of X-linked variants

Yoko Inoue[1,2], Osamu Machida[1,3], Yosuke Kita[4], Toshiyuki Yamamoto[1,2]

[1] Division of Gene Medicine, Graduate School of Medical Science, Tokyo Women's Medical University, Tokyo, Japan;
[2] Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan;
[3] Department of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan;
[4] Department of Psychology, Faculty of Letters, Keio University, Tokyo, Japan.

SUMMARY The guidelines provided by American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (AMP) (ACMG/AMP guidelines) suggest a framework for the classification of clinical variants. However, the interpretations can be inconsistent, with each definition sometimes proving to be ambiguous. In particular, there can be difficulty with interpretation of variants related to the X-linked recessive trait. To confirm whether there are biases in the interpretation of inherited traits, we reanalyzed variants reported prior to the release of the ACMG/AMP guidelines. As expected, the interpretation ratio as pathogenic or likely pathogenic was significantly lower for variants related to the X-linked recessive trait. Evaluation of variants related to the X-linked recessive trait, hence, need to consider whether the variant is identified only in males in accordance with the X-linked recessive trait. The ACMG/AMP guidelines should be revised to eliminate the bias revealed in this study.

Keywords ACMG/AMP guidelines, X-linked recessive, interpretation, sequence variant, diagnostic odyssey

1. Introduction

When medical diagnoses are difficult to achieve based on just the clinical information, patients and their families often experience a "diagnostic odyssey" that requires unnecessary medical evaluations (*1*). The longer the diagnostic odyssey, the greater the disadvantage to the patients and their families. Patients subjected to a diagnostic odyssey may have rare and undiagnosed genomic disorders that can only be identified using genomic analyses. Rare diseases only occur in a fraction of the general population. However, collectively, they comprise approximately 7,000 different disorders, the majority of which have a genetic origin (*2*). Thus, systematic and comprehensive genomic analyses to identify the causative genetic variants of rare and undiagnosed diseases would assist patients and their families (*3*).

Massive parallel sequencing analyses using next generation sequencing to detect causative variants of Mendelian disorders in undiagnosed patients have led to the identification of unprecedented numbers of genomic variants. Almost all variants have been unrelated to the disease diagnosis. Thus, for Mendelian disorders, the identification of one or two disease-causing sequence variants typically represents a bottleneck in the filtering of many sequence variants (*4*). This filtering step generally uses population data, computational predictive data, variant types, gene-specific information, variant segregation, and functional data (*5*). After narrowing down the potential candidate variants, it needs to be determined whether these variants are indeed causative. This requires a process of systematic interpretation. In 2008, the American College of Medical Genetics and Genomics (ACMG) issued recommendations for interpretative categories of sequence variants (*6*). In 2015, a workgroup of the ACMG and the Association of Molecular Pathology (AMP) provided defined terms and detailed variant classification guidance as updated standards and guidelines for the interpretation of sequence variants (*7*).

The ACMG/AMP guidelines are recommended for the interpretation of sequence variants in Mendelian disorders. Using the guidelines, variants are classified into five categories: pathogenic, likely pathogenic, uncertain significance (VUS), likely benign, and benign (*8*). However, the interpretations can be inconsistent, with each definition sometimes proving to be ambiguous, and molecular geneticists can have a bias favoring overestimation of pathogenicity (*9*). Therefore,

it is necessary to share the nuances that enable more accurate variant interpretations to be obtained among molecular geneticists through professional training (*10*). The guidelines in fact mention that it is necessary to develop more focused guidance regarding the classification of variants in specific genes (*7*). Indeed, hearing loss-specific guidelines have been established, and these have resolved discrepancies in variation classification, leading to more consistent results for patients in need of an accurate diagnosis (*11*).

In the past decade, we have participated in research to identify the genomic background of patients with undiagnosed neurodevelopmental disorders (*12*). In this research project, we experienced difficulties in determining disease-causing variants related to X-linked genes, and we considered whether the ACMG/AMP guidelines may exhibit biases in the scoring system depending on the mode of inheritance. Here, we evaluated the scoring system of the ACMG/AMP guidelines for inheritance patterns, and we considered whether there is a better way to interpret such sequence variants.

2. Materials and Methods

Variants reported as disease-related prior to the establishment of the ACMG/AMP guidelines were reanalyzed to assess whether there were any discrepancies in the scoring using the ACMG/AMP guidelines depending on the differences in the inheritance traits, including autosomal dominant, autosomal recessive, and X-linked. The variants reported prior to the establishment of the ACMG/AMP guidelines are not considered to be affected by the ACMG/AMP guidelines. Variants were selected from reports published in international journals with an impact factor greater than 4 at the time of analyses. In the variant interpretation step, InterVar, a bioinformatics software tool for clinical interpretation of genetic variants by the ACMG/AMP guidelines (*https://wintervar.wglab.org/*), was used as the reference (*13*). Web-based tools, including wANNOVAR (*https://wannovar.wglab.org/*) (*14,15*), gnomAD (*https://gnomad.broadinstitute.org/*), and ClinVar (*https://www.ncbi.nlm.nih.gov/clinvar/*), were also used. Finally, two or more curators confirmed the scoring based on the ACMG/AMP guidelines.

According to the obtained results of the variants previously reported as disease-causing prior to 2015, the proportion of the variants classified as pathogenic or likely pathogenic was aggregated according to the inheritance manner and statistically analyzed by Fisher's Exact Test and Yate's chi-squared test.

If the proportion of X-linked variants determined to be pathogenic or likely pathogenic was significantly lower, we hypothesized that an additional score specific for X-linked variants might eliminate the difference from other inheritance mechanisms and added a new score for Pathogenicity of Strong Evidence (PS score) or Pathogenicity of Moderate Evidence (PM score) in cases where the target patient was a male and females carrying the same variant did not exhibit any clinical symptoms except for in specific cases. We further examined whether the above difference could be eliminated by the addition of new scores when the target patient was a male, and females carrying the same variant did not exhibit any clinical symptoms except for in specific cases.

3. Results

We selected the reports published prior to 2015. For variants associated with the autosomal dominant trait, 158 variants of 13 genes were selected from 7 reports (*16-22*). All scores in accordance with the ACMG/AMP guidelines are summarized in Supplemental Table S1 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=113*). Among the evaluated variants, 143 (90.5%) were interpreted as pathogenic or likely pathogenic (Table 1). For the autosomal recessive trait, 109 variants of 17 genes were selected from 11 reports (*23-33*). All scores are presented in Supplemental Table S2 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=113*). Among these, 93 variants (85.3%) were interpreted as pathogenic or likely pathogenic (Table 1). For the X-linked recessive trait, 105 variants of 35 genes were selected from 9 reports (*34-42*). All scores are presented in Supplemental Table S3 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=113*). Among these, 42 variants (40.0%) were interpreted as pathogenic or likely pathogenic (Table 1). There were significant differences between autosomal dominant versus X-linked, and autosomal recessive versus X-linked. However, there was no significant difference between the autosomal dominant and autosomal recessive traits.

As the ratio of the variants interpreted as pathogenic or likely pathogenic was significantly low in case of the X-linked recessive trait, we examined whether those difference can be compensated after additions of a new PS score (PSX) or PM score (PMX) as described above. By these modifications, the ratio of the variants interpreted as pathogenic or likely pathogenic changed (Supplemental Tables S4 & S5, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=113*). However, the statistical analysis still revealed a significant difference between X-linked and others (Table 1).

These results are demonstrated as a bar graph for better understanding (Figure 1).

4. Discussion

The ACMG/AMP guidelines for interpretation of nucleotide variants were published in 2015 (*7*).

Table 1. Summary of the interpretation of the variants and the results of statiscial analyses

Items	Total number of the variants	Number of the variants interpreted as		Fisher's Exact Test *p* values		Chi-squared values Yates' correction	
		Pathogenic/ Likely pathogenic	Others	versus AD	versus AR	versus AD	versus AR
Autosomal dominant trait (AD)	158	143 (90.5%)	15 (9.5%)	NA	0.27	NA	1.2
Autosomal recessive trait (AR)	109	93 (85.3%)	16 (14.7%)	0.27	NA	1.2	NA
X-linked recessive trait (XLR)	105	42 (40.0%)	63 (60.0%)	$2.2 \times 10^{16*}$	$3.7 \times 10^{12*}$	74.7^{***}	45.2^{***}
X-linked recessive trait (plus PS score)	105	72 (68.6%)	33 (31.4%)	$1.4 \times 10^{5*}$	0.00534^{*}	18.9^{***}	7.6^{**}
X-linked recessive trait (plus PM score)	105	62 (59.0%)	43 (41.0%)	$2.6 \times 10^{9*}$	$1.7 \times 10^{5*}$	34.5^{***}	17.2^{***}

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, NA, not applicable.

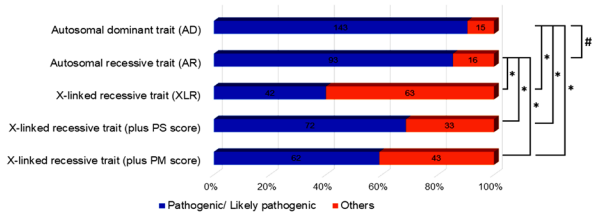


Figure 1. Distribution of the interpretation of the variants for each inheritance trait represented by a 100% stacked chart. The numbers in the boxes indicate the corresponding numbers of the variants. *, Statistically significant ($p < 0.05$); #, no significant difference.

These guidelines define the basic method for variant interpretation. The interpretations provided by these guidelines are crude, and the interpretation of each score is somewhat ambiguous. Establishment of an individual interpretation method corresponding to each disease or gene is, therefore, recommended (*5*).

The ACMG/AMP guidelines provide a framework for clinical variant classification (*7*). However, in the process of applying the ACMG/AMG guidelines to the classification of thousands of variants, many groups have identified several areas in which the guidelines lack specificity or are subject to ambiguous or contradictory interpretation (*43*). Najafi *et al.* analyzed the variants related to fibrillinopathies, and they noted that it is necessary to pay attention to the possibility that disease-related variants are included in population-based information (*4*). Furthermore, disease-specific guidelines have been published, specifically for MYH-associated inherited cardiomyopathy (*44*), RASopathy (*45*), hearing loss (*11*), and Alport syndrome (*46*). Nonetheless, best practice guidelines specific for X-linked recessive rare disorders have not yet been reported.

In this study, we evaluated variants reported before the publication of the ACMG/AMP guidelines. As a result, the interpretation ratio as pathogenic or likely pathogenic was significantly lower in variants related to the X-linked recessive trait. This indicates the existence of a bias between inheritance traits. Most of the variants related to the rare disorders in the autosomal dominant trait were occurred as the consequence of de novo. The de novo variants correspond to "PS2", indicating strong evidence of pathogenicity. Therefore, variants associated with autosomal dominant inheritance are more likely to be interpreted as pathogenic/likely pathogenic. For variants related to autosomal recessive inheritance, "PM3" can be assigned if another pathogenic variant was present in the trans-related allele. Therefore, many variants related to the autosomal recessive trait have been interpreted as either pathogenic or likely pathogenic.

In comparison, it is difficult to interpret variants related to the X-linked recessive trait as pathogenic or likely pathogenic because there are no specific scores for variants involved in the X-linked recessive inheritance. De Luca *et al.* suggested that assessing pathogenicity is more challenging in X-linked cases (*47*). For X-linked variants, segregation analysis has been recommended as a powerful tool to further confirm pathogenicity for early-onset and high-penetrance disorders. The identification of the variant in several affected male family members together with their healthy or mildly affected carrier mothers is in strong support for pathogenicity. Thus, we considered a specific score for variants that may be related to the X-linked recessive inheritance.

When variants associated with the X-linked recessive trait were identified only in male patients and the carrier females did not exhibit any related symptoms, we temporarily assigned a PS or a PM score, and the

ratio of interpretation as pathogenic/likely pathogenic increased. However, the significant difference was not eliminated (Figure 1). Considering the situation of each variant, some variants were inappropriately interpreted as pathogenic after the addition of a PS score, even for variants that should be interpreted as benign. Therefore, assignment of a PS score may be excessive, and assigning a PM score may be more appropriate.

In conclusion, we confirmed the bias of the ACMG/AMP guidelines regarding inheritance traits. Evaluation of variants related to the X-linked recessive trait should consider whether the variant was identified only in males in accordance with the X-linked recessive trait. The bias revealed in this study should be eliminated by future revision of the ACMG/AMP guidelines.

Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Wu AC, McMahon P, Lu C. Ending the diagnostic odyssey-Is whole-genome sequencing the answer? JAMA Pediatr. 2020; 174:821-822.
2. Umlai UI, Bangarusamy DK, Estivill X, Jithesh PV. Genome sequencing data analysis for rare disease gene discovery. Brief Bioinform. 2022; 23:bbab363.
3. Takahashi Y, Date H, Oi H, Adachi T, Imanishi N, Kimura E, Takizawa H, Kosugi S, Matsumoto N, Kosaki K, Matsubara Y; IRUD Consortium, Mizusawa H. Six years' accomplishment of the Initiative on Rare and Undiagnosed Diseases: nationwide project in Japan to discover causes, mechanisms, and cures. J Hum Genet. 2022. doi: 10.1038/s10038-022-01025-0.
4. Najafi A, Caspar SM, Meienberg J, Rohrbach M, Steinmann B, Matyas G. Variant filtering, digenic variants, and other challenges in clinical sequencing: a lesson from fibrillinopathies. Clin Genet. 2020; 97:235-245.
5. Kim YE, Ki CS, Jang MA. Challenges and considerations in sequence variant interpretation for mendelian disorders. Ann Lab Med. 2019; 39:421-429.
6. Richards CS, Bale S, Bellissimo DB, Das S, Grody WW, Hegde MR, Lyon E, Ward BE; Molecular Subcommittee of the ACMG Laboratory Quality Assurance Committee. ACMG recommendations for standards for interpretation and reporting of sequence variations: Revisions 2007. Genet Med. 2008; 10:294-300.
7. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehm HL; ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015; 17:405-424.
8. Bahcall OG. Genetic testing. ACMG guides on the interpretation of sequence variants. Nat Rev Genet. 2015; 16:256-257.
9. Amendola LM, Muenzen K, Biesecker LG, *et al.* Variant classification concordance using the ACMG-AMP Variant Interpretation Guidelines across nine genomic implementation research studies. Am J Hum Genet. 2020; 107:932-941.
10. Strande NT, Brnich SE, Roman TS, Berg JS. Navigating the nuances of clinical sequence variant interpretation in Mendelian disease. Genet Med. 2018; 20:918-926.
11. Patel MJ, DiStefano MT, Oza AM, *et al.* Disease-specific ACMG/AMP guidelines improve sequence variant interpretation for hearing loss. Genet Med. 2021; 23:2208-2212.
12. Yamamoto T, Imaizumi T, Yamamoto-Shimojima K, *et al.* Genomic backgrounds of Japanese patients with undiagnosed neurodevelopmental disorders. Brain Dev. 2019; 41:776-782.
13. Li Q, Wang K. InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. Am J Hum Genet. 2017; 100:267-280.
14. Chang X, Wang K. wANNOVAR: annotating genetic variants for personal genomes via the web. J Med Genet. 2012; 49:433-436.
15. Yang H, Wang K. Genomic variant annotation and prioritization with ANNOVAR and wANNOVAR. Nat Protoc. 2015; 10:1556-1566.
16. Alvarez-Dominguez JR, Amosova O, Fresco JR. Self-catalytic DNA depurination underlies human β-globin gene mutations at codon 6 that cause anemias and thalassemias. J Biol Chem. 2013; 288:11581-11589.
17. Bombelli F, Stojkovic T, Dubourg O, Echaniz-Laguna A, Tardieu S, Larcher K, Amati-Bonneau P, Latour P, Vignal O, Cazeneuve C, Brice A, Leguern E. Charcot-Marie-Tooth disease type 2A: from typical to rare phenotypic and genotypic features. JAMA Neurol. 2014; 71:1036-1042.
18. Di Gregorio E, Borroni B, Giorgio E, *et al.* ELOVL5 mutations cause spinocerebellar ataxia 38. Am J Hum Genet. 2014; 95:209-217.
19. Lalani SR, Safiullah AM, Fernbach SD, *et al.* Spectrum of CHD7 mutations in 110 individuals with CHARGE syndrome and genotype-phenotype correlation. Am J Hum Genet. 2006; 78:303-314.
20. O'Roak BJ, Vives L, Fu W, *et al.* Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science. 2012; 338:1619-1622.
21. Roberts ME, Riegert-Johnson DL, Thomas BC, Rumilla KM, Thomas CS, Heckman MG, Purcell JU, Hanson NB, Leppig KA, Lim J, Cappel MA. A clinical scoring system to identify patients with sebaceous neoplasms at risk for the Muir-Torre variant of Lynch syndrome. Genet Med. 2014; 16:711-716.
22. Türkmen S, Gillessen-Kaesbach G, Meinecke P, *et al.* Mutations in NSD1 are responsible for Sotos syndrome, but are not a frequent finding in other overgrowth phenotypes. Eur J Hum Genet. 2003; 11:858-865.
23. Cottenie E, Kochanski A, Jordanova A, *et al.* Truncating and missense mutations in IGHMBP2 cause Charcot-Marie Tooth disease type 2. Am J Hum Genet. 2014; 95:590-601.
24. Ehmke N, Caliebe A, Koenig R, *et al.* Homozygous and compound-heterozygous mutations in TGDS cause Catel-Manzke syndrome. Am J Hum Genet. 2014; 95:763-770.
25. Gersting SW, Kemter KF, Staudigl M, Messing DD, Danecka MK, Lagler FB, Sommerhoff CP, Roscher AA, Muntau AC. Loss of function in phenylketonuria is caused by impaired molecular motions and conformational instability. Am J Hum Genet. 2008; 83:5-17.

26. Hussain MS, Battaglia A, Szczepanski S, *et al.* Mutations in CKAP2L, the human homolog of the mouse Radmis gene, cause Filippi syndrome. Am J Hum Genet. 2014; 95:622-632.
27. Kopajtich R, Nicholls TJ, Rorbach J, *et al.* Mutations in GTPBP3 cause a mitochondrial translation defect associated with hypertrophic cardiomyopathy, lactic acidosis, and encephalopathy. Am J Hum Genet. 2014; 95:708-720.
28. Law R, Dixon-Salazar T, Jerber J, *et al.* Biallelic truncating mutations in FMN2, encoding the actin-regulatory protein Formin 2, cause nonsyndromic autosomal-recessive intellectual disability. Am J Hum Genet. 2014; 95:721-728.
29. Malik S, Percin FE, Bornholdt D, Albrecht B, Percesepe A, Koch MC, Landi A, Fritz B, Khan R, Mumtaz S, Akarsu NA, Grzeschik KH. Mutations affecting the BHLHA9 DNA-binding domain cause MSSD, mesoaxial synostotic syndactyly with phalangeal reduction, Malik-Percin type. Am J Hum Genet. 2014; 95:649-659.
30. Ohlenbusch A, Henneke M, Brockmann K, Goerg M, Hanefeld F, Kohlschütter A, Gärtner J. Identification of ten novel mutations in patients with eIF2B-related disorders. Hum Mutat. 2005; 25:411.
31. Sosnay PR, Siklosi KR, Van Goor F, *et al.* Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet. 2013; 45:1160-1167.
32. Thomas AC, Williams H, Setó-Salvia N, *et al.* Mutations in SNX14 cause a distinctive autosomal-recessive cerebellar ataxia and intellectual disability syndrome. Am J Hum Genet. 2014; 95:611-621.
33. Zhang K, Jordan MB, Marsh RA, Johnson JA, Kissell D, Meller J, Villanueva J, Risma KA, Wei Q, Klein PS, Filipovich AH. Hypomorphic mutations in PRF1, MUNC13-4, and STXBP2 are associated with adult-onset familial HLH. Blood. 2011; 118:5794-5798.
34. Hirata H, Nanda I, van Riesen A, *et al.* ZC4H2 mutations are associated with arthrogryposis multiplex congenita and intellectual disability through impairment of central and peripheral synaptic plasticity. Am J Hum Genet. 2013; 92:681-695.
35. Homan CC, Kumar R, Nguyen LS, Haan E, Raymond FL, Abidi F, Raynaud M, Schwartz CE, Wood SA, Gecz J, Jolly LA. Mutations in USP9X are associated with X-linked intellectual disability and disrupt neuronal cell migration and growth. Am J Hum Genet. 2014; 94:470-478.
36. Kato M, Das S, Petras K, *et al.* Mutations of ARX are associated with striking pleiotropy and consistent genotype-phenotype correlation. Hum Mutat. 2004; 23:147-159.
37. Martínez-Montero P, Muñoz-Calero M, Vallespín E, Campistol J, Martorell L, Ruiz-Falcó MJ, Santana A, Pons R, Dinopoulos A, Sierra C, Nevado J, Molano J. PLP1 gene analysis in 88 patients with leukodystrophy. Clin Genet. 2013; 84:566-571.
38. Piton A, Redin C, Mandel JL. XLID-causing mutations and associated genes challenged in light of data from large-scale human exome sequencing. Am J Hum Genet. 2013; 93:368-383.
39. Ramser J, Ahearn ME, Lenski C, Yariz KO, Hellebrand H, von Rhein M, Clark RD, Schmutzler RK, Lichtner P, Hoffman EP, Meindl A, Baumbach-Reardon L. Rare missense and synonymous variants in UBE1 are associated with X-linked infantile spinal muscular atrophy. Am J Hum Genet. 2008; 82:188-193.
40. Rosenberg EH, Almeida LS, Kleefstra T, deGrauw RS, Yntema HG, Bahi N, Moraine C, Ropers HH, Fryns JP, deGrauw TJ, Jakobs C, Salomons GS. High prevalence of SLC6A8 deficiency in X-linked mental retardation. Am J Hum Genet. 2004; 75:97-105.
41. Royer G, Hanein S, Raclin V, Gigarel N, Rozet JM, Munnich A, Steffann J, Dufier JL, Kaplan J, Bonnefont JP. NDP gene mutations in 14 French families with Norrie disease. Hum Mutat. 2003; 22:499.
42. Vulto-van Silfhout AT, de Vries BB, van Bon BW, *et al.* Mutations in MED12 cause X-linked Ohdo syndrome. Am J Hum Genet. 2013; 92:401-406.
43. Nykamp K, Anderson M, Powers M, Garcia J, Herrera B, Ho YY, Kobayashi Y, Patil N, Thusberg J, Westbrook M, Topper S. Sherloc: a comprehensive refinement of the ACMG-AMP variant classification criteria. Genet Med. 2017; 19:1105-1117.
44. Kelly MA, Caleshu C, Morales A, *et al.* Adaptation and validation of the ACMG/AMP variant classification framework for MYH7-associated inherited cardiomyopathies: recommendations by ClinGen's Inherited Cardiomyopathy Expert Panel. Genet Med. 2018; 20:351-359.
45. Gelb BD, Cavé H, Dillon MW, Gripp KW, Lee JA, Mason-Suares H, Rauen KA, Williams B, Zenker M, Vincent LM. ClinGen's RASopathy Expert Panel consensus methods for variant interpretation. Genet Med. 2018; 20:1334-1345.
46. Savige J, Storey H, Watson E, *et al.* Consensus statement on standards and guidelines for the molecular diagnostics of Alport syndrome: refining the ACMG criteria. Eur J Hum Genet. 2021; 29:1186-1197.
47. De Luca C, Race V, Keldermans L, Bauters M, Van Esch H. Challenges in molecular diagnosis of X-linked Intellectual disability. Br Med Bull. 2020; 133:36-48.

Received June 10, 2022; Revised July 27, 2022; Accepted August 4, 2022.

**Address correspondence to:*
Toshiyuki Yamamoto, Institute of Medical Genetics, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ward, Tokyo 162-8666, Japan.
E-mail: yamamoto.toshiyuki@twmu.ac.jp

Released online in J-STAGE as advance publication August 10, 2022.

Original Article

DOI: 10.5582/irdr.2022.01062

Knowledge and perception of inborn errors of metabolism (IEMs) among healthcare students at a selected public university in Klang Valley, Malaysia

Shi Hui Liew[1], Jing Ying Lim[2,3], Hanis Mastura Yahya[1,3], Roslee Rajikan[1,3,*]

[1] Nutritional Science Program, Faculty of Health Science, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia;
[2] Dietetics Program, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia;
[3] Centre of Healthy Aging and Wellness (H-Care), Faculty of Health Sciences, Universiti Kebangsaan Malaysia.

SUMMARY Healthcare providers play an important role in improving the health of Inborn Error of Metabolism (IEM) patients. However, IEM knowledge level among local healthcare students has yet to be determined. Thus, the aim of this study is to assess the knowledge and perception of IEM among local healthcare students. An online self-administered questionnaire was distributed to 378 students across the Faculty of Health Science, Pharmacy and Dentistry from a selected public university in *Lembah Klang*, Malaysia. For knowledge, a score of 1 is assigned to each correct answer with a maximum total score of 14. Likert scale was used to determine their perception of IEM. The total mean score of IEM knowledge among healthcare students is 5.8. There was no significant difference of mean score of IEM knowledge among the students from the Faculty of Health Science (6.1 ± 2.7), Pharmacy (5.5 ± 2.6) and Dentistry (5.8 ± 2.8). However, the score of knowledge is observed to be significantly different by ethnicity, religion and family history of IEM ($p < 0.05$). Furthermore, students with experience of meeting an IEM patient and attending IEM classes scored higher than those with no experience ($p < 0.05$). Most of the healthcare students (89.5%) perceived their knowledge to be insufficient and very poor. Majority of the students from faculty of pharmacy (70.8%) agreed that the IEM course should be mandatory compared to health sciences and dentistry ($p < 0.05$). This study identified an overall inadequacy of knowledge of IEM among healthcare students. There is a pressing need to improve the IEM-related knowledge and awareness of Malaysian healthcare students. This can be accomplished by incorporating online classes that emphasizes the treatment and management of IEMs in the university curriculum.

Keywords knowledge, perception, healthcare, inborn errors metabolism

1. Introduction

Inborn errors of metabolism (IEM) denote a large group of rare genetic disorders resulting from an enzyme defect in biochemical and metabolic pathways (*1*). Although individual IEMs are rare, they are common collectively and have a global prevalence of 50.9 per 100,000 live births (*2*). Although IEM cases are rare in Malaysia, the true number of IEM patients remains unknown as the country lacks a central IEM patient registry and IEM-related awareness among healthcare providers is low (*3,4*).

The management of IEMs is divided into two parts: diagnostics and long-term care (*5*). New-born screening (NBS) is a useful tool with which to detect IEMs in early life (*6*). Basic laboratory tests; such as blood or urinalysis; can be used in conjunction with clinical evaluation to obtain a differential diagnosis or confirm a diagnosis (*7*). Meanwhile, the long-term care and treatment of IEM patients involves collaboration of healthcare professionals from multiple disciplines; such as physicians, nurses, pharmacists, dietitians, laboratory practitioners, speech therapists, occupational therapists, dentists, and social workers (*8-14*). A multidisciplinary team is essential and crucial to provide IEM patients with better quality health care in terms of treatment, psychological support, and educational resources (*15*).

Healthcare providers play an important role in recognizing and making referrals for appropriate patient management (*16*). However, there seems to be difficulty obtaining supportive treatment for IEM patients (*17*) due to a lack of IEM-related knowledge (*3*). For instance, a

majority (73.6%) of healthcare professionals in Hong Kong were unaware of the availability of IEM screening (*18*). Furthermore, because many healthcare students report insufficient IEM-related knowledge, they struggle to distinguish rare IEMs from other common diseases (*19,20*). A Saudi Arabian study also reported an overall lack of IEM-related knowledge, especially among students in non-medical faculties (*21,22*).

Healthcare students are also more likely to underrate the epidemiology and burden of IEMs (*23*). One study reported that healthcare students have little IEM-related knowledge and that the majority of them agreed that healthcare funding should prioritize common diseases over IEMs as the latter only affects a small portion of the population (*24*). Moreover, the majority of healthcare students are unprepared to care for IEM patients (*19,20*).

Despite the current circumstances, only a limited number of studies have examined factors that influence IEM-related knowledge among healthcare students. Several studies have concluded that individuals with higher levels of education as well as prior knowledge of genetics had more IEM-related knowledge (*25,26*). Female participants were also found to have higher levels of IEM-related knowledge. This may be due to their natural ability to produce offspring, which increases concerns (*i.e.*, maternal instincts) of prenatal genetic testing (*21,22,27*). Other factors; such as a family history of genetic disorders; was also found to contribute to higher levels of IEM-related knowledge (*21*).

A search of the literature through electronic bibliographic databases, such as PubMed®/MEDLINE (National Library of Medicine), ScienceDirect® (Elsevier) and google scholar revealed that currently, studies to assess the knowledge of IEM patients were conducted in Saudi Arabia and Poland and there was no study conducted among the Asian population as well as Malaysian healthcare students. Therefore, the purpose of this present study was twofold: *i*) to assess the knowledge and perception of IEMs among healthcare students from the faculties of health sciences, pharmacy, and dentistry, and *ii*) to determine the mean difference in IEM-related knowledge scores according to varying prespecified sociodemographic factors and other factors among healthcare students.

2. Materials and Methods

2.1. Study design

This present study conducted a cross-sectional survey using convenience sampling. A sub-sample of 378 healthcare students from three different faculties; namely, the Faculty of Health Sciences, the Faculty of Pharmacy, and the Faculty of Dentistry; were recruited. The inclusion criteria included undergraduate healthcare students from three different faculties who were able to read and understand either Malay or English. An online survey was distributed via social media, mailing lists, and online discussion groups between August and October 2020. This research was approved by the Research Ethics Committee of the National University of Malaysia (approval number: PPI/111/8/JEP-2020-346). Informed consent was obtained from all participants via online forms. All participants were also informed that participation in the present study was voluntary and that all data would be anonymous and confidential.

2.2. Questionnaire

A self-administered questionnaire that consisted of four sections and 47 items was adapted from extant literature (*19,22*) (Supplemental Table S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=111*).

Section I collected the demographic information of the participants, such as gender, age, ethnicity, religion, faculty, year of study, marital status, declaration of a family history of IEMs, and previous encounters with IEM patients, if any. Section II evaluated the IEM-related knowledge of the students. It included 14 single-best answer closed-ended questions on the causes, epidemiology, symptoms, diagnosis, management, and organizational issues of IEMs. A score of one was assigned to each correct answer and zero for each wrong answer, with a maximum total score of 14 for the entire questionnaire. The total score was tabulated by summing up the scores of the 14 questions on IEM-related knowledge. Section III included multiple-answer closed-ended questions that required students to select IEMs from a list of 24 IEMs and non-IEM diseases. This assessed their ability to recognise IEM names among other diseases. Section IV included six questions on self-perceived knowledge and opinions on IEMs.

Prior to data collection, the content validity of the survey instrument was established. The content validity index (CVI) was measured based on the opinions of three experts who have experience teaching IEMs or evaluating IEM-related courses. The content validity form contained four domains and 47 items on IEM-related knowledge and perception. The panel of industry experts were requested to rate the degree of relevance, clarity, simplicity, and ambiguity of each item on a scale of one to four, where a score of one denoted that an item was irrelevant, lacked clarity, lacked simplicity, or was not easily understood and a score of four indicated that the item was highly relevant, very clear, very simple, and easily understood. As the relevance of all 47 items achieved an I-CVI score of 1.00, they were all retained based on the established criteria (*28*). The CVI score in terms of clarity, simplicity, and ambiguity was 0.98. Item 9 was modified according to the recommendations of a panel member. The questionnaire was then forward translated to Malay. A pilot study was conducted at

the Kuala Lumpur campus of the National University of Malaysia (UKM) with 10 students of different sociodemographic backgrounds. The respondents were asked if they had any questions or if they did not understand any of the words used in the questionnaire. They were also asked to share the rationale behind the answer that they provided based on the instructions given after filling in the form. In the second revision, minor changes were made to the questionnaire based on the feedback received from these 10 students.

2.3. Statistical analysis

The collected data was analyzed using Statistical Package for the Social Sciences (SPSS) version 25.0. The categorical variables were described in frequencies and percentages while the continuous variables were described as means and standard deviations. A score of one was assigned to correct answers and zero to incorrect answers or the "I do not know" option of the 14 questions on IEM-related knowledge. The total score on IEM-related knowledge was summed up, with scores ranging between 0 to 14 for each respondent. Descriptive statistics were then used to describe the sociodemographic characteristics of the respondents. The difference in the mean IEM-related knowledge scores according to pre-specified sociodemographic factors and other factors was tested using the independent-samples *t*-test and the one-way analysis of variance (one-way ANOVA). Pearson's chi-squared test was then used to determine the association between the two variable categories. A p value of < 0.05 was deemed statistically significant.

3. Results

3.1. Sociodemographic characteristics

A total of 378 valid respondents completed the survey. Most of the respondents were from the Faculty of Health Sciences (40.8%) while the others were from the Faculty of Dentistry (31.2%) and the Faculty of Pharmacy (28.0%). Table 1 displays the sociodemographic characteristics of respondents from the faculties of health sciences, pharmacy, and dentistry. The average age was 22.1 ± 1.5 years and

Table 1. Sociodemographic characteristics across the Faculties of Health Sciences, Pharmacy and Dentistry

Variables	Faculties, *n* (%)			Total, *n* (%) (*n* = 378)
	Health Science (*n* = 154)	Pharmacy (*n* = 106)	Dentistry (*n* = 118)	
Age				
18-21	52 (33.8)	36 (34.0)	46 (39.0)	134 (35.4)
22-24	96 (62.3)	68 (64.1)	65 (55.1)	229 (60.6)
> 25	6 (3.9)	2 (1.9)	7 (5.9)	15 (4.0)
Gender				
Male	36 (23.4)	28 (26.4)	36 (30.5)	100 (26.5)
Female	118 (76.6)	78 (73.6)	82 (69.5)	278 (73.5)
Ethnicity				
Malay	58 (37.7)	53 (50.0)	64 (54.2)	175 (46.3)
Chinese	83 (53.9)	30 (28.3)	36 (30.6)	149 (39.4)
India	11 (7.1)	14 (13.2)	11 (9.3)	36 (9.5)
Others	2 (1.3)	9 (8.5)	7 (5.9)	18 (4.8)
Religion				
Islam	60 (39.0)	59 (55.6)	66 (55.9)	185 (48.9)
Buddha	76 (49.4)	29 (27.4)	33 (28.0)	138 (36.5)
Hindu	9 (5.8)	11 (10.4)	11 (9.3)	31 (8.2)
Others	9 (5.8)	7 (6.6)	8 (6.8)	24 (6.4)
Marital Status				
Single	153 (99.4)	106 (100.0)	118 (100.0)	377 (99.7)
Married	1 (0.6)	0 (0.0)	0 (0.0)	1 (0.3)
Year of Study				
1-2	56 (36.4)	44 (41.5)	45 (38.1)	145 (38.4)
≥ 3	98 (63.6)	62 (58.5)	73 (61.9)	233 (61.6)
Have you ever heard the term IEM?				
Yes	71 (46.1)	24 (22.6)	42 (35.6)	137(36.2)
No	83 (53.9)	82 (77.4)	76 (64.4)	241(63.8)
Have you ever met an IEM patient?				
Yes	14 (9.1)	6 (5.7)	9 (7.6)	29 (7.7)
No	98 (63.6)	65 (61.3)	73 (61.9)	236 (62.4)
I do not know	42 (27.3)	35 (33.0)	36 (30.5)	113 (29.9)
Is anyone in your family suffering from IEM?				
Yes	0 (0.0)	3 (2.8)	1 (0.9)	4 (1.0)
No	154 (100.0)	103 (97.2)	117 (99.1)	374 (99.0)
Have you had any classes about IEM during your studies?				
Yes	57 (37.0)	15 (14.2)	31 (26.3)	103 (27.3)
No	82 (53.3)	81 (76.4)	65 (55.1)	228 (60.3)
I do not know	15 (9.7)	10 (9.4)	22 (18.6)	47 (12.4)

Table 2. Students' knowledge about Inborn Error of Metabolism (IEM)

Item	Correct *n* (%)	Incorrect *n* (%)	Don't know *n* (%)
Causes, Epidemiology, Symptom of IEM			
1. IEM can be caused by infection (yes)	34 (9.0)	173 (45.8)	171 (45.2)
2. IEM can be caused by enzyme deficiency (yes)	273 (72.2)	6 (1.6)	99 (26.2)
3. Symptoms of IEM appear usually at Neonatal/Pediatric/Adult age (Neonatal age)	256 (67.7)	122 (32.3)	-
4. Symptom IEM may include: (Anemia/ Hypoglycemia/ Hepatomegaly/Mental retardation/ Acid/base imbalance/ Coma/ Seizures/ Hearing and visual impairment (all above)	16 (4.2)	362 (95.8)	-
5. Person with positive family history are at higher risk of having IEM (yes)	299 (79.1)	9 (2.4)	70 (18.5)
Diagnosis and treatment of IEM			
6. Some IEM can be diagnosed by using a blood sample only (yes)	99 (26.2)	55 (14.6)	224 (59.2)
7. Diagnosis of some IEM can be done prenatally (yes)	182 (48.1)	31 (8.2)	165 (43.7)
8. Patients diagnosed with IEM should be isolated (no)	276 (73.0)	11 (2.9)	91 (24.1)
9. Some IEM can be controlled by avoidance of some foods (yes)	201 (53.2)	35 (9.2)	142 (37.6)
10. Liver transplantation is an option in management of some IEM (yes)	173 (45.8)	14 (3.7)	191 (50.5)
11. Gene therapy can be a helpful tool for treatment of some IEM (yes)	237 (62.7)	8 (2.1)	133 (35.2)
12. There is no available treatment for most IEM (no)	66 (17.5)	58 (15.3)	254 (67.2)
Organizational Issues of IEM			
13. There is central register for patient IEM in Malaysia (no)	5 (1.3)	102 (27.0)	271 (71.7)
14. There is a national plan for IEM in Malaysia (yes)	80 (21.2)	10 (2.6)	288 (76.2)

ranged between 18 and 31 years old. More women (73.5%) participated in this present study than men (26.5%). A vast majority of the respondents were single (99.7%). 62.4% of the respondents reported that they had never met an IEM patient while only one percent reported a family history of IEMs. Moreover, most of the respondents (60.3%) reported that they had never attended any classes on IEMs during their studies.

3.2. Knowledge of IEM-related topics

The answers seen in Table 2 indicate that a majority of the respondents answered questions on basic IEM-related knowledge correctly (items 2 and 3). However, less than 10% were able to provide correct answers when the questions were more in-depth; such as the specific symptoms of IEMs (item 4) and infections that may cause IEMs (item 1). Furthermore, less than 50% answered items that evaluated their understanding of IEM-related diagnoses, treatments, and organizational issues correctly (items 6, 7, 10, 12, 13, and 14).

As seen in Figure 1, most of the respondents were able to correctly identify IEMs, with the exception of Gaucher disease (46.6%) and Lesch-Nyhan syndrome (49.5%). The most easily recognised IEMs were biotinidase deficiency (83.1%), phenylketonuria (83.1%), and urea cycle disorders (83.1%). Less than 50% of the respondents misidentified non-IEM diseases as IEMs. Of this number, 38.9% of the respondents misidentified Crohn's Disease as an IEM.

A further inferential analysis was conducted to determine if differences existed among the multiple pre-specified sociodemographic variables and other variables in terms of the mean IEM-related knowledge score of the students. As seen in Table 3, ethnicity,

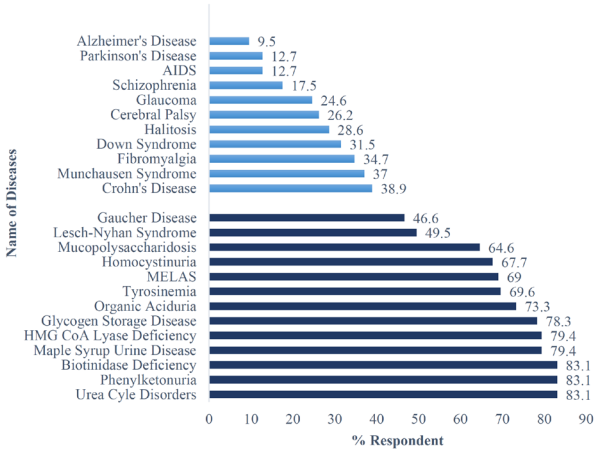


Figure 1. Proportion of respondents indicating different name of diseases (IEM and non-IEM) as IEM.

religion, and a family history of IEMs significantly affected IEM-related knowledge scores. Malay students (5.4 ± 2.7) scored significantly lower than Chinese students (6.4 ± 2.6) ($p < 0.05$) while Muslim students (5.4 ± 2.7) scored significantly lower than Buddhist students (6.5 ± 2.5) ($p < 0.05$). Students with a family history of IEMs (7.0 ± 0.8) scored significantly higher than their counterparts without a family history of IEMs (5.8 ± 2.7) ($p < 0.05$). Apart from that, students who had encountered IEM patients or attended IEM classes during their studies scored significantly higher than counterparts without experience or who answered "I do not know" ($p < 0.05$). Overall, the mean IEM-related knowledge score was 5.8 out of 14. Health sciences students scored the highest (6.1 ± 2.7) followed by dentistry (5.8 ± 2.8) and pharmacy (5.5 ± 2.6) students. Interestingly, 46.1% of health sciences students reported

Table 3. Difference of mean score of IEM knowledge according to sociodemographic and other variables

Variables	Mean ± SD	*p* value
Sociodemographic		
Age		0.190
18-21	5.7 ± 2.8	
22-24	5.8 ± 2.7	
> 25	7.1 ± 1.9	
Gender		0.774
Male	6.0 ± 3.1	
Female	6.1 ± 2.7	
Ethnicity		0.020*
Malay	5.4 ± 2.7[a]	
Chinese	6.4 ± 2.6[b]	
India	5.5 ± 3.0[ab]	
Others	5.8 ± 3.1[ab]	
Religion		0.003**
Islam	5.4 ± 2.7[a]	
Buddha	6.5 ± 2.5[b]	
Hindu	5.7 ± 3.0[ab]	
Others	5.4 ± 3.1[ab]	
Year of study		0.073
1-2	5.5 ± 2.6	
> 3	6.0 ± 2.8	
Faculty		0.253
Health Science	6.1 ± 2.7	
Pharmacy	5.5 ± 2.6	
Dentistry	5.8 ± 2.8	
Other Variables		
Have you ever met an IEM patient?		< 0.001***
Yes	7.5 ± 2.0[a]	
No	6.1 ± 2.6[b]	
I do not know	4.7 ± 2.8[c]	
Is anyone in your family suffering from IEM?		0.036*
Yes	7.0 ± 0.8	
No	5.8 ± 2.7	
Have you had any classes about IEM during your studies?		< 0.001***
Yes	7.7 ± 1.9[a]	
No	5.3 ± 2.5[b]	
I do not know	4.0 ± 3.1[c]	

*$p < 0.05$ - significant using Independent t-test for comparison between 2 groups and one-way ANOVA for comparison between three groups. ***$p < 0.001$; **$p < 0.01$ – significant using one-way ANOVA. Superscript a,b,c indicate a significant mean difference for groups ($p < 0.05$, Post-Hoc Turkey test).

coming across the term "IEMs" while only 22.6% and 35.6% of pharmacy and dentistry students reported the same, respectively ($p < 0.05$). However, the mean IEM-related knowledge score did not vary significantly between the three faculties.

There was no difference in the mean IEM-related knowledge score in terms of age, gender, or year of study. Marital status was exempted from analysis as a disproportionate number of the respondents were single (99.7%) while only 0.3% were married. This would yield invalid results.

3.3. Perception of IEMs

The majority of students (88.7%) expressed an interest in increasing their IEM-related knowledge, with the Internet reported as the most common source (54.4%) (Figure 2). As seen in Table 4, student perception of

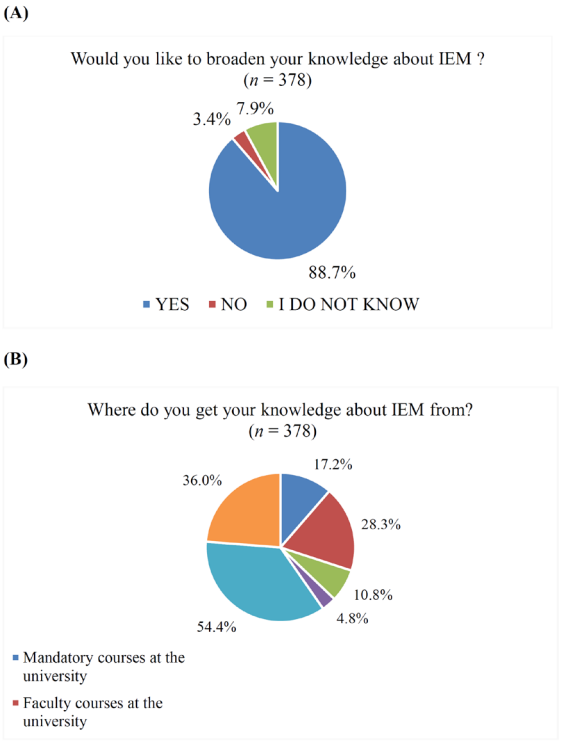


Figure 2. Student perceptions of (A) increasing their knowledge and (B) source of information on IEM.

IEM-related knowledge was also evaluated. Most of the healthcare students (89.5 %) believed that they had little IEM-related knowledge while 60.3% agreed that IEM courses should be a mandatory part of the school curricula. It is noteworthy that more than half of the pharmacy students (70.8%) and health sciences students (66.2%) agreed that IEM courses should be a mandatory part of the course curricula but only 43.2% of dentistry students agreed. As such, there was a significant difference in opinion between the groups ($p < 0.001$). In terms of preparation to care for an IEM patient, only 42.0% of students were prepared to care for an IEM patient.

4. Discussion

As IEMs are becoming more common in Malaysia, its management and treatment has become increasingly important to the Malaysian healthcare system (*4*). This present study discovered an overall insufficiency of IEM-related knowledge among healthcare students, especially in terms of diagnoses, management, and organisational issues of IEMs. These findings were similar to that of a recent Saudi Arabian study which concluded that both medical and non-medical students lacked the knowledge with which to diagnose and manage IEMs (*22*). This was also consistent with the findings of several other studies, which reported that the majority of healthcare students also lacked knowledge on the organizational issues of rare disorders,

Table 4. Students' self-perceived knowledge and opinion regarding Inborn Error of Metabolism (IEM)

Item	Health Science *n* = 154 (%)	Pharmacy *n* = 106 (%)	Dentistry *n* = 118 (%)	Total *n* = 378 (%)	*p* value
16. How would you rate your knowledge about IEM?					0.186
Very good/good	23 (14.9)	6 (5.7)	11 (9.3)	40 (10.6)	
Insufficient	62 (40.3)	50 (47.2)	53 (44.9)	165 (43.7)	
Very poor	69 (44.8)	50 (47.2)	54 (45.8)	173 (45.8)	
17. Do you think that there should be mandatory course on IEM in course curricula?					< 0.001*
Strongly agree/agree	102 (66.2)	75 (70.8)	51 (43.2)	228 (60.3)	
Neutral	46 (29.9)	31 (29.2)	62 (52.5)	139 (36.8)	
Strongly disagree/Disagree	6 (3.9)	0 (0.0)	5 (4.2)	11 (2.9)	
18. Do you feel prepared for caring over a patient with an IEM?					0.784
Strongly agree/agree	70 (45.5)	40 (37.7)	49 (41.5)	53 (14.0)	
Neutral	50 (32.5)	39 (36.8)	43 (36.4)	132 (34.9)	
Strongly disagree/Disagree	34 (22.1)	27 (25.5)	26 (22.0)	31 (8.3)	

*$p < 0.001$ – Significant using Pearson's chi-square's test

including IEMs (*19,20*). This indicated that students lacked concern or awareness of the launch of the 2019 Malaysian National Medicines Policy regarding palliative care services for IEM patients (*29*).

Infections are thought to potentially contribute to an accumulation of genetic mutations in mitochondrial DNA, which results in one IEM in particular; mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) (*30*). The findings of this present study were similar to that of extant studies on medical and non-medical faculties which showed that these students were less likely to recognize the modes of IEMs transmission; such as infections (*31*).

The interfaculty factor did not significantly alter the mean IEM-related knowledge score of the students. This finding was similar to that of a Polish study which reported that although the percentage of students who had encountered rare IEM terms varied significantly between the faculties of medicine (92%), health sciences (82%), and pharmacy (68.8%), the mean IEM-related knowledge scores were similar across the three faculties (*23*). In terms of year of study and level of education, the findings of this present study were also similar to that of other studies because these factors did not significantly affect the IEM-related knowledge score. One potential explanation for this phenomenon may be that these students acquired IEM-related knowledge out of sheer interest or intellectual curiosity (*19*).

This present study found that ethnicity, religion, and family history of IEMs affected the IEM-related knowledge score. Furthermore, Muslim students were found to score significantly lower than Buddhist students. This contradicted the findings of Chapman *et al.* (2019) who found no significant correlation between the IEM-related knowledge of Muslim and Buddhist participants (*25*). This present study also found that students with a family history of IEMs had more IEM-related knowledge. This finding was similar to that of another study which concluded that a family history of genetic disorders was one of the key determinants of acquiring more knowledge on IEMs (*21*). This was because most individuals with hereditary diseases have, at the very least, some knowledge of the disease in question (*32*).

This present study also found that students who had attended IEM classes or interacted with IEM patients had more IEM-related knowledge than those who did not. This was in agreement with an earlier observation that healthcare students with prior IEM-related knowledge and experience or involvement in scientific societies tend to have more IEM-related knowledge (*23,31,33*).

A majority of the respondents to this present study reported obtaining IEM-related knowledge from the Internet. This finding differed from that of a recent study which concluded that, in descending order, lectures and the Internet were the primary sources of IEM-related knowledge (*31*). This emphasized the importance of implementing a formal curriculum on IEMs through verified and reliable websites or online classes for healthcare students in the future. Furthermore, a higher proportion of pharmacy and health sciences students believed that IEM classes should be compulsory in the university curricula. The significance difference in perception between the students from the three different faculties may be because pharmacy and health sciences students are more likely to utilize IEM-related knowledge during clinical practice than dentistry students. Furthermore, the treatment of IEMs mainly involves pharmacotherapy treatments, in which a pharmacist plays an important role (*11*). Apart from that, other allied healthcare professionals; such as dietitians, physiotherapists, and occupational therapists; are a part of the holistic approach of treating and caring for IEM patients (*34*).

Most of the respondents to this present study expressed a willingness to expand their IEM-related knowledge. This was consistent with that of a previous study (*19*). However, less than 50% of respondents felt prepared to care for an IEM patient. Therefore, the results of this present study confirmed that healthcare students were unenthusiastic about caring for IEM patients because less than 5% of the respondents were adequately prepared to care for IEM patients. This figure was even lower in extant studies and may be due to a lack of IEM-related knowledge among healthcare students (*19,20*).

Some of the limitations of this present study included students that were allowed to complete the survey without monitoring or adhering to time restrictions. This meant that respondents could refer to materials or other sources prior to answering the questionnaire. However, the anonymous nature of the questionnaire is believed to have mitigated this issue.

To the best of our knowledge, this present study was the first to assess the knowledge and perception of Malaysian healthcare students on IEMs. It revealed that there is a pressing need to improve IEM-related knowledge and awareness of Malaysian healthcare students. This can be accomplished by incorporating online classes that emphasizes the treatment and management of IEMs in the university curriculum. Future studies should examine the key determinants that influence IEM-related knowledge and perception. It is hoped that this present study serves as a stepping stone to increasing the number of future studies on other healthcare and medical faculties as well as increasing the IEM-related knowledge of future healthcare professionals in the country.

Acknowledgements

We thank to everyone who helped in the article writing process.

Funding: This project was supported by a grant from the Fundamental Research Grant Scheme (FRGS/1/2020/SS0/UKM/02/14). The authors would like to extend their gratitude to everyone who helped in the article writing process. The authors confirm independence from the sponsors; the sponsors have not influenced the content of the article.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Ezgu F. Inborn Errors of Metabolism. In: Advances in Clinical Chemistry (Makowski G, ed. Elsevier Inc, Turkey, 2016; pp. 195-250.
2. Waters D, Adeloye D, Woolham D, Wastnedge E, Patel S, Rudan I. Global birth prevalence and mortality from inborn errors of metabolism: a systematic analysis of the evidence. J Glob Health. 2018; 8:021102.
3. Shafie AA, Supian A, Hassali MAA, Ngu LH, Thong MK, Ayob H, Chaiyakunapruk N. Rare disease in Malaysia: Challenges and solutions. PLoS One. 2020; 15:e0230850.
4. Yunus ZM, Rahman SA, Choy YS, Keng WT, Ngu LH. Pilot study of newborn screening of inborn error of metabolism using tandem mass spectrometry in Malaysia: outcome and challenges. J Pediatr Endocrinol Metab. 2016; 29:1031-1039.
5. Vernon HJ. Inborn errors of metabolism: advances in diagnosis and therapy. JAMA Pediatrics. 2015; 169:778-782.
6. Ombrone D, Giocaliere E, Forni G, Malvagia S, la Marca G. Expanded newborn screening by mass spectrometry: New tests, future perspectives. Mass Spectrom Reviews. 2016; 35:71-84.
7. Guerrero RB, Salazar D, Tanpaiboon P. Laboratory diagnostic approaches in metabolic disorders. Ann Transl Med. 2018; 6:470.
8. Ballikaya E, Yildiz Y, Sivri HS, Tokatli A, Dursun A, Olmez S, Coskun T, Uzamis Tekcicek M. Oral health status of children with phenylketonuria. J Pediatr Endocrinol Metab. 2020; 33:361-365.
9. Scanlan PM. A review of bachelor's degree medical laboratory scientist education and entry level practice in the United States. EJIFCC. 2013; 24:5-13.
10. Lamoureux MF, Tingley K, Kronick JB, *et al.* Metabolic clinic atlas: organization of care for children with inherited metabolic disease in Canada. JIMD Rep. 2015; 21:15-22.
11. Harthan AA. An Introduction to Pharmacotherapy for Inborn Errors of Metabolism. J Pediatr Pharmacol Ther. 2018; 23:432-446.
12. Echeverri OY, Guevara JM, Espejo-Mojica ÁJ, Ardila A, Pulido N, Reyes M, Rodriguez-Lopez A, Alméciga-Díaz CJ, Barrera LA. Research, diagnosis and education in inborn errors of metabolism in Colombia: 20 years' experience from a reference center. Orphanet J Rare Dis. 2018; 13:141.
13. Tanpaiboon P. Practical management of lysosomal storage disorders (LSDs). Transl Sci Rare Dis. 2019; 4:133-157.
14. Yuskiv N, Potter BK, Stockler S, Ueda K, Giezen A, Cheng B, Langley E, Ratko S, Austin V, Chapman M, Chakraborty P, Collet JP, Pender A; Canadian Inherited Metabolic Diseases Research Network (CIMDRN). Nutritional management of phenylalanine hydroxylase (PAH) deficiency in pediatric patients in Canada: a survey of dietitians' current practices. Orphanet J Rare Dis. 2019; 14:7.
15. De Castro M, Turner C, Kirmse B. Practical recommendations for the transition to adulthood for the adolescent with a genetic diagnosis. Special emphasis on inborn errors of metabolism. Transl Sci Rare Dis. 2019; 2019:159-168.
16. Agana M, Frueh J, Kamboj M, Patel DR, Kanungo S. Common metabolic disorder (inborn errors of metabolism) concerns in primary care practice. Ann Transl Med. 2018; 6:469.
17. Tejada-Ortigosa EM, Flores-Rojas K, Moreno-Quintana L, Muñoz-Villanueva MC, Pérez-Navero JL, Gil-Campos M. Necesidades sanitarias y socioeducativas de niños con enfermedades raras de tipo metabólico y sus

familias: estudio cualitativo en un hospital de tercer nivel [Health and socio-educational needs of the families and children with rare metabolic diseases: Qualitative study in a tertiary hospital]. An Pediatr (Engl Ed). 2019; 90:42-50. (in Spanish)
18. Mak CM, Law EC, Lee HH, *et al.* The first pilot study of expanded newborn screening for inborn errors of metabolism and survey of related knowledge and opinions of health care professionals in Hong Kong. Hong Kong Med J. 2018; 24:226-237.
19. Walkowiak D, Domaradzki J. Needs assessment study of rare diseases education for nurses and nursing students in Poland. Orphanet J Rare Dis. 2020; 15:167.
20. Domaradzki J, Walkowiak D. Medical students' knowledge and opinions about rare diseases: A case study from Poland. Intractable Rare Dis Res. 2019; 8:252-259.
21. Mitra AK, Al-Enezi K. Knowledge, attitude, and satisfaction of university students regarding premarital screening programs in Kuwait. European Journal of Environment and Public Health. 2017; 1:1-11.
22. Alqrache A. Knowledge and awareness of metabolic inborn errors among male and female students at King Abdulaziz University – Rabigh. The Egyptian Journal of Medical Education. 2020; 4:1-5.
23. Jonas K, Waligóra M, Hołda M, Sulicka-Grodzicka J, Strach M, Podolec P, Kopeć G. Knowledge on rare diseases among health care students – the effect of targeted education. Przegl Epidemiol. 2017; 71:80-89.
24. Ramalle-Gomara E, Ruiz E, Quinones C, Andres S, Iruzubieta J, Gil-de-Gomez J. General knowledge and opinion of future health care and non-health care professionals on rare diseases. J Eval Clin Pract. 2015; 21:198-201.
25. Chapman R, Likhanov M, Selita F, Zakharov I, Smith-Woolley E, Kovas Y. New literacy challenge for the twenty-first century: genetic knowledge is poor even among well educated. J Community Genet. 2019; 10:73-84.
26. Schmidlen TJ, Scheinfeldt L, Zhaoyang R, Kasper R, Sweet K, Gordon ES, Keller M, Stack C, Gharani N, Daly MB, Jarvis J, Christman MF. Genetic knowledge among participants in the coriell personalized medicine collaborative. J Genet Couns. 2016; 25:385-394.
27. Olwi D, Merdad L, Ramadan E. Knowledge of genetics and attitudes toward genetic testing among college students in Saudi Arabia. Public Health Genomics. 2016; 19:260-268.
28. Polit DF, Beck CT, Owen SV. Is the CVI an acceptable indicator of content validity? Appraisal and recommendations. Res Nurs Health. 2007; 30:459-467.
29. Ministry of Health Malaysia. Policy and strategic plan 2019-2030: National Palliative Care. 2019. *https://www.moh.gov.my/moh/resources/Polisi/BUKU_NATIONAL_PALLIATIVE_CARE_POLICY_AND_STRATEGY_PLAN_2019-2030.pdf* (accessed February 7 2022)
30. Ryzhkova AI, Sazonova MA, Sinyov VV, Galitsyna EV, Chicheva MM, Melnichenko AA, Grechko AV, Postnov AY, Orekhov AN, Shkurat TP. Mitochondrial diseases caused by mtDNA mutations: a mini-review. Ther Clin Risk Manag. 2018; 14:1933-1942.
31. Shareef J, Sridhar SB, Shariff A, Sabah MM, Hameed MY. Inborn errors of metabolism in the United Arab Emirates: Are our future healthcare providers knowing enough about it—A cross-sectional study. Journal of Applied Pharmaceutical Science. 2021; 11:093-098.
32. Claassen L, Henneman L, Janssens AC, Wijdenes-Pijl M, Qureshi N, Walter FM, Yoon PW, Timmermans DR. Using family history information to promote healthy lifestyles and prevent diseases; a discussion of the evidence. BMC Public Health. 2010; 10:248.
33. Ugwu NI. Sickle cell disease: Awareness, knowledge and attitude among undergraduate students of a Nigerian tertiary educational institution. Asian Journal of Medical Sciences. 2016; 7:87-92.
34. Burgard P. A holistic approach to the patients/ Families with inborn errors of metabolism. J Mother Child. 2020; 24:65-72.

Received May 29, 2022; Revised July 27, 2022; Accepted August 5, 2022.

**Address correspondence to:*
Roslee Rajikan, Dietetics Programme & Centre of Healthy Aging and Wellness (H-Care), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300, Kuala Lumpur, Malaysia.
E-mail: roslee@ukm.edu.my

Released online in J-STAGE as advance publication August 10, 2022.

Original Article

DOI: 10.5582/irdr.2022.01077

Identification of potential core genes and miRNAs in pediatric ACC *via* bioinformatics analysis

Chunyan Fang[1,§], Yulong Ye[2,§], Fangyue Wang[1], Yifeng Shen[1], Yaodong You[1,*]

[1] TCM Regulating Metabolic Diseases Key Laboratory of Sichuan Province, Hospital of Chengdu University of Traditional Chinese Medicine, Chengdu, Sichuan, China;
[2] Tea Research Institute, Sichuan Academy of Agricultural Sciences, Chengdu, Sichuan, China.

SUMMARY Pediatric adrenocortical carcinomas (ACC) are rare aggressive neoplasms with heterogeneous prognosis, and often produce a most lethal malignant tumor, whereas its aetiology is still unclear. The aim of the present study was to identify the factors responsible for the development of pediatric ACC, a better understanding of the disease, and investigate new molecular biomarkers and therapeutic targets. To identify the key genes and miRNAs linked to pediatric ACC, as well as their potential molecular mechanisms, the GSEGSE75415 and GSE169253 microarray datasets were analyzed. A total of 329 differentially produced genes (DEGs) and 187 differentially produced miRNAs (DEMs) were obtained after analyzing the GSEGSE75415 and GSE169253 datasets, respectively. Next, 3,359 genes were obtained by overlapping the target mRNAs of DEMs. Following protein-protein interaction network and Gene Ontology analysis, the ten nodes with the highest degrees were screened as hub genes. Among them, the highly expressed hub genes, *MAPK1* and *EP300*, were associated with a worse overall survival. Additionally, hsa-miR-376, hsa-miR-148, hsa-miR-139, and hsa-miR-1305 were strongly associated with poorer survival. We proposed that the hub genes (*MAPK1*, *EP300*, hsa-miR-376, hsa-miR-148, hsa-miR-139, and hsa-miR-1305) may have a definite impact on cellular proliferation and migration in adrenocortical tumors. The roles of these hub genes in adrenocortical tumors may provide novel insight to improve the diagnosis and treatment of patients with pediatric ACC.

Keywords pediatric adrenocortical carcinomas, hub genes, DEGs, microRNAs, prognosis

1. Introduction

Almost 3% to 10% of the population has been reported to present adrenal tumors, and most adrenal tumors are benign (*1,2*). Conversely, ACCs originating in the outer part of the adrenal gland, are an ultra-rare endocrine malignancy, and lethal malignancies with poor overall survival (*3*). The highest incidence of ACC is age 1 to 4, and 40 to 50 in two time periods of life. Adult ACC is a rare cancer with a reported incidence of 0.7-2 cases per million people/year worldwide (*4*). Pediatric ACC is even rarer with a reported incidence of 0.2-0.3 cases per million people/year worldwide (*5*). Because of its rarity, pediatric ACC is less studied than adult ACC, which may be an obstacle to conducting clinical trials and determining accurate guidelines for the clinical management of pediatric ACC.

ACC in children present as a unique entity, signs and symptoms hyperfunctioning, because of the hypersecretion of sex hormones, cortisol, or aldosterone hypersecretion or mixed endocrine syndromes (*6,7*). It has been reported that pediatric ACC more often has a cancer predisposing familial genetic basis, such as the Li–Fraumeni syndrome, the germline P53 mutations, carney complex, and the Beckwith–Wiedemann syndrome (*6*). The most important treatment of ACC is surgery which is the only mode of therapy documented as effective for treating pediatric ACC, and moreover several adjuvant therapies are used depending on grade and stage of the tumor to lengthen overall survival (*8*). Unfortunately, the prognosis of ACC is still usually poor due to the late stage at diagnosis. Thus, it is necessary to identify accurate biomarkers for pediatric ACC treatment to facilitate the accurate early stages of diagnosis for the pediatric ACC cases, and therefore for the early discovery and treatment of pediatric ACC cases.

MicroRNAs (miRNAs) are small non-coding regulatory RNA molecules 19 to 25 nucleotides in size that regulate post-transcriptional repression of target genes. A single miRNA can target hundreds of mRNAs

by recognizing the 3′untranslated region (UTR) of target miRNAs. Studies have shown that about 60% of human genes are regulated by miRNAs, indicating that miRNA play key roles in a variety of processes, such as embryogenesis, maintenance of tissue homeostasis, tissue repair and carcinogenesis. And the great majority of studies have found that the abnormal expression of miRNAs correlates with a greater risk of carcinogenesis, including colon cancer, breast cancer, prostate cancer, lung cancer, cholangiocarcinoma, uterine leiomyoma, ovarian cancer, *etc*. (*9*). Hence, it is urgent and necessary to explore novel therapeutic targets for the treatment of pediatric ACC

In the present study, we selected two gene expression datasets (GSEGSE75415, and GSE169253), which were downloaded from the Gene Expression Omnibus (GEO) database, to obtain differentially expressed genes (DEGs) and differentially expressed microRNAs (DEMs) between pediatric adrenocortical tumors and normal adrenal glands. Differentially expressed miRNAs were identified by integrating multiple bioinformatics analysis methods. Then, functional enrichment and network analyses were applied to identify target genes of differentially expressed miRNAs. Subsequently, we established a protein-protein interaction (PPI) network to identify hub genes related to ACC. The expression values of these hub genes were determined using the online database UALCAN. Survival analysis of these hub genes was performed using the online database Gene Expression Profiling Interactive Analysis (GEPIA). Our findings serve as a valuable resource to further explore the mechanisms of pediatric ACC development and progression, and provide potentially effective diagnostic markers and therapeutic targets for pediatric ACC.

2. Materials and Methods

2.1. Identification of DEGs and DEMs

Two gene expression profiles, GSE75415 and GSE169253, were obtained from the GEO database (*http://www.ncbi.nlm.nih.gov/geo/*). The array data of GSE75415 comprising 24 pediatric tumors and 7 normal adrenal glands was submitted by West AN *et al*. (*10*). GSE169253 consisted of 37 adrenocortical tumors and 9 non-neoplastic adrenal controls contributed by Veronez LC *et al*. (*11*). DEGs were obtained from the GEO database by GEO2R analysis (*http://www.ncbi.nlm.nih.gov/geo/geo2r/*). Adjusted $p < 0.05$ and log fold-change (|logFC|) > 2.0 were set as the DEG cutoff criterion. Adjusted $p < 0.05$ and |logFC| > 1.0 were set as the DEM cutoff criterion.

2.2. Target prediction of the DEMs and screening of the key oncogenes

The online tool miRDB (*http://mirdb.org/*) and TargetScanHuman 7.2 (*https://www.targetscan.org/vert_72/*) were used to predict the mRNAs potentially targeted by the DEMs acquired from the GSE169253 dataset. The criterion of any target was a matching score = 1. The intersection of all targets was obtained through a Venn diagram. Given all DEMs were downregulated, we screened all the upregulated DEMs from the GSE75415 dataset. The intersection of DEGs and the targets of DEMs represented the key oncogenes associated with ACC.

2.3. Gene Ontology (GO) and Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

GO analysis is a commonly used method for large-scale functional enrichment research; gene functions can be classified into biological process (BP), molecular function (MF) and cellular component (CC). KEGG is a widely used database that stores data on genomes, biological pathways, diseases, chemical substances and drugs. GO annotation and KEGG pathway enrichment analyses of the DEGs identified in this study were performed using DAVID tools. $p < 0.05$ was considered to indicate a statistically significant difference.

2.4. Protein-protein interaction (PPI) enrichment analysis

PPI enrichment analysis was carried out on the key oncogenes using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database (*https://string-db.org/*), and a PPI network was generated. Only physical interactions (physical score > 0.4) in STRING were used. Subsequently, the PPI network was visualized by Cytoscape software (version 3.7.1; *www.cytoscape.org/*). Nodes with a higher degree of connectivity tend to be more essential in maintaining the stability of the entire network. CytoHubba (version 0.1) (*12*), a plugin in Cytoscape, was used to calculate the degree of each protein node. In our study, the top ten genes were identified as hub genes.

2.5. Expression profiles of hub genes based on tumor histology and survival analysis

UALCAN (http://UALCAN.path.uab.edu) and GEPIA (*http: //gepia.cancer-pku.cn/*) are user-friendly, interactive web resource for analyzing cancer transcriptome data. According to the median expression of a particular gene, the patients with ACC were split into high and low expression groups. The overall survival (OS) of ACC patients was evaluated using GEPIA (*13*). $p < 0.05$ was considered to indicate a statistically significant result.

2.6. Exosomal miRNA-mRNA network model construction

By using miRDIP v4.1 online tool, the interactions

between the key mRNAs and key exosomal miRNAs were obtained. Then, the interactions showing high confidence class were chosen for construction of exosomal miRNA-mRNA network by using Cytoscape v3.7.2. The mRNAs in the network were considered as hub mRNAs in this study.

3. Results

3.1. ACC-associated DEGs and DEMs

The gene expression profiles GSE75415and GSE169253 were selected in this study. Based on the criteria of $P < 0.05$ and |logFC|> 2.0, a total of 329 DEGs were identified from GSE75415. Of these, 125 were up-regulated, and 204 were down-regulated in pediatric tumors compared with normal adrenals (Figure 1A). A total of 106 DEMs are upregulated, and 81 DEMs are down-regulated in adrenocortical tumors (Figure 1B). All of these DEMs were identified from GSE169253. We selected five up-regulated DEMs and down-regulate DEMs respectively as hub exosomal miRNAs, with the largest differential multiples, to predict miRNA target genes (Table 1).

3.2. Target genes of the DE miRNAs

In order to reduce the false positive rate of software prediction results, the targets of the above 10 miRNAs were acquired by two miRNA target prediction softwares, miRDB and TargetscanHuman7.2. The results show that there are 10, 29, 138, 298 and 505 target genes in five up-regulated miRNA, hsa-miR-1915-5p, hsa-miR-615-5p, hsa-miR-587, hsa-miR-611 and hsa-miR-452-3p, respectively (Figure 2); There are 393, 293, 15, 1526 and 152 target genes in five down-regulated miRNA, hsa-miR-630, hsa-miR-575, hsa-miR-572, hsa-miR-1305 and hsa-miR-139-3P, respectively (Figure 3).

3.3. Functional and pathway enrichment analyses of target genes

The functional categories of the identified proteins were analyzed with Blast2GO software based on their Gene Ontology (GO) annotations. The enriched GO terms were divided into Biological Processes (BP), Cellular Component (CC), and Molecular Function (MF) ontology terms. The 921 genes identified in five up-regulated miRNAs could be classified into 1,472 categories based

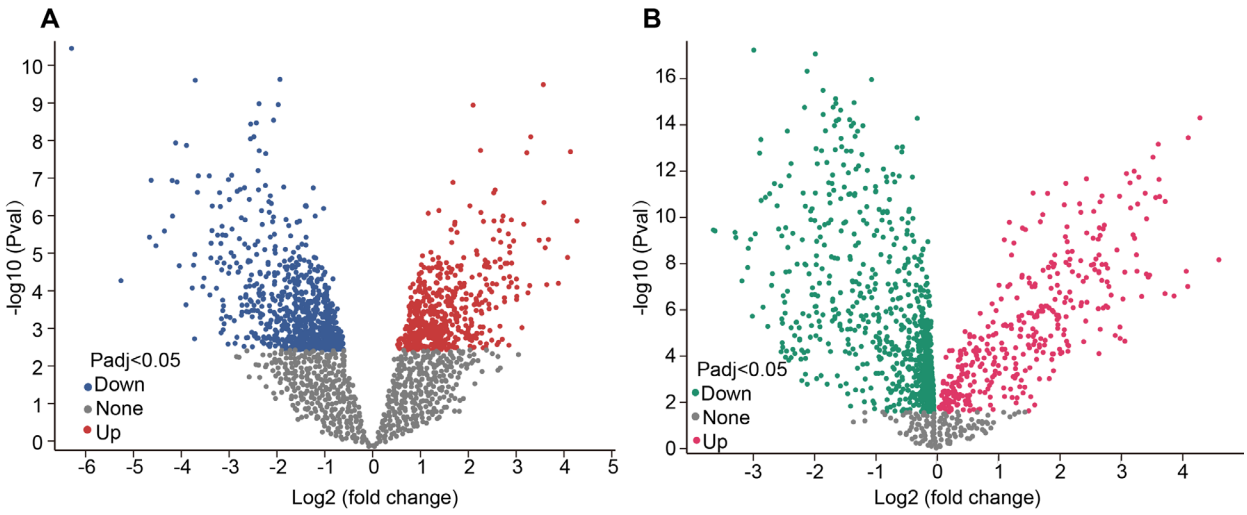


Figure 1. Volcano plots of differentially expressed mRNAs (DE mRNAs, in theGSE75415 dataset) (A), and miRNA (DE-miRs, in the 169253 dataset) (B). DE, differentially expressed.

Table 1. The largest differential expression of miRNA in adrenocortical tumors and non-neoplastic adrenal controls

Items	miRNA_ID	log2(fold change)	adj. *p* value	*p* value
Up-regulated miRNA	hsa-miR-376a	4.605	1.06E-07	8.12E-09
	hsa-miR-21	4.296	1.30E-12	6.45E-15
	hsa-miR-376c	4.073	1.04E-06	1.13E-07
	hsa-miR-377	4.073	2.81E-07	2.52E-08
	hsa-miR-148a	3.73	7.15E-10	2.52E-11
Down-regulated miRNA	hsa-miR-630	-3.635	7.91E-09	4.25E-10
	hsa-miR-575	-3.283	9.65E-09	5.37E-10
	hsa-miR-572	-3.076	2.03E-07	1.74E-08
	hsa-miR-1305	-3.067	3.82E-08	2.59E-09
	hsa-miR-139-3p	-2.977	1.57E-14	7.92E-18

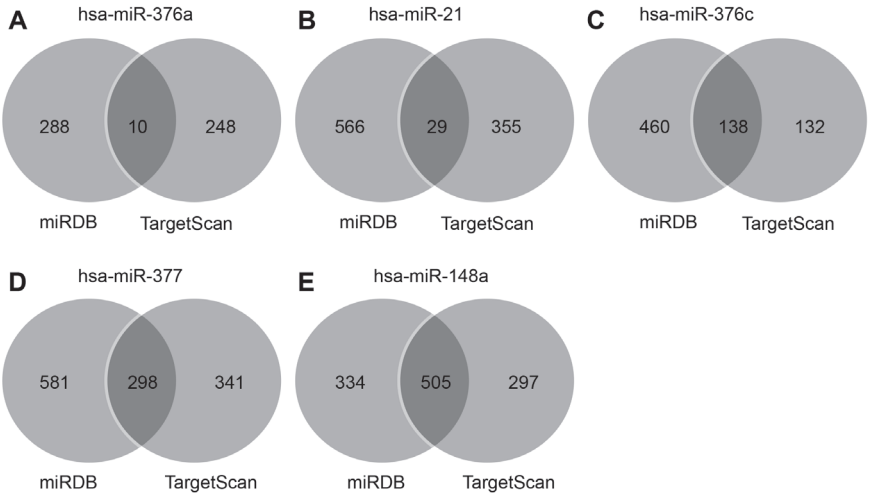


Figure 2. Target gene prediction of five miRNA with the largest up-regulated expression.

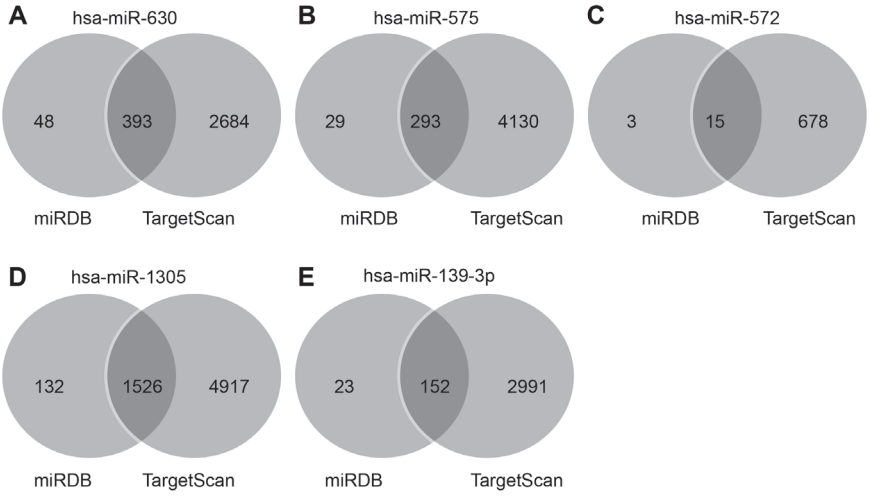


Figure 3. Target gene prediction of five miRNAs with the largest down-regulated expression.

on their annotated BP, and most of them were distributed in neuron projection development, vasculature development, neuron projection morphogenesis, plasma membrane bounded cell projection morphogenesis, cell projection morphogenesis, cell part morphogenesis, blood vessel development, cell morphogenesis, cellular component morphogenesis, and tube morphogenesis, implying that these processes play a significant roles for the occurrence of ACC in children. For CC, the postsynapse, axon, neuron to neuron synapse, dendrite, asymmetric synapse, and dendritic tree were the largest groups of genes. DNA-binding transcription activator activity, RNA polymerase II-specific, DNA-binding transcription activator activity, phosphotransferase activity, alcohol group as acceptor, protein kinase activity, and kinase activity were the most abundant MF categories, which implied that transcription activator activity and kinase activity are vital for carcinogenesis of ACC (Table 2).

The results of GO analysis indicated that the target genes of down-regulated miRNAs were mainly enriched in BPs, including head development, brain development, neuron projection development, and cell morphogenesis. CC analysis showed that the target genes of down-regulated miRNAs were significantly enriched in Golgi apparatus subcompartment axon, cytoplasmic stress granule, and intracellular protein-containing complex. For MF ontology, these genes were mainly enriched in chromatin binding, protein domain specific binding, nucleoside-triphosphatase activity, and kinase activity (Table 3).

To obtain an overview of the function of target genes of DEMs, the gene identifications were used to search KEGG pathway database using KAOBAS 2.0. The genes involved in Pathways in cancer, PI3K-Akt signaling pathway, Breast cancer, Proteoglycans in cancer, FoxO signaling pathway and MAPK signaling pathway were the most abundant pathways of the target genes of up-regulated DEMs (Figure 4A). Nevertheless, the target genes of down-regulated DEMs were also mainly enriched in FoxO signaling pathway, Pathways in cancer, PI3K-Akt signaling pathway, MAPK signaling pathway,

Table 2. Significantly enriched Go terms of the target genes of up-regulated miRNAs

Category	Term	Description	Count	*p* value
BP term	GO:0031175	neuron projection development	641	1E-23
	GO:0001944	vasculature development	526	1E-22
	GO:0048812	neuron projection morphogenesis	454	1E-21
	GO:0120039	plasma membrane bounded cell projection morphogenesis	458	1E-21
	GO:0048858	cell projection morphogenesis	462	1E-21
	GO:0032990	cell part morphogenesis	481	1E-20
	GO:0001568	blood vessel development	505	1E-20
	GO:0000902	cell morphogenesis	670	1E-19
	GO:0032989	cellular component morphogenesis	574	1E-19
	GO:0035239	tube morphogenesis	663	1E-19
CC term	GO:0098794	postsynapse	614	1E-19
	GO:0030424	axon	631	1E-19
	GO:0098984	neuron to neuron synapse	347	1E-17
	GO:0030425	dendrite	611	1E-15
	GO:0032279	asymmetric synapse	323	1E-15
	GO:0097447	dendritic tree	613	1E-15
	GO:0099572	postsynaptic specialization	341	1E-15
	GO:0097060	synaptic membrane	373	1E-14
	GO:0014069	postsynaptic density	318	1E-14
	GO:0048471	perinuclear region of cytoplasm	721	1E-13
MF term	GO:0001228	DNA-binding transcription activator activity, RNA polymerase II-specific	462	1E-17
	GO:0001216	DNA-binding transcription activator activity	466	1E-17
	GO:0016773	phosphotransferase activity, alcohol group as acceptor	674	1E-16
	GO:0004672	protein kinase activity	565	1E-16
	GO:0016301	kinase activity	730	1E-15
	GO:0004674	protein serine/threonine kinase activity	430	1E-13
	GO:0019904	protein domain specific binding	687	1E-13
	GO:0004712	protein serine/threonine/tyrosine kinase activity	446	1E-13
	GO:0008134	transcription factor binding	595	1E-12
	GO:0106310	protein serine kinase activity	361	1E-12

Table 3. Significantly enriched Go terms of the target genes of down-regulated miRNAs

Category	Term	Description	Count	*p* value
BP term	GO:0060322	head development	789	1E-16
	GO:0007420	brain development	744	1E-14
	GO:0031175	neuron projection development	641	1E-14
	GO:0000902	cell morphogenesis	670	1E-12
	GO:0000278	mitotic cell cycle	605	1E-11
	GO:0048812	neuron projection morphogenesis	454	1E-11
	GO:0007017	microtubule-based process	786	1E-11
	GO:1903047	mitotic cell cycle process	514	1E-11
	GO:0051301	cell division	503	1E-11
	GO:0120039	plasma membrane bounded cell projection morphogenesis	458	1E-11
CC term	GO:0098791	Golgi apparatus subcompartment	384	1E-12
	GO:0030424	axon	631	1E-12
	GO:0010494	cytoplasmic stress granule	81	1E-11
	GO:0140535	intracellular protein-containing complex	753	1E-11
	GO:0005813	centrosome	620	7.94328E-10
	GO:0000139	Golgi membrane	662	1.25893E-09
	GO:0016607	nuclear speck	413	3.98107E-09
	GO:0005802	trans-Golgi network	261	3.98107E-09
	GO:0036464	cytoplasmic ribonucleoprotein granule	244	1.99526E-08
	GO:0005819	spindle	425	2.51189E-08
MF term	GO:0003682	chromatin binding	586	1E-11
	GO:0019904	protein domain specific binding	687	1E-10
	GO:0017111	nucleoside-triphosphatase activity	699	1.99526E-10
	GO:0016301	kinase activity	730	3.16228E-10
	GO:0016773	phosphotransferase activity, alcohol group as acceptor	674	5.01187E-10
	GO:0008134	transcription factor binding	595	7.94328E-10
	GO:0070717	poly-purine tract binding	29	1.25893E-09
	GO:0003924	GTPase activity	337	1.58489E-09
	GO:0003712	transcription coregulator activity	497	1.58489E-09
	GO:0016462	pyrophosphatase activity	754	3.16228E-09

and axon guidance (Figure 4B).

3.4. PPI network construction and the analysis of hub genes

A total of 900 nodes and 4,223 edges were mapped in the PPI network of the targets of up-regulated DEMs (Figure 5A). The 10 nodes with the highest degrees, including phosphatase and tensin homolog (*PTEN*), phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (*PIK3CA*), mitogen-activated protein kinase 1 (*MAPK1*), estrogen receptor 1 (*ESR1*), and erb-b2 receptor tyrosine kinase 3 (*ERBB3*) were screened as hub genes (Table 4).

There were 1,555 nodes and 7,712 edges, which mapped in the PPI network of the targets of down-regulated DEMs (Figure 5B). The 10 nodes with the highest degrees of these genes were: tumor protein p53 (*TP53*), E1A binding protein p300 (*EP300*), KRAS proto-oncogene, GTPase (*KRAS*), DDB1 and CUL4 associated factor 10 (*DCAF10*), and phosphatase and tensin homolog (*PTEN*) (Table 5).

3.5. Expression profiles of the hub genes and survival analysis

To investigate the expression and prognostic values of the ten potential hub genes, the GEPIA bioinformatics analysis platform was used. We found that the nine hub genes were significantly (all p-value < 0.05) associated

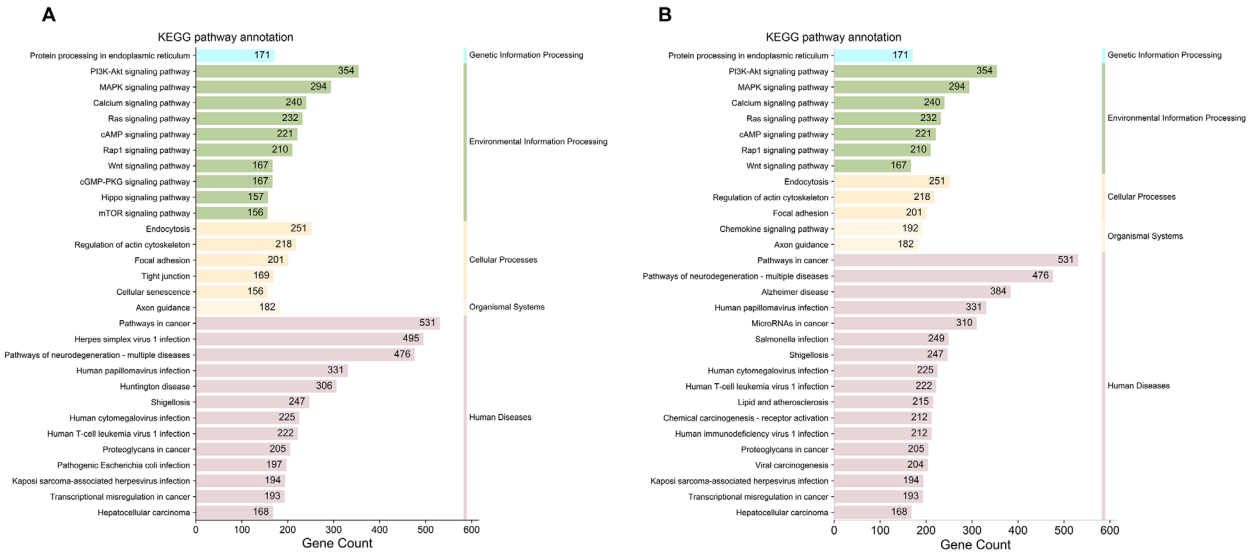


Figure 4. KEGG pathways analysis of identified genes.

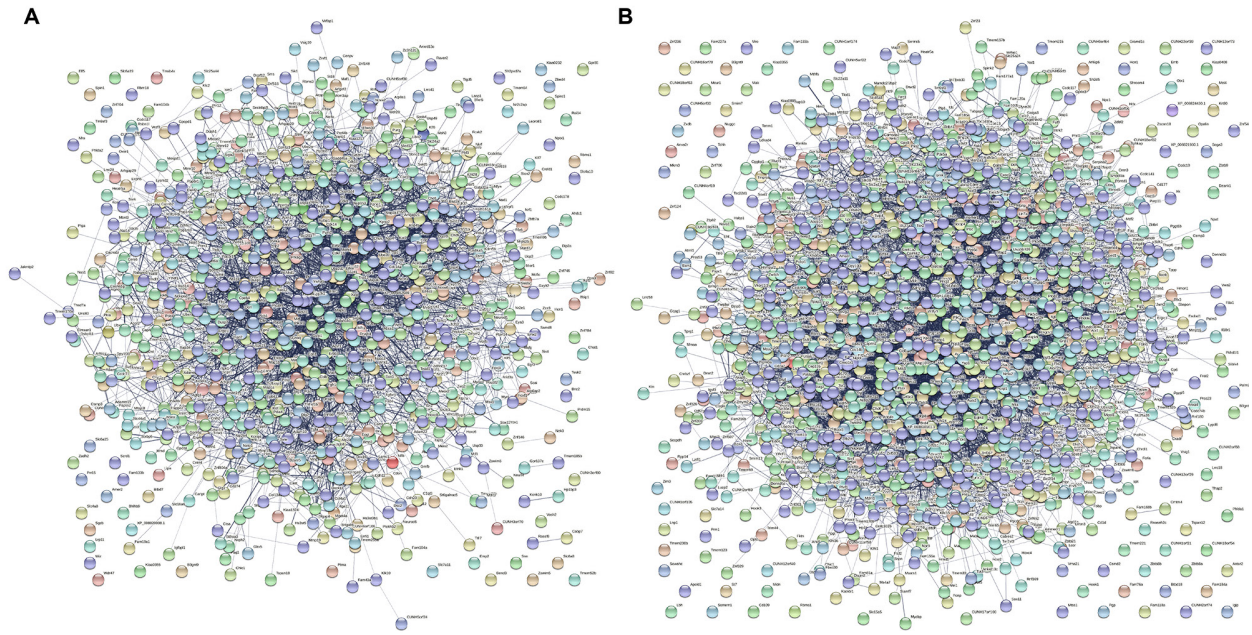


Figure 5. PPI network of the of the target genes of DEMs.

Table 4. Top five key genes with the highest degrees of connectivity in the target genes of up-regulated DEMs

Gene	Gene description	Degree
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	64
MAPK1	mitogen-activated protein kinase 1	63
ESR1	estrogen receptor 1	55
ERBB3	erb-b2 receptor tyrosine kinase 3	54
NRAS	NRAS proto-oncogene, GTPase	52

Table 5. Top five key genes with the highest degrees of connectivity in the target genes of down-regulated DEMs

Gene	Gene description	Degree
TP53	tumor protein p53	137
EP300	E1A binding protein p300	118
KRAS	KRAS proto-oncogene, GTPase	89
DCAF10	DDB1 and CUL4 associated factor 10	80
PTEN	phosphatase and tensin homolog	79

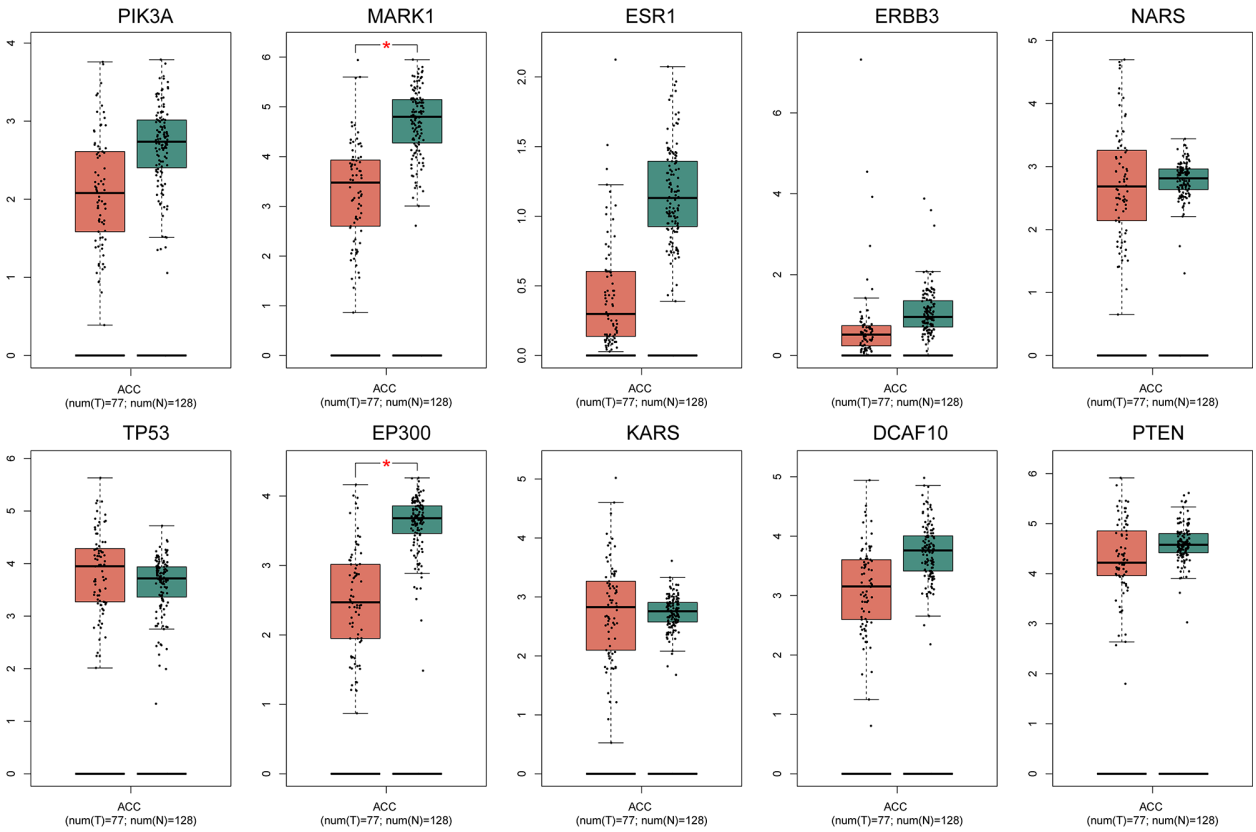


Figure 6. Gene Expression Profiling Interactive Analysis for overall survival associated with the expression of the ten hub genes in patients with adrenocortical carcinoma. Red line represents high expression, and blue line represents low expression.

with shorter survival duration of the adrenocortical carcinoma (Figure 6). *MARP1* and *EP300* were significant, and we found that the high expression of hub genes was associated with an unfavorable OS of patients with adrenocortical carcinoma (Figure 7).

3.6. Validation of the hub exosomal miRNAs

As shown in Figure 8A, an exosomal miRNA-mRNA network consisting of four miRNAs (hsa-miR-376, hsa-miR-148, hsa-miR-139, and hsa-miR-1305) and 6 mRNAs (*ESR1, PTEN, NARS, ERBB3, MAPK1*, and *KARS*) was constructed. These mRNAs and miRNAs were respectively considered to be hub mRNAs and hub exosomal miRNAs that might play crucial roles in ACC development *via* exosomes. As shown in Figure 8B, every index in different times had no significant difference from that in primary carcinoma ($p > 005$). Then, we explored the value of the hub exosomal miRNAs as diagnosis biomarkers in ACC by performing ROC curves and calculating the area under the curves (AUCs) [95% confidence intervals (CIs)]. The AUCs

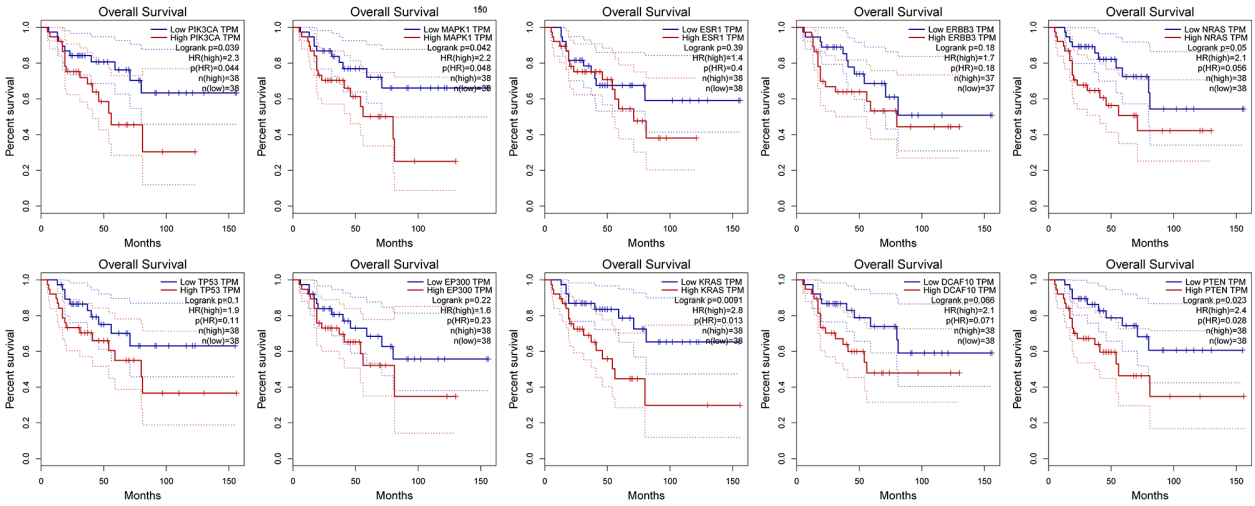


Figure 7. Expression values of the top hub genes in adrenocortical carcinoma and non-adrenocortical carcinoma.

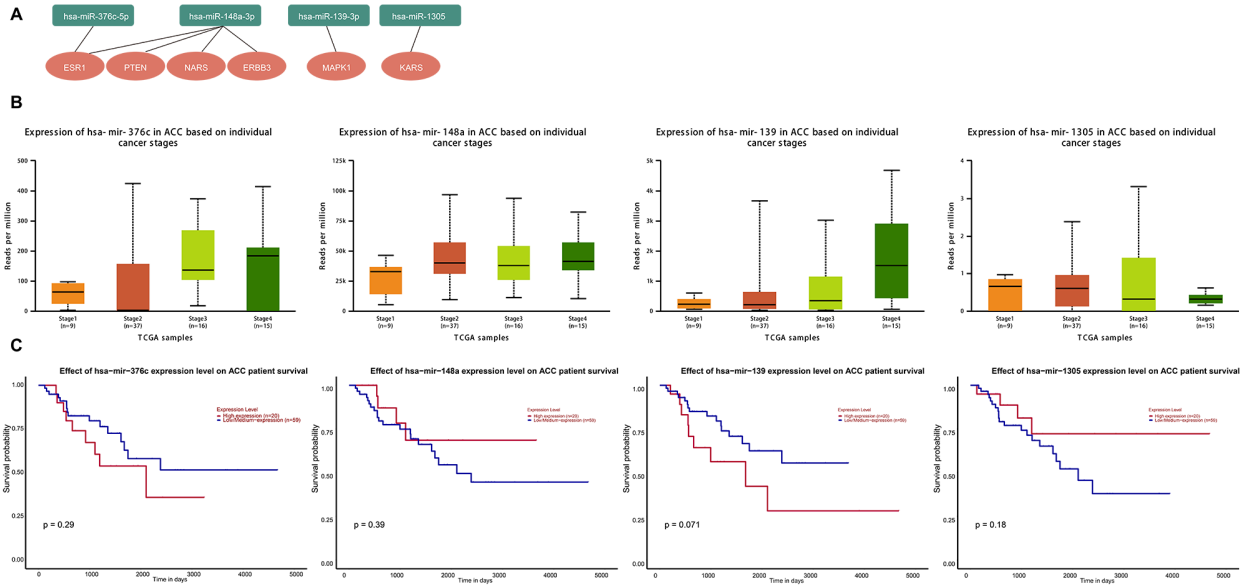


Figure 8. Validation of the hub exosomal miRNAs. A Exosomal miRNA-mRNA network. B Expression of Hub genes in primary ACC and metastatic ACC. C Results for the overall survival (OS) analysis of the hub mRNAs.

of hsa-miR-376, hsa-miR-148, hsa-miR-139, and hsa-miR-1305 were respectively 0.29, 0.39, 0.071, and 0.18, which proved that the four hub exosomal miRNAs can well distinguish tumor and normal samples.

4. Discussion

Pediatric ACC is rare aggressive neoplasms with heterogeneous prognosis, and often is a most lethal malignant tumor. It is usually discovered and diagnosed at its advanced stage. Despite extensive efforts, identifying reliable prognostic factors for pediatric patients with ACC remains a challenge. MicroRNA (miRNA) signatures have been associated with cancer diagnosis, treatment response, and prognosis of several types of cancer. However, the role of miRNAs has been poorly explored in pediatric ACC. Therefore, it is important to develop a miRNA-mRNA network that drives the mechanisms of pediatric ACC for identifying potential biomarkers for improving the diagnostic accuracy of pediatric ACC.

In this study, through comprehensive analysis of the open access miRNA and mRNA data for pediatric ACC from GEO, we identified 329 DEGs and 187 DEMs that were differentially expressed in pediatric tumors and normal adrenal glands based on the GSE75415 dataset and GSE169253 dataset, respectively. A total of 106 DEMs are upregulated, and 81 DEMs are down-regulated in adrenocortical tumors. All of these DEMs were identified from GSE169253. We selected five up-regulated DEMs and down-regulated DEMs respectively as hub miRNAs, with the largest differential multiples, to predict their target mRNAs. Next, 3,359 genes were obtained by overlapping the target mRNAs, which could

ensure to get the most potential mRNAs associated with pediatric ACC progression. The genes identified in five up-regulated miRNAs were enriched in "neuron projection development", "vasculature development", and "neuron projection morphogenesis", while the genes identified in five down-regulated miRNAs were mainly enriched in "head development", "brain development", and "neuron projection development". Moreover, all these target genes of DEMs were involved in "Pathways in cancer", "PI3K-Akt signaling pathway", "MAPK signaling pathway", and "FoxO signaling pathway". Then, we identified ten high-degree hub genes by constructing the PPI network, including *PIK3CA, MAPK1, ESR1, ERBB3, NRAS, TP53, EP300, KRAS, DCAF10*, and *PTEN*. We predicted the association between the expression of the hub genes and the prognosis of ACC patients. Based on GEPIA, the overexpression of all hub genes was related to an unfavorable prognosis in patients with ACC. Among them, *MAPK1* and *EP300* were significantly up-regulated in pediatric tumors. Finally, we performed intersection of differentially expressed miRNAs with hub genes. After finishing the intersection, a total of 4 candidate miRNAs were obtained to distinguish tumor and normal samples, including hsa-miR-376, hsa-miR-148, hsa-miR-139, and hsa-miR-1305.

Recent studies have found that MAPK1 is highly expressed in many tumors, including liver cancer, renal cell carcinoma, prostate cancer, lung cancer, and so on (*14-17*). The abnormal-expression of MAPK in tumors leads to an activation of the MAPK pathway, which is a highly conserved module that is involved in various cellular functions, including cell proliferation, differentiation and migration. This pathway involves besides RAF kinases and upstream GTPases of the RAS family, the mitogen-activated extracellular signal-regulated kinases 1/2 (MEK1/2) and extracellular signal-regulated kinases 1/2 (ERK1/2) (*18*). It is hyperactivated in a large variety of tumors, and many of its elements have been identified as oncogenes. These observations have generated a profound interest in targeting this pathway as a therapeutic option for cancer (*19*). *EP300* is known to participate in a variety of cellular functions including DNA repair, cell growth and differentiation, apoptosis, cell cycle regulation and chromatin remodeling, all of which are consistent with a tumor suppressor role (*20*). EP300 acetylation of TP53 in response to DNA damage regulates its DNA binding and transcription functions (*21*). Therefore, *MAPK1* and *EP300* in ACC are worthy of more attention.

Members of the miR-376 cluster are transcribed as one transcript bearing multiple hairpin structures that undergo RNA editing at multiple sites prior to being processed into individual pre-miRNAs. miR-376a has two major editing sites located at +4 in 5p seed sequence and +44 in 3p seed sequence (*22,23*). These editing sites show high levels of modification frequencies in specific regions of the brain compared to other tissues. It is consistent with our results that the genes identified in DEMs were mainly enriched in head development and neuron projection development. The altered expression of hsa-miR-148 was found in various tumors, including gastric cancer, pancreatic cancer, lung cancer and gastric cancer, *etc*. (*24-27*). It has been largely through directly targeting key players of integrin signaling like ITGA5, ROCK1, and PI3KCA/p110α as well as NRAS, which controlled the pathway involved in tumor growth and metastasis (*26*). Cristina Montero-Conde reported that differential expression analysis revealed a consistent hsa-miR-139-5p down expression in primary carcinomas from patients with recurrent/metastatic disease compared to disease-free patients, indicating that hsa-miR-139 was associated with recurrent disease independent of genetic background (*28*). Thus, miRna differential expression analysis between prognostic classes identify hsa-miR-139-5p as a disease outcome marker. Yinjie Su indicated that circRIP2 sponges miR-1305 to elevate Tgf-β2 in bladder cancer cells (*29*). Morever, Weliu reported that ASB16-AS1 promotes cell proliferation, migration, invasion *via* binding miR-1305 with Wnt2, and enhancing the Wnt/β-catenin pathway in cervical cancer (*30*).

5. Conclusions

Based on our results and above-mentioned literature, we suppose that there are multiple regulatory axes related to ACC development in intracellular communications mediated by miRNA and mRNA. The current study efficiently identified several candidate targets (*MAPK1, EP300, hsa-miR-376, hsa-miR-148, hsa-miR-139*, and *hsa-miR-1305*) that can potentially serve as biomarkers in the diagnosis of pediatric ACC, and may be potential targets for seminoma therapy. These findings provide a new direction for diagnosis and treatment of pediatric ACC.

Funding: This work was supported by the National Natural Science Foundation of China [No. 81973647] and the Xinglin scholar discipline promotion talent program of Chengdu University of traditional Chinese medicine [No. BSH2021018].

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. NIH state-of-the-science statement on management of the clinically inapparent adrenal mass ("incidentaloma"). NIH Consens State Sci Statements. 2002; 19:1-25.
2. Thampi A, Shah E, Elshimy G, Correa R. Adrenocortical carcinoma: a literature review. Transl Cancer Res. 2020; 9:1253-1264.
3. Bilimoria KY, Shen WT, Elaraj D, Bentrem DJ,

Winchester DJ, Kebebew E, Sturgeon C. Adrenocortical carcinoma in the United States: treatment utilization and prognostic factors. Cancer. 2008; 113:3130-3136.
4. Else T, Kim AC, Sabolch A, Raymond VM, Kandathil A, Caoili EM, Jolly S, Miller BS, Giordano TJ, Hammer GD. Adrenocortical carcinoma. Endocr Rev. 2014; 35:282-326.
5. Mahendraraj K, Lau CSM, Sidhu K, Chamberlain RS. Adrenocortical carcinoma in adults and children: a population-based outcomes study involving 1,623 patients from the Surveillance, Epidemiology, and End Result (SEER) Database (1973-2012). Clin Surg. 2016; 1:1017.
6. Ribeiro RC, Figueiredo B. Childhood adrenocortical tumours. Eur J Cancer. 2004; 40:1117-1126.
7. Michalkiewicz E, Sandrini R, Figueiredo B, Miranda EC, Caran E, Oliveira-Filho AG, Marques R, Pianovski MA, Lacerda L, Cristofani LM, Jenkins J, Rodriguez-Galindo C, Ribeiro RC. Clinical and outcome characteristics of children with adrenocortical tumors: a report from the International Pediatric Adrenocortical Tumor Registry. J Clin Oncol. 2004; 22:838-845.
8. From the American Association of Neurological Surgeons (AANS), American Society of Neuroradiology (ASNR), Cardiovascular and Interventional Radiology Society of Europe (CIRSE), *et al*. Multisociety consensus quality improvement revised consensus statement for endovascular therapy of acute ischemic stroke. Int J Stroke. 2018; 13:612-632.
9. Lee YS, Dutta A. MicroRNAs in cancer. Annu Rev Pathol. 2009; 4:199-227.
10. West AN, Neale GA, Pounds S, Figueredo BC, Rodriguez Galindo C, Pianovski MA, Oliveira Filho AG, Malkin D, Lalli E, Ribeiro R, Zambetti GP. Gene expression profiling of childhood adrenocortical tumors. Cancer Res. 2007; 67:600-608.
11. Veronez LC, Fedatto PF, Correa CAP, *et al*. MicroRNA expression profile predicts prognosis of pediatric adrenocortical tumors. Pediatr Blood Cancer. 2022; 69:e29553.
12. Chin CH, Chen SH, Wu HH, Ho CW, Ko MT, Lin CY. cytoHubba: identifying hub objects and sub-networks from complex interactome. BMC Syst Biol. 2014;8 Suppl 4:S11.
13. Tang Z, Li C, Kang B, Gao G, Li C, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017; 45:W98-W102.
14. Hu ZQ, Zhou SL, Li J, Zhou ZJ, Wang PC, Xin HY, Mao L, Luo CB, Yu SY, Huang XW, Cao Y, Fan J, Zhou J. Circular RNA sequencing identifies circASAP1 as a key regulator in hepatocellular carcinoma metastasis. Hepatology. 2020; 72:906-922.
15. Hao JF, Chen P, Li HY, Li YJ, Zhang YL. Effects of LncRNA HCP5/miR-214-3p/MAPK1 molecular network on renal cell carcinoma cells. Cancer Manag Res. 2020; 12:13347-13356.
16. Hu D, Jiang L, Luo S, Zhao X, Hu H, Zhao G, Tang W. Development of an autophagy-related gene expression signature for prognosis prediction in prostate cancer patients. J Transl Med. 2020; 18:160.
17. Zhang ZY, Gao XH, Ma MY, Zhao CL, Zhang YL, Guo SS. CircRNA_101237 promotes NSCLC progression *via* the miRNA-490-3p/MAPK1 axis. Sci Rep. 2020; 10:9024.
18. Kunz M. Oncogenes in melanoma: an update. Eur J Cell Biol. 2014; 93:1-10.
19. Drosten M, Barbacid M. Targeting the MAPK pathway in KRAS-driven tumors. Cancer Cell. 2020; 37:543-550.
20. Gayther SA, Batley SJ, Linger L, Bannister A, Thorpe K, Chin SF, Daigo Y, Russell P, Wilson A, Sowter HM, Delhanty JD, Ponder BA, Kouzarides T, Caldas C. Mutations truncating the EP300 acetylase in human cancers. Nat Genet. 2000; 24:300-303.
21. Yuan ZM, Huang Y, Ishiko T, Nakada S, Utsugisawa T, Shioya H, Utsugisawa Y, Yokoyama K, Weichselbaum R, Shi Y, Kufe D. Role for p300 in stabilization of p53 in the response to DNA damage. J Biol Chem. 1999; 274:1883-1886.
22. Yang Y, Okada S, Sakurai M. Adenosine-to-inosine RNA editing in neurological development and disease. RNA Biol. 2021; 18:999-1013.
23. Kawahara Y, Zinshteyn B, Sethupathy P, Iizasa H, Hatzigeorgiou AG, Nishikura K. Redirection of silencing targets by adenosine-to-inosine editing of miRNAs. Science. 2007; 315:1137-1140.
24. Nie F, Liu T, Zhong L, Yang X, Liu Y, Xia H, Liu X, Wang X, Liu Z, Zhou L, Mao Z, Zhou Q, Chen T. MicroRNA-148b enhances proliferation and apoptosis in human renal cancer cells *via* directly targeting MAP3K9. Mol Med Rep. 2016; 13:83-90.
25. Liu GL, Liu X, Lv XB, Wang XP, Fang XS, Sang Y. miR-148b functions as a tumor suppressor in non-small cell lung cancer by targeting carcinoembryonic antigen (CEA). Int J Clin Exp Med. 2014; 7:1990-1999.
26. Cimino D, De Pittà C, Orso F, *et al*. miR148b is a major coordinator of breast cancer progression in a relapse-associated microRNA signature by targeting ITGA5, ROCK1, PIK3CA, NRAS, and CSF1. FASEB J. 2013; 27:1223-1235.
27. Friedrich M, Pracht K, Mashreghi MF, Jäck HM, Radbruch A, Seliger B. The role of the miR-148/-152 family in physiology and disease. Eur J Immunol. 2017; 47:2026-2038.
28. Montero-Conde C, Graña-Castro O, Martín-Serrano G, *et al*. Hsa-miR-139-5p is a prognostic thyroid cancer marker involved in HNRNPF-mediated alternative splicing. Int J Cancer. 2020; 146:521-530.
29. Su Y, Feng W, Shi J, Chen L, Huang J, Lin T. circRIP2 accelerates bladder cancer progression *via* miR-1305/Tgf-β2/smad3 pathway. Mol Cancer. 2020; 19:23.
30. Liu W, Zhuang R, Feng S, Bai X, Jia Z, Kapora E, Tan W. Long non-coding RNA ASB16-AS1 enhances cell proliferation, migration and invasion *via* functioning as a ceRNA through miR-1305/Wnt/β-catenin axis in cervical cancer. Biomed Pharmacother. 2020; 125:109965.

Received June 27, 2022; Revised August 14, 2022; Accepted August 23, 2022.

[§]These authors contributed equally to this work.
**Address correspondence to:*
Yaodong You, TCM Regulating Metabolic Diseases Key Laboratory of Sichuan Province, Hospital of Chengdu University of Traditional Chinese Medicine, No. 39 shi-er-qiao Road, Chengdu, Sichuan 610072, China.
E-mail: yyd110@163.com

Released online in J-STAGE as advance publication August 30, 2022.

Brief Report

DOI: 10.5582/irdr.2022.01065

Interstitial deletions in the proximal regions of 6q: 12 original cases and a literature review

Osamu Machida[1,2], Keiko Yamamoto Shimojima[3,4], Takashi Shiihara[5], Satoshi Akamine[6], Ryutaro Kira[6], Yuiko Hasegawa[7], Eriko Nishi[7], Nobuhiko Okamoto[7], Satoru Nagata[2], Toshiyuki Yamamoto[1,4,*]

[1] Department of Genetic Medicine, Division of Advanced Biomedical Sciences, Graduate School of Medicine, Tokyo Women's Medical University, Tokyo, Japan;
[2] Department of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan;
[3] Department of Transfusion Medicine and Cell Processing, Tokyo Women's Medical University, Tokyo, Japan;
[4] Institute of Medical Genetics, Tokyo Women's Medical University, Tokyo, Japan;
[5] Department of Pediatric Neurology, Gunma Children's Medical Center, Gunma, Japan;
[6] Department of Pediatric Neurology, Fukuoka Children's Hospital, Fukuoka, Japan;
[7] Department of Medical Genetics, Osaka Women's and Children's Hospital, Izumi, Japan.

SUMMARY Interstitial microdeletions in the proximal region of the long arm of chromosome 6 are rare. Herein we have reported 12 patients with developmental delays associated with interstitial microdeletions in 6q ranging from q12 to q22. The microdeletions were detected by chromosomal microarray testing. To confirm the clinical significance of these deletions, genotype-phenotype correlation analysis was performed using genetic and predicted loss-of-function data. *SIM1* was recognized as the gene responsible for developmental delay, particularly in Prader-Willi syndrome-like phenotypes. Other genes possibly related to developmental delay were *ZNF292*, *PHIP*, *KCNQ5*, and *NUS1*. To further establish the correlation between the genotype and phenotype, more patient information is required.

Keywords chromosomal microarray testing, 6q interstitial deletions, developmental delay

1. Introduction

Interstitial microdeletions in the proximal region of the long arm of chromosome 6 are rare. In 1997, Hopkins *et al.* reported three new cases and reviewed 57 previously reported cases of partial deletions on 6q and classified them into three phenotypic groups: proximal [del(6)(q11–q16)], intermediate [del(6)(q15–q25)], and distal [del(6)(q25–qter)] (*1*). Although there were some characteristic features unique to each phenotypic group, recognizable clinical entities were not established at that time. In 2007, Klein *et al.* reported three patients with 6q deletions (*2*). Two of them were obese and showed signs of hypotonia and developmental delay, which were described as Prader-Willi syndrome (PWS)-like phenotypes; *SIM1* (MIM* 603128) located in the deleted region, was suspected to be a candidate gene for the PWS-like phenotype. In 2012, Rosenfeld *et al.* reported 12 new cases of interstitial 6q deletions and four of the cases had PWS-like phenotypes (*3*). These findings suggest that there is a correlation between the occurrence of the PWS-like phenotype and *SIM1* deletion. This is further supported by other reports of patients with similar genotypic and phenotypic findings (*4,5*).

Similarly, deletions in certain regions were associated with characteristic findings. So far, in our ongoing research on genomic copy number analysis using chromosomal microarray testing, we have encountered 12 patients who showed interstitial microdeletions in 6q. Herein, the genotype-phenotype correlation was assessed.

2. Patients and Methods

This study was performed in accordance with the Declaration of Helsinki and approved by the ethics committee of this institution. Written informed consent was obtained from the families of the patients. Blood samples were collected from the patients; additionally, blood samples were also collected from the parents of the patients when it was necessary for further investigation. Genomic copy number variations were analyzed using the Agilent Microarray system (Agilent Technologies, Santa Clara, CA, USA), as previously described (*6*). Based on the results, patients with 6q interstitial microdeletions were included in this study.

The clinical information of the patients was obtained from their attending doctors. The correlation between the genotype and phenotype was then investigated using a genome map in which the deleted regions were depicted (Figure 1). Gene information was evaluated using Online Mendelian Inheritance in Man® (OMIM; *https://www.omim.org/*). Predicted loss-of-function was also used for the evaluation. The genomic coordinate referred to was the GRCh37/hg19 genome build.

3. Results and Discussion

Interstitial deletions involving the long arm of chromosome 6 were identified in 12 patients: six males and six females, ranging in age from 1 to 21 years (Table 1). Although all the patients showed developmental delays, the extent of developmental delay varied among the patients. The deleted regions are shown on the genome map in Figure 1. For further evaluation, we summarized the genes from the deleted regions and the probability of loss-of-function intolerance (pLI), shown in Supplemental Table S1 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=112*) (*7*). The candidate genes listed were those related to autosomal dominant traits and had a pLI score of 1. The genes listed are shown in Figure 1, in which genome map is captured from the UCSC genome browser (*https://genome.ucsc.edu/*).

The first patient was an 8-year-9-month-old girl (patient 1) who was born by cesarean section owing to an unequal infant pelvis. The patient showed distinctive features. Her development was delayed; the patient started walking independently at 2 years of age and speaking at 4 years of age. At 6 years of age, she developed epilepsy, for which she had to take antiepileptic drugs. A radiological examination of the brain revealed no abnormalities.

The second patient, a 13-month-old boy (patient 2), could turn over, but not sit up; this indicated delayed motor function development. Furthermore, he had language delay. Physical examination revealed distinctive features, which included coarse scalp hair. Magnetic resonance imaging (MRI) of the brain showed no apparent abnormalities.

The third patient was a 17-month-old girl (patient 3) who could not walk unaided, which indicated a developmental delay. She also showed growth deficiency with -2 SD parameters and microcephaly with -3 SD parameters.

The prenatal history of a 21-year-old male patient (patient 4) revealed amniotic fluid overload in the 8th month of pregnancy. There were several congenital anomalies, such as congenital duodenal atresia, inguinal hernia, and cleft palate. In addition, there were also urogenital anomalies present that included bilateral hypoplastic kidneys, renal enuresis, and cryptorchidism. An external strabismus was observed later. His motor skills were limited to sitting, and he had not yet acquired substantial language skills; this indicated a severe psychomotor developmental delay. He developed epilepsy and neuropsychiatric features, including sleep disorder and self-injurious behavior, such as hitting his eyes with his hands. Episodes of hypothermia were also observed.

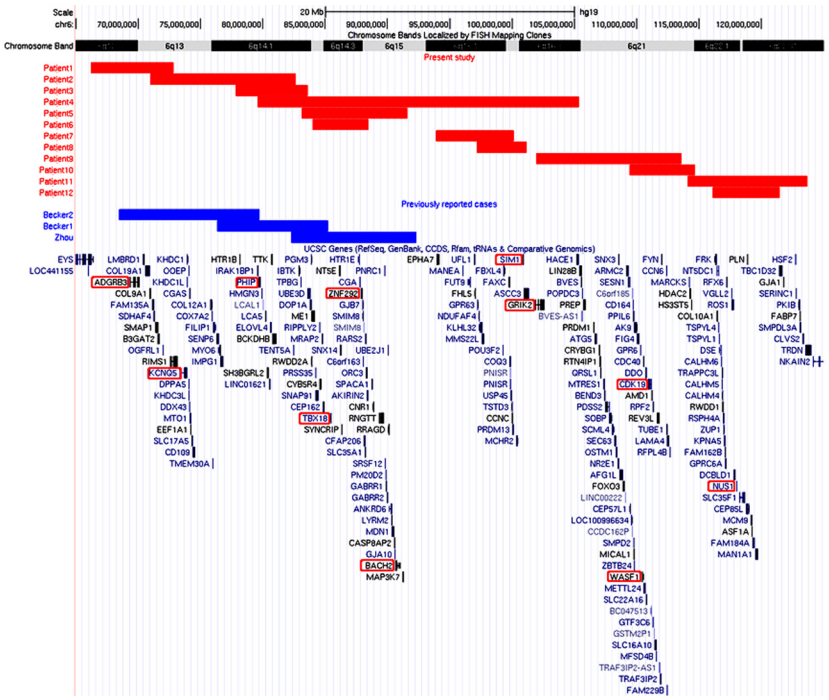


Figure 1. Genome map of 6q captured from the UCSC genome browser. Regions of the identified deletions are depicted by custom tracks with rectangles; the red and blue are for regions of the deletions identified in this study and previous studies, respectively. The genes discussed in the text are highlighted using red circles.

Table 1. Clinical features of the patients in this study

Items	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11	Patient 12
Age	8y9m	1y2m	1y5m	21y	1y6m	3y2m	2y3m	16y4m	5y7m	3y10m	4y10m	13y9m
Gender	Female	Male	Female	Male	Male	Male	Female	Female	Male	Female	Male	Female
Chromosome band	6q12q13	6q13q14.1	6q14.1	6q14.1q16.3	6q14.1q15	6q14.2q15	6q16.1	6q16.1q16.3	6q16.3q21	6q21q22.1	6q21q22.31	6q22.1q22.31
Start*	65,485,026	67,176,848	76,392,293	72,891,116	81,135,362	84,025,024	92,902,137	96,533,420	98,273,562	108,670,734	113,020,897	114,656,502
End*	73,838,151	85,971,380	84,606,179	110,736,632	93,665,050	88,452,718	101,255,262	101,724,035	117,068,093	115,143,797	125,341,725	123,074,922
Delition Size (Mb)	8.4	18.8	8.2	37.8	12.5	4.4	8.4	5.2	18.8	6.5	12.3	8.4
Gestational age	NA	39w1d	39w3d	38w0d	NA	40w6d	39w5d	38w4d	37w6d	39w6d	39w0d	40w5d
Birth weight	NA	2976	3135	1970	NA	3194	2860	3048	2156	3554	3165	2595
Birth length	NA	47.6	49.5	NA	NA	50.5	48	NA	46	52	49.1	46.2
Birth occipitofrontal circumference	NA	32.9	33	NA	NA	35	30.4	NA	32	34	32.5	31
Developmental delay	+	+	+	+	+	+	+	±	+	+	+	+
DQ/IQ			DQ 79			DQ 38	DQ below 35	IQ 76	DQ 35	DQ60	DQ below 35	DQ54
Growth deficiency	-	-	+	NA	-	-	-	-	+	-	-	-
Microcephaly	-	-	+	-	-	-	-2.6SD	-	-	-	+	-
Epilepsy	+	-	-	+	-	-	-	-	-	-	+	-
MRI brain abnormalities	-	-	NA	NA	+	-	-	-	NA	+	NA	+
Gastrointestinal anomalies	-	-	-	+	+	-	-	-	+	-	-	-
Genital anomalies	-	-	-	+	+	+	-	-	-	-	-	-
Distinctive facia features												
Low-ser-ears	-	+	+	-	-	+	+	-	-	-	+	-
Epicanthus	-	-	+	-	-	+	-	-	-	+	+	-
Blepharophimosis	-	-	-	-	+	-	+	-	-	-	-	-
Micrognathia	-	+	+	-	+	+	+	-	+	-	-	-
Flat nasal bridge	-	-	-	-	-	+	-	-	-	-	+	-
Wide ala nasi	-	-	-	-	-	-	-	-	-	+	-	-
Hypertelorism	-	-	-	-	-	+	+	-	-	+	+	-
Other findings				Hypoplastic kidney, renal enuresis		Cryptorchidism, ptosis		Hypothyroidism, precocious puberty, obesity				

y, years; m, months; w, weeks; s, days; NA, not available, DQ, developmental quotient; IQ, inteligent quotient, *Genomic cordinate corresponds to GRCh37/hg19.

The fifth patient, an 18-month-old boy (patient 5), had no remarkable perinatal history. The patient showed psychomotor developmental delays from early infancy. There were several abnormal findings, such as blephaophimosis, small jaw, hypertrichosis on the back, tapered phalanges, long first toe, accessory ears, abnormal dentition, hypodontia, soft larynx, and left optic nerve hypoplasia. Gastroesophageal reflux disease was also observed. Brain MRI showed corpus callosum hypoplasia and delayed myelination. However, there were no abnormal results from the laboratory tests for inborn errors of metabolism, mitochondrial disease, Pompe's disease, Fabry disease, and mucopolysaccharidosis. Auditory testing revealed no abnormalities.

Motor development in a 3-year-old boy (patient 6) was delayed; he achieved head control at 8 months, independent sitting at 12 months, crawling at 12 months, and independent walking at 32 months. His language development was severely delayed, and he could not speak comprehensibly. He showed distinctive features that included flat occiput, hypertelorism, epicanthus, bilateral ptosis, strabismus, flat basal bridge, low-set ears, small mouth, short neck, and bilateral cryptorchidism.

The seventh patient, a 27-month-old girl (patient 7), had a small jaw as a distinctive feature. She could crawl, but could not grasp or speak significantly, indicating psychomotor developmental delay. Ophthalmological examination revealed interocular dissection. A neurological examination revealed generalized hypotonia. Brain MRI showed no abnormalities. Moreover, there were no abnormalities found upon routine laboratory examination. She is now 15 years of age. The patient had severe intellectual disability with microcephaly and was not obese.

The eighth patient was a 16-year-4-month-old girl (Patient 8) who was diagnosed with psychomotor developmental delay and hypothyroidism at 14 months of age. She developed precocious puberty and was treated with leuplin, but later she became amenorrheic. This patient was 151 cm tall (-1.3 SD) and weighed 79 kg (+2.6 SD), which indicated obesity.

The ninth patient was a 5-year-7-month-old boy (patient 9) who showed developmental delay. At 2 years of age, he started to walk independently and had no significant speech, indicating delayed development. The patient had a small jaw and was short in stature. Gastroesophageal reflux disease, sleep apnea syndrome, and scoliosis were also observed.

The tenth patient, a 3-year-10-month-old girl (patient 10), had occipital flatness, bilateral eye openings, lamina propria, auricular deformity, low nasal apex, wide nasal bridge, bilateral middle fingers, and curly hair. Brain MRI showed corpus callosum hypoplasia. At the time of examination, her height, weight, and occipitofrontal circumference were 107 cm (+2.3 SD), 18.1 kg (+1.7 SD), and 47.3 cm (-1.2 SD), respectively; this indicated a relatively large stature.

A 4-year-10-month-old boy (patient 11) had sufficient head and neck control; however, he was not ambulatory and has no significant speech, indicating a developmental delay. Physical examination revealed microcephaly, occipital flatness, bilateral eye openings, lamina propria, low auricular position, flat nasal dorsum, cleft palate, and a small mandible. He experienced epileptic seizures, with loss of consciousness for a few seconds, and was taking antiepileptic drugs.

The twelfth patient, a 13-year-9-month-old girl (patient 12), had developmental delay hand tremors. Brain MRI revealed an enlarged cerebellar fissure. EEG abnormalities were also observed. The patient did not experience any epileptic seizures.

According to the genome map (Figure 1), the deleted regions in two of the patients (patients 4 and 7) included *SIM1*, which was confirmed to contribute to obesity and PWS-like features when loss-of-function variants were present in the coding region (*8*). Indeed, patient 7 showed PWS-like phenotypes, including obesity; however, patient 4 showed a much more severe developmental delay than that of patient 7. The PWS-like phenotypes could not be distinguished owing to the large region of deletion in 6q, that was observed in this study.

Additionally, patient 4 exhibited urinary tract abnormalities. The overlapping regions of the deletions in patients 4, 5, and 6 included *TBX18* (MIM* 604613), which is related to "congenital anomalies of kidney and urinary tract" (MIM# 143400). However, *TBX18* variants identified in humans with congenital anomalies of kidney and urinary tract exert a dominant-negative effect rather than haploinsufficiency (*9*). Thus, we considered that the urinary tract abnormalities observed in patient 4 were not related to the deletion of *TBX18*, which explained why patient 5 did not show any urinary tract abnormalities.

The overlapping regions of deletion in patients 4, 5, and 6 included *ZNF292* (MIM* 616213), which is related to "intellectual developmental disorder, autosomal dominant 64" (MIM# 619188). Therefore, the haploinsufficiency of *ZNF292* may have contributed to the developmental delay observed in patients 4 and 5 (*10,11*). Furthermore, Zhou *et al.* (2017) reported a patient with developmental delay in association with an overlapping region of a deletion from 6q14.1 to 6q15; this suggested that the haploinsufficiency of *ZNF292* is responsible for the delayed development (*12*).

Additionally, the overlapping region of the deletions in patients 4 and 5 included *BACH2* (MIM* 605394), which is related to "immunodeficiency 60 and autoimmunity" (IMD60) (MIM# 618394). Previous reports determined that missense variations of *BACH2* contributed to the dominant negative effects; therefore, *BACH2* was excluded from this analysis (*13*).

The shortest region of overlapping deletions was observed in patients 2, 3, and 4. This region included *PHIP* (MIM* 612870), which is known to cause Chung-Jansen syndrome (CHUJANS; MIM# 617991), a clinical

entity characterized by global developmental delay in infants, impaired intellectual development or learning difficulties, behavioral abnormalities, dysmorphic features, and obesity (*14*). Thus, it is plausible that *PHIP* haploinsufficiency contributed to the neurodevelopmental delay observed in patients 2, 3, and 4.

Becker *et al.* reported two patients with microdeletions in 6q (*15*). One of the patients (patient 2) showed a more proximal deletion than that observed in the patient reported by Zhou *et al.* (*12*). The deleted region included *ADGRB3* (MIM* 602684). *ADGRB3* is a member of the adhesion-G protein-coupled receptor family and is mostly expressed in the brain (*16*). Although the pLI score of *ADGRB3* was "1", which indicated intolerance for haploinsufficiency, previously reported *ADGRB3* variants were biallelic. Thus, it is unknown whether *ADGRB3* is related to the developmental delay observed in patient 1.

The haploinsufficiency of the other genes located in the proximal regions of 6q may be related to the clinical features observed in this study. *KCNQ5* (MIM * 607357), located on 6q13, is related to "intellectual developmental disorder, autosomal dominant 46" (MIM# 617601) (*17*). Loss-of-function variants in *KCNQ5* were predicted to lower the seizure threshold by decreasing the repolarization reserve; therefore, it is possible that *KCNQ5* haploinsufficiency contributed to the clinical features that were observed in patient 2.

Additionally, *GRIK2* (MIM* 138244) was located in the deleted regions in patients 4 and 9. It is related to "neurodevelopmental disorder with impaired language and ataxia and with or without seizures" (MIN# 619580). However, the identified variants of *GRIK2* were related to gain-of-function (*18*); thus, the haploinsufficiency of *GRIK2* would not have contributed to the clinical features of these patients.

WASF1 (MIM* 605035) and *CDK19* (MIM* 614720) were in the deleted regions that were observed in patients 9 and 10. *WASF1* is responsible for "neurodevelopmental disorder with absent language and variable seizures" (MIM# 618707). Previously reported *WASF1* variants were nonsense variants, and they were considered to have dominant negative effects, which were a consequence of evading nonsense mediated decay (*19*). *CDK19* is associated with "developmental and epileptic encephalopathy 87" (MIM# 618916). All previously reported variants of *CDK19* were dominant negative missense variants that resulted (*20*). Therefore, it is difficult to attribute the developmental delay observed in patients 8 and 9 to *WASF1* or *CDK19* haploinsufficiency.

The overlapping region of the deletions observed in patients 9 and 10 was proposed as the etiology for acro-cardio-facial syndrome (MIM 600460) (*21,22*); however, none of the patients showed any symptoms related to acro-cardio-facial syndrome.

Furthermore, *NUS1* (MIM* 610463) was located in the deleted regions observed in patients 10 and 11. *NUS1* is responsible for "intellectual developmental disorder, autosomal dominant 55, with seizures" (MIM# 617831). Because previously reported *NUS1* variants were related to loss of function, haploinsufficiency was considered the pathogenic mechanism (*23*).

4. Conclusion

Among the genes in the long arm of chromosome 6 (chr6:65,485,026-125,341,725), only five genes (*SIM1*, *ZNF292*, *PHIP*, *KCNQ5*, and *NUS1*) were considered to be related to the developmental delay observed in the patients reported in this study.

Acknowledgements

We would like to express our gratitude to the patients and their families for their cooperation.

Funding: This study was supported by KAKENHI (Grant Numbers 21K07873) from the Japan Society for the Promotion of Science, Initiative on Rare and Undiagnosed Diseases (grant number 20ek0109301) from the Japan Agency for Medical Research and Development (AMED), and a grant from the Ministry of Health, Labor, and Welfare Japan.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Hopkin RJ, Schorry E, Bofinger M, Milatovich A, Stern HJ, Jayne C, Saal HM. New insights into the phenotypes of 6q deletions. Am J Med Genet. 1997; 70:377-386.
2. Klein OD, Cotter PD, Moore MW, Zanko A, Gilats M, Epstein CJ, Conte F, Rauen KA. Interstitial deletions of chromosome 6q: genotype-phenotype correlation utilizing array CGH. Clin Genet. 2007; 71:260-266.
3. Rosenfeld JA, Amrom D, Andermann E, *et al.* Genotype-phenotype correlation in interstitial 6q deletions: a report of 12 new cases. Neurogenetics. 2012; 13:31-47.
4. Izumi K, Housam R, Kapadia C, Stallings VA, Medne L, Shaikh TH, Kublaoui BM, Zackai EH, Grimberg A. Endocrine phenotype of 6q16.1-q21 deletion involving SIM1 and Prader-Willi syndrome-like features. Am J Med Genet A. 2013; 161a:3137-3143.
5. Vignoli A, Scornavacca GF, Peron A, La Briola F, Canevini MP. Interstitial 6q microdeletion syndrome and epilepsy: a new patient and review of the literature. Am J Med Genet A. 2013; 161a:2009-2015.
6. Yamamoto T, Wilsdon A, Joss S, Isidor B, Erlandsson A, Suri M, Sangu N, Shimada S, Shimojima K, Le Caignec C, Samuelsson L, Stefanova M. An emerging phenotype of Xq22 microdeletions in females with severe intellectual disability, hypotonia and behavioral abnormalities. J Hum Genet. 2014; 59:300-306.
7. Fabre A, Mancini J. No preferential mode of inheritance for highly constrained genes. Intractable Rare Dis Res. 2022; 11:25-28.

8. Bonnefond A, Raimondo A, Stutzmann F, *et al.* Loss-of-function mutations in SIM1 contribute to obesity and Prader-Willi-like features. J Clin Invest. 2013; 123:3037-3041.
9. Vivante A, Kleppa MJ, Schulz J, *et al.* Mutations in TBX18 cause dominant urinary tract malformations *via* transcriptional dysregulation of ureter development. Am J Hum Genet. 2015; 97:291-301.
10. Engwerda A, Frentz B, den Ouden AL, Flapper BCT, Swertz MA, Gerkes EH, Plantinga M, Dijkhuizen T, van Ravenswaaij-Arts CMA. The phenotypic spectrum of proximal 6q deletions based on a large cohort derived from social media and literature reports. Eur J Hum Genet. 2018; 26:1478-1489.
11. Mirzaa GM, Chong JX, Piton A, *et al.* De novo and inherited variants in ZNF292 underlie a neurodevelopmental disorder with features of autism spectrum disorder. Genet Med. 2020; 22:538-546.
12. Zhou Q, Wu XH, Yang YC, Zou CC. Clinical Features in Patients with Microdeletion at 6q14.1-q15. Indian J Pediatr. 2017; 84:883-886.
13. Afzali B, Grönholm J, Vandrovcova J, *et al.* BACH2 immunodeficiency illustrates an association between super-enhancers and haploinsufficiency. Nat Immunol. 2017; 18:813-823.
14. Webster E, Cho MT, Alexander N, Desai S, Naidu S, Bekheirnia MR, Lewis A, Retterer K, Juusola J, Chung WK. De novo PHIP-predicted deleterious variants are associated with developmental delay, intellectual disability, obesity, and dysmorphic features. Cold Spring Harb Mol Case Stud. 2016; 2:a001172.
15. Becker K, Di Donato N, Holder-Espinasse M, *et al.* De novo microdeletions of chromosome 6q14.1-q14.3 and 6q12.1-q14.1 in two patients with intellectual disability - further delineation of the 6q14 microdeletion syndrome and review of the literature. Eur J Med Genet. 2012; 55:490-497.
16. Scuderi C, Saccuzzo L, Vinci M, Castiglia L, Galesi O, Salemi M, Mattina T, Borgione E, Città S, Romano C, Fichera M. Biallelic intragenic duplication in ADGRB3 (BAI3) gene associated with intellectual disability, cerebellar atrophy, and behavioral disorder. Eur J Hum Genet. 2019; 27:594-602.
17. Lehman A, Thouta S, Mancini GMS, *et al.* Loss-of-function and gain-of-function mutations in KCNQ5 cause intellectual disability or epileptic encephalopathy. Am J Hum Genet. 2017; 101:65-74.
18. Guzmán YF, Ramsey K, Stolz JR, Craig DW, Huentelman MJ, Narayanan V, Swanson GT. A gain-of-function mutation in the GRIK2 gene causes neurodevelopmental deficits. Neurol Genet. 2017; 3:e129.
19. Ito Y, Carss KJ, Duarte ST, *et al.* De novo truncating mutations in WASF1 cause intellectual disability with seizures. Am J Hum Genet. 2018; 103:144-153.
20. Chung HL, Mao X, Wang H, *et al.* De novo variants in CDK19 are associated with a syndrome involving intellectual disability and epileptic encephalopathy. Am J Hum Genet. 2020; 106:717-725.
21. Milani D, Cagnoli GA, Baccarin M, Alfei E, Guerneri S, Esposito S. Insights into 6q21-q22: Refinement of the critical region for acro-cardio-facial syndrome. Congenit Anom (Kyoto). 2016; 56:187-189.
22. Shukla A, Hebbar M, Harms FL, Kadavigere R, Girisha KM, Kutsche K. Phenotypic variability in patients with interstitial 6q21-q22 microdeletion and Acro-Cardio-Facial syndrome. Am J Med Genet A. 2016; 170:2998-3003.
23. Hamdan FF, Myers CT, Cossette P, *et al.* High rate of recurrent de novo mutations in developmental and epileptic encephalopathies. Am J Hum Genet. 2017; 101:664-685.

Received June 8, 2022; Revised July 27, 2022; Accepted August 3, 2022.

**Address correspondence to:*
Toshiyuki Yamamoto, Institute of Medical Genetics, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku-ward, Tokyo 162-8666, Japan.
E-mail: yamamoto.toshiyuki@twmu.ac.jp

Released online in J-STAGE as advance publication August 10, 2022.

Brief Report

DOI: 10.5582/irdr.2022.01063

When LUCA met gnomAD: genetic constraints on universal genes in humans

Alexandre Fabre[1,2,*], Julien Mancini[3,4]

[1] Aix Marseille Univ, INSERM, MMG, Marseille, France;
[2] APHM, Multidisciplinary Pediatrics Department, Timone Enfant Hospital, Marseille, France;
[3] Aix-Marseille Univ, INSERM, IRD, ISSPAM, SESSTIM, Marseille, France;
[4] APHM, BIOSTIC, Hop Timone, Marseille, France.

SUMMARY LUCA, the last universal common ancestor, is the hypothetical most recent common ancestor of the three domains of life which share the universal genes (UG). It seems interesting to evaluate whether the UG phylogeny has had an impact on current Human gene constraints. A list of human homologs of UG was retrieved from the eggNOG database. We analyzed this LUCA gene (LG) group, and a random sample of 500 genes from the gnomAD database (RG group). Gene constraint metrics were retrieved from gnomAD and associations with Mendelian diseases and modes of inheritance were retrieved from OMIM. The LG group consisted of 277 genes and the RG group, 492 (8 genes were in LG group). 38.6% of the genes in the LG group and 25.2% of the genes in the RG group were associated with a Mendelian disease ($p < 0.0001$). The mode of inheritance was more often autosomal recessive (69.0 *vs.* 50.5%), and less often autosomal dominant (19.0 *vs.* 31.3%), or mixed (6.0 *vs.* 12.1%) for those associated with the LG group ($p = 0.048$). The LG group was significantly more constrained for missense variants (MOEUF, 0.919 *vs*. 0.997, $p < 0.0001$) and was borderline significantly more constrained for loss-of-function variants (LOEUF, 0.872 *vs.* 0.947, $p = 0.051$). These results suggest that the UG in humans differs from the rest of the genome in terms of constraints and associated Mendelian diseases. It suggests that phylogenic data can explain some of the characteristics of human genes and could help in interpreting variants.

Keywords LUCA, gnomAD, last universal common ancestor, gene constraints

1. Introduction

LUCA (the last universal common ancestor) is the hypothetical most recent common ancestor of the three domains of life, archaea, bacteria and eukarya (*1*). LUCA's nature is elusive – presumed to be hyper-thermophilic to mesophilic for example (*1-3*) – because it is a reconstruction, based on comparisons of the genomes of current living organisms. While the size of LUCA's genome is unknown, it is assumed to at least contain the limited set of genes found across all three domains of life, sometimes referred to as the ancestral genetic core of cells or universal genes.

The genome aggregation database (gnomAD) contains more than 100,000 human exomes and genomes, along with annotations including constraint metrics that quantify the relative intolerance to variation of each protein-coding gene. These constraint metrics are calculated as the ratio of observed to expected synonymous, missense and loss-of-function variants, lower scores indicating more constrained genes. They have been used to interpret next generation-sequencing data, notably in the context of Mendelian diseases (*4*), and are presumed to be a reflection of natural selection (*5*)

In this study, we investigated whether the phylogenetic characteristics of LUCA genes are reflected in specific levels of genetic constraints or frequencies of associated Mendelian diseases.

2. Material and Methods

The gnomAD constraint metric by gene table (*6*) was downloaded from the gnomAD website (*https://gnomad.broadinstitute.org/downloads*, file "pLoF Metrics by Gene TSV"). The list of human homologs of universal genes (the LUCA gene (LG) group) was retrieved from the eggNOG database (*7*) (*http://eggnogdb.embl.de/*) using the clusters of orthologous groups (COGs) described by Harris *et al.* (*8*), Ciccarelli *et al.* (*9*), et Puigbò *et al.* 2009 (*10*) as being representative

of universal genes. The functional categories and number of human homologs per COG were recorded (Supplemental Table S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=103*). The LG group was analyzed in comparison with the random gene (RG) group, a random sample of 500 of the 19,704 genes in the gnomAD table.

The variables considered for each gene were the genetic constraint metrics (the synonymous, missense and loss-of-function observed/expected upper bound fractions, the SOEUF, MOEUF and LOEUF, respectively) and chromosome localization. Manual searches were performed for each gene on the Online Inheritance in Man (OMIM) website (*11*) between 15 October 2019 and 5 May 2020 for each of the included genes. The data retrieved were the existence of an associated Mendelian disease (non-diseases and multifactorial disorders were not considered), and for each disease, the recorded mode of inheritance (autosomal dominant, autosomal recessive or X-linked). For genes associated with multiple phenotypes, the number of associated Mendelian diseases was also recorded. and the mode of inheritance was recorded as mixed if it varied between phenotypes. All statistical analyses were performed with SPSS.

No ethics approval was required under French law as the study only involved data analysis. Database data were used in accordance with the corresponding data use agreements.

3. Results and Discussion

Among the 80, 36 and 102 COGs respectively described by Harris *et al*., Ciccarelli *et al.*, and Puigbò *et al.* (*8-10*), as being representative of universal genes, 120 were unique and 33 were common to the three lists (Figure 1). Fourteen had no human homolog (COG0073, COG0250, COG0540 and COG0071 from Harris *et al.* (*8*) and COG0136, COG0195, COG0492, COG0575, COG0358, COG0455, COG0527, COG0528, COG1080 and COG2812 from Puigbò *et al.* (*10*) and three human

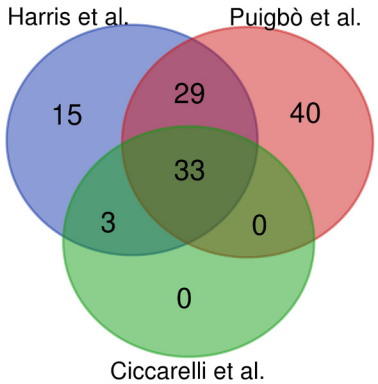


Figure 1. Venn diagram of the clusters of orthologous groups (COGs) retrieved from Harris *et al.*, Ciccarelli *et al.*, and Puigbò *et al.* Diagram prepared using the website *https://bioinformatics.psb.ugent.be/webtools/Venn/*

genes were identified as homologs for two COGs (YARS, COG0143 and COG0162; NME8, COG0105 and COG0526; and EPRS, COG0008 and COG0442). The mean number of homologs per COG was 5.2 (SD, 5.9; range, 1-23), giving a total of 277 genes in the LG group. For the RG group, eight of the 500 initially selected genes were discarded because they also appeared in the LG group. The final number of genes analyzed was therefore 769 (277 in the LG group and 492 in the RG group).

The OMIM database is 17.5% smaller than gnomAD (16,253 *vs.* 19,704 genes). Eighteen (6.5%) of the genes in the LG group and 99 (20.1%) of the genes in the RG group were not listed in the OMIM database ($p < 0.0001$). Among genes present in the OMIM database, 100/259 (38.6%) of those in the LG group and 99/393 (25.2%) of those in the RG group were associated with a Mendelian disease ($p < 0.0001$). The mode of inheritance was more often autosomal recessive and less often autosomal dominant or mixed for diseases associated with the LG group (69.0 *vs.* 50.5%, 19.0 *vs.* 31.3%, and 6.0 *vs.* 12.1%, respectively, $p = 0.048$; Table 1). The LG group was significantly more constrained for missense variants (MOEUF, 0.919 *vs.* 0.997, $p < 0.0001$) and was borderline significantly more constrained for loss-of-function variants (LOEUF, 0.872 *vs.* 0.947, $p = 0.051$). Limiting the analysis to COGs with five or fewer homologs (because only one COG each had 6, 7, 9, 12 and 23 homologs), the number of homologs per COG was not significantly correlated with the SOEUF ($\rho = -0.09$, 95% CI [−0.22, 0.04], $p = 0.17$), MOEUF ($\rho = 0.13$, 95% CI [−0.01, 0.25], $p = 0.061$) or the LOEUF ($\rho = 0.11$, 95% CI [−0.02, 0.24], $p = 0.095$) of the genes (Figure 2).

The analysis was repeated for the LGmin group, consisting of 62 genes associated with the 31 COGs common to all three lists (mean number of homologs per COG, 2.5; SD, 1.1; range, 1-5; details in Table 1). Comparisons with the RG group showed the same, if slightly stronger trends as observed for the full LG group, with a higher proportion of genes present in the OMIM and associated with a Mendelian disease than in the RG group. The LGmin group was significantly more constrained for synonymous variants and missense variants but was not significantly more constrained for loss-of-function variants (Table 1). The number of homologs per COG was significantly but weakly correlated with the MOEUF ($\rho = 0.38$, 95% CI [0.1249, 0.5713], $p = 0.004$), and the LOEUF of the LGmin genes ($\rho = 0.30$, 95% CI [0.05, 0.52], $p = 0.02$), and non-significantly correlated with the SOEUF ($\rho = -0.2153$, 95% CI [−0.45, 0.04], $p = 0.1$).

One possible explanation for these results is that the genes in the two groups belong to different functional categories. For example, 45.7% of those in the RG group are of unknown function (218/ 477 as 15 gene of RG group are not present in eggNOG database), whereas none of those in the LG group are; and conversely, while

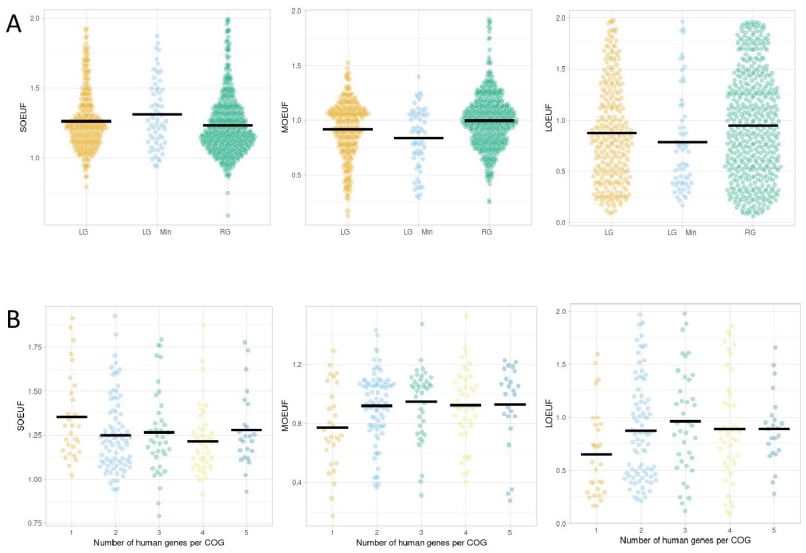


Figure 2. (A) Distributions of synonymous, missense, and loss-of-function observed/expected upper bound fractions (respectively SOEUF, MOEUF and LOEUF for LUCA genes (LG group, n = 277; orange), consensus LUCA genes (LGmin group, n = 62, blue), and a random selection of human gene (RG group, n = 492, green). **(B)** SOEUF, MOEUF, and LOEUF scores of LUCA genes as a function of the number of genes in the corresponding COG (cluster or orthologous groups). Figure prepared using the website *https://huygens.science.uva.nl/PlotsOfData*

Table 1. Gene characteristics according to groups

Items	LUCA genes (LG group)	Consensus LUCA genes (LGmin group)	Random selection of genes (RG group)	LG *vs.* RG	LGmin *vs.* RG
Genes	277	62	492		
Present in the OMIM database	259 (93.5%)	58 (93.5%)	393 (79.9%)	$p < 0.0001$	$p = 0.009$
Associated with Mendelian disease in the OMIM database	100 (38.6%)	27 (46.6%)	99 (25.2%)	$p < 0.0001$	$p = 0.001$
Autosomal dominant inheritance	19 (19%)	7 (25.9%)	31 (31.3%)		
Autosomal recessive inheritance	69 (69%)	17 (63%)	50 (50.5%)	$p = 0.048$	$p = 0.782$
Autosomal recessive and dominant inheritance	6 (6%)	2 (7.4%)	12 (12.1%)		
X-linked inheritance	6 (6%)	1 (3.7%)	6 (6.1%)		
Mean number of OMIM phenotypes per gene associated with a Mendelian disease (SD)	1.25 (0.626)	1.19 (0.396)	1.34 (0.641)	$p = 0.3$	$p = 0.225$
Mean SOEUF (SD)	1.263 (0.209)	1.312 (0.241)	1.235 (0.214)	$p = 0.079$	$p = 0.009$
Mean MOEUF (SD)	0.919 (0.252)	0.837 (0.277)	0.997 (0.248)	$p < 0.0001$	$p < 0.0001$
Mean LOEUF (SD)	0.872 (0.481)	0.787 (0.493)	0.947 (0.512)	$p = 0.051$	$p = 0.021$

LUCA, last universal common ancestor; OMIM, Online Inheritance in Man; SD, standard deviation; SOEUF, synonymous observed/expected upper bound fraction; MOEUF, missense observed/expected upper bound fraction; LOEUF, loss-of-function observed/expected upper bound fraction.

only 9 genes in the RG group (1.9%) are involved in translation, ribosomal structure and biogenesis, 131 (47.3%) of those in the LG group are. We therefore performed the same analysis considering each functional group separately.

Subgroup analysis was performed for the 12 functional categories found in both groups and containing more than 10 genes (C, CO, E, F, G, H, J, K, L, M, O, U). The MOEUF and LOEUF values for the LG group were lower than those in the RG group in 8/12 functional categories (C, G, J, K, L, M, O and U), with statically significant differences for M and U in terms of MOEUF and LOEUF and for K in terms of MOEUF. The results for the SOEUF metric were more variable, with values obtained for the LG group being lower in 6 categories but higher in the 6 others. The number of associated Mendelian diseases was non-significantly higher in the LG group for 7 functional categories (G, H, I, J, K, M, O), and significantly higher for the L category, and the same as in the RG group for the U category (Supplemental Table S2, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=104*).

This is, to our knowledge, the first study of genetic constraint in the putative ancestral core of the human genome. We found that these LUCA genes were slightly more constrained than a random sample of genes for missense and loss-of-function variants, and less constrained for synonymous variants. Whereas LUCA genes were found to be more frequently associated with Mendelian diseases, strangely, the mode of inheritance was more frequently autosomal recessive (69.0% *vs.* 50.5%) and less frequently autosomal dominant (19.0 *vs.* 31.3%) than it was for diseases associated with the randomly selected genes. Genes with lower LOEUFs tend to be haploinsufficiency genes and less commonly autosomal recessive (*6*). However, the mean LOEUF of the LUCA genes (0.872) is well above the threshold below which genes are usually considered constrained (0.35) (*12*). The fact that a higher proportion of universal genes are associated with autosomal recessive diseases, suggests that ancient genes are more constrained but have become more tolerant of heterozygous loss-of-function.

The fact that the analysis in terms of eggNOG functional categories produced the same results suggests that our results are not an artefact due to the large proportion of LUCA genes linked to translation, ribosomal structure and biogenesis or due to the ~50% of randomly selected genes being of unknown function.

Unsurprisingly, since gene duplication has been an important force in evolution (*13*), most COGs were associated with several human genes. It could have been assumed that constraints on the two genes would differ after duplication, one being more constrained and the other less as a new function is acquired (*14*). However, the variations in MOEUF, LOEUF and SOEUF values were huge even when the corresponding COG was only associated with a single gene, and the number of homologs per COG was only weakly correlated with these metrics, and thus the effect does not seem to be important.

In conclusion, these preliminary results suggest that the ancestral core differs from the rest of the human genome in terms of genetic constraint and associated Mendelian diseases. An interesting line of research may be to use phylogenic data to uncover whether these universal genes can explain some of the characteristics of human genes and help in interpreting variation in a clinical setting.

Acknowledgements

We thank Pr. Céline Brochier-Armanet for useful discussion which lead to this work. We thank Paul Guerry (GreenGrow Scientific) for editing the article.

Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Forterre P, Gribaldo S, Brochier C. Luca: the last universal common ancestor. Med Sci (Paris). 2005; 21:860-865. (in French)
2. Palacios-Pérez M, José MV. The evolution of proteome: From the primeval to the very dawn of LUCA. Biosystems. 2019; 181:1-10.
3. Catchpole RJ, Forterre P. The evolution of reverse gyrase suggests a nonhyperthermophilic last universal common ancestor. Mol Biol Evol. 2019; 36:2737-2747.
4. Bennett CA, Petrovski S, Oliver KL, Berkovic SF. ExACtly zero or once: A clinically helpful guide to assessing genetic variants in mild epilepsies. Neurol Genet. 2017; 6; 3:e163.
5. Fuller ZL, Berg JJ, Mostafavi H, Sella G, Przeworski M. Measuring intolerance to mutation in human genetics. Nat Genet. 2019; 51:772-776.
6. Karczewski KJ, Francioli LC, Tiao G, *et al.* The mutational constraint spectrum quantified from variation in 141,456 humans. Nature. 2020; 581:434-443.
7. Huerta-Cepas J, Szklarczyk D, Heller D, Hernández-Plaza A, Forslund SK, Cook H, Mende DR, Letunic I, Rattei T, Jensen LJ, von Mering C, Bork P. eggNOG 5.0: a hierarchical, functionally and phylogenetically annotated orthology resource based on 5090 organisms and 2502 viruses. Nucleic Acids Res. 2019; 47:D309-D314.
8. Harris JK, Kelley ST, Spiegelman GB, Pace NR. The genetic core of the universal ancestor. Genome Res. 2003; 13:407-412.
9. Ciccarelli FD, Doerks T, von Mering C, Creevey CJ, Snel B, Bork P. Toward automatic reconstruction of a highly resolved tree of life. Science. 2006; 311:1283-1287.
10. Puigbò P, Wolf YI, Koonin EV. Search for a 'Tree of Life' in the thicket of the phylogenetic forest. J Biol. 2009; 8:59.
11. McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University (Baltimore, MD), Online Mendelian Inheritance in Man, OMIM®. *https://omim.org* (accessed December 6, 2021)
12. Francioli L, Tiao G, Karczewski K, Solomonson M, Watts N. gnomAD v2.1 *https://macarthurlab.org/2018/10/17/gnomad-v2-1/* (accessed December 6, 2021).
13. Magadum S, Banerjee U, Murugan P, Gangapur D, Ravikesavan R. Gene duplication as a major force in evolution. J Genet. 2013; 92:155-161.
14. Wagner A. Selection and gene duplication: a view from the genome. Genome Biol. 2002; 3:reviews1012.

Received June 1, 2022; Revised June 24, 2022; Accepted July 11, 2022.

**Address correspondence to:*
Alexandre Fabre, Pediatric Multidisciplinary Department, Timone Enfant Hospital, APHM, 264 Rue Saint Pierre 13005 Marseille, France.
E-mail: alexandre.fabre@ap-hm.fr

Released online in J-STAGE as advance publication July 22, 2022.

Communication

DOI: 10.5582/irdr.2022.01069

Attention should be paid to acute hepatitis of unknown etiology in children

Guangbin Chen[1], Hongzhou Lu[2,*]

[1] Department of Pharmacy, Shenzhen Third People's Hospital, Shenzhen, Guangdong, China;
[2] National Clinical Research Center for Infectious Diseases, Shenzhen Third People's Hospital, Shenzhen, Guangdong, China.

SUMMARY Since April 5, 2022, an increase in cases of severe acute hepatitis of unknown etiology among children with no underlying conditions was first reported in the United Kingdom (UK). Testing excluded viral hepatitis types A, B, C, D, and E and other known common and uncommon infectious and non-infectious causes of acute hepatitis. As of May 26, 2022, 650 cases of acute hepatitis of unknown etiology in children have been reported in at least 33 countries worldwide, with 99 additional cases pending classification. Here, the current prevalence of this condition around the world, a hazard analysis, possible causes, the risk of an outbreak in China, and advice on prevention have been briefly reviewed.

Keywords adenovirus infection, viral hepatitis, healthy children, acute hepatitis of unknown etiology in children, epidemic

1. Introduction

Since January 2022, cases of acute hepatitis of unknown etiology in previously healthy children have been reported in Europe, the USA, Japan and other regions and countries, causing widespread concern around the world. These cases excluded hepatitis viruses A, B, C, D, and E and other known common and uncommon infectious and non-infectious causes of acute hepatitis (*1,2*). There is no standard term for these cases of hepatitis. Here, the term "acute hepatitis of unknown etiology in children" as recommended by the World Health Organization (WHO) has been adopted. The current prevalence of this condition around the world, a hazard analysis, possible causes, the risk of an outbreak in China, and advice on prevention have been briefly reviewed.

2. Global epidemic situation

On April 5, 2022, there were reports of 10 cases of severe acute hepatitis of unknown etiology in children in the United Kingdom, and these cases involved children < 10 years of age. As of May 26, 2022, 650 cases of acute hepatitis of unknown etiology in children have been reported in at least 33 countries worldwide, with 99 additional cases pending classification (*3*). Compared to the last notification by the WHO on April 23, 2022, the number of cases increased from 169 to 650, and the number of countries reporting cases increased from 22 to 33. Of 650 probable cases, 222 (34.2%) were reported in the UK, the first country where they were reporter. Two hundred and sixteen cases (33.2%) were reported in the United States. In addition, Japan has reported 31 cases, which is the most in Asia. Canada has reported 10 cases and Mexico has reported 10 cases as well. Of the 33 countries that reported cases, 22 (66.7%) are in Europe, where 374 cases were reported. Other European countries besides the UK that have reported high numbers of cases include: Spain (29 cases), Italy (27), Belgium (14), the Netherlands (14), Portugal (11), Sweden (9), and Ireland (7) (Figures 1 and 2).

Of the 650 cases of acute hepatitis of unknown etiology in children, children in 9 cases (1.38%) died. At least 38 children (5.85%) required a liver transplant. The majority of reported cases (n = 490; 75.4%) involve children under 5 years of age. Of 156 patients on which information is available, 22 were critically ill and admitted to the ICU and 14 underwent a liver transplant.

As of June 9, 2022, 402 cases have been reported in Europe according to the European Centre for Disease Prevention and Control (ECDC). Cases were reported in the United Kingdom (224), Spain (36), Italy (31), Belgium (14), the Netherlands (14), Portugal (15), Sweden (9), Ireland (13), Poland (8), Denmark (7), France (7), Greece (6), and Norway (5) (*4*). No cases have been reported in China to date (Figure 3).

Epidemiologically, the vast majority of reported cases are unrelated. Moreover, there were no familial clusters

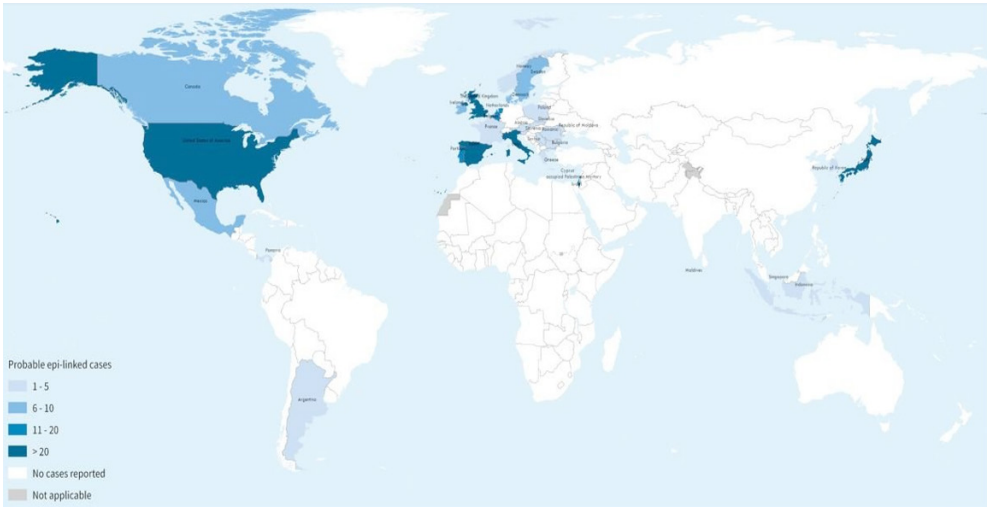


Figure 1. Distribution of probable cases of acute hepatitis of unknown etiology in children by country in five WHO Regions as of May 26, 2022 (*n* = 650). From the WHO.

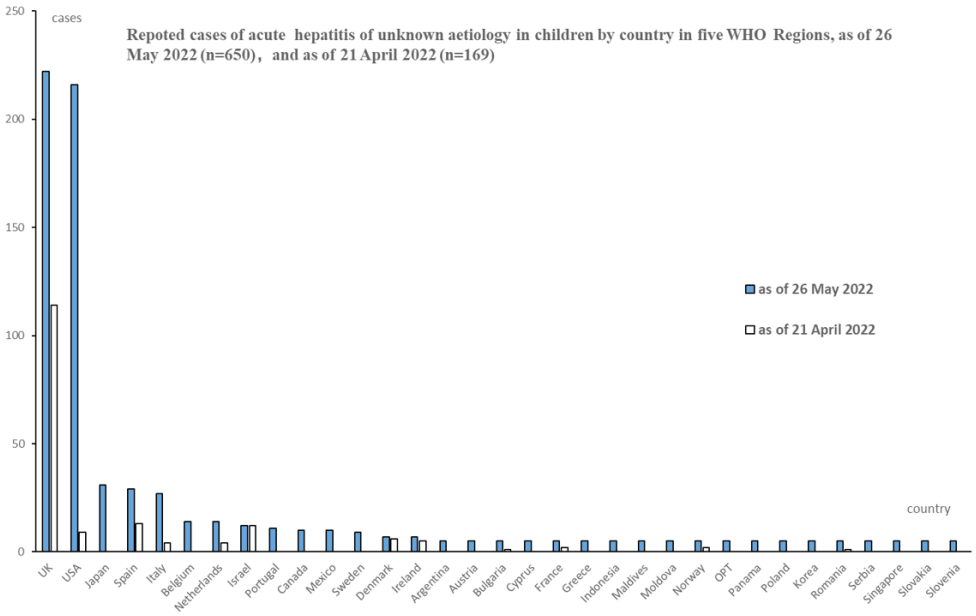


Figure 2. Reported cases of acute hepatitis of unknown etiology in children by country in five WHO Regions as of May 26, 2022 (*n* = 650) and as of April 21, 2022 (*n* = 169). From the WHO.

of the condition. The children were reported in different countries, and no travel history associated with the cases was noted. At present, most cases reported worldwide are sporadic, with no clusters or evidence of human-to-human transmission of infection. Although the risk at the global level was assessed as moderate, the WHO does not recommend travel restrictions for countries with cases (*3*).

3. Hazard analysis

The clinical characteristics of acute hepatitis of unknown etiology in children are as follows: An age of onset ranging from 1 month to 16 years of age, being previously healthy, and mostly involving children under 5 years of age. The main manifestations the condition include nausea, vomiting, diarrhea, jaundice, pale stool, and drowsiness. A fever has been noted in a few cases, most patients have no respiratory symptoms, ALT and AST levels are higher than 500 IU/L (higher than 2000 IU/L in some cases), and most cases have a good prognosis, but a few children develop liver failure and require a liver transplant (*3,5,6*).

There have been sporadic cases of acute hepatitis of unknown cause in children in the past, but the reason why this condition has aroused global attention now is because: 1) The number of children with acute hepatitis of unknown etiology and the number of countries reporting cases have increased rapidly in a short period of time. For example, only 169 cases were reported in 22 countries on April 23, 2022, while 650 cases were reported in 33 countries on May 26, 2022. Moreover,

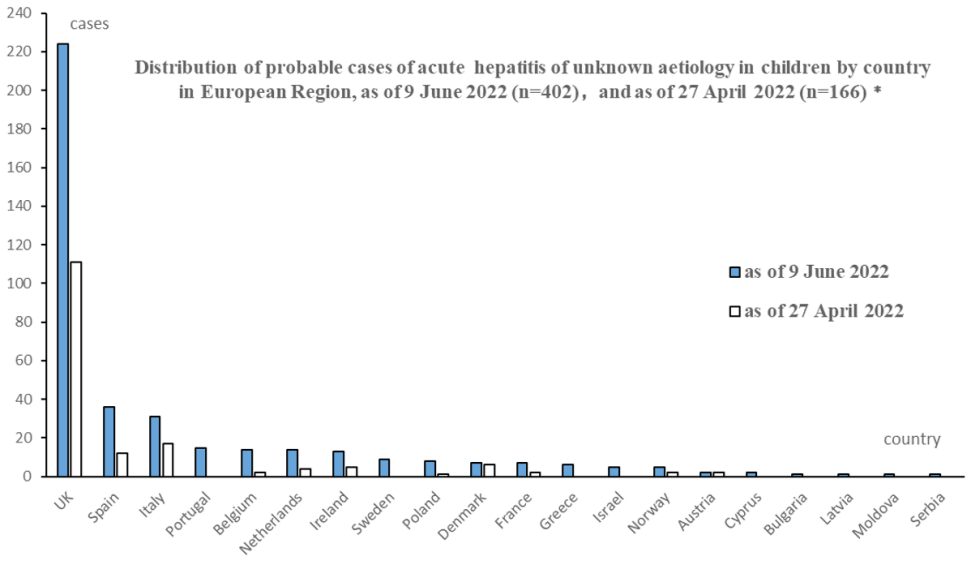


Figure 3. Reported cases of acute hepatitis of unknown etiology in children by country in European Region as of June 9, 2022 (*n* = 402) and as of April 27, 2022 (*n* = 166). From the ECDC. *Cases in the UK as of April 20, 2022.

due to the limited capacity to monitor the condition in some countries and regions, the true number of cases may be underestimated. 2) Compared to the previously reported cases of acute hepatitis of unknown etiology in children, cases being reported now are clinically severe, with a higher proportion of patients developing acute liver failure, requiring a liver transplant, and having a higher mortality. 3) The etiology is unknown, precluding assessment of the potential for further transmission. 4) This condition appeared during the COVID-19 epidemic and an association with SARS-CoV-2 cannot be completely ruled out. 5) Children have a limited ability to defend against disease, and this is particularly true for children under 5 years of age. Children are known to have a more limited organ reserve than adults; their condition changes rapidly and illnesses are more likely to be fatal. 6) Thus far, clusters of cases have not been noted, but human-to-human transmission cannot be completely ruled out yet. Therefore, attention should be paid to acute hepatitis of unknown etiology in children.

4. Possible causes

The exact etiology is unknown, and hepatitis viruses A, B, C, D, and E were excluded in these cases. According to the ECDC, specimens were collected in 293 cases and tested for adenovirus, of which 158 (53.9%) were positive. A total of 273 children with acute hepatitis of unknown cause were identified by PCR for SARS-COV-2, of which 29 (10.6%) tested positive. Serological tests for SARS-COV-2 were performed in 47 cases, of which 30 (63.8%) were positive (*3*). To the extent known, adenoviruses mainly cause respiratory infections and rarely cause severe acute hepatitis. According to the ECDC, an infectious agent (including adenovirus or a novel variant adenovirus or a new variant of SARS-CoV-2 or other pathogen) remains the most plausible cause, but a drug, a toxin, environmental exposure, *etc*. cannot be completely ruled out (*7*). Many children under the age of 10 in Europe have not been vaccinated against COVID-19, so acute hepatitis of unknown etiology in children is not considered to be related to COVID-19 vaccine so far.

Since liver biopsy specimens were not pathologically diagnosed in most cases, there is no direct grounds for considering the condition to be adenovirus hepatitis. Therefore, a confirmed diagnosis of adenovirus hepatitis based only on test results of specimens from the respiratory tract, digestive tract, and blood is difficult. Previously, the majority of confirmed cases of adenovirus hepatitis have been reported in immunocompromised or immunosuppressive adults, such as patients with lymphoma or patients receiving immunosuppressive agents while undergoing a liver transplant (*8-10*). In those cases, a liver biopsy was pathologically diagnosed, *e.g*., electron microscopy of biopsied liver tissue revealed adenovirus particles.

There are a few cases of acute hepatitis caused by adenovirus in children in the literature, where adenovirus hepatitis was confirmed by pathological results of a liver biopsy. In Canada, a 20-month-old boy with atypical malformed rhabdomyoma (ATRT) underwent a liver biopsy after admission. Adenovirus hepatitis was confirmed when electron microscopy revealed adenovirus particles, and the patient was positive for adenovirus according to PCR (*11*). A liver biopsy is difficult to perform in children because they are not as capable of tolerating the procedure as adults and they cannot hold their breath.

In addition to adenoviruses, Epstein-Barr viruses, enteroviruses, rhinoviruses, metapneumoviruses, and respiratory syncytial viruses were also reported

to associated with hepatitis in children (*13*). Other rare pathogens causing hepatitis in children include human parvovirus B19, rotavirus, Boca virus, respiratory envelope virus, cytomegalovirus, varicella-zoster virus, and measles virus (*13-18*). In addition to microbial pathogens, other conditions that cause hepatitis in children include Wilson's disease, hereditary hemochromatosis (HH), hereditary hyperbilirubinemia, an α-1 antitrypsin deficiency, drug-induced liver failure, Reye syndrome, cystic fibrosis, Alagille syndrome, and tyrosinemia type I, *etc*..

5. Risk assessment in China

Based on the currently available information, an outbreak of acute hepatitis of unknown etiology in children in China is unlikely in the short term. Possible reasons for this may be: 1) Current epidemiological data have revealed no evidence of human-to-human transmission, with 650 cases distributed across 33 countries; 2) China's strict and scientific COVID-19 epidemic prevention policy ensures that imported cases are prevented. Chinese Customs will continue to take strict measures to ensure entry-exit health quarantines, enhance multi-department joint prevention and control, and strictly prevent the risk of imported epidemics. 3) Good hand hygiene, wearing masks, etiquette when coughing, social distancing and other measures to combat COVID-19 have been well understood and implemented by the general public. Common respiratory infections in children, as an example, have been significantly reduced since the implementation of COVID-19 prevention policies in China. At present, China's experience with the prevention and control of COVID-19 and improvements in public health awareness have greatly helped to prevent acute hepatitis of unknown etiology in children. 4) Practice has proven that the dynamic zero-COVID approach safeguards people's health best. These epidemic prevention policies are conducive to the prevention and control of acute hepatitis of unknown etiology in children. However, the possibility of acute hepatitis of unknown cause in children appearing in China in the future cannot be completely ruled out.

6. Advice on prevention and control

Although an outbreak of acute hepatitis of unknown cause in children in China is unlikely in the short term, physicians still need to be vigilant and pay close attention to WHO and ECDC reports. According to the recommendations of the Chinese Health Commission, the following preventive measures should be taken: 1) Children should avoid going to crowded public places with poor ventilation, and droplet contact and fecal-oral transmission should be interrupted; 2) Children should receive adequate sleep and nutrition; 3) Children's outdoor clothes and objects they regularly touch should be washed, they should wear a mask, and they should practice good hand hygiene and maintain social distancing. In addition, children with symptoms such as vomiting and diarrhea should stay at home for 48 hours until symptoms disappear before returning to school or kindergarten. Healthcare workers, and especially pediatric medical staff, should be on high alert for clinical symptoms of hepatitis in children. Suspected cases of acute hepatitis of unknown etiology in children should be reported to the Centers for Disease Control (CDC) as soon as possible and affected children should be sent to the hospital for testing to promptly identify the cause of hepatitis. Health authorities should assemble experts to study and discuss prevention and treatment strategies and to formulate guidelines to facilitate diagnosis, case investigation and reporting, and clinical management of acute liver failure in children. Health authorities should be prepared for any possible outbreak.

Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. World Health Organization (April 15, 2022). Disease Outbreak News: Acute hepatitis of unknown aetiology - the United Kingdom of Great Britain and Northern Ireland. *https://www.who.int/emergencies/disease-outbreak-news/item/acute-hepatitis-of-unknown-aetiology---the-united-kingdom-of-great-britain-and-northern-ireland* (accessed June 3, 2022).
2. World Health Organization (April 23, 2022). Disease Outbreak News: Multi-Country – Acute, severe hepatitis of unknown origin in children. *https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON376* (accessed June 3, 2022).
3. World Health Organization (May 27, 2022). Disease Outbreak News: Acute hepatitis of unknown aetiology in children-Multi-country. *https://www.who.int/emergencies/disease-outbreak-news/item/DON-389* (accessed June 3, 2022).
4. European Centre for Disease Prevention and Control/ WHO Regional Office for Europe. Hepatitis of Unknown Aetiology in Children, Joint Epidemiological overview, 10 June, 2022. *https://www.ecdc.europa.eu/en/increase-severe-acute-hepatitis-cases-unknown-aetiology-children* (accessed June 3, 2022).
5. European Centre for Disease Prevention and Control. Increase in severe acute hepatitis cases of unknown aetiology in children – 28 April 2022. ECDC: Stockholm; 2022. *https://www.ecdc.europa.eu/sites/default/files/documents/RRA-20220420-218-erratum.pdf* (accessed June 3, 2022).
6. The United Kingdom Health Security Agency (UKHSA), Guidance note including recommended tests, available at *https://www.gov.uk/government/publications/hepatitis-increase-in-acute-cases-of-unknown-aetiology-in-children/increase-in-acute-hepatitis-cases-of-unknown-aetiology-in-children* (accessed June 3, 2022).

7. European Centre for Disease Prevention and Control. Increase in severe acute hepatitis cases of unknown aetiology in children. *https://www.ecdc.europa.eu/sites/default/files/documents/RRA-20220420-218-erratum.pdf* (accessed June 3, 2022).
8. Ronan BA, Agrwal N, Carey EJ, De Petris G, Kusne S, Seville MT, Blair JE, Vikram HR. Fulminant hepatitis due to human adenovirus. Infection. 2014; 42:105-111.
9. Rothenberg M, Cheung R, Ahmed A. Adenovirus-induced acute liver failure. Dig Dis Sci. 2009; 54:218-221.
10. Kerensky T, Hasan A, Schain D, Trikha G, Liu C, Rand K, Soldevila-Pico C, Gupte A. Histopathologic resolution of adult liver transplantation adenovirus hepatitis with cidofovir and intravenous immunoglobulin: A case report. Transplant Proc. 2013; 45:293-296.
11. McKillop SJ, Belletrutti MJ, Lee BE, Yap JY, Noga ML, Desai SJ, Sergi C. Adenovirus necrotizing hepatitis complicating atypical teratoid rhabdoid tumor. Pediatr Int. 2015; 57:974-977.
12. Baker JM, Buchfellner M, Britt W, *et al*. Acute hepatitis and adenovirus infection among children - Alabama, October 2021-February 2022. MMWR Morb Mortal Wkly Rep. 2022;71:638-640.
13. Leon LAA, Alves ADR, Garcia RCNC, Melgaço JG, de Paula VS, Pinto MA. Parvovirus B19 Infection in a Fatal Case of Acute Liver Failure. Pediatr Infect Dis J. 2017; 36:e355-e358.
14. Ishige M, Fuchigami T, Furukawa M, Kobayashi H, Fujiki R, Ogawa E, Ishige N, Sasai H, Fukao T, Hashimoto K, Inamo Y, Morioka I. Primary carnitine deficiency with severe acute hepatitis following rotavirus gastroenteritis. J Infect Chemother. 2019; 25(:913-916.
15. Haytoğlu Z, Canan O. Bocavirus viremia and hepatitis in an immunocompetent child. Balkan Med J, 2017; 34: 281-283.
16. Kirin BK, Topić RZ, Dodig S. Hepatitis during respiratory syncytial virus infection--a case report. Biochem Med (Zagreb). 2013; 23:112-116.
17. Han SB, Seo YE, Kim SK, Lee JW, Lee DG, Chung NG, Cho B, Kang JH, Kim HK, Jung ES. Varicella with rapidly progressive hepatitis presenting with multiple hepatic nodules in a child with acute leukemia. J Infect Chemother. 2016; 22:822-825.
18. Gur I, Shapira Y, Amitai Y, Shvil I. Renal failure, hepatitis and encephalitis following measles. Isr J Med Sci. 1984; 20:441-2.

Received June 5, 2022; Revised June 24, 2022; Accepted June 27, 2022.

**Address correspondence to:*
Hongzhou Lu, Department of Infectious Diseases, National Clinical Research Center for Infectious Diseases, Shenzhen Third People's Hospital, Shenzhen 518112, Guangdong Province, China.
E-mail: luhongzhou@fudan.edu.cn

Released online in J-STAGE as advance publication June 29, 2022.

Letter

DOI: 10.5582/irdr.2022.01076

A case of hilar biliary cystadenoma with elevated IgG4 levels

Zushun Chen, Haiming Lu, Jingxuan Xu, Liang Ma*

Hepatobiliary Surgery, Guangxi Medical University Cancer Hospital, Nanning, Guangxi, China.

SUMMARY Cholangiocytic adenoma in the hilar bile duct is rare, and elevated IgG4 at the same time is extremely rare. This situation has not been reported in the literature. Nonetheless, the current case involved hilar biliary cystadenoma with elevated IgG4 levels. A 66-year-old man presented at this hospital with dark tea-colored urine. Preoperative imaging studies suggested hilar cholangiocarcinoma. This case demonstrates the difficulty of preoperative diagnosis of benign hilar lesions and the rarity of two combined benign lesions. A point of contention is whether this case should be treated with surgery or hormone therapy.

Keywords biliary cystadenoma, hilar cholangiocarcinoma, IgG4, immunoglobulin G4-related cholangitis, jaundice

Biliary cystadenoma is a rare benign cystic lesion of the liver originating from the bile duct epithelium, and it accounts for less than 5% of all hepatic cystic lesions (*1*). Its pathogenesis is still unclear; its congenital cause may be related to the abnormal development of the vagus bile duct in the embryonic stage (*2*), and its acquired causes are mainly related to liver cirrhosis and oral contraceptives (*3*). The symptoms of this disease are mostly atypical, and they often differ substantially depending on the location and size of the tumor. Moreover, there are no effective serological tumor markers. For special types of bile duct cystadenomas, the clinical features may even overlap with those of hilar cholangiocarcinoma and IgG4-related sclerosing cholangitis, which can easily lead to misdiagnosis. Here, a case of hilar bile duct cystadenoma with elevated IgG4 is reported to improve the understanding of this disease.

A 66-year-old man visited a local hospital in September 2019 due to "dark tea-colored urine for longer than a month." Imaging studies suggested hilar cholangiocarcinoma, and inflammatory lesions were not excluded. Total bilirubin was 96.1 umol/L, direct bilirubin was 67.8 umol/L, autoimmune-related antibodies were negative, and the serological tumor markers AFP, CA199, and CA125 were within the normal range. In July 2020, total bilirubin was 76.0 umol/L, and direct bilirubin was 44.1 umol/L. Afterwards, ERCP was performed and a common bile duct stent was placed. Biliary cytology pathology: (hilar bile duct) a small number of atypical glandular cells, no typical cancer cells. The patient visited this hospital in August, and bilirubin and serological tumor markers were checked as before. CT, MRI, and MRCP revealed stent shadows in the lumen of the common hepatic duct. The hepatic hilum ended abruptly, the left and right hepatic ducts were separated, the bile duct wall had thickened, and suspicious soft tissue masses were evident in the hepatic hilar region (Figure 1A).

Imaging studies could not rule out hilar cholangiocarcinoma. Autoimmune-related cholangitis was considered, but the serum IgG4 level was 1.670 g/L (Figure 1C), and imaging studies revealed no other organ lesions. A left hepatectomy with caudate lobe resection was performed on August 21, 2020. A postoperative histopathological examination revealed biliary cystadenoma and negativity for IgG4 (Figure 1B). The patient was discharged 14 days after surgery, and further testing revealed that IgG4 had returned to normal (Figure 1C).

Hilar bile duct cystadenoma and elevated IgG4 is a rare condition, and it has not been reported previously. Ghazale *et al*. reported that one-third of patients preoperatively diagnosed with hilar cholangiocarcinoma were diagnosed with IgG4-related cholangitis after surgery (*4*). At present, there are no accurate diagnostic criteria for IgG4-related cholangitis and obtaining a pathological diagnosis via a biopsy is difficult, causing a delay in diagnosis. The serum IgG4 level has certain limitations as a diagnostic criterion because some patients with IgG4-related cholangitis have only a slight increase (between 1 and 4 times the normal value); moreover, up to 15% of patients with cholangiocarcinoma also have elevated serum levels of IgG4 (*5*). According to the diagnostic criteria for

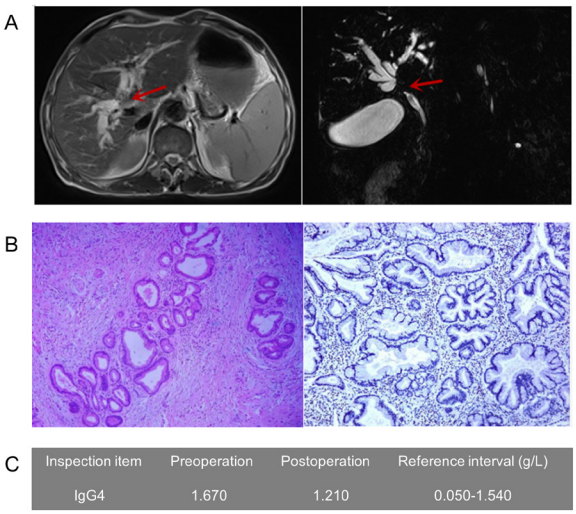


Figure 1. (A), MRI and MRCP showing the confluence of the left and right hepatic ducts and a left bile duct mass; (B), Postoperative pathology showing the dilated bile ducts and negativity for IgG4; (C), Level of IgG4 pre- and post-operatively.

IgG4-related cholangitis devised in Japan in 2012 (*6.7*): (*i*) characteristic changes in biliary imaging; (*ii*) serum IgG4 levels higher than the normal upper limit (≥1.35 g/L); (*iii*) lesions in other organs as well; and (*iv*) histopathological characteristics: ① infiltration of landmark lymphocytes or plasma cells; ② infiltration of IgG4-positive plasma cells (>10 IgG4-positive plasma cells per high-power field); ③ spoke-shaped fibrosis; and ④ obliterative phlebitis.

The current case involved a slight increase in serum IgG4, a histology revealing no infiltration of IgG4-positive cells, and no diffuse intrahepatic bile duct lesions. The lesions overlapped cystadenoma lesions, and both were located in the hilar bile duct. The postoperative decrease in IgG4 to normal is also a feature of this case that deserves attention. However, a postoperative pathological examination of the cystadenomas did not suggest that IgG4 was elevated in diseased tissue, indicating that this case may have little relationship to IgG4. Postoperatively, IgG4 decreased below the normal level, which may be related to the resection of the lesion and bile drainage. The local inflammatory reaction subsided, and it needs to be reviewed regularly after surgery. Previous studies have reported that slight elevation of IgG4 may occur in bile duct inflammation and bile duct malignancies (*2,6*), and this clinical feature was also evident in the current case. Imaging revealed no lesions in the pancreas, which is the most frequently infiltrated organ in IgG4-related cholangitis, or other organs, so a histological biopsy was not clinically indicated. The malignant transformation rate of intrahepatic bile duct cystadenoma is about 30% (*8*), and it is considered to be a precancerous lesion, so once it is found, it needs to be treated. Hilar invasion should be treated with gallbladder and extrahepatic bile duct resection. Even after local resection, extrahepatic bile duct cystadenoma still has a recurrence rate of 50%, and the tumor should be completely resected to achieve a negative resection margin before performing cholangioenterostomy (*9*).

In summary, hormone therapy is the treatment of choice for isolated IgG4-related cholangitis (*10,11*). When cystadenoma is also present, however, whether hormone therapy will delay or accelerate the malignant transformation of the cystadenoma is unclear. As corroborated by the literature, our center considers cystadenoma to be a precancerous lesion. Hilar cholangiocarcinoma could not be ruled out before surgery, so surgical resection was performed in the current case. This strategy warrants discussion, and it needs to be studied in large-scale studies with a follow-up.

Funding: None.

Conflict of Interest: The authors have no conflicts of interest to disclose.

References

1. Ishak K, Willis G, Cummins S, Bullock A. Biliary cystadenoma and cystadenocarcinoma. Report of 14 cases and review of the literature. Cancer. 1977; 39:322-338.
2. Zen Y, Fujii T, Itatsu K, Nakamura K, Konishi F, Masuda S, Mitsui T, Asada Y, Miura S, Miyayama S, Uehara T, Katsuyama T, Ohta T, Minato H, Nakanuma Y. Biliary cystic tumors with bile duct communication: a cystic variant of intraductal papillary neoplasm of the bile duct. Mod Pathol. 2006; 19:1243-1254.
3. Vogt D, Henderson J, Chmielewski E. Cystadenoma and cystadenocarcinoma of the liver: A single center experience. JJ Am Coll Surg. 2005; 200:727-733.
4. Ghazale A, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, Topazian MD, Clain JE, Pearson RK, Petersen BT, Vege SS, Lindor K, Farnell MB. Immunoglobulin G4-associated cholangitis: Clinical profile and response to therapy. Gastroenterology. 2008; 134:706-715.
5. Oseini AM, Chaiteerakij R, Shire AM, Ghazale A, Kaiya J, Moser CD, Aderca I, Mettler TA, Therneau TM, Zhang L, Takahashi N, Chari ST, Roberts LR. Utility of serum immunoglobulin G4 in distinguishing immunoglobulin G4-associated cholangitis from cholangiocarcinoma. Hepatology. 2011; 54:940-948.
6. Okazaki K, Uchida K, Koyabu M, Miyoshi H, Ikeura T, Takaoka M. IgG4 cholangiopathy: Current concept, diagnosis, and pathogenesis. J Hepatol. 2014; 61:690-695.
7. Ohara H, Okazaki K, Tsubouchi H, *et al*. Clinical diagnostic criteria of IgG4-related sclerosing cholangitis 2012. J Hepatobiliary Pancreat Sci. 2012; 19:536-542.
8. Teoh A, Ng S, Lee K, Lai P. Biliary cystadenoma and other complicated cystic lesions of the liver: Diagnostic and therapeutic challenges. World Journal of Surgery. 2006; 30:1560-1566.
9. Davies W, Chow M, Nagorney D. Extrahepatic biliary cystadenomas and cystadenocarcinoma. Ann Surg. 1995;

222:619-625.
10. Liu W, Chen W, He X, Qu Q, Hong T, Li B. Poor response of initial steroid therapy for IgG4-related sclerosing cholangitis with multiple organs affected. Medicine. 2017; 96:e6400.
11. Smit W, Culver E, Chapman R. New thoughts on immunoglobulin g4- related sclerosing cholangitis. Clin Liver Dis. 2016; 20:47-65.

Received July 18, 2022; Revised August 16, 2022; Accepted August 21, 2022.

*Address correspondence to:
Liang Ma, Hepatobiliary Surgery, Guangxi Medical University Cancer Hospital, No. 71 Hedi Road, Nanning, Guangxi, 530021, China.
E-mail: chemzs@163.com

Released online in J-STAGE as advance publication August 25, 2022.

IRDR
Intractable & Rare Diseases Research

Guide for Authors

1. Scope of Articles

Intractable & Rare Diseases Research (Print ISSN 2186-3644, Online ISSN 2186-361X) is an international peer-reviewed journal. Intractable & Rare Diseases Research devotes to publishing the latest and most significant research in intractable and rare diseases. Articles cover all aspects of intractable and rare diseases research such as molecular biology, genetics, clinical diagnosis, prevention and treatment, epidemiology, health economics, health management, medical care system, and social science in order to encourage cooperation and exchange among scientists and clinical researchers.

2. Submission Types

Original Articles should be well-documented, novel, and significant to the field as a whole. An Original Article should be arranged into the following sections: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and References. Original articles should not exceed 5,000 words in length (excluding references) and should be limited to a maximum of 50 references. Articles may contain a maximum of 10 figures and/or tables. Supplementary Data are permitted but should be limited to information that is not essential to the general understanding of the research presented in the main text, such as unaltered blots and source data as well as other file types.

Brief Reports definitively documenting either experimental results or informative clinical observations will be considered for publication in this category. Brief Reports are not intended for publication of incomplete or preliminary findings. Brief Reports should not exceed 3,000 words in length (excluding references) and should be limited to a maximum of 4 figures and/or tables and 30 references. A Brief Report contains the same sections as an Original Article, but the Results and Discussion sections should be combined.

Reviews should present a full and up-to-date account of recent developments within an area of research. Normally, reviews should not exceed 8,000 words in length (excluding references) and should be limited to a maximum of a maximum of 10 figures and/or tables and 100 references. Mini reviews are also accepted, which should not exceed 4,000 words in length (excluding references) and should be limited to a maximum of 5 figures and/or tables and 50 references.

Policy Forum articles discuss research and policy issues in areas related to life science such as public health, the medical care system, and social science and may address governmental issues at district, national, and international levels of discourse. Policy Forum articles should not exceed 3,000 words in length (excluding references) and should be limited to a maximum of 5 figures and/or tables and 30 references.

Communications are short, timely pieces that spotlight new research findings or policy issues of interest to the field of global health and medical practice that are of immediate importance. Depending on their content, Communications will be published as "Comments" or "Correspondence". Communications should not exceed 1,500 words in length (excluding references) and should be limited to a maximum of 2 figures and/or tables and 20 references.

Editorials are short, invited opinion pieces that discuss an issue of immediate importance to the fields of global health, medical practice, and basic science oriented for clinical application. Editorials should not exceed 1,000 words in length (excluding references) and should be limited to a maximum of 10 references. Editorials may contain one figure or table.

News articles should report the latest events in health sciences and medical research from around the world. News should not exceed 500 words in length.

Letters should present considered opinions in response to articles published in *Intractable & Rare Diseases Research* in the last 6 months or issues of general interest. Summaries of research results and sharing of experiences in clinical practice and basic research (findings based on case reports, clinical pictures, *etc.*) can also be published as Letters. Letters should not exceed 800 words in length and may contain a maximum of 10 references. Letters may contain one figure or table.

3. Editorial Policies

For publishing and ethical standards, *Intractable & Rare Diseases Research* follows the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals (*http://www.icmje.org/recommendations*) issued by the International Committee of Medical Journal Editors (ICMJE), and the Principles of Transparency and Best Practice in Scholarly Publishing (*https://doaj.org/bestpractice*) jointly issued by the Committee on Publication Ethics (COPE), the Directory of Open Access Journals (DOAJ), the Open Access Scholarly Publishers Association (OASPA), and the World Association of Medical Editors (WAME).

Intractable & Rare Diseases Research will perform an especially prompt review to encourage innovative work. All original research will be subjected to a rigorous standard of peer review and will be edited by experienced copy editors to the highest standards.

Ethics: *Intractable & Rare Diseases Research* requires that authors of reports of investigations in humans or animals indicate that those studies were formally approved by a relevant ethics committee or review board. For research involving human experiments, a statement that the participants gave informed consent before taking part (or a statement that it was not required and why) should be indicated. Authors should also state that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013). When reporting experiments on animals, authors should indicate whether the institutional and national guide for the care and use of laboratory animals was followed.

Conflict of Interest: All authors are required to disclose any actual or potential conflict of interest including financial interests or relationships with other people or organizations that might raise questions of bias in the work reported. If no

conflict of interest exists for each author, please state "There is no conflict of interest to disclose".

Submission Declaration: When a manuscript is considered for submission to *Intractable & Rare Diseases Research*, the authors should confirm that 1) no part of this manuscript is currently under consideration for publication elsewhere; 2) this manuscript does not contain the same information in whole or in part as manuscripts that have been published, accepted, or are under review elsewhere, except in the form of an abstract, a letter to the editor, or part of a published lecture or academic thesis; 3) authorization for publication has been obtained from the authors' employer or institution; and 4) all contributing authors have agreed to submit this manuscript.

Cover Letter: The manuscript must be accompanied by a cover letter prepared by the corresponding author on behalf of all authors. The letter should indicate the basic findings of the work and their significance. The letter should also include a statement affirming that all authors concur with the submission and that the material submitted for publication has not been published previously or is not under consideration for publication elsewhere. The cover letter should be submitted in PDF format. For example of Cover Letter, please visit: Download Centre (*https://www.irdrjournal.com/downcentre*).

Copyright: When a manuscript is accepted for publication in *Intractable & Rare Diseases Research*, the transfer of copyright is necessary. A JOURNAL PUBLISHING AGREEMENT (JPA) form will be e-mailed to the authors by the Editorial Office and must be returned by the authors as a scan. Only forms with a hand-written signature are accepted. This copyright will ensure the widest possible dissemination of information. Please note that your manuscript will not proceed to the next step in publication until the JPA Form is received. In addition, if excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article.

Peer Review: *Intractable & Rare Diseases Research* uses single-blind peer review, which means that reviewers know the names of the authors, but the authors do not know who reviewed their manuscript. The external peer review is performed for research articles by at least two reviewers, and sometimes the opinions of more reviewers are sought. Peer reviewers are selected based on their expertise and ability to provide high quality, constructive, and fair reviews. For research manuscripts, the editors may, in addition, seek the opinion of a statistical reviewer. Consideration for publication is based on the article's originality, novelty, and scientific soundness, and the appropriateness of its analysis.

Suggested Reviewers: A list of up to 3 reviewers who are qualified to assess the scientific merit of the study is welcomed. Reviewer information including names, affiliations, addresses, and e-mail should be provided at the same time the manuscript is submitted online. Please do not suggest reviewers with known conflicts of interest, including participants or anyone with a stake in the proposed research; anyone from the same institution; former students, advisors, or research collaborators (within the last three years); or close personal contacts. Please note that the Editor-in-Chief may accept one or more of the proposed reviewers or may request a review by other qualified persons.

Language Editing: Manuscripts prepared by authors whose native language is not English should have their work proofread by a native English speaker before submission. If not, this might delay the publication of your manuscript in *Intractable & Rare Diseases Research*.

The Editing Support Organization can provide English proofreading, Japanese-English translation, and Chinese-English translation services to authors who want to publish in *Intractable & Rare Diseases Research* and need assistance before submitting a manuscript. Authors can visit this organization directly at *http://www.iacmhr.com/iac-eso/support.php?lang=en*. IAC-ESO was established to facilitate manuscript preparation by researchers whose native language is not English and to help edit works intended for international academic journals.

4. Manuscript Preparation

Manuscripts are suggested to be prepared in accordance with the "Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals", as presented at *http://www.ICMJE.org*.

Manuscripts should be written in clear, grammatically correct English and submitted as a Microsoft Word file in a single-column format. Manuscripts must be paginated and typed in 12-point Times New Roman font with 24-point line spacing. Please do not embed figures in the text. Abbreviations should be used as little as possible and should be explained at first mention unless the term is a well-known abbreviation (*e.g.* DNA). Single words should not be abbreviated.

Title page: The title page must include 1) the title of the paper (Please note the title should be short, informative, and contain the major key words); 2) full name(s) and affiliation(s) of the author(s), 3) abbreviated names of the author(s), 4) full name, mailing address, telephone/fax numbers, and e-mail address of the corresponding author; and 5) conflicts of interest (if you have an actual or potential conflict of interest to disclose, it must be included as a footnote on the title page of the manuscript; if no conflict of interest exists for each author, please state "There is no conflict of interest to disclose"). Please visit Download Centre and refer to the title page of the manuscript sample.

Abstract: The abstract should briefly state the purpose of the study, methods, main findings, and conclusions. For articles that are Original Articles, Brief Reports, Reviews, or Policy Forum articles, a one-paragraph abstract consisting of no more than 250 words must be included in the manuscript. For Communications, Editorials, News, or Letters, a brief summary of main content in 150 words or fewer should be included in the manuscript. Abbreviations must be kept to a minimum and non-standard abbreviations explained in brackets at first mention. References should be avoided in the abstract. Three to six key words or phrases that do not occur in the title should be included in the Abstract page.

Introduction: The introduction should be a concise statement of the basis for the study and its scientific context.

Materials and Methods: The description should be brief but with sufficient detail to enable others to reproduce the experiments. Procedures that have been published

previously should not be described in detail but appropriate references should simply be cited. Only new and significant modifications of previously published procedures require complete description. Names of products and manufacturers with their locations (city and state/country) should be given and sources of animals and cell lines should always be indicated. All clinical investigations must have been conducted in accordance with Declaration of Helsinki principles. All human and animal studies must have been approved by the appropriate institutional review board(s) and a specific declaration of approval must be made within this section.

Results: The description of the experimental results should be succinct but in sufficient detail to allow the experiments to be analyzed and interpreted by an independent reader. If necessary, subheadings may be used for an orderly presentation. All figures and tables must be referred to in the text.

Discussion: The data should be interpreted concisely without repeating material already presented in the Results section. Speculation is permissible, but it must be well-founded, and discussion of the wider implications of the findings is encouraged. Conclusions derived from the study should be included in this section.

Acknowledgments: All funding sources should be credited in the Acknowledgments section. In addition, people who contributed to the work but who do not meet the criteria for authors should be listed along with their contributions.

References: References should be numbered in the order in which they appear in the text. Citing of unpublished results, personal communications, conference abstracts, and theses in the reference list is not recommended but these sources may be mentioned in the text. In the reference list, cite the names of all authors when there are fifteen or fewer authors; if there are sixteen or more authors, list the first three followed by *et al*. Names of journals should be abbreviated in the style used in PubMed. Authors are responsible for the accuracy of the references. The EndNote Style of *Intractable & Rare Diseases Research* could be downloaded at **EndNote** (*https://www.irdrjournal.com/examples/Intractable_Rare_Diseases_Research.ens*).

Examples are given below:

Example 1 (Sample journal reference):

Inagaki Y, Tang W, Zhang L, Du GH, Xu WF, Kokudo N. Novel aminopeptidase N (APN/CD13) inhibitor 24F can suppress invasion of hepatocellular carcinoma cells as well as angiogenesis. Biosci Trends. 2010; 4:56-60.

Example 2 (Sample journal reference with more than 15 authors):

Darby S, Hill D, Auvinen A, *et al*. Radon in homes and risk of lung cancer: Collaborative analysis of individual data from 13 European case-control studies. BMJ. 2005; 330:223.

Example 3 (Sample book reference):
Shalev AY. Post-traumatic stress disorder: Diagnosis, history and life course. In: Post-traumatic Stress Disorder, Diagnosis, Management and Treatment (Nutt DJ, Davidson JR, Zohar J, eds.). Martin Dunitz, London, UK, 2000; pp. 1-15.

Example 4 (Sample web page reference):

World Health Organization. The World Health Report 2008 – primary health care: Now more than ever. *http://www.who.int/whr/2008/whr08_en.pdf* (accessed September 23, 2010).

Tables: All tables should be prepared in Microsoft Word or Excel and should be arranged at the end of the manuscript after the References section. Please note that tables should not in image format. All tables should have a concise title and should be numbered consecutively with Arabic numerals. If necessary, additional information should be given below the table.

Figure Legend: The figure legend should be typed on a separate page of the main manuscript and should include a short title and explanation. The legend should be concise but comprehensive and should be understood without referring to the text. Symbols used in figures must be explained. Any individually labeled figure parts or panels (A, B, *etc*.) should be specifically described by part name within the legend.

Figure Preparation: All figures should be clear and cited in numerical order in the text. Figures must fit a one- or two-column format on the journal page: 8.3 cm (3.3 in.) wide for a single column, 17.3 cm (6.8 in.) wide for a double column; maximum height: 24.0 cm (9.5 in.). Please make sure that the symbols and numbers appeared in the figures should be clear. Please make sure that artwork files are in an acceptable format (TIFF or JPEG) at minimum resolution (600 dpi for illustrations, graphs, and annotated artwork, and 300 dpi for micrographs and photographs). Please provide all figures as separate files. Please note that low-resolution images are one of the leading causes of article resubmission and schedule delays.

Units and Symbols: Units and symbols conforming to the International System of Units (SI) should be used for physicochemical quantities. Solidus notation (*e.g*. mg/kg, mg/mL, mol/mm^2/min) should be used. Please refer to the SI Guide *www.bipm.org/en/si/* for standard units.

Supplemental data: Supplemental data might be useful for supporting and enhancing your scientific research and *Intractable & Rare Diseases Research* accepts the submission of these materials which will be only published online alongside the electronic version of your article. Supplemental files (figures, tables, and other text materials) should be prepared according to the above guidelines, numbered in Arabic numerals (*e.g*., Figure S1, Figure S2, and Table S1, Table S2) and referred to in the text. All figures and tables should have titles and legends. All figure legends, tables and supplemental text materials should be placed at the end of the paper. Please note all of these supplemental data should be provided at the time of initial submission and note that the editors reserve the right to limit the size and length of Supplemental Data.

5. Submission Checklist

The Submission Checklist will be useful during the final checking of a manuscript prior to sending it to *Intractable & Rare Diseases Research* for review. Please visit Download

Centre and download the Submission Checklist file.

6. Online Submission

Manuscripts should be submitted to *Intractable & Rare Diseases Research* online at *https://www.irdrjournal.com*. The manuscript file should be smaller than 5 MB in size. If for any reason you are unable to submit a file online, please contact the Editorial Office by e-mail at *office@irdrjournal.com*

7. Accepted Manuscripts

Proofs: Galley proofs in PDF format will be sent to the corresponding author *via* e-mail. Corrections must be returned to the editor (*office@irdrjournal.com*) within 3 working days.

Offprints: Authors will be provided with electronic offprints of their article. Paper offprints can be ordered at prices quoted on the order form that accompanies the proofs.

Page Charge: No page charges will be levied to authors for the publication of their article except for reprints.

Misconduct: *Intractable & Rare Diseases Research* takes seriously all allegations of potential misconduct and adhere to the ICMJE Guideline (*http://www.icmje.org/recommendations*) and COPE Guideline (*http://publicationethics.org/files/Code_of_conduct_for_journal_editors.pdf*). In cases of suspected research or publication misconduct, it may be necessary for the Editor or Publisher to contact and share submission details with third parties including authors' institutions and ethics committees. The corrections, retractions, or editorial expressions of concern will be performed in line with above guidelines.

(As of February 2022)

Intractable & Rare Diseases Research
Editorial and Head Office
Pearl City Koishikawa 603,
2-4-5 Kasuga, Bunkyo-ku,
Tokyo 112-0003, Japan.
E-mail: office@irdrjournal.com