ISSN 2186-3644 Online ISSN 2186-361X

IRDR

Intractable & Rare Diseases Research

Volume 2, Number 3
August, 2013

IRCA BSSA

IACMHR

www.irdrjournal.com

IRDR

Intractable & Rare Diseases Research

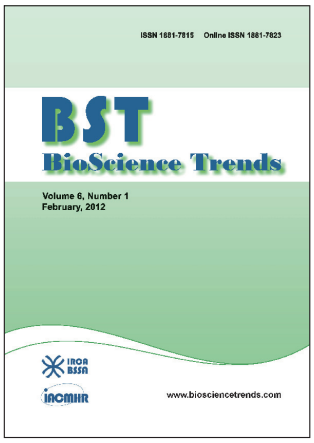
ISSN: 2186-3644
Online ISSN: 2186-361X
CODEN: IRDRA3
Issues/Year: 4
Language: English
Publisher: IACMHR Co., Ltd.

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, Shandong Academy of Medical Sciences, and Shandong Rare Disease Association.

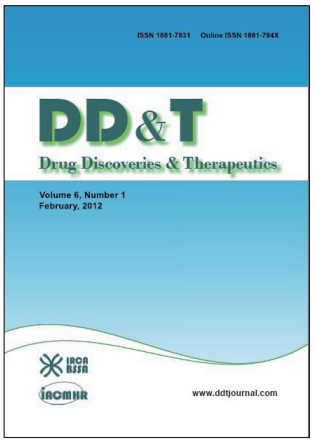
Intractable & Rare Diseases Research devotes to publishing the latest and most significant research in intractable and rare diseases. Articles cover all aspects of intractable and rare diseases research such as molecular biology, genetics, clinical diagnosis, prevention and treatment, epidemiology, health 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, News, and Letters on all aspects of the field of intractable and rare diseases research. All contributions should seek to promote international collaboration.

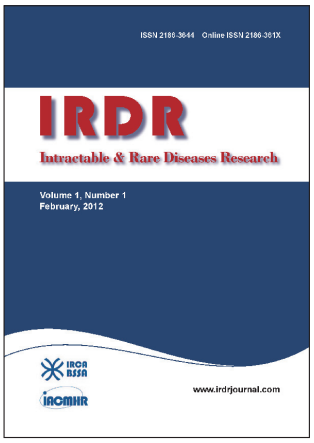
IRCA-BSSA Group Journals



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

Tel: +81-3-5840-9968, Fax: +81-3-5840-9969
E-mail: office@irdrjournal.com
URL: www.irdrjournal.com

Editorial Board

CONTENTS

Volume 2, Number 3, 2013

Policy Forum

Reviews

Brief Report

Commentary

(*This journal was partially supported by a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science.*)

Policy Forum

DOI: 110.5582/irdr.2013.v2.3.72

Newborn screening and related policy against Phenylketonuria in China

Lin Mei[1], Peipei Song[1,2], Lingzhong Xu[1,*]

[1] *Department of Health Care Management and Maternal and Child Health, Shandong University, Ji'nan, Shandong, China;*
[2] *Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.*

Summary **Phenylketonuria (PKU) is a treatable and preventable inherited metabolic disease. The overall incidence of PKU in China is 1/11,144. Newborn screening is an effective method of controlling PKU. In1981, the Chinese Government initiated a newborn screening program and the number of newborns screened for PKU in China has risen each year. This review describes the current status of laws and regulations related to newborn screening for PKU in China and it identifies how China's newborn screening program has improved as a result of these laws and regulations. Specific measures and regulations, such as those implemented by government, follow-up services, and government coverage of expenses, have been implemented in different areas where they have yielded good results. These measures and regulations may serve as a reference for other areas of China. However, measures and regulations regarding newborn screening in China still face challenges. Prenatal health examinations and national financial support are expected to play a more significant role in newborn screening for PKU in the future.**

Keywords: Phenylketonuria (PKU), inherited metabolic disease, incidence, newborn screening, measures and regulations

1. Introduction

Phenylketonuria (PKU) is usually caused by a deficiency of phenylalanine hydroxylase and results in severe mental retardation and neurobehavioral abnormalities. The overall incidence of PKU worldwide varies widely in different human populations. According to the National Institute of Child Health and Human Development, PKU occurs in approximately 1 in 15,000 births in the United States (*1*). However, incidence varies worldwide in different ethnic populations. A high incidence is reported in Turkey (1/2,600), while countries such as Finland and Japan have extremely low rates, with fewer than one case of PKU in 100,000 births (*2,3*). PKU is a treatable and preventable inherited metabolic disease (*4*). Newborn screening combined with a Phe-restricted therapeutic diet throughout childhood can help to control PKU in most patients (*5*). Studies have shown that a higher rate of newborn screening and earlier treatment can lead to a better prognosis for patients (*6-8*).

In China, the overall incidence of PKU is 1/11,144 (*9*). In 1981, the Chinese Government instituted a newborn screening program by testing dried blood samples (DBS); this program is focused primarily on congenital hypothyroidism (CH) and PKU. From1985 to 2006, a total of 13.66 million newborns had been tested for PKU and 1,170 cases had been confirmed, for a PKU rate of 1/11,680 (*10*). Prior to 2011, a total of 35.79 million DBS from newborns had been tested for PKU and 3,082 cases of PKU had been confirmed, for a PKU rate of 1/11,614 (*11*). According to available data (*10,12*), the number of newborns screened for PKU increased markedly after 1999 (Figure 1).

The current review has described laws and regulations related to newborn screening for PKU in China. Based on an analysis of the current status of and challenges faced by newborn screening for PKU, this review seeks to provide insight into the future prospects of newborn screening for PKU in China.

**Address correspondence to:*
Dr. Lingzhong Xu, Department of Health Care Management and Maternal and Child Health, NO.110 mailbox, Shandong University, 44 Wenhuaxi Road, 250012 Ji'nan, China.
E-mail: lzxu@sdu.edu.cn

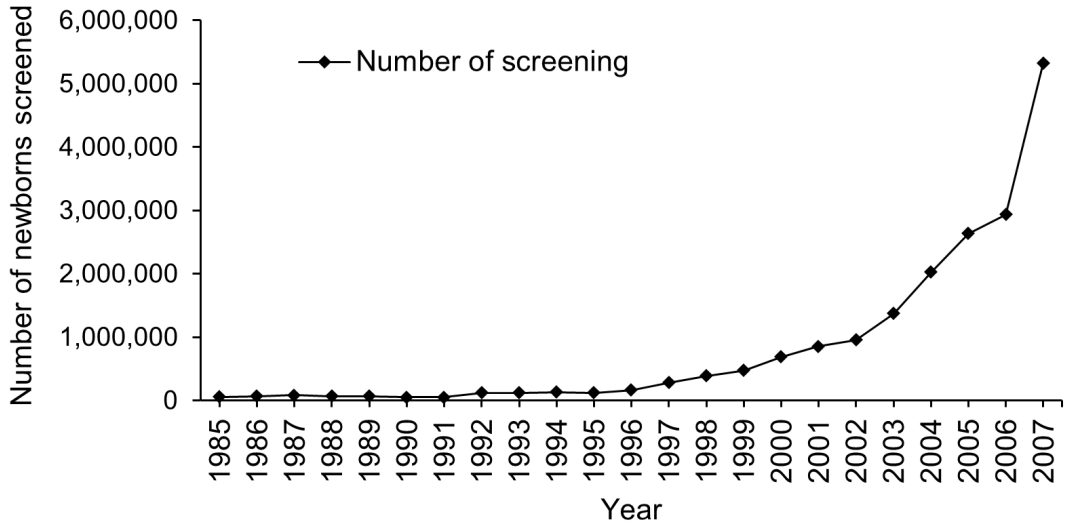


Figure 1. Annual number of newborns screened from 1985 to 2007.

2. Program of newborn screening for PKU in China

2.1. *Laws and regulations related to the program of newborn screening for PKU*

As mentioned earlier, a newborn screening program was instituted by the Chinese Government in 1981. Screening for PKU is a part of that screening program. The very first screening took place in Shanghai in October 1981, with 14 maternity hospitals participating (*13*). Since then, the Chinese Government has drafted a series of laws and regulations to enhance the extent of newborn screening (Table 1). These laws and regulations have identified the importance of newborn screening in law, stipulated that PKU be screened for, included newborn screening into basic maternal and infant health care services, established a protocol and technical specifications for China's newborn screening (in order to improve the quality of PKU screening and testing), and specified the objectives of newborn screening program, including PKU screening (*14-18*). The rate of newborn screening in Eastern China should reach 90% by 2012 and 95% by 2015, that in middle China should reach 50% by 2012 and 80% by 2015, and that in Western China should reach 40% by 2012 and 60% by 2015.

The program of newborn screening for PKU had made significant process with the help of those laws and regulations. There were only 3 screening centers in the 1980s, when newborn screening had just started, and yet the number increased to 46 in 2002 and then to 179 by the end of 2009 (*11*). The percentage of newborns covered by the newborn screening program also increased from 3.86% in 2003 to 59.01% in 2009 (*11*). In addition to these laws and regulations, specific measures and regulations regarding PKU that have been implemented in cities and provinces of China warrant attention.

2.2. *Implementation by government*

Local government directives stipulating that newborn screening for PKU must be instituted helped to expand the coverage of newborn screening. Shanghai is one of first cities in China that instituted newborn screening for PKU; the percentage of newborns screened in Shanghai reached 97% in 2008 (*19*). In Guangzhou, the rate of newborn screening reached 99.0% and coverage by the city's Newborn Screening Network reached 99.3% in 2008 (*20*). One commonality of the measures and regulations regarding newborn screening in these two cities is that screening "must be instituted".

In 1996, the Standing Committee of the Shanghai People's Congress drafted Regulations on Maternal and Infant Health Care in Shanghai (*21*). According to article 35 of the Regulations, the Shanghai Government directed that a newborn screening program be instituted in the city. In Guangzhou, the Standing Committee of the Guangdong People's Congress also drafted Regulations on Management of Maternal and Infant Health Care in 1998 (*22*). Article 20 of Guangdong's Regulations stipulates that Guangdong Province must institute a newborn screening program and the Regulations also propose that screening include PKU testing. These two sets of regulations ensure that these areas are legally obligated to institute newborn screening including PKU testing. To some extent, implementation by government did enhance the rate of newborn screening for PKU in these areas.

2.3. *Follow-up services*

Follow-up services also play an important role during

Table 1. Features of laws and regulations related to newborn screening for PKU in China

Year	Law or regulation	Enacted by	Features	*Ref.*
National laws or regulations				
1994	Law on Maternal and Infant Health Care	The Central People's Government of the People's Republic of China	Identified the importance of newborn screening for the first time in law.	(*14*)
2001	Measures Implementing the Law on Maternal and Infant Health Care in China	The Central People's Government of the People's Republic of China	Included newborn screening in maternal and infant health care services.	(*15*)
2009	Measures for the Management of Newborn Screening	National Health and Family Planning Commission of the People's Republic of China	Specified that PKU be screened for and provided a protocol for China's newborn screening program.	(*16*)
2009	Plans for a Newborn Screening Program	National Health and Family Planning Commission of the People's Republic of China	Specified the objectives of the newborn screening program.	(*17*)
2010	Technical Specifications for Newborn Screening	National Health and Family Planning Commission of the People's Republic of China	Seeks to improve the quality of PKU screening and test.	(*18*)
Local laws or regulations				
1996	Regulations on Maternal and Infant Health Care in Shanghai	Standing Committee of the Shanghai People's Congress	Stipulatedthat the newborn screening program must be implemented in Shanghai.	(*21*)
1998	Regulations on Management of Maternal and Infant Health Care	Standing Committee of the Guangdong People's Congress	Stipulated that the newborn screening program must be implemented in Guangdong and proposed that screening include PKU testing.	(*22*)
2004	Working Proposal for Management of Maternal and Infant Health Care in Shanghai Major Projects and Components of Maternal and Infant Health Care in Shanghai	Health Bureauof the City of Shanghai	Indicated the time limit for PKU screening.	(*23*)
2006	Measures for the Management of Newborn Screening in Guangzhou	Health Department of Guangdong Province	Stipulated that children confirmed to have PKU can receive treatment for free until 8 years of age.	(*26*)
2007	Proposed Implementation of Newborn Screening for Genetic and Metabolic Diseases	Health Bureauof the City of Shanghai	Specified follow-up services for patients with PKU.	(*24*)

the process of newborn screening for PKU. The Health Bureau of the City of Shanghai drafted a Working Proposal for Management of Maternal and Infant Health Care in Shanghai and Major Projects and Components of Maternal and Infant Health Care in Shanghai in 2004 (*23*). In 2007, the Health Bureau of the City of Shanghai drafted measures for Proposed Implementation of Newborn Screening for Genetic and Metabolic Diseases (*24*). These directives specified aspects of newborn screening for PKU and follow-up services: *i*) 1-2 visits must be made within 28 days of birth; *ii*) DBS for PKU testing must be collected within 72 hours of birth and the DBS must be sent to a designated laboratory within 24 hours; *iii*) PKU testing must be performed by the laboratory within 2 days of receiving the DBS and screening results must be submitted within 5 days; *iv*) parents must be informed of potentially positive results within 1 day, the newborn must be brought to the laboratory for further testing, and final results must be submitted within 7-10 days. By offering these follow-up services, Shanghai has more effectively screened newborns for PKU, and follow-up services have contributed somewhat to the percentage of newborns covered by the screening program.

2.4. *Government coverage of expenses*

Economic factors play an important role in newborn screening for PKU. HongKong has a screening rate higher than 95%, and the government covers expenses for newborn screening (*25*). The Health Bureau of the City of Guangzhou drafted its own regulations on newborn screening directing that children confirmed to have PKU can receive the treatment from the Newborn Screening Center of the City of Guangzhou for free until 8 years of age (*26*). According to a report by the Health Bureau of the City of Guangzhou, 15 patients with PKU had received free treatment prior to 2005, for a treatment rate of 100% (*27*). To some extent, government coverage of expenses can actually encourage parents to have their newborn screened. Experience in HongKong and Guangzhou may serve as a reference for other areas of China.

3. Challenges faced by and prospects for measures and regulations regarding newborn screening in China

The number and percentage of newborns screened

for PKU in China has increased significantly thanks to national laws and regulations and specific local measures and regulations. These specific measures and regulations, such as those implemented by government, follow-up services, and government coverage of expenses, may serve as a reference and can be implemented in other areas. However, there still are some challenges faced by existing newborn screening, and more effective measures and regulations need to be implemented in China.

Prenatal health examinations Prenatal health examinations are expected to play an important role in the prevention of PKU. Effective prevention of birth defects can be classified into three levels: primary prevention (avoiding causes of congenital abnormalities), secondary prevention (early detection of congenital abnormalities), and tertiary prevention (surgical intervention to repair congenital abnormalities) (*28*). The most important stage, primary prevention, has not openly contributed to the fight against PKU. Fees for prenatal health examinations are now completely covered by the state (*29*), but there are no reports of PKU being detected during prenatal health examinations.

National financial support National financial support is still relatively weak. Fees for PKU screening and treatment can significantly influence whether parents have their newborn screened, and yet only 40% provinces of China cover some of the fees for newborn screening for PKU. Funds to cover fees come from the new rural Cooperative Medical System (CMS) (such as in Guangdong, Guizhou, and Sichuan) or government expenditures (such as in Shanghai and Jiangsu) (*30*). In addition, the existing policy of free treatment should also be improved. The revenue from newborn screening in Guangzhou has been used to treat patients with PKU for free, and yet the cost of free treatment is only 5.5% of actual revenue (*27*). The duration of subsidized treatment could be lengthened from 8 years of age to a longer period.

In conclusion, the number and percentage of newborns screened for PKU in China increased significantly after 1981. Experience with implementation by government, follow-up services, and government coverage of expenses may benefit other areas in China. However, prenatal health examinations and financial support are expected to play a more significant role in newborn screening for PKU in the future.

References

1. Targum SD, Lang W. Neurobehavioral problems associated with phenylketonuria. Psychiatry (Edgmont). 2010; 7:29-32.
2. Donati A, Vincenzi C, Tosti A. Acute hair loss in phenylketonuria. J Eur Acad Dermatol Venereol. 2009; 23:613-615.
3. Williams RA, Mamotte CD, Burnett JR. Phenylketonuria: An inborn error of phenylalanine metabolism. Clin Biochem Rev. 2008; 29:31-41.
4. Bickel H, Gerrard J, Hickmans EM. Influence of phenylalanine intake on phenylketonuria. Lancet. 1953; 265:812-813.
5. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. Lancet. 2010; 376:1417-1427.
6. WHO. Prevention of hearing impairment. *http://www.who.int/entity/pbd/deafness/en/english.pdf* (accessed August 7, 2013).
7. Olusanya BO. Highlights of the new WHO report on newborn and infant hearing screening and implications for developing countries. Int J Pediatr Otorhinolaryngol. 2011; 75:745-748.
8. Huang LH, Zhang L, Tobe RY, *et al*. Cost-effectiveness analysis of neonatal hearing screening program in China: Should universal screening be prioritized? BMC Health Serv Res. 2012; 12:97.
9. Zhou YA, Ma YX, Zhang QB, Gao WH, Liu JP, Yang JP, Zhang GX, Zhang XG, Yu L. Mutations of the phenylalanine hydroxylase gene in patients with phenylketonuria in Shanxi, China. Genet Mol Biol. 2012; 35:709-713.
10. Xu YH, Qin YF, Zhao ZY. Retrospective study on neonatal screening for congenital hypothyroidism and phenylketonuria in China in the past 22 years. Zhonghua Er Ke Za Zhi. 2009; 47:18-22. (in Chinese)
11. Shi XT, Cai J, Wang YY, Tu WJ, Wang WP, Gong LM, Wang DW, Ye YT, Fang SG, Jing PW. Newborn screening for inborn errors of metabolism in mainland China: 30 years of experience. JIMD Rep. 2012; 6:79-83.
12. Gu X, Wang Z, Ye J, Han L, Qiu W. Newborn screening in China: Phenylketonuria, congenital hypothyroidism and expanded screening. Ann Acad Med Singapore. 2008; 37:107-104.
13. Chen RG, Chen HY, Shi SZ, *et al*. Preliminary report on neonatal screening for hypothyroidism, phenylketonuria and galactosemia. Shanghai Medical Journal. 1983; 6:344-347. (in Chinese)
14. The Central People's Government of the People's Republic of China. Law on Maternal and Infant Health Care in China. *http://www.gov.cn/banshi/2005-08/01/content_18943.htm* (accessed August 7, 2013) (in Chinese)
15. The Central People's Government of the People's Republic of China. Measures implementing the Law on Maternal and Infant Health Care in China. *http://www.gov.cn/banshi/2005-08/01/content_19126.htm* (accessed August 7, 2013) (in Chinese)
16. NHFPC. Measures for the Management of Newborn Screening. *http://www.moh.gov.cn/fzs/s3576/200903/c53a0c97e68740c286ceb96e9ac56280.shtml* (accessed August 8, 2013) (in Chinese)
17. NHFPC. Plans for a Newborn Screening Program. *http://www.moh.gov.cn/cmsresources/mohfybjysqwss/cmsrsdocument/doc6649.doc* (accessed August 8, 2013) (in Chinese)
18. NHFPC. Technical Specifications for Newborn Screening. *http://www.moh.gov.cn/cmsresources/mohfybjysqwss/cmsrsdocument/doc10798.doc* (accessed August 8, 2013) (in Chinese)
19. Health Bureau of the City of Shanghai. 2008 Joint Committee on Public Health Efforts in Shanghai. *http://www.smhb.gov.cn/website/b/37912.shtml* (accessed August 9, 2013) (in Chinese)
20. Newborn Screening Center of the City of Guangzhou. Overview of Newborn Screening in Guangdong

Province. *http://www.gdnsn.com/qiansengaikuan/qsgk.htm* (accessed August 9, 2013) (in Chinese)
21. The Standing Committee of the Shanghai People's Congress. Regulations on Maternal and Infant Health Care in Shanghai. *http://www.shanghai.gov.cn/shanghai/node2314/node3124/node3125/node3127/userobject6ai250.html* (accessed August 10, 2013) (in Chinese)
22. The Standing Committee of the Guangdong People's Congress. Regulations on Management of Maternal and Infant Health Care in Guangdong Province. *http://www.gdnsn.com/falifagui/gdsmybjtl.htm* (accessed August 10, 2013) (in Chinese)
23. Health Bureau of the City of Shanghai. Working Proposal for Management of Maternal and Infant Health Care in Shanghai and Major projects and Components of Maternal and Infant Health Care in Shanghai. *http://www.smhb.gov.cn/website/b/31847.shtml* (accessed August 11, 2013) (in Chinese)
24. Health Bureau of the City of Shanghai. Proposed Implementation of Newborn Screening for Genetic and Metabolic Diseases. *http://www.smhb.gov.cn/upload/UserFile/Wonders/xxgk/gkml/zcfg/gfwj/fybj/userobject7ai1372/00000000.doc* (accessed August 11, 2013) (in Chinese)
25. Padilla CD, Therrell BL. Newborn screening in the Asia Pacific region. J Inherit Metab Dis. 2007; 30:490-506.
26. Health Department of Guangzhou Province. Measures for the Management of Newborn Screening in Guangzhou. *http://www.gdnsn.com/falifagui/gzscbf.htm* (accessed August 11, 2013) (in Chinese)
27. Guangdong Newborn Screening Network. Summary of Experiences with Newborn Screening for and Free Treatment of PKU in Guangzhou. *http://www.gdnsn.com/qiansengaikuan/pkuzl.htm* (accessed August 12, 2013) (in Chinese)
28. Czeizel AE. Birth defects are preventable. Int J Med Sci. 2005; 2:91-92.
29. NHFPC. Announcement that the Ministry of Finance is Encouraging State Coverage of All Fees for Prenatal Health Examinations. *http://www.moh.gov.cn/fys/s3589/201307/0ca30a6cbe7146fba86b0bf9211be576.shtml* (accessed August 12, 2013) (in Chinese)
30. Liu Y, Gao L, Yu EL, Zhu J. Investigation and analysis on screening expenses of neonatal diseases in China. Maternal and Child Health Care of China. 2011; 26:325-328. (in Chinese)

(Received July 19, 2013; Reviesed August 20, 2013; Accepted August 23, 2013)

Review

DOI: 110.5582/irdr.2013.v2.3.77

Acute liver failure and liver transplantation

Nobuhisa Akamatsu[1], Yasuhiko Sugawara[2,*], Norihiro Kokudo[2]

[1] *Department of Hepato-biliary-pancreatic Surgery, Saitama Medical Center, Saitama Medical University, Kawagoe, Saitama, Japan;*
[2] *Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.*

Summary **Acute liver failure (ALF) is defined by the presence of coagulopathy (International Normalized Ratio ≥ 1.5) and hepatic encephalopathy due to severe liver damage in patients without pre-existing liver disease. Although the mortality due to ALF without liver transplantation is over 80%, the survival rates of patients have considerably improved with the advent of liver transplantation, up to 60% to 90% in the last two decades. Recent large studies in Western countries reported 1, 5, and 10-year patient survival rates after liver transplantation for ALF of approximately 80%, 70%, and 65%, respectively. Living donor liver transplantation (LDLT), which has mainly evolved in Asian countries where organ availability from deceased donors is extremely scarce, has also improved the survival rate of ALF patients in these regions. According to recent reports, the overall survival rate of adult ALF patients who underwent LDLT ranges from 60% to 90%. Although there is still controversy regarding the graft type, optimal graft volume, and ethical issues, LDLT has become an established treatment option for ALF in areas where the use of deceased donor organs is severely restricted.**

Keywords: Acute liver failure, treatment, liver transplantation, living donor liver transplantation (LDLT), optimal graft volume

1. Introduction

Acute liver failure (ALF), sometimes called "fulminant hepatic failure" or "acute hepatic necrosis", is defined by the presence of coagulopathy (International Normalized Ratio ≥ 1.5) and hepatic encephalopathy due to severe liver damage in patients without pre-existing liver disease (*1-4*). The clinical symptoms are complicated, and include cerebral edema, coagulopathy, renal failure, metabolic disturbance, hemodynamic instability, and susceptibility to infection. Until recently, ALF has had a high mortality rate (widely reported to be > 80%) (*5*), but with the improved recognition of this entity, more aggressive medical therapy, intensive care, and especially the advent of orthotopic liver transplantation (OLT) as a radical treatment option, the survival rate has considerably improved (*2,6-10*).

Here we review ALF with a focus on OLT for adult ALF patients, to describe the importance of OLT in the treatment of this critical disease entity. Additionally, living donor liver transplantation (LDLT) for ALF is separately reviewed.

2. Overview of acute liver failure

2.1. *Definitions*

In 1970, ALF was classically defined as fulminant hepatic failure in patients with no prior liver disease in which rapidly deteriorating hepatocellular function ensued with associated encephalopathy within 8 weeks of the initial presentation (*11*). The syndrome was redefined by O'Gradey *et al*. in 1993, who used the term ALF to describe a clinical syndrome in which encephalopathy occurs between 8 and 28 days after the onset of jaundice (*12*). They suggested further sub-classification comprising three distinct syndromes depending on the jaundice-to-encephalopathy time interval, thus categorizing liver failure as hyperacute

**Address correspondence to:*
Dr. Yasuhiko Sugawara, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: yasusuga-tky@umin.ac.jp

(onset within 1 week), acute (between 8 days and 4 weeks), and subacute (between 29 days and 12 weeks). This sub-classification reflects not only the cause of the disease and probable complications, but also the differences in the survival rate for these groups, with the hyperacute group paradoxically having the best prognosis (*13*).

2.2. *Epidemiology and etiology*

ALF is rare and represents a syndrome, rather than a specific disease, with multiple causes that vary in course and outcome, making its precise incidence difficult to establish. Reports from developed countries suggest an overall incidence of between 1 and 6 per million people per year (*7,14,15*). A recent nationwide survey in Japan revealed an incidence of 0.9 per million people per year (*16*). It was once estimated to account for 0.1% of all deaths and 6% of all liver-related deaths in the United States (*17*).

The etiology of ALF varies widely depending on geographic location, patient profile, and year of the report. Whereas in a historical series from the 1980s, viral hepatitis was the most common etiology of ALF in the United States (*18*), the results of a recent multicenter study of ALF identified acetaminophen overdose as the most frequent cause in the United States (46% of cases) (*3*) as well as in European countries (*7*). On the other hand, in Africa and Asia, viral hepatitis remains the leading cause of ALF, and cases resulting from hepatitis E as well as hepatitis A and B are common (*16,19,20*). Table 1 shows the various etiologies in the United States, Europe, Japan, and other countries (*3,7,16,20-24*).

Identification of the cause of ALF is critically important because the etiology influences the prognosis and management (*25*), with outcome being much better for patients with ALF associated with acetaminophen, pregnancy, or hepatitis A (> 50-90%) than for those with seronegative hepatitis, idiosyncratic drug reactions, or Wilson's disease (< 10-20%) (*9*). On the other hand, the etiology of ALF remains indeterminate or unknown in approximately 14%, 33%, and 30% of cases in the United States, Europe, and Japan, respectively.

2.3. *Clinical features and their management*

Acute liver failure frequently causes multiple organ failure, while the initial presenting symptoms are usually non-specific, and include fatigue, malaise, anorexia, nausea, abdominal pain, fever, and jaundice (*26*). These symptoms finally lead to the development of encephalopathy and/or coma, although the rates of progression vary case by case, which necessitates urgent decision making regarding the use of the only effective treatment for this disease entity; emergent liver transplantation (*27,28*).

2.3.1. *Encephalopathy and cerebral edema*

The most lethal complication associated with ALF is the development of encephalopathy and cerebral edema, the mechanism and the actual percentage of which are still poorly understood (*29*). Recent clinical and experimental studies suggest an important role for the increased concentrations of circulating neurotoxins, especially, ammonia (*30*), with reports of a relation between the development of high grades of encephalopathy and arterial ammonia concentration (*31,32*). Cerebral edema occurs in nearly 80% of patients who progress to Grade 4 hepatic encephalopathy, leading to intracranial hypertension with subsequent irreversible ischemic brain damage or brainstem herniation, and accounting for up to 50% of ALF mortality (*33,34*). Intracranial pressure (ICP) should be monitored directly and more aggressively managed in patients who are deemed candidates for liver transplantation (*35,36*). Cerebral perfusion pressure, defined as systemic blood pressure minus ICP, must be maintained above 40 mmHg. Classical treatments to reduce ICP comprise sedation (*37*), hypertonic saline (*38*), and mannitol (*39*). In patients with increased ICP resistant to standard medical therapy, mild to moderate hypothermia (32-33°C) is also effective as a bridge to liver transplantation (*40*).

Table 1. Etiologies of acute liver failure worldwide

Contries and regions	Drug-induced (%)		Viral (%)			Unknown (%)	Other (%)
	Acetaminophen	Non-acetaminophen	HAV	HBV	HEV		
USA 1998-2007 (*3*)	46	11	3	7		14	19
Europe 2004-2009 (*7*)	14	12	19			33	22
UK 1999-2008 (*21*)	57	11	2	5	1	17	7
Germany 1996-2005 (*22*)	15	14	4	18		21	28
Japan 2004-2009 (*16*)	15		3	40		30	12
Korea 2000-2009 (*23*)	29		15	29		10	17
India 1989-1996 (*24*)	0	1	2	15	44	31	7
Sudan 2003-2004 (*20*)	0	8	0	22	5	38	27

Abbreviations: HAV, hepatitis A virus; HBV, hepatitis B virus; HEV, hepatitis E virus.

High-flow hemodiafiltration, using a high-performance polysulfone membrane coupled with plasma exchange with 40 to 50 units of fresh frozen plasma (FFP) per session is also useful for improving coma status or protecting against irreversible brain damage in patients scheduled for liver transplantation (*41*).

2.3.2. *Coagulopathy*

Because the liver plays a central role in the synthesis of almost all of the coagulation factors, severe coagulopathy is always encountered in ALF patients. Coagulopathy occurs not only due to the decreased synthesis of clotting factors, but also to an increase in peripheral consumption and at least some degree of disseminated intravascular coagulation and thrombocytopenia (*42*), which sometimes results in spontaneous hemorrhage, *i.e.*, from the gastrointestinal tract or cerebrovascular system (*43,44*). In contrast, there are abnormalities in both the coagulation and fibrinolytic pathways and recent data suggest the defects are balanced, that is, there is a relative preservation of hemostasis unless the platelet count is extremely low (*45,46*). Theoretically, prophylactic treatment with FFP in the absence of bleeding is unadvised for two reasons; first, the decrease in prothrombin time after FFP administration decreases the accuracy with which prognosis can be judged and second, FFP administration results in a volume load that might deteriorate renal function and increase ICP (*25,47*). In clinical practice, however, FFP infusion and platelet transfusion are sometimes inevitable, especially before invasive procedures, and correction of coagulopathy should be determined on a case by case basis (*28*).

2.3.3. *Renal failure and metabolic abnormalities*

Many factors encountered in ALF patients, such as the direct toxic effect of an ingested agent, volume depletion, and systemic hypoperfusion, co-existing sepsis, and hepatorenal syndrome can cause acute renal failure (*48*). Acute renal failure complicates ALF in 40% to 50% of cases and is significantly associated with a poor prognosis (*49-51*). When dialytic support is required, continuous hemodialysis is preferable to intermittent support to maintain the hemodynamic stability and decrease the cerebral perfusion pressure (*52*). Hypoglycemia due to decreased glycogenolysis and gluconeogenesis, and metabolic acidosis are often encountered as a result of massive liver necrosis (*53*).

2.3.4. *Cardiopulmonary and hemodynamic complications*

Cardiopulmonary complications and hemodynamic instability are conspicuous clinical sequelae of ALF, characterized by vasodilation, low systemic vascular resistance, and hypotension, with a compensatory increase in cardiac output (*48*). This hemodynamic status results in lower cerebral perfusion pressure, worsened encephalopathy, and pulmonary edema, which often preclude liver transplantation (*27*). Treatment goals are to perform volume resuscitation, provide supportive care with vasopressors, and increase oxygenation by mechanical ventilation (*25,28*).

2.3.5. *Infection*

The milieu of inflammation and necrosis of the liver predispose ALF patients to the development of infection, which means that patients with ALF are functionally immunosuppressed. Bacterial or fungal infection, particularly of the respiratory and urinary tracts, is common (occurring in up to 80% of patients), which worsens encephalopathy (*54*), and is the cause of death in 10% to 37% of cases (*55-58*). Bacteremia is observed in 20% to 80% of ALF patients (*2*). Additionally, patients with ALF are at substantial risk for sepsis caused by reticuloendothelial dysfunction and decreased opsonization (*59*). The mortality rate of patients with sepsis and septic shock is 59% and 98%, respectively (*60*). The empiric use of antibiotics, however, is controversial. Prophylactic antibiotics decrease the number of infections, but they do not alter the overall outcome, and may result in frequent fungal infections (*56*). Fungal infection, particularly *Candida albicans*, develops in up to one-third of ALF patients, and is a poor prognostic sign associated with high mortality, thereby precluding liver transplantation (*55,58*). Serial blood cultures for bacteria and fungi should be performed, and the administration of anti-infectives (both antibacterial and antifungal) is recommended for patients with significant isolates on surveillance cultures, refractory hypotension, or clinical evidence of systemic inflammatory response syndrome (*28*).

2.4. *Prognostic scores and indication for liver transplantation*

The most commonly used criteria to absolutely exclude ALF patients from liver transplantation, although these vary by transplant center, include age older than 70 years; certain malignancies outside of the liver; severe cardiac, lung, or multiple organ failure; severe infection; uncontrolled septic shock; and brain death (*61*).

The essentials for selecting patients for liver transplantation are the accurate identification of those in need, as well as those who will benefit from liver transplantation. Generally, there are two approaches to listing patients with ALF for liver transplantation. The first is to use some set of indicators of a poor prognosis without liver transplantation, and the second is to list all patients with encephalopathy and make the decision at the time a donor organ becomes available (*9*).

It is critical to promptly and accurately identify those patients most likely to benefit from emergent liver

Table 2. Prognostic scoring systems for acute liver failure

Variables	King's College Criteria non-Acetaminophen	King's College Criteria Acetaminophen	Clichy	MELD	APACHE II	Japanese guideline
Age	×		×		×	×
Etiology	×					
Encephalopathy		×	×		×	×
Arterial pH		×			×	
Onset of encephalopathy	×					×
Factor V			×			
PT-INR	×	×		×		×
Serum creatinine		×		×	×	
Serum bilirubin	×			×		×
Direct/indirect bilirubin ratio						×
Serum sodium and potassium					×	
White blood cell count					×	
Hematocrit					×	
Serum alanine aminotransferase						
Serum cholinesterase						
Vital signs (BT, BP, HR, RR)					×	
Oxygenation					×	

Abbreviations: APACHE, acute physiology and chronic health evaluation; BT, body temperature; BP, blood pressure; HR, heart rate; MELD, model for end-stage liver disease; PT-INR, prothrombin time-international normalized ratio; RR, respiratory ratio.

transplantation, as overall only 15% to 20% of ALF patients survive spontaneously (*5,62,63*). The risks of emergent transplantation in the context of deteriorating multiple organ failure must be balanced against the possibility of survival with continued medical supportive care alone. In addition, early recognition of irreversible ALF is important so that transplantation can be performed before the complications of ALF contraindicate transplantation. Because of the current organ scarcity, short-term risks of surgery, and requirement of lifelong immunosuppression, it is equally important to recognize reversible ALF so as not to perform unnecessary transplantations (*64*).

Many prognostic scoring systems have been reported worldwide (*50,65-70*). The major prognostic models listed in Table 2 are extracted from our previous report (*71*). Based on separate cohorts examining the accuracy of these models, the King's College Criteria are superior and the use of this prognostic model is prevalent worldwide (*62,72*). Two meta-analysis have been performed on the King's College Criteria. One included 18 studies with 1105 patients and yielded an overall specificity of 93% and sensitivity of 88% (*73*). The other meta-analysis included 14 studies with 1960 patients and yielded a specificity of 95% and a sensitivity of 58% (*74*). Three studies evaluated MELD score, which has been introduced and widely validated for the prediction of prognosis of chronic liver disease, in comparison with the King's College Criteria (*66,75,76*). One of the studies found that the MELD score was superior to the King's College Criteria (*66*). A recently described modification of the MELD score replaced bilirubin with cytokeratin 18 (a marker of cell injury), which yielded peak sensitivity and specificity of 81% and 82%, respectively, in 68 consecutive patients with ALF due to various etiologies (*77*). None of the currently available prognostic scoring systems, however, consistently demonstrates reliable accuracy in predicting the outcome of ALF and the subsequent need for liver transplantation. Therefore, the American Association for the Study of Liver Diseases recommends that none of these systems be relied on independently, and that all ALF patients with encephalopathy be considered for transplantation (*25*).

Among the factors listed in Table 2, the most important factors for predicting survival from ALF are the degree of encephalopathy, patient age, and the cause of ALF (*1*). Other factors reported to be associated with the prognosis of ALF include severity of systemic inflammatory response syndrome (*54,60,78*), serum alpha fetoprotein levels (*79*), serum phosphate levels (*80,81*), findings from computed tomography scans of the liver (*82-84*), 99mTc-GSA (*85*), cytokine levels (*86*), and serum lactate levels (*87-89*).

3. Liver transplantation for acute liver failure

Liver transplantation, the only proven therapy for ALF, has revolutionized the management of ALF. ALF accounted for 8% of indications for liver transplantation in Europe during 1988-2009 (*7*), and 7% in the US during 1999-2008 (90). With the advent of OLT as a treatment option, survival rates of ALF patients have improved considerably, up to 60% to 92% in large centers over the last two decades (*3,7,8,63*), which is remarkable given that the rate was below 20% before the OLT era (*5*). Although the survival rate of OLT for ALF patients was previously lower than that for other diseases (*91,92*), a recent report demonstrated comparable results of OLT for ALF and chronic liver diseases (*3*). Even in Western countries where OLT

with deceased donors is aggressively performed for ALF patients, death without transplantation occurs in 20% to 30% of cases (*3,93*). According to a multicenter study, even when patients with ALF are managed in large-volume centers, the OLT performance rate ranges between 41% to 72%, and the median wait time for a graft is 5 days (*94*). This is due to the rapid progression of ALF and persistent organ shortages.

Current results of OLT for ALF are satisfactory considering the natural history of this disease entity, the multi-organ involvement, the emergent context, and the lack of other effective treatments. To date the 1-year and 5-year patient survival rates documented in several studies range between 74% and 81%, and 61% and 76%, respectively (*7,63,93,95-98*), and two studies reported 10-year patient survival rates of 69% and 63% (*7,97*).

Multiple factors influence the outcome after liver transplantation. In some centers, the etiology of the underlying disease correlates with outcome. The best results were achieved for Wilson's disease and the worst for idiosyncratic drug reactions (*15,93*). Recipient age has a considerable impact on the outcome of liver transplantation for ALF, with postoperative mortality more than doubled in those over 50 years of age (*93,95*). Studies over United Network for Organ Sharing (UNOS) database (*98*) and European Liver Transplant Registry (ELTR) database (*7*) found that age over 50 is an independent risk factor for impaired outcome. The third main factor that determines outcome is the quality of the graft used. Impaired outcomes occur in recipients receiving size-reduced (*7,93,99*), ABO-incompatible (*7,93,99*), or steatotic grafts (*63,93*). Impaired graft function is poorly tolerated in acutely sick recipients and might predispose to the development of sepsis – the main cause of postoperative death. In earlier reports of emergent liver transplantation for ALF, graft quality was sacrificed to avoid waiting list mortality, which resulted in the primary non-function rates as high as 13% (*96*). Higher risk grafts, such as severely steatotic or partial grafts, result in worse survival in ALF. The use of ABO-incompatible grafts is most frequently reported for liver transplantation for ALF, which ranges from 13% to 29% and is associated with worse outcomes, including impaired patient/graft survival and complications (*63,96,100-103*).

Four risk factors were identified following an analysis of 1457 recipients who underwent liver transplantation for ALF in the US, a BMI > 30, serum creatinine > 2 mg/dL, recipient age over 50 years, and a history of life-support (*98*). A recent large study from ELTR among 4903 recipients who underwent liver transplantation for ALF found that recipient age > 50 years, ABO-incompatible transplant, acetaminophen-related ALF, ALF due to other known causes, and reduced-size graft were independent risk factors for patient/graft survival (*7*).

4. Living donor liver transplantation for acute liver failure patients

Organ scarcity is serious in Asia, where the deceased donor liver transplantation (DDLT) rate remains below 5 per million population (*104*). According to a recent report in Japan (*16*), only 24% of ALF patients underwent liver transplantation, and death without transplantation occurred around 40%, and more than 98% of liver transplants are from living donors, although organ procurement from brain-dead donors was legalized in 1997. Recently, the Intractable Liver Diseases Study Group of Japan reported that the overall 1-year, 5-year, and 10-year survival rates of ALF patients after LDLT were 79%, 74%, and 73%, respectively, in Japan (*105*).

LDLT for ALF was initially performed only in children, but since the first report of successful LDLT for adult ALF patients by Kato and colleagues (*106*), LDLT has become widely accepted as the alternative treatment of choice for adult ALF patients, mainly in Asian countries, but also in Western countries (*8*). Controversy remains regarding LDLT itself in regions where organs from deceased donors are fairly available, especially under urgent situations, but LDLT seems to have become an established treatment for ALF patients in Asia.

Although it has been suggested that the results of emergent LDLT are inferior to those of elective transplantation (*107*), there are as yet no conclusive data indicating that emergent LDLT is an ineffective or inferior treatment compared to emergent DDLT or elective liver transplants. Compared with cadaveric liver grafts, live donor grafts offer distinct advantages in that patients can receive a graft relatively early and graft function is excellent because the cold ischemia time is short if an adequate graft size is obtained (*108*). Large-volume centers report that outcomes of LDLT and DDLT are comparable for ALF patients (*23,109-116*). The timely availability of a liver graft in LDLT should be emphasized, because in the LDLT series for ALF reviewed here there has never been a neurologic death secondary to severe hepatic encephalopathy, except for two cases of the Seoul group (*110*), in contrast to the relatively high incidence of recipient death due to encephalopathy in the DDLT setting (*117*). Yet, beyond these results, many ALF patients without an appropriate donor have died, and, in the future, an increased prevalence of DDLT donors is expected in Asian countries (*16,105*). Considering the burden on live donors, LDLT will never replace DDLT where cadaveric organs are fairly available, but the addition of LDLT to a DDLT program may further improve the management of ALF, even in Western countries (*108*).

4.1. *Donor and graft selection*

LDLT should be considered only in situations in which

the risk to the donor is justified by the expectation of an acceptable outcome in the recipient. The potential live organ donor should be an adult who is mentally competent, willing to donate, free from coercion, medically and psychosocially suitable, and fully informed of the risks, benefits, and alternative treatments available to the recipient (*118*). Apart from the medical workup of the donor and volumetric/ anatomic evaluation of the graft, ethical matters should be especially emphasized in urgent LDLT situations, such as for ALF, because donors do not have adequate time for contemplation and reflection, and most donors are likely to be close relatives who are influenced by the imminent death of the patient (*119*). Ethical issues include ensuring donor safety, avoiding donor coercion, and determining whether adequate informed consent can be obtained in this highly charged emergent setting. Donor safety, of course, is the primary measure of success. The ethical justification for LDLT cannot be based on the absence of donor complications and deaths because that is an unattainable goal. Therefore, its justification is dependent on continuous demonstration of overwhelming recipient benefit and reasonable donor safety (*108,120*).

Most countries consider 18 or 20 years to be the minimum age for independent decision-making regarding organ donation, but the upper age limit of the live donor differs by center, from 55 to 65 years (*107,110-112*). Similarly, the politics regarding the requisite relationship between the donor and recipient vary widely around the world according to different political and cultural standards. The relation to the recipient is usually limited to within the second or third degree of consanguinity (*121*); some countries, however, require no familial relation between the donor and recipient (*122*).

Selection of the graft type and appropriate graft size are critical problems in LDLT for adult ALF patients, in terms of both recipients and donors. It has been postulated that, because ALF is not associated with pre-existing portal hypertension, patients with ALF may tolerate smaller grafts than patients with chronic liver disease. In favor of the recipients outcome, to minimize the problem of a small-for-size graft, many centers have adopted the use of right liver grafts (*123*), although complications are much more common in those donating a right liver than a left liver (*124,125*). Generally, the lower limit of a live donor graft is set at 35% to 40% of the recipient standard liver volume (SLV) or at 0.8% to 1.0% of a graft-to-recipient weight ratio (GRWR).

The estimated risk of mortality and morbidity currently associated with live donor hepatectomy is 0.4% and 35%, respectively (*126*). Although surgeons should always try to decrease both morbidity and mortality, the present level of donor risk is considered acceptable in public opinion (*127*).

4.2. *Reports from high-volume LDLT centers*

Recently, Yamashiki *et al.* (*105*) performed a Japanese nationwide survey on 209 recipients who underwent LDLT for ALF. LDLT was performed a median of 4 days after the onset of encephalopathy. The right liver was utilized in 105 cases (50%), while the left liver was used in 99 cases (47%). The 1-year, 5-year, and 10-year survival rates were 79%, 74%, and 73%, respectively. Prognostic factors associated with patient survival were older recipient, older donor, and ABO-incompatible transplant.

The Kyoto group (*107*) reported 34 LDLT for ALF patients, including 15 adults. Among the 15 adult recipients, 3 received auxiliary partial orthotopic liver transplants (APOLT), 8 received a right liver graft, and 4 received a left liver graft. The minimum GRWR was 0.8%, but the optimal estimated GRWR was 1.0%. If the GRWR of the left liver was less than 0.8%, APOLT was considered. The median time from referral to transplant was 2.0 days (range 0-7 days). The overall 1-year and 3-year survival rate of this population, including 3 APOLT cases and the pediatric cases, was 59%.

The Kyushu group (*111*) primarily chose the left liver graft if it was over 35% of the recipient SLV. The right liver graft without the middle hepatic vein was otherwise chosen when the remnant liver volume was greater than 35% of the total liver volume of the donor. LDLT was performed in 42 ALF patients, including 3 pediatric cases and 39 adult cases (31 with left liver and 8 with right liver). The left liver grafts weighed 442 g (range 260-750 g), representing 42% (range 23-64%) of the SLV of recipients, and the right liver grafts weighed 605 g (range 505-735 g), representing 51% (range 37-57) of the SLV of recipients. The mean interval from onset to LDLT was 5 days. The overall 1-year, 5-year, and 10-year patient survival rates were 80%, 68%, and 68%, respectively.

The Tokyo group (*112*) utilized a right liver graft, a left liver graft, and a right lateral sector graft, with a lower limit of 40% of the recipient SLV, provided that the remnant liver volume of the donor over 30% of the total liver volume. LDLT was performed in 36 ALF patients, including 4 pediatric patients, with 18 right liver grafts, 16 left liver grafts, and 2 right lateral sector grafts. The cold ischemic time was 1.9 h (range 0.3-4.2 h). The weight of the grafts was 503 g (range 276-777 g), which corresponded to 46% (range 22-75%) of the recipient SLV. The overall 1-year and 5-year patient survival rates were 94% and 87%, respectively.

The Hong Kong group (*109*) emphasized the advantage of right liver grafts for ALF patients. Although they reported successful LDLT for an ALF patient with a minimum graft volume (25% of the estimated SLV, GRWR of 0.6%), this case suffered from massive ascites and required a long hospital stay (*128*). Based on their experience with this small-for-

size graft in an ALF case, they reported that the graft should be the donor's right lobe larger than 40% of the recipient SLV, and that the remnant donor's left liver should be greater than 30% of the total liver volume. LDLT was performed in 16 adult patients with ALF with extended right liver grafts from a family member. The median time from listing to transplant was 2 days (range 0.6-9 days) for ALF patients. The median cold ischemia time was 2.2 h (range 1.3-3.7 h). The grafts weighed 615 g (range 430-950) and represented 48% (range 39-89%) of the estimated SLV of the recipients. The overall 1-year survival rate was 88%.

The Seoul group (*23,110,129*) used a left liver graft, right liver graft (without middle hepatic vein), and a dual graft to achieve a GRWR of at least 0.8, provided that the remnant left liver of the donor was over 30% of the total liver volume. The dual graft comprised two left liver grafts or a combination of right and left hemiliver grafts. LDLT was performed in 124 patients with ALF (median age 40 (range 28-49)), including 100 cases with a right liver graft, 16 cases with dual grafts, and 8 cases with a left liver graft. The median GRWR was 1.0 (range 0.9-1.2). The median interval from admission to LDLT was 4 days (range 2-7 days). The overall 1-year and 5-year patient survival rates were 79% and 75%, respectively.

The US A2ALL study group recently reported the results of LDLT for ALF (*130*). Of 1201 LDLT candidates, LDLT was indicated for ALF in only 14 cases (1%). Among those, 10 finally received LDLT, 3 received DDLT, and 1 spontaneously recovered and was removed from the waiting list. The overall survival rate for LDLT was 70%.

5. Conclusion

The advent of liver transplantation has dramatically improved the outcome of patients with ALF, but ALF remains both a challenging and still-too-common cause of mortality. Although numerous medical therapies have been investigated, liver transplantation is the only proven effective treatment for ALF patients. LDLT has remarkably improved the overall survival rate of ALF patients living in regions where organ donation from brain-dead donors is extremely scarce, achieving an outcome comparable to that of DDLT. LDLT might also be valuable to increase the donor pool in Western countries.

References

1. Bernal W, Auzinger G, Dhawan A, Wendon J. Acute liver failure. Lancet. 2010; 376:190-201.
2. Lee WM. Recent developments in acute liver failure. Best Pract Res Clin Gastroenterol. 2012; 26:3-16.
3. Lee WM, Squires RH Jr, Nyberg SL, Doo E, Hoofnagle JH. Acute liver failure: Summary of a workshop. Hepatology. 2008; 47:1401-1415.
4. Lee WM, Stravitz RT, Larson AM. Introduction to the revised American Association for the Study of Liver Diseases Position Paper on acute liver failure 2011. Hepatology. 2012; 55:965-967.
5. Bernuau J, Rueff B, Benhamou JP. Fulminant and subfulminant liver failure: Definitions and causes. Semin Liver Dis. 1986; 6:97-106.
6. Bernal W, Hyyrylainen A, Gera A, Audimoolam VK, McPhail MJ, Auzinger G, Rela M, Heaton N, O'Grady JG, Wendon J, Williams R. Lessons from look-back in acute liver failure? A single centre experience of 3300 patients. J Hepatol. 2013; 59:74-80.
7. Germani G, Theocharidou E, Adam R, *et al*. Liver transplantation for acute liver failure in Europe: Outcomes over 20 years from the ELTR database. J Hepatol. 2012; 57:288-296.
8. Liou IW, Larson AM. Role of liver transplantation in acute liver failure. Semin Liver Dis. 2008; 28:201-209.
9. O'Grady J. Liver transplantation for acute liver failure. Best Pract Res Clin Gastroenterol. 2012; 26:27-33.
10. Ostapowicz G, Fontana RJ, Schiodt FV, *et al*. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med. 2002; 137:947-954.
11. Trey C, Davidson CS. The management of fulminant hepatic failure. Prog Liver Dis. 1970; 3:282-298.
12. O'Grady JG, Schalm SW, Williams R. Acute liver failure: Redefining the syndromes. Lancet 1993; 342:273-275.
13. O'Grady JG, Williams R. Classification of acute liver failure. Lancet. 1993; 342:743.
14. Bower WA, Johns M, Margolis HS, Williams IT, Bell BP. Population-based surveillance for acute liver failure. Am J Gastroenterol. 2007; 102:2459-2463.
15. Brandsaeter B, Höckerstedt K, Friman S, Ericzon BG, Kirkegaard P, Isoniemi H, Olausson M, Broome U, Schmidt L, Foss A, Bjøro K. Fulminant hepatic failure: Outcome after listing for highly urgent liver transplantation-12 years experience in the nordic countries. Liver Transpl. 2002; 8:1055-1062.
16. Oketani M, Ido A, Nakayama N, Takikawa Y, Naiki T, Yamagishi Y, Ichida T, Mochida S, Onishi S, Tsubouchi H; Intractable Hepato-Biliary Diseases Study Group of Japan. Etiology and prognosis of fulminant hepatitis and late-onset hepatic failure in Japan: Summary of the annual nationwide survey between 2004 and 2009. Hepatol Res. 2013; 43:97-105.
17. Hoofnagle JH, Carithers RL Jr, Shapiro C, Ascher N. Fulminant hepatic failure: Summary of a workshop. Hepatology. 1995; 21:240-252.
18. Sass DA, Shakil AO. Fulminant hepatic failure. Liver Transpl. 2005; 11:594-605.
19. Acharya SK, Dasarathy S, Kumer TL, Sushma S, Prasanna KS, Tandon A, Sreenivas V, Nijhawan S, Panda SK, Nanda SK, Irshad M, Joshi YK, Duttagupta S, Tandon RK, Tandon BN. Fulminant hepatitis in a tropical population: Clinical course, cause, and early predictors of outcome. Hepatology. 1996; 23:1448-1455.
20. Mudawi HM, Yousif BA. Fulminant hepatic failure in an African setting: Etiology, clinical course, and predictors of mortality. Dig Dis Sci. 2007; 52:3266-3269.
21. Bernal W, Auzinger G, Wendon J. Prognostic utility of the bilirubin lactate and etiology score. Clin Gastroenterol Hepatol. 2009; 7:249.
22. Hadem J, Tacke F, Bruns T, Langgartner J, Strnad P,

Denk GU, Fikatas P, Manns MP, Hofmann WP, Gerken G, Grünhage F, Umgelter A, Trautwein C, Canbay A; Acute Liver Failure Study Group Germany. Etiologies and outcomes of acute liver failure in Germany. Clin Gastroenterol Hepatol. 2012; 10:664-669 e2.
23. Jin YJ, Lim YS, Han S, Lee HC, Hwang S, Lee SG. Predicting survival after living and deceased donor liver transplantation in adult patients with acute liver failure. J Gastroenterol. 2012; 47:1115-1124.
24. Khuroo MS, Kamili S. Aetiology and prognostic factors in acute liver failure in India. J Viral Hepat. 2003; 10:224-231.
25. Polson J, Lee WM; American Association for the Study of Liver Disease. AASLD position paper: The management of acute liver failure. Hepatology. 2005; 41:1179-1197.
26. Chung RT, Stravitz RT, Fontana RJ, Schiodt FV, Mehal WZ, Reddy KR, Lee WM. Pathogenesis of liver injury in acute liver failure. Gastroenterology. 2012; 143:e1-e7.
27. Bernal W, Auzinger G, Sizer E, Wendon J. Intensive care management of acute liver failure. Semin Liver Dis. 2008; 28:188-200.
28. Stravitz RT, Kramer AH, Davern T, Shaikh AO, Caldwell SH, Mehta RL, Blei AT, Fontana RJ, McGuire BM, Rossaro L, Smith AD, Lee WM; Acute Liver Failure Study Group. Intensive care of patients with acute liver failure: Recommendations of the U.S. Acute Liver Failure Study Group. Crit Care Med. 2007; 35:2498-2508.
29. Vaquero J, Butterworth RF. Mechanisms of brain edema in acute liver failure and impact of novel therapeutic interventions. Neurol Res. 2007; 29:683-690.
30. Bjerring PN, Eefsen M, Hansen BA, Larsen FS. The brain in acute liver failure. A tortuous path from hyperammonemia to cerebral edema. Metab Brain Dis. 2009; 24:5-14.
31. Bhatia V, Singh R, Acharya SK. Predictive value of arterial ammonia for complications and outcome in acute liver failure. Gut. 2006; 55:98-104.
32. Bernal W, Hall C, Karvellas CJ, Auzinger G, Sizer E, Wendon J. Arterial ammonia and clinical risk factors for encephalopathy and intracranial hypertension in acute liver failure. Hepatology. 2007; 46:1844-1852.
33. Ede RJ, Williams RW. Hepatic encephalopathy and cerebral edema. Semin Liver Dis. 1986; 6:107-118.
34. Gazzard BG, Portmann B, Murray-Lyon IM, Williams R. Causes of death in fulminant hepatic failure and relationship to quantitative histological assessment of parenchymal damage. Q J Med. 1975; 44:615-626.
35. Lidofsky SD, Bass NM, Prager MC, Washington DE, Read AE, Wright TL, Ascher NL, Roberts JP, Scharschmidt BF, Lake JR. Intracranial pressure monitoring and liver transplantation for fulminant hepatic failure. Hepatology. 1992; 16:1-7.
36. Vaquero J, Fontana RJ, Larson AM, Bass NM, Davern TJ, Shakil AO, Han S, Harrison ME, Stravitz TR, Muñoz S, Brown R, Lee WM, Blei AT. Complications and use of intracranial pressure monitoring in patients with acute liver failure and severe encephalopathy. Liver Transpl. 2005; 11:1581-1589.
37. Ellis AJ, Wendon JA, Williams R. Subclinical seizure activity and prophylactic phenytoin infusion in acute liver failure: A controlled clinical trial. Hepatology. 2000; 32:536-541.
38. Murphy N, Auzinger G, Bernel W, Wendon J. The effect of hypertonic sodium chloride on intracranial pressure in patients with acute liver failure. Hepatology. 2004; 39:464-470.
39. Canalese J, Gimson AE, Davis C, Mellon PJ, Davis M, Williams R. Controlled trial of dexamethasone and mannitol for the cerebral oedema of fulminant hepatic failure. Gut. 1982; 23:625-629.
40. Vaquero J. Therapeutic hypothermia in the management of acute liver failure. Neurochem Int. 2012; 60:723-735.
41. Sekido H, Matsuo K, Takeda K, Ueda M, Morioka D, Kubota T, Tanaka K, Endo I, Togo S, Shimada H. Usefulness of artificial liver support for pretransplant patients with fulminant hepatic failure. Transplant Proc. 2004; 36:2355-2356.
42. O'Grady JG, Langley PG, Isola LM, Aledort LM, Williams R. Coagulopathy of fulminant hepatic failure. Semin Liver Dis. 1986; 6:159-163.
43. Gazzard BG, Clark R, Borirakchanyavat V, Williams R. A controlled trial of heparin therapy in the coagulation defect of paracetamol-induced hepatic necrosis. Gut. 1974; 15:89-93.
44. MacDougall BR, Williams R. H2-receptor antagonist in the prevention of acute upper gastrointestinal hemorrhage in fulminant hepatic failure: A controlled trial. Gastroenterology. 1978; 74:464-465.
45. Hugenholtz GC, Adelmeijer J, Meijers JC, Porte RJ, Stravitz RT, Lisman T. An unbalance between von Willebrand factor and ADAMTS13 in acute liver failure: Implications for hemostasis and clinical outcome. Hepatology. 2013; 58:752-761.
46. Stravitz RT, Lisman T, Luketic VA, Sterling RK, Puri P, Fuchs M, Ibrahim A, Lee WM, Sanyal AJ. Minimal effects of acute liver injury/acute liver failure on hemostasis as assessed by thromboelastography. J Hepatol. 2012; 56:129-136.
47. Gazzard BG, Henderson JM, Williams R. Early changes in coagulation following a paracetamol overdose and a controlled trial of fresh frozen plasma therapy. Gut. 1975; 16:617-620.
48. Bihari DJ, Gimson AE, Williams R. Cardiovascular, pulmonary and renal complications of fulminant hepatic failure. Semin Liver Dis. 1986; 6:119-128.
49. Ring-Larsen H, Palazzo U. Renal failure in fulminant hepatic failure and terminal cirrhosis: A comparison between incidence, types, and prognosis. Gut. 1981; 22:585-591.
50. O'Grady JG, Alexander GJ, Hayllar KM, Williams R. Early indicators of prognosis in fulminant hepatic failure. Gastroenterology. 1989; 97:439-445.
51. Jain S, Pendyala P, Varma S, Sharma N, Joshi K, Chawla Y. Effect of renal dysfunction in fulminant hepatic failure. Trop Gastroenterol. 2000; 21:118-120.
52. Davenport A, Will EJ, Davidson AM. Improved cardiovascular stability during continuous modes of renal replacement therapy in critically ill patients with acute hepatic and renal failure. Crit Care Med. 1993; 21:328-338.
53. Schneeweiss B, Pammer J, Ratheiser K, *et al*. Energy metabolism in acute hepatic failure. Gastroenterology. 1993; 105:1515-1521.
54. Vaquero J, Polson J, Chung C, Helenowski I, Schiodt FV, Reisch J, Lee WM, Blei AT. Infection and the progression of hepatic encephalopathy in acute liver failure. Gastroenterology. 2003; 125:755-764.
55. Rolando N, Harvey F, Brahm J, Philpott-Howard J,

Alexander G, Casewell M, Fagan E, Williams R. Fungal infection: A common, unrecognised complication of acute liver failure. J Hepatol. 1991; 12:1-9.
56. Rolando N, Harvey F, Brahm J, Philpott-Howard J, Alexander G, Gimson A, Casewell M, Fagan E, Williams R. Prospective study of bacterial infection in acute liver failure: An analysis of fifty patients. Hepatology. 1990; 11:49-53.
57. Rolando N, Philpott-Howard J, Williams R. Bacterial and fungal infection in acute liver failure. Semin Liver Dis. 1996; 16:389-402.
58. Wade JJ, Rolando N, Hayllar K, Philpott-Howard J, Casewell MW, Williams R. Bacterial and fungal infections after liver transplantation: An analysis of 284 patients. Hepatology. 1995; 21:1328-1336.
59. Canalese J, Gove CD, Gimson AE, Wilkinson SP, Wardle EN, Williams R. Reticuloendothelial system and hepatocytic function in fulminant hepatic failure. Gut. 1982; 23:265-269.
60. Rolando N, Wade J, Davalos M, Wendon J, Philpott-Howard J, Williams R. The systemic inflammatory response syndrome in acute liver failure. Hepatology. 2000; 32:734-739.
61. Samuel D, Bismuth H. Transplantation in patients with fulminant hepatitis. In: Transplantation of the Liver, 3rd Edition (Maddrey W, Schiff E,Sorrell M, Eds). Lippincott Williams & Williams, Philadelphia, USA, 2001; pp. 361-370.
62. Shakil AO, Kramer D, Mazariegos GV, Fung JJ, Rakela J. Acute liver failure: Clinical features, outcome analysis, and applicability of prognostic criteria. Liver Transpl. 2000; 6:163-169.
63. Bismuth H, Samuel D, Castaing D, Adam R, Saliba F, Johann M, Azoulay D, Ducot B, Chiche L. Orthotopic liver transplantation in fulminant and subfulminant hepatitis. The Paul Brousse experience. Ann Surg. 1995; 222:109-119.
64. Wlodzimirow KA, Eslami S, Chamuleau RA, Nieuwoudt M, Abu-Hanna A. Prediction of poor outcome in patients with acute liver failure-systematic review of prediction models. PLoS One. 2012; 7:e50952.
65. Bernuau J, Goudeau A, Poynard T, Dubois F, Lesage G, Yvonnet B, Degott C, Bezeaud A, Rueff B, Benhamou JP. Multivariate analysis of prognostic factors in fulminant hepatitis B. Hepatology. 1986; 6:648-651.
66. Yantorno SE, Kremers WK, Ruf AE, Trentadue JJ, Podesta LG, Villamil FG. MELD is superior to King's college and Clichy's criteria to assess prognosis in fulminant hepatic failure. Liver Transpl. 2007; 13:822-828.
67. Mitchell I, Bihari D, Chang R, Wendon J, Williams R. Earlier identification of patients at risk from acetaminophen-induced acute liver failure. Crit Care Med. 1998; 26:279-284.
68. Mochida S, Nakayama N, Matsui A, Nagoshi S, Fujiwara K. Re-evaluation of the Guideline published by the Acute Liver Failure Study Group of Japan in 1996 to determine the indications of liver transplantation in patients with fulminant hepatitis. Hepatol Res. 2008; 38:970-979.
69. Takahashi Y, Kumada H, Shimizu M, *et al*. A multicenter study on the prognosis of fulminant viral hepatitis: Early prediction for liver transplantation. Hepatology. 1994; 19:1065-1071.
70. Yoshiba M, Sekiyama K, Inoue K, Yamada M, Kako M, Nagai K, Takatori M, Iwabuchi S, Sumino Y, Tanaka K, Hakozaki Y, Hasegawa K, Shibuya A. Accurate prediction of fulminant hepatic failure in severe acute viral hepatitis: Multicenter study. J Gastroenterol. 2002; 37:916-921.
71. Akamatsu N, Sugawara Y. Acute liver failure and living donor liver transplantation. Hepatol Res. 2008; 38 (Suppl 1):S60-S71.
72. Bailey B, Amre DK, Gaudreault P. Fulminant hepatic failure secondary to acetaminophen poisoning: A systematic review and meta-analysis of prognostic criteria determining the need for liver transplantation. Crit Care Med. 2003; 31:299-305.
73. McPhail MJ, Wendon JA, Bernal W. Meta-analysis of performance of Kings's College Hospital Criteria in prediction of outcome in non-paracetamol-induced acute liver failure. J Hepatol. 2010; 53:492-499.
74. Craig DG, Ford AC, Hayes PC, Simpson KJ. Systematic review: Prognostic tests of paracetamol-induced acute liver failure. Aliment Pharmacol Ther. 2010; 31:1064-1076.
75. Schmidt LE, Larsen FS. MELD score as a predictor of liver failure and death in patients with acetaminophen-induced liver injury. Hepatology. 2007; 45:789-796.
76. Zaman MB, Hoti E, Qasim A, Maguire D, McCormick PA, Hegarty JE, Geoghegan JG, Traynor O. MELD score as a prognostic model for listing acute liver failure patients for liver transplantation. Transplant Proc. 2006; 38:2097-2098.
77. Bechmann LP, Jochum C, Kocabayoglu P, Sowa JP, Kassalik M, Gieseler RK, Saner F, Paul A, Trautwein C, Gerken G, Canbay A. Cytokeratin 18-based modification of the MELD score improves prediction of spontaneous survival after acute liver injury. J Hepatol. 2010; 53:639-647.
78. Miyake Y, Sakaguchi K, Iwasaki Y, Ikeda H, Makino Y, Kobashi H, Araki Y, Ando M, Kita K, Shiratori Y. New prognostic scoring model for liver transplantation in patients with non-acetaminophen-related fulminant hepatic failure. Transplantation. 2005; 80:930-936.
79. Schiødt FV, Ostapowicz G, Murray N, Satyanarana R, Zaman A, Munoz S, Lee WM. Alpha-fetoprotein and prognosis in acute liver failure. Liver Transpl. 2006; 12:1776-1781.
80. Schmidt LE, Dalhoff K. Serum phosphate is an early predictor of outcome in severe acetaminophen-induced hepatotoxicity. Hepatology. 2002; 36:659-665.
81. Chung PY, Sitrin MD, Te HS. Serum phosphorus levels predict clinical outcome in fulminant hepatic failure. Liver Transpl. 2003; 9:248-253.
82. Shakil AO, Jones BC, Lee RG, Federle MP, Fung JJ, Rakela J. Prognostic value of abdominal CT scanning and hepatic histopathology in patients with acute liver failure. Dig Dis Sci. 2000; 45:334-339.
83. Itai Y, Sekiyama K, Ahmadi T, Obuchi M, Yoshiba M. Fulminant hepatic failure: Observation with serial CT. Radiology. 1997; 202:379-382.
84. Yamagishi Y, Saito H, Ebinuma H, Kikuchi M, Ojiro K, Kanamori H, Tada S, Horie Y, Kato S, Hibi T. A new prognostic formula for adult acute liver failure using computer tomography-derived hepatic volumetric analysis. J Gastroenterol. 2009; 44:615-623.
85. Monoe T, Nomura T, Nakao H, Sobue S, Mizuno Y, Nojiri S, Kanematsu T, Senda K, Ohmi H, Ohara H, Joh T, Itoh K, Itoh M. Prognostic indicators estimated by

(99m)Tc-GSA in acute liver damage. Hepatol Res. 2005; 31:153-159.
86. Nagaki M, Iwai H, Naiki T, Ohnishi H, Muto Y, Moriwaki H. High levels of serum interleukin-10 and tumor necrosis factor-alpha are associated with fatality in fulminant hepatitis. J Infect Dis. 2000; 182:1103-1108.
87. Macquillan GC, Seyam MS, Nightingale P, Neuberger JM, Murphy N. Blood lactate but not serum phosphate levels can predict patient outcome in fulminant hepatic failure. Liver Transpl. 2005; 11:1073-1079.
88. Bernal W. Lactate is important in determining prognosis in acute liver failure. J Hepatol. 2010; 53:209-210.
89. Bernal W, Donaldson N, Wyncoll D, Wendon J. Blood lactate as an early predictor of outcome in paracetamol-induced acute liver failure: A cohort study. Lancet. 2002; 359:558-563.
90. 2009 Annual report of the U.S. organ procurement and transplantation network and the scientific registry of transplant recipients: Transplant data 1999-2008. U.S. Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation. *http://www.ustransplant.org/annual_reports/current* (accessed July 1, 2013).
91. Hanto DW, Fishbein TM, Pinson CW, Olthoff KM, Shiffman ML, Punch JD, Goodrich NP. Liver and intestine transplantation: Summary analysis, 1994-2003. Am J Transplant. 2005; 5:916-933.
92. Adam R, McMaster P, O'Grady JG, *et al*. Evolution of liver transplantation in Europe: Report of the European Liver Transplant Registry. Liver Transpl. 2003; 9:1231-1243.
93. Bernal W, Cross TJ, Auzinger G, Sizer E, Heneghan MA, Bowles M, Muiesan P, Rela M, Heaton N, Wendon J, O'Grady JG. Outcome after wait-listing for emergency liver transplantation in acute liver failure: A single centre experience. J Hepatol. 2009; 50:306-313.
94. Eghtesad B, Bronsther O, Irish W, Casavilla A, Abu-Elmagd K, Van Thiel D, Tzakis A, Fung JJ, Starzl TE. Disease gravity and urgency of need as guidelines for liver allocation. Hepatology. 1994; 20:56S-62S.
95. Wigg AJ, Gunson BK, Mutimer DJ. Outcomes following liver transplantation for seronegative acute liver failure: Experience during a 12-year period with more than 100 patients. Liver Transpl. 2005; 11:27-34.
96. Farmer DG, Anselmo DM, Ghobrial RM, *et al*. Liver transplantation for fulminant hepatic failure: Experience with more than 200 patients over a 17-year period. Ann Surg. 2003; 237:666-675; discussion 675-676.
97. Chan G, Taqi A, Marotta P, Levstik M, McAlister V, Wall W, Quan D. Long-term outcomes of emergency liver transplantation for acute liver failure. Liver Transpl. 2009; 15:1696-1702.
98. Barshes NR, Lee TC, Balkrishnan R, Karpen SJ, Carter BA, Goss JA. Risk stratification of adult patients undergoing orthotopic liver transplantation for fulminant hepatic failure. Transplantation. 2006; 81:195-201.
99. Burroughs AK, Sabin CA, Rolles K, *et al*. 3-month and 12-month mortality after first liver transplant in adults in Europe: Predictive models for outcome. Lancet. 2006; 367:225-232.
100. Detre K, Belle S, Beringer K, Daily OP. Liver transplantation for fulminant hepatic failure in the United States: October 1987 through December 1991. Clin Transplant. 1994; 8:274-280.
101. Toso C, Al-Qahtani M, Alsaif FA, Bigam DL, Meeberg GA, James Shapiro AM, Bain VG, Kneteman NM. ABO-incompatible liver transplantation for critically ill adult patients. Transpl Int. 2007; 20:675-681.
102. Bjøro K, Ericzon BG, Kirkegaard P, Höckerstedt K, Söderdahl G, Olausson M, Foss A, Schmidt LE, Isoniemi H, Brandsaeter B, Friman S. Highly urgent liver transplantation: Possible impact of donor-recipient ABO matching on the outcome after transplantation. Transplantation. 2003; 75:347-353.
103. Farges O, Kalil AN, Samuel D, Saliba F, Arulnaden JL, Debat P, Bismuth A, Castaing D, Bismuth H. The use of ABO-incompatible grafts in liver transplantation: A life-saving procedure in highly selected patients. Transplantation. 1995; 59:1124-1133.
104. de Villa VH, Lo CM, Chen CL. Ethics and rationale of living-donor liver transplantation in Asia. Transplantation. 2003; 75(3 Suppl):S2-S5.
105. Yamashiki N, Sugawara Y, Tamura S, Nakayama N, Oketani M, Umeshita K, Uemoto S, Mochida S, Tsubouchi H, Kokudo N. Outcomes after living donor liver transplantation for acute liver failure in Japan: Results of a nationwide survey. Liver Transpl. 2012; 18:1069-1077.
106. Kato T, Nery JR, Morcos JJ, Gyamfi AR, Ruiz P, Molina EG, Tzakis AG. Successful living related liver transplantation in an adult with fulminant hepatic failure. Transplantation. 1997; 64:415-417.
107. Uemoto S, Inomata Y, Sakurai T, Egawa H, Fujita S, Kiuchi T, Hayashi M, Yasutomi M, Yamabe H, Tanaka K. Living donor liver transplantation for fulminant hepatic failure. Transplantation. 2000; 70:152-157.
108. Carlisle EM, Angelos P, Siegler M, Testa G. Adult living-related liver donation for acute liver failure: Is it ethically appropriate? Clin Transplant. 2011; 25:813-820.
109. Liu CL, Fan ST, Lo CM, Yong BH, Fung AS, Wong J. Right-lobe live donor liver transplantation improves survival of patients with acute liver failure. Br J Surg. 2002; 89:317-322.
110. Park SJ, Lim YS, Hwang S, Heo NY, Lee HC, Suh DJ, Yu E, Lee SG. Emergency adult-to-adult living-donor liver transplantation for acute liver failure in a hepatitis B virus endemic area. Hepatology. 2010; 51:903-911.
111. Ikegami T, Taketomi A, Soejima Y, Yoshizumi T, Sanefuji K, Kayashima H, Shimada M, Maehara Y. Living donor liver transplantation for acute liver failure: A 10-year experience in a single center. J Am Coll Surg 2008; 206:412-418.
112. Matsui Y, Sugawara Y, Yamashiki N, Kaneko J, Tamura S, Togashi J, Makuuchi M, Kokudo N. Living donor liver transplantation for fulminant hepatic failure. Hepatol Res. 2008; 38:987-996.
113. Kilic M, Aydin U, Noyan A, Arikan C, Aydogdu S, Akyildiz M, Karasu Z, Zeytunlu M, Alper M, Batur Y. Live donor liver transplantation for acute liver failure. Transplantation. 2007; 84:475-479.
114. Wu YM, Ho MC, Hu RH, Ko WJ, Yang PM, Lai MY, Lee PH. Liver transplantation for acute hepatic failure. Transplant Proc. 2004; 36:2226-2227.
115. Miwa S, Hashikura Y, Mita A, Kubota T, Chisuwa H, Nakazawa Y, Ikegami T, Terada M, Miyagawa S, Kawasaki S. Living-related liver transplantation for patients with fulminant and subfulminant hepatic failure. Hepatology. 1999; 30:1521-1526.

116. Yuan D, Liu F, Wei YG, Li B, Yan LN, Wen TF, Zhao JC, Zeng Y, Chen KF. Adult-to-adult living donor liver transplantation for acute liver failure in China. World J Gastroenterol. 2012; 18:7234-7241.
117. Bronster DJ, Emre S, Boccagni P, Sheiner PA, Schwartz ME, Miller CM. Central nervous system complications in liver transplant recipients – incidence, timing, and long-term follow-up. Clin Transplant. 2000; 14:1-7.
118. Wright L, Faith K, Richardson R, Grant D; Joint Centre for Bioethics, University of Toronto, Toronto, Ont. Ethical guidelines for the evaluation of living organ donors. Can J Surg. 2004; 47:408-413.
119. Marcos A, Ham JM, Fisher RA, Olzinski AT, Shiffman ML, Sanyal AJ, Luketic VA, Sterling RK, Posner MP. Emergency adult to adult living donor liver transplantation for fulminant hepatic failure. Transplantation. 2000; 69:2202-2205.
120. Northup PG, Berg CL. Living donor liver transplantation: The historical and cultural basis of policy decisions and ongoing ethical questions. Health Policy. 2005; 72:175-185.
121. Sugawara Y, Makuuchi M. Living donor liver transplantation: Present status and recent advances. Br Med Bull. 2005; 75-76:15-28.
122. Adams PL, Cohen DJ, Danovitch GM, *et al*. The nondirected live-kidney donor: Ethical considerations and practice guidelines: A National Conference Report. Transplantation. 2002; 74:582-589.
123. Saidi RF, Jabbour N, Li Y, Shah SA, Bozorgzadeh A. Is left lobe adult-to-adult living donor liver transplantation ready for widespread use? The US experience (1998-2010). HPB (Oxford). 2012; 14:455-460.
124. Ghobrial RM, Freise CE, Trotter JF, Tong L, Ojo AO, Fair JH, Fisher RA, Emond JC, Koffron AJ, Pruett TL, Olthoff KM; A2ALL Study Group. Donor morbidity after living donation for liver transplantation. Gastroenterology. 2008; 135:468-476.
125. Shin M, Song S, Kim JM, Kwon CH, Kim SJ, Lee SK, Joh JW. Donor morbidity including biliary complications in living-donor liver transplantation: Single-center analysis of 827 cases. Transplantation. 2012; 93:942-948.
126. Barr ML, Belghiti J, Villamil FG, Pomfret EA, Sutherland DS, Gruessner RW, Langnas AN, Delmonico FL. A report of the Vancouver Forum on the care of the live organ donor: Lung, liver, pancreas, and intestine data and medical guidelines. Transplantation. 2006; 81:1373-1385.
127. Neuberger J, Farber L, Corrado M, O'Dell C. Living liver donation: A survey of the attitudes of the public in Great Britain. Transplantation. 2003; 76:1260-1264.
128. Lo CM, Fan ST, Chan JK, Wei W, Lo RJ, Lai CL. Minimum graft volume for successful adult-to-adult living donor liver transplantation for fulminant hepatic failure. Transplantation. 1996; 62:696-698.
129. Lee SG, Ahn CS, Kim KH. Which types of graft to use in patients with acute liver failure? (A) Auxiliary liver transplant (B) Living donor liver transplantation (C) The whole liver. (B) I prefer living donor liver transplantation. J Hepatol. 2007; 46:574-578.
130. Campsen J, Blei AT, Emond JC, Everhart JE, Freise CE, Lok AS, Saab S, Wisniewski KA, Trotter JF; Adult-to-Adult Living Donor Liver Transplantation Cohort Study Group. Outcomes of living donor liver transplantation for acute liver failure: The adult-to-adult living donor liver transplantation cohort study. Liver Transpl. 2008; 14:1273-1280.

(Received July 7, 2013; Accepted July 18, 2013)

Review

DOI: 110.5582/irdr.2013.v2.3.88

Idiopathic pulmonary fibrosis: The current status of its epidemiology, diagnosis, and treatment in China

Haibo Huang, Xiaonu Peng*, Chongwen Zhong

Department of Thoracic Surgery, Yantai Yuhuangding Hospital, Medical College of Qingdao University, Yantai, Shandong, China.

Summary **Idiopathic pulmonary fibrosis (IPF) is a type of intractable and rare disease, and its epidemiology in China is still unclear. The diagnosis and treatment of IPF has received considerable attention and two editions of guidelines on IPF diagnosis and treatment have been published by the Chinese Society of Respiratory Diseases. Treatment of IPF with Traditional Chinese Medicine (TCM) has been widely investigated in China and several types of TCM extracts are reported to be effective in animal models. One effective treatment is lung transplantation; this treatment has been successfully performed in China, yielding satisfactory long-term survival.**

Keywords: Prevalence, guideline, therapy, Traditional Chinese Medicine, lung transplantation

1. Introduction

Idiopathic pulmonary fibrosis (IPF) is a rare disease characterized by chronic, progressive fibrosing interstitial pneumonia of unknown etiology. Although the exact morbidity is still unclear due to the lack of large-scale studies, IPF is reported to have no distinct geographical or ethnical distribution (*1*). Because of China's huge population, the country is estimated to have the largest number of patients with IPF. IPF is a type of intractable disease. No specific pharmacologic therapies to treat IPF have been found to date. IPF is progressive, irreversible, and associated with an extremely poor prognosis. The pulmonary function of patients with IPF will progressively deteriorate as the disease progresses, and patients have a median survival time of 2 to 3 years (*2*). The current article briefly reviews the current status of IPF epidemiology, diagnosis, and treatment in China.

2. Search strategy

Wanfang Database, an electronic database of Chinese medicine, was searched using the keyword "idiopathic pulmonary fibrosis" combined with "diagnosis" or "treatment" or "epidemiology" to identify all relevant literature published in Chinese journals since January 1980. The full text of each article was reviewed and the article was selected if pertinent.

3. General condition and epidemiology

The first article about IPF in Chinese academic journals was a description of the condition in Japan in 1980 (*3*). The first Chinese case was reported in 1981 (*4*). IPF was first known as Hamman-Rich syndrome and then later known as cryptogenic fibrosing alveolitis (CFA) before its current appellation of IPF. The terms CFA and IPF have both been used in China but IPF is now preferred.

Different studies have reported a varying prevalence of IPF from 1.25 to 27.9 cases per 100,000 (*5-12*), as shown in Figure 1. A study in the United Kingdom estimated that the incidence of IPF increased by 11% annually from 1991 to 2003 (*13*).

Most data on IPF were from North America, Europe, and Japan. To date, no epidemiological studies of IPF have been conducted in China and the exact prevalence of IPF in China is still unknown. There is very little documentation on the epidemiology of rare diseases in China because there are no systems to register rare diseases (*14*). This is partly because of the delays in Chinese epidemiology, unbalanced economic development, and China's large population. Another key reason for the lack of information on IPF in China is

**Address correspondence to:*
Dr. Xiaonu Peng, Department of Thoracic Surgery, Yantai Yuhuangding Hospital, No. 20, Yuhuangding Donglu, Yantai, Shandong, China.
E-mail: doctorhuanghaibo@outlook.com

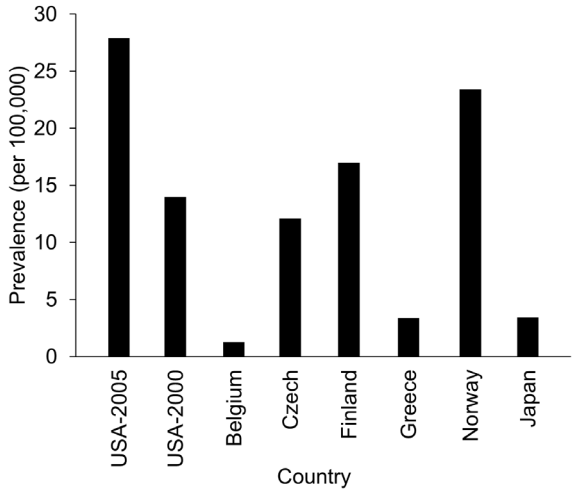


Figure 1. Prevalence of idiopathic pulmonary fibrosis in different countries and according to different studies.

because the former gold standard for IPF diagnosis (*15*), a surgical biopsy, is not readily accepted in China. To Chinese patients, the risk of diminished health caused by a diagnostic procedure is greater than the benefit of a surgical biopsy. Because of the absence of effective treatments, Chinese patients prefer experimental therapy to a diagnostic procedure.

Some epidemiological characteristics of IPF can be ascertained indirectly from several studies in China. A study (*16*) conducted by the Chinese Society of Respiratory Diseases at ten hospitals found that those hospitals treated 697 cases of IPF from 1990 to 2003; these cases accounted for about 25.5% of all cases of diffuse interstitial lung disease (DILD). In another multicenter study in Tianjin (*17*), patients with DILD accounted for about 39.5% of patients in Respiratory Medicine and 1.51% of all inpatients in 2009. Some epidemiologic characteristics of IPF in China have been noted, such as one diagnostic criterion listed in the 2002 guidelines for the diagnosis and treatment of IPF from the Chinese Society of Respiratory Diseases (*18*). IPF usually develops past middle age and males are more susceptible than females at a rate of about 2 to 1. IPF is rarely seen in children. Smoking (*19*) and environmental factors, like metal and wood dust exposure (*20*), have been reported to be major risk factors for IPF. Smoking is strongly associated with IPF, particularly for individuals with a history of smoking more than 20 cigarettes a day over 20 years (20 pack-years). China is known to have the largest population of smokers. According to one study in 2002, the prevalence of smoking among males was 66% and the prevalence of smoking among females was 3.08% (*21*). As the largest developing country, labor protections are still developing in China and the risk of metal and wood dust exposure remains high. Thus, China will have a higher incidence of IPF compared to developed countries, and further large-scale epidemiologic studies are needed.

4. Diagnosis

Usual interstitial pneumonia (UIP) is the characteristic pattern of IPF. UIP has distinctive findings in both pathological and radiological examinations. However, a number of other conditions can also cause the changes associated with UIP. The most common are connective tissue disease and certain drug toxicities. Before a diagnosis of IPF is made, these conditions should be ruled out.

For a long time, there was debate about whether evidence of UIP in a radiological examination could be used to diagnose IPF without pathological confirmation. In China, the first paper on the diagnosis of IPF was published in 1984 (*22*). In this paper, the author posited that a surgical lung biopsy was not necessarily needed for patients suspected of having IPF. In a trial version of IPF guidelines drafted by the Chinese Society of Respiratory Diseases in 1994 (*23*), both pathological and radiological evidence of UIP were considered diagnostic criteria. The guidelines deemed pathological evidence of UIP to be confirmed IPF and they deemed radiological evidence of UIP to be clinically diagnosed IPF. The guidelines recommended that both be treated for IPF.

In 2002, the Chinese Society of Respiratory Diseases revised its guidelines. In the new guidelines (*18*), the criteria for pathologically diagnosed IPF did not change but the criteria for clinical diagnosis of IPF did change. Four major diagnostic criteria and four minor diagnostic criteria were listed. The major criteria included ruling out of other possible diseases based on the patient's medical history, restrictive ventilatory functional disturbance or gas interchange disturbance, radiological evidence of UIP and ruling out of other diseases based on bronchoalveolar lavage fluid (BALF) or a transbronchial lung biopsy (TBLB). The minor criteria included being over 50 years of age, a history of symptoms for three months or longer, insidious onset of unexplained dyspnea on exertion, and bibasilar inspiratory ("Velcro") crackles. All four major diagnostic criteria and at least 3 minor criteria should be met.

Performing a lung biopsy when IPF is suspected has not been readily accepted in China because of the potential for IPF to worsen, the invasiveness of the procedure, and its cost. Although the exact rate of lung biopsies in China has not been reported, it is estimated to be lower than 10%. Nearly all reports of IPF involved the clinical diagnostic criteria in the guidelines drafted by the Chinese Society of Respiratory Diseases. Only a few reports of surgically diagnosed IPF described a pathological diagnosis reached incidentally as a result of surgery to treat another disease, such as spontaneous pneumothorax. One study reviewed the ability of high-resolution computed tomography (HRCT)-guided lung needle biopsy to diagnose IPF. The technique had an accuracy of 76.2% (*24*). Another

study (*25*) retrospectively analyzed 39 cases of IPF and compared the 2002 guidelines of the Chinese Society of Respiratory Diseases and 2000 guidelines of the American Thoracic Society (ATS); the study noted that the guidelines coincided at a rate of 84.6%.

In the West, the first guidelines for IPF were drafted by the ATS, European Respiratory Society (ERS), and the American College of Chest Physicians (ACCP) in 2000 (*15*). These guidelines recommended a surgical lung biopsy for most patients to reach a correct diagnosis. However, one study (*26*) found that the radiological pattern of UIP was nearly always consistent with pathological evidence of UIP. Thus, the guidelines on IPF drafted by the ATS, ERS, the Japanese Respiratory Society (JRS), and the Latin American Thoracic Association (ALAT) in 2011 no longer recommend a surgical lung biopsy for patients with a UIP pattern on HRCT (*27*).

5. Treatment

5.1. *Pharmacological therapies*

Because of its unknown pathogenesis, most pharmacologic treatments of IPF assume that IPF is caused by inappropriate inflammation and subsequent injury and fibrosis of pulmonary alveoli. Thus, corticosteroids, immunosuppressive or cytotoxic agents, and antifibrotic agents have long been used to treat IPF. To date, however, pharmacologic therapies to treat IPF have failed to offer promise.

The 1994 trial guidelines for the diagnosis and treatment of IPF (*23*) recommended corticosteroids as a first-line therapy and immunosuppressive or cytotoxic agents were recommended as a second-line therapy. Because of the possible side effects of these drugs, personalized therapy and timely dose adjustment according to test results were also recommended. After several years of clinical use, these regimens were found to temporarily improve symptoms but not improve long-time survival. The 2002 guidelines of the Chinese Society of Respiratory Diseases (*18*) recommended a combination of corticosteroids with immunosuppressive or cytotoxic agents. Courses of treatment and indices of their effectiveness were also described. The revised guidelines also mentioned many types of new drugs, like N acetylcysteine, γ interferon, colchicine, erythromycin, and pirfenidone. Because of the lack of clinical evidence, however, they were not recommended.

After years of clinical experience and advances in evidence-based medicine, the 2011 guidelines on IPF diagnosis and treatment from the ATS (*27*) indicated that corticosteroid monotherapy, colchicine, acetylcysteine, γ interferon, bosentan, etanercept, and the combination of corticosteroids with immunosuppressive agents were not recommended. Only acetylcysteine combined with corticosteroids and azathioprine, acetylcysteine monotherapy, anticoagulant monotherapy, and pirfenidone monotherapy were recommended in a minority of IPF cases.

Traditional Chinese Medicine (TCM) in the form of both herbal compounds and single herb extracts has been reported to have some effect on IPF (*28*). The 1994 trial guidelines for the diagnosis and treatment of IPF recommended TCM therapy as an experimental therapy (*23*). TCM is a reflection of the Chinese people's long struggle against disease. TCM is readily accepted in China and nearly all patients with IPF had received TCM therapy at one point. Because there are no large-scale, multicenter, randomized, double-blind, parallel-treatment, placebo-controlled studies of the effects of TCM, its exact effects are still debated. TCM has not been readily accepted in the West because clear evidence of its pharmacological mechanisms is lacking. That said, the development of TCM extraction and purification techniques has resulted in several studies (*29-41*) reporting that some single herb extracts of TCM had some effect in animal models of IPF (Table 1).

5.2. *Oxygen therapy and palliative therapy*

The patient's pulmonary function will deteriorate rapidly as IPF progresses. In the end stages, severe hypoxia will develop even when at rest. The 1994 trial guidelines for the diagnosis and treatment of IPF (*23*) recommended that patients with end-stage IPF not receive drug therapy but only oxygen therapy and palliative therapy. The 2011 guidelines of the ATS recommended long-term oxygen therapy for patients with IPF and clinically significant resting hypoxemia (*27*).

Because nearly all patients will develop severe hypoxia in the end stages of IPF, oxygen therapy and palliative therapy are crucial. However, there are still no Chinese guidelines on the indications for oxygen therapy, inhaled oxygen flow, and the duration of oxygen therapy. One study (*42*) retrospectively analyzed 5 patients with end-stage IPF who received oxygen therapy. The study found that high-flow oxygen therapy was not suitable and the oxygen flow needed to be adjusted depending on blood oxygen saturation. In China, however, most patients with end-stage IPF receive oxygen therapy at home. They would have difficulty monitoring their blood oxygen saturation. Thus, instructing patients with end-stage IPF in use of home oxygen therapy is still a major problem.

5.3. *Lung transplantation*

Lung transplantation is one form of highly effective treatment for many types of end-stage lung disease. Lung transplantation has made great advances in recent years. Lung transplantation to treat IPF first resulted in successful long-term survival in 1983 (*43*). Since then, IPF has become the primary indication for lung

Table 1. Single-herb TCM reported to be effective in treating IPF in animal models

Common name	Latin name	Major active ingredients	Main pharmacologic function	*Ref.*
Danshen root	*Radix salvia miltiorrhiza*	Salvianolic acid A and B, polysaccharide	Scavenges oxygen radicals, anti-lipid peroxidation	*29*
Chuanxiongzine	*Ligustrazine*	Tetramethylpyrazine	Inhibits fibroblast cell proliferation, down-regulates CTGF expression	*30*
Chinese angelica	*Angelica sinensis*	5-Hydroxymethyl-furfural, polysaccharides	Scavenges oxygen free radicals, down-regulates CTGF expression	*31,32*
Huangqi, Radix astragali	*Astragalus membranaceus*	Polysaccharides, saponins, and flavonoids	Down-regulates the expression of MMP-2 and TIMP-1	*33*
Sanqi, pseudo-ginseng	*Panax notoginseng*	Panax notoginseng saponins	Down-regulates the expression of hydroxyproline and CTGF	*34*
Ginkgo Leaf	*Folium Ginkgo*	Flavonoid, terpenoid	Down-regulates the expression of CTGF, inhibit AT II epithelial cell apoptosis	*35,36*
Fourstamen Stephania root/radix	*Stephania tetrandra*	Tetrandrine	Blocks calcium channels and calmodulin	*37*
Common three-wingnut root/radix	*Tripterygium wilfordii*	Triptolide and diterpenoids	Anti-inflammatory and anti-oxidant	*38*
Sha shen, root of straight ladybell	*Adenophora stricta*	Triterpenoids and β-sitosterol	Down-regulates the expression of TGF-β1 and TNF-*α*	*39*
Chinese caterpillar fungus	*Cordyceps sinensis*	Cordyceps polysaccharide, cordycepin, ergosterol	Reduces inflammatory cell infiltration and fibroblast deposition, regulates the balance of MMP-9/TIMP-1	*40*
Turmeric, Jianghuang	*Curcuma longa*	Curcumin, demethoxycurcumin, and bisdemethoxycurcumin	Induces the apoptosis of abnormal lung fibroblasts	*41*

Abbreviations: AT, alveolar type; CTGF, connective tissue growth factor; MMP-2, matrix metalloproteinases-2; MMP-9, matrix metalloproteinases-9; TIMP-1, tissue inhibitor of metalloproteinases-1; TGF-β1, transforming growth factor; TNF-*α*, tumor necrosis factor-*α*.

Figure 2. Long-term survival after lung transplantation.

transplantation and about 37% of lung transplants prior to 2009 were to treat IPF (*44*). Lung transplantation to treat IPF was recommended by both the 1994 trial guidelines for the diagnosis and treatment of IPF and the 2011 guidelines of the ATS.

The largest lung transplantation center in China is in Jiangsu Province. There, 131 patients underwent lung transplantation from September 2002 to December 2011. Of these patients, 59 (45%) were diagnosed as having end-stage IPF (*45*). Patients had a 1-year survival rate of 69.5%, a 3-year survival rate of 51.2%, and a 5-year survival rate of 34.7%, with a median survival time of 42 months. In the West, lung transplantation to treat IPF (*46*) has yielded significant benefits in terms of survival. Currently, patients have a 1-year survival rate of 74%, a 5-year survival rate of 45%, and a 10-year survival rate of 22%. A comparison of survival rates is shown in Figure 2.

Therapies to treat IPF are rarely effective and disease progression is inevitable. To date, lung transplant remains the only viable treatment option offering long-term survival, with a 5-year survival rate of 45% and a 10-year survival rate of 22% (*46*). This is very encouraging for patients with IPF. However, only a few patients with IPF in China have the opportunity to receive a lung transplant to improve their quality of life and prolong their survival due to the expense of the procedure and lack of donors. From 1978 to 2010, only about 244 lung transplants were performed at about 20 facilities in China (*47*). This cannot meet the demands of the large estimated population of patients with IPF. In addition, progression of fibrosis in the native lungs after lung transplantation is also a major challenge (*46*).

6. Conclusion

IPF is a type of rare disease, and little is known about its epidemiologic characteristics in China. Because of China's huge population, the country is estimated to have the largest population of patients with IPF.

Doctors in China have continually focused on this intractable disease and they have drafted two editions of guidelines for IPF diagnosis and treatment. However, the current state of IPF diagnosis and treatment remains unsatisfactory. To date, there are no specific pharmacological therapies for IPF. CTM is widely used in China and is reported to have some effect on IPF, but more large-scale studies and randomized, placebo-controlled studies are needed, as are studies of the pharmacological mechanisms of TCM. Home oxygen therapy is widely accepted in China. Lung transplantation to treat IPF has been successfully performed in China, resulting in long-term survival.

References

1. Mannino DM, Etzel RA, Parrish RG. PPulmonary fibrosis deaths in the United States, 1979-1991. An analysis of multiple-cause mortality data. Am J Respir Crit Care Med. 1996; 153:1548-1552.
2. Rudd RM, Prescott RJ, Chalmers JC, Johnston ID; Fibrosing Alveolitis Subcommittee of the Research Committee of the British Thoracic Society. British Thoracic Society Study on cryptogenic fibrosing alveolitis: Response to treatment and survival. Thorax. 2007; 62:62-66.
3. Zhu GG. Idiopathic pulmonary fibrosis. Progress in Japanese Medicine. 1980; 1:9-11. (in Chinese)
4. Peng DH, Xu HY. Idiopathic pulmonary fibrosis (Hamman-Rich syndrome): A case report and review of the data. Medicine and Pharmacology of YunNan. 1981; 10-12. (in Chinese)
5. Fernández Pérez ER, Daniels CE, Schroeder DR, St Sauver J, Hartman TE, Bartholmai BJ, Yi ES, Ryu JH. Incidence, prevalence, and clinical course of idiopathic pulmonary fibrosis: A population-based study. Chest. 2010; 137:129-137.
6. Raghu G, Weycker D, Edelsberg J, Bradford WZ, Oster G. Incidence and prevalence of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med. 2006; 174:810-816.
7. Thomeer MJ, Costabe U, Rizzato G, Poletti V, Demedts M. Comparison of registries of interstitial lung diseases in three European countries. Eur Respir J. 2001; 32(suppl):114-118.
8. Kolek V. Epidemiology of cryptogenic fibrosing alveolitis in Moravia and Silesia. Acta Univ Palacki Olomuc Fac Med. 1994;137:49-50.
9. Hodgson U, Laitinen T, Tukiainen P. Nationwide prevalence of sporadic and familial idiopathic pulmonary fibrosis: Evidence of founder effect among multiplex families in Finland. Thorax. 2002; 57:338-342.
10. Karakatsani A, Papakosta D, Rapti A, Antoniou KM, Dimadi M, Markopoulou A, Latsi P, Polychronopoulos V, Birba G, Ch L, Bouros D; Hellenic Interstitial Lung Diseases Group. Epidemiology of interstitial lung diseases in Greece. Respir Med. 2009; 103:1122-1129.
11. Von Plessen C, Grinde O, Gulsvik A. Incidence and prevalence of cryptogenic fibrosing alveolitis in a Norwegian community. Respir Med. 2003; 97:428-435.
12. Ohno S, Nakaya T, Bando M, Sugiyama Y. Idiopathic pulmonary fibrosis – results from a Japanese nationwide epidemiological survey using individual clinical records. Respirology. 2008; 13:926-928.
13. Gribbin J, Hubbard RB, Le Jeune I, Smith CJ, West J, Tata LJ. Incidence and mortality of idiopathic pulmonary fibrosis and sarcoidosis in the UK. Thorax. 2006; 61:980-985.
14. Zhao H, Cui YZ, Zhou XY, Pang JX, Zhang XM, Xu SQ, Han JX. Study and analysis of the state of rare disease research in Shandong Province, China. Intractable Rare Dis Res. 2012; 1:161-166.
15. American Thoracic Society. Idiopathic pulmonary fibrosis: diagnosis and treatment. International consensus statement. American Thoracic Society (ATS), and the European Respiratory Society (ERS). Am J Respir Crit Care Med. 2000; 161:646-664.
16. Kang J, Kong LF. Proceedings of the 6th National Conference on Respiratory Diseases of the Chinese Medical Association. Chinese Journal of Internal Medicine. 2005; 44:154-156. (in Chinese)
17. Wei LQ, Peng SC, Cao J, Liu GY, Lai YP, Jia W, Zheng H, Li LY, Xiao YY, Li N, Zhang NX. A multicenter study of the clinical characteristics of diffuse interstitial lung diseases and their diagnosis and treatment. Chinese Journal of General Practice. 2012; 15:2521-2524. (in Chinese)
18. Chinese Society of Respiratory Diseases. Draft guidelines for the diagnosis and treatment of IPF. Chinese Journal of Internal Medicine. 2002; 41:498-500. (in Chinese)
19. Hubbard R, Lewis S, Richards K, Johnston I, Britton J. Occupational exposure to metal or wood dust and aetiology of cryptogenic fibrosing alveolitis. Lancet. 1996; 347:284-289.
20. Iwai K, Mori T, Yamada N, Yamaguchi M, Hosoda Y. Idiopathic pulmonary fibrosis. Epidemiologic approaches to occupational exposure. Am J Respir Crit Care Med. 1994; 150:670-675.
21. Yang GH, Ma JM, Liu N, Zhou LN. Smoking and passive smoking of Chinese, 2002. Chin J Epidemiol. 2005; 26:77-83. (in Chinese)
22. Lei ZZ. The diagnosis of idiopathic pulmonary disease. Chinese Journal of Postgraduates of Medicine. 1984; 5:6-7. (in Chinese)
23. Chinese Society of Respiratory Diseases. Trial guidelines for the diagnosis and treatment of idiopathic pulmonary fibrosis. Chin J Tuberc Respir Dis. 1994; 17:8-8. (in Chinese)
24. Liu JH, Bao YW. The diagnostic value of HRCT-guided lung needle biopsy for 21 cases of Idiopathic pulmonary fibrosis. Fujian Medical Journal. 2005; 27:68-69. (in Chinese)
25. Zhao YB, Tong ZY, Ao R, Li ZH, Hou XM. Retrospective analysis of 39 cases of idiopathic pulmonary fibrosis. Journal of China Medical University. 2003; 32:77-78. (in Chinese)
26. FFlaherty KR, King TE Jr, Raghu G, Lynch JP 3rd, Colby TV, Travis WD, Gross BH, Kazerooni EA, Toews GB, Long Q, Murray S, Lama VN, Gay SE, Martinez FJ. Idiopathic interstitial pneumonia: What is the effect of a multidisciplinary approach to diagnosis? Am J Respir Crit Care Med. 2004; 170:904-910.
27. Raghu G, Collard HR, Egan JJ, *et al*. An Official ATS/ERS/JRS/ALAT Statement: Idiopathic Pulmonary Fibrosis: Evidence-based Guidelines for Diagnosis and Management. Am J Respir Crit Care Med. 2011; 183:788-824.
28. Chen YY, Fan XM. Advances in the treatment of IPF

with Traditional Chinese Medicine. Chinese Journal of Clinicians (Electronic Edition). 2010; 4:130-132. (in Chinese)
29. Lu SJ, Liu YN, Wang HZ. Study of the protective function of salvia miltiorrhiza on bleomycin-induced pulmonary fibrosis in a rat model. Chinese Journal of Clinical Pharmacology and Therapeutics. 2005; 10:514-517. (in Chinese)
30. Jiang Y, Li W, Chen M. Effect of ligustrazine on CTGF expression and collagen deposition in rats with pulmonary fibrosis. Chinese Journal of General Practice. 2008; 6:1215-1216. (in Chinese)
31. Chai WS, Li YC, Wang HX, Guo M, Li YQ. The effects of Angelica sinensis on pulmonary fibrosis in rats. Chinese Pharmacological Bulletin. 2003; 19: 819-822. (in Chinese)
32. Guo GS, Wang H. Effects of Angelica sinensis on fibrosis in rats with bleomycin-induced pulmonary fibrosis. Medical Journal of Wuhan University. 2007; 28:560-563. (in Chinese)
33. Peng Q, Xin JB, Su LP, Xiang F, Ma WL, Wang F. Effect of Radix astragali on expression of MMP-2 and TIMP-1 in rats with pulmonary fibrosis. ACTA MED Univ Sci Technol Huazhong. 2007; 36:35-37. (in Chinese)
34. Quan Y, Xia QM, Li FX, Li HY, Zhang DT. Effects of Panax notoginseng on pulmonary fibrosis and CTGF expression in rats. Medical Journal of National Defending Forces in Southwest China. 2009; 19:23-25. (in Chinese)
35. Huang YM, Chen XL, Xu N, Liu W, Qi J, Liu JH, Ai J. Effects of Ginkgo biloba extract on the expression of connective tissue growth factor in the initial stage of fibrosis in lungs of rats. Chinese Journal of Pathophysiology. 2007; 23:1130-1132. (in Chinese)
36. Xu ZX, Ding MQ, Chen R, Wang P, Fei Y, Liu X. Effects of Ginkgo biloba extract on apoptotic changes in AT-II cells in rats with bleomycin-induced pulmonary fibrosis. Liaoning Journal of Traditional Chinese Medicine. 2008; 35:1312-1314. (in Chinese)
37. Chen YF, Xi JZ, Gao CY, Liu JY. The effects of tetrandrine on the calmodulin system in rats with pulmonary fibrosis. Journal of Xinxiang Medical College. 2003; 20:83-85. (in Chinese)
38. Ren YX, Zhou R, Tang W, Wang WH, Li YC, Yang YF, Zuo JP. (5R)-5-hydroxytriptolide (LLDT-8) protects against bleomycin-induced lung fibrosis in mice. Acta Pharmocol Sin. 2007; 28:518-525.
39. Yao L, Sheng L, Li DS, Ma Z. The effects of adenophora stricta on the expression of TGF-β1 and TNF-α in pulmonary fibrosis of rats. Traditional Chinese Medicinal Research. 2007; 20:20-22. (in Chinese)
40. Chen M, Cheung FW, Chan MH, Hui PK, Ip SP, Ling YH, Che CT, Liu WK. Protective roles of Cordyceps on lung fibrosis in cellular and rat models. J Ethnopharmacol. 2012; 143:448-454.
41. Gao W, Zhang DP, Hua YF. Effect of curcumin on proliferation and apoptosis of pulmonary fibroblasts. The Journal of Practical Medicine. 2008; 24:2393-2396. (in Chinese)
42. Chen XY. Oxygen therapy and care for 5 patients with idiopathic pulmonary fibrosis. Journal of Clinical Pulmonary Medicine. 2010; 15:1213-1213. (in Chinese)
43. Toronto Lung Transplant Group. Unilateral lung transplantation for pulmonary fibrosis. N Engl J Med. 1986; 314:1140-1145.
44. Christie JD, Edwards LB, Aurora P, Dobbels F, Kirk R, Rahmel AO, Stehlik J, Taylor DO, Kucheryavaya AY, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: Twenty-sixth Official Adult Lung and Heart-Lung Transplantation Report-2009. J Heart Lung Transplant. 2009; 28:1031-1049.
45. Liu D, Mao WJ, Chen JY. A single center's experience with lung transplantation for idiopathic pulmonary fibrosis. Chinese Academic Conference on Organ Transplantation. 2012; 25:213-213. (in Chinese)
46. Elicker BM, Golden JA, Ordovas KG, Leard L, Golden TR, Hays SR. Progression of native lung fibrosis in lung transplant recipients with idiopathic pulmonary fibrosis. Respir Med. 2010; 104:426-433.
47. Mao W, Chen J, Zheng M, Wu B, Zhu Y. Initial experience of lung transplantation at a single center in China. Transplant Proc. 2013; 45:349-355.

(Received July 2 2013; Reviesed July 29, 2013;Accepted August 8, 2013)

Review

DOI: 110.5582/irdr.2013.v2.3.94

The current clinical aspects of idiopathic portal hypertension

Tomohiro Tanaka[1], Yasuhiko Sugawara[2,*], Norihiro Kokudo[2]

[1] *Organ Transplantation Service, The University of Tokyo Hospital, Tokyo, Japan;*
[2] *Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan.*

Summary **Idiopathic portal hypertension (IPH) comprises disorders developing increased portal pressure in the absence of cirrhosis: the clear mechanisms to explain this disease are still not well recognized. IPH usually suggests a benign prognosis, but sometimes is complicated with severe hemorrhage due to ruptured esophageal varices, or massive splenomegaly. Conventional treatments for those complications for patients with cirrhosis usually works when diverted to patients with IPH, although some of those patients might require liver transplantation if the treatment fails. However, there are few consistent treatment strategies for IPH itself, its complications or the indications for liver transplantation. In this mini review, we summarize the clinical manifestations and several potential theories to explain the etiology, as well as the current treatment options for IPH.**

Keywords: Idiopathic portal hypertension (IPH), non-cirrhotic portal fibrosis, nodular regenerative hyperplasia (NRH), Idiopathic noncirrhotic portal hypertension

1. Introduction

Portal hypertension is a clinical manifestation which is defined as the presense of a porto-caval venous pressure gradient > 5 mmHg (*1*). The most common cause of portal hypertension is liver cirrhosis. There are, however, a variety of disorders which develop portal hypertension without cirrhosis: Idiopathic portal hypertension (IPH) is one of the most important etiologies of portal hypertension without cirrhosis. The name IPH was first proposed by Boyer by excluding liver cirrhosis from Banti syndrome in 1967 (*2*).

IPH is usually regarded as a disorder with feasible prognosis, and is mainly managed by supportive treatment such as endoscopic, radiological and/or surgical management for esophageal varices and/or splenomegaly. However, it has been reported that IPH sometimes leads to poor prognosis due to gastrointestinal hemorrhage and/or liver failure (*3*), which would result in liver transplantation or death.

**Address correspondence to:*
Dr. Yasuhiko Sugawara, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan.
E-mail: yasusuga-tky@umin.ac.jp

Importantly, the etiology and mechanisms to explain IPH are still uncertain. The main scope of this review is to provide an overview of IPH, including its potential etiologies, clinical manifestations and treatment options.

2. Terminology and Epidemiology

Japanese study groups usually use the term of "IPH", whereas Indian researchers prefer to use the term non-cirrhotic portal fibrosis for the name of this disorder. Groups in Western countries have proposed terms such as nodular regenerative hyperplasia (NRH), hepatoportal sclerosis (*4*), or Idiopathic noncirrhotic portal hypertension (*5*).

In contrast to its high prevalence in India, IPH is comparatively a rare disorder in Western countries (*6*). Presumably because of that reason, there are limited recent literatures published to investigate the epidemiology of IPH. Although the amount of literature quoting the epidemiology of this disease remains little, slight male predominance is reported (*7*).

3. Etiology

The definite etiology of IPH is still uncertain, although there are several theories on the potential pathogenesis of IPH. These theories include immunological

disorders, infections, and genetic variants. There is another potential theory that IPH is associated with prothrombotic disorders ("Thrombosis theory") (*8,9*), but this theory is very controversial and still needs to be investigated: however, the pathogenesis of IPH seems to be multifactorial.

3.1. *Immunological disorders*

Disease in patients with IPH is sometimes complicated by progressive systemic sclerosis (PSS), systemic lupus erythematosus (SLE) and/or mixed connective tissue disease (MCTD), and hyper-gammaglobulinemia and autoantibodies are frequently seen which suggests underlying autoimmunological disorders in IPH (*10-12*). It has been reported that the strong expression of HLA-DR or VCMA-1 in endothelial cells of portal branches were seen in patients with IPH, which suggests the vascular endothelial cell could be the target of autoimmunity (*13,14*).

3.2. *Infections*

Chronic exposure to antigenemia of intestinal origin causes inflammatory reactions in portal tracts, which may trigger the histopathological changes that eventually result in IPH. This theory could be supported by the fact that IPH is frequently seen in patients from comparatively low socioeconomic areas, because abdominal infection at birth or in early childhood may play an important role to establish this potential mechanism (*15*). In addition, injection of Escherichia coli into the portal vein in an animal model was reported to contribute to the development of IPH-like symptoms (*16*).

3.3. *Genetic Disorders*

There are several reports mentioning that patients with IPH sometimes are observed with familial aggregation (*17,18*) or among a group of patients with congenital disorders such as "Adams-Oliver" syndrome (*19,20*) or Turner's syndrome (*21*). This fact suggests that IPH could be associated with a potential genetic background.

4. Diagnosis and clinical manifestations

The diagnosis of IPH is a challenge, as there are no guidelines or consensus on the diagnosis of this disease, so far. Difficulties also are related to the fact that this disease is rarely seen in daily practice, and physicians who first see portal hypertension usually suspect underlying liver cirrhosis (*22*).

Patients might present with episodes such as gastrointestinal bleeding (esophageal varices) and/or left upper quadrant discomfort/pain (splenomegaly). It is rare to see significant ascites at the initial presentation even in the context of developed portal hypertension. Those patients could have abnormal liver tests, and undergo ultrasonography. The findings of ultrasonography for patients with IPH are characterized by a thick wall of the portal vein and a nodular liver surface (*23-25*), which is, however, frequently seen in those with liver cirrhosis as well. Differentiation between IPH and liver cirrhosis seems difficult without liver biopsy. Recent reports suggested the usefulness of transient elastgraphy as a potencial surrogate of liver biopsy by estimating liver stiffness (*5*).

As for the prognosis of IPH, it has been considered to be a benign disorder as long as the main complications such as esophageal varices and hypersplenism are successfully controlled, mainly because of preserved liver synthetic functions. The 5-year death rate was reported to be close to 0% (*6*), however, a more recent study showed an inferior survival rate (78% survival rate at 5 years) compared to the general population (*26*). The mortality due to acute hemorrhage in IPH is significantly lower than that observed in those with liver cirrhosis (*8,27,28*), however, the management of esophageal varices is one of the most important factors in following those with IPH, as variceral bleeding is the dominant cause of death in these patients (*29*). Once the patients with IPH develop ascitis, the prognosis is reported to be poor (*26*). As for the predictive factors of survival of patients with IPH, Eapen *et al*. recently has reported that older age at first presentation with IPH, hepatic encephalopathy, and portal vein thrombosis were associated with reduced transplant-free survival *(30*). Several reports have been published mentioning liver transplantation in patients with IPH: the indications were unsuccessful treatment for portal hypertension, hepatic encephalopathy, hepatopulmonary syndrome, and/or liver failure (*22,31*).

5. Pathological findings of IPH

The liver is often atrophic due to drop off of the peripheral liver. Those findings are usually seen in the late stage of IPH, not in the early stage. Along with liver atrophy, several stages of portal sclerosis could appear. Portal vein or its medium/large branch could be dilated or thickened. Because of the abnormal intrahepatic blood flow, hyperplastic nodules such as nodular regenerative hyperplasia (NRH) or partial nodular transformation (PNT) are often seen. At autopsy, obliterating thrombosis in medium to large portal branches is extensively detected. Based on these findings, Nakamura *et al*. proposed a staging system for IPH which is graded from 1 to 4 according to the degree of disease progression based on the histopathological findings (*32*).

6. Treatment options

As noted above, the main complications of IPH are

represented as esophageal varices and hypersplenism.

6.1. *Esophageal varices, gastric varices and portal hypersentive gastropathy*

Most of (90%) the patients with IPH develop esophageal varices (*33*). The esophageal varices in IPH patients are characterized by several specific features. As the wall of the variceal vein (as well as portal vein) is thicker than the varices seen in cirrhotic patients, the varices rarely show a red-color sign. The rate of rupture should be less in IPH patients than cirrhotic patients. Firm evidence of treatment of esophageal varices in IPH patients is limited. Endoscopic sclerotherapy was reported to be effective in controlling ruptured esophageal varices (*34*). Endoscopic variceal ligation (EVL) is not recognized as the standard of care in this setting due to lack of scientific evidence so far, although it is widely applied in the clinical situation because of the proven superiority of EVL to sclerotherapy in controlling esophageal varices of cirrhotic patients (*35*).

Portal hypertension in IPH patients, again, is characterized by the preserved liver synthetic function, and the mechanisms of portal hypertension in cirrhotic patients are different because of a liver that remains functioning: hyper-dynamic mesenteric circulation and imbalance in vasoactive mediators should not be observed in IPH. This means that conventional medical treatment for cirrhotic patients such as beta-blockers (*36*) and angiotensin II receptor antagonists (*37*) should not be applied equally to patients with IPH due to this mechanism difference. There is one small study which reported the inferiority of medical therapy to endoscopic ligation for the prevention of rebleeding in those with IPH (*38*).

Gastric varices and portal hypertensive gastropathy are (PHG) less frequently seen in IPH patients than esophageal varices. Gastric varices was reported to be found in one fourth of the Indian IPH patiens (*39*). In patients with liver cirrhosis, nonselective beta-blockers have been shown to suppress blood flow of gastric mucosa, and seems effective in preventing recurrent bleeding from PHG (*40*).

6.2. *Hypersplenism*

Pancytopenia and severe hypersplenism is now considered as one of the most important indications for splenectomy or partial splenic embolizationin (PSE), because those therapies have been demonstrated to decrease portal hypertension in these patients (*41, 42*).

7. Conclusion

IPH is a heterogenous disorder with varying clinical pictures, including (a)symptomatic splenomegaly and variceal bleeding. The etiology IPH still needs to be clarified, and there is no consensus on treatment options for IPH itself or complications such as variceal bleeding or massive splenomegaly. The patients have a relatively well-preserved liver function, but further investigation of its etiology and more detailed clinical characterization is warranted, because some group of patients with IPH may develop into a critical prognosis which might require liver transplantation.

References

1. Sanyal AJ, Bosch J, Blei A, Arroyo V. Portal hypertension and its complications. Gastroenterology. 2008; 134:1715-1728.
2. Boyer JL, Sen Gupta KP, Biswas SK, Pal NC, Basu Mallick KC, Iber FL, Basu AK. Idiopathic portal hypertension. Comparison with the portal hypertension of cirrhosis and extrahepatic portal vein obstruction. Ann Intern Med. 1967; 66:41-68.
3. Sawada S, Sato Y, Aoyama H, Harada K, Nakanuma Y. Pathological study of idiopathic portal hypertension with an emphasis on cause of death based on records of Annuals of Pathological Autopsy Cases in Japan. J Gastroenterol Hepatol. 2007; 22:204-209.
4. Mikkelsen WP, Edmondson HA, Peters RL, Redeker AG, Reynolds TB. Extra- and intrahepatic portal hypertension without cirrhosis (hepatoportal sclerosis). Ann Surg. 1965; 162:602-620.
5. Schouten JN, Garcia-Pagan JC, Valla DC, Janssen HL. Idiopathic noncirrhotic portal hypertension. Hepatology. 2011; 54:1071-1081.
6. Sarin SK, Kapoor D. Non-cirrhotic portal fibrosis: Current concepts and management. J Gastroenterol Hepatol. 2002; 17:526-534.
7. Sama SK, Bhargava S, Nath NG, Talwar JR, Nayak NC, Tandon BN, Wig KL. Noncirrhotic portal fibrosis. Am J Med. 1971; 51:160-169.
8. Hillaire S, Bonte E, Denninger MH, Casadevall N, Cadranel JF, Lebrec D, Valla D, Degott C. Idiopathic non-cirrhotic intrahepatic portal hypertension in the West: A re-evaluation in 28 patients. Gut. 2002; 51:275-280.
9. Matsutani S, Maruyama H, Akiike T, Kobayashi S, Yoshizumi H, Okugawa H, Fukuzawa T, Kimura K, Saisho H. Study of portal vein thrombosis in patients with idiopathic portal hypertension in Japan. Liver Int. 2005; 25:978-983.
10. Nakanuma Y, Nonomura A, Hayashi M, *et al*. Pathology of the liver in "idiopathic portal hypertension" associated with autoimmune disease. The Ministry of Health and Welfare Disorders of Portal Circulation Research Committee. Acta Pathol Jpn. 1989; 39:586-592.
11. Saito K, Nakanuma Y, Takegoshi K, Ohta G, Obata Y, Okuda K, Kameda H. Non-specific immunological abnormalities and association of autoimmune diseases in idiopathic portal hypertension. A study by questionnaire. Hepatogastroenterology. 1993; 40:163-166.
12. Tsuneyama K, Harada K, Katayanagi K, Watanabe K, Kurumaya H, Minato H, Nakanuma Y. Overlap of idiopathic portal hypertension and scleroderma: Report of two autopsy cases and a review of literature. J Gastroenterol Hepatol. 2002; 17:217-223.
13. Terada T, Nakanuma Y, Obata H. HLA-DR expression on the microvasculature of portal tracts in idiopathic portal

hypertension. Immunohistochemical characteristics and relation to portal phlebosclerosis. Arch Pathol Lab Med. 1991; 115:993-997.
14. Yamaguchi N, Tokushige K, Haruta I, Yamauchi K, Hayashi N. Analysis of adhesion molecules in patients with idiopathic portal hypertension. J Gastroenterol Hepatol. 1999; 14:364-369.
15. Sarin SK. Non-cirrhotic portal fibrosis. Gut. 1989; 30:406-415.
16. Kono K, Ohnishi K, Omata M, Saito M, Nakayama T, Hatano H, Nakajima Y, Sugita S, Okuda K. Experimental portal fibrosis produced by intraportal injection of killed nonpathogenic Escherichia coli in rabbits. Gastroenterology. 1988; 94:787-796.
17. Sarin SK, Mehra NK, Agarwal A, Malhotra V, Anand BS, Taneja V. Familial aggregation in noncirrhotic portal fibrosis: A report of four families. Am J Gastroenterol. 1987; 82:1130-1133.
18. Barnett JL, Appelman HD, Moseley RH. A familial form of incomplete septal cirrhosis. Gastroenterology. 1992; 102:674-678.
19. Schwarzler P, Carvalho JS, Senat MV, Masroor T, Campbell S, Ville Y. Screening for fetal aneuploidies and fetal cardiac abnormalities by nuchal translucency thickness measurement at 10-14 weeks of gestation as part of routine antenatal care in an unselected population. Br J Obstet Gynaecol. 1999; 106:1029-1034.
20. Girard M, Amiel J, Fabre M, Pariente D, Lyonnet S, Jacquemin E. Adams-Oliver syndrome and hepatoportal sclerosis: Occasional association or common mechanism? Am J Med Genet A. 2005; 135:186-189.
21. Roulot D, Degott C, Chazouillères O, Oberti F, Calès P, Carbonell N, Benferhat S, Bresson-Hadni S, Valla D. Vascular involvement of the liver in Turner's syndrome. Hepatology. 2004; 39:239-247.
22. Krasinskas AM, Eghtesad B, Kamath PS, Demetris AJ, Abraham SC. Liver transplantation for severe intrahepatic noncirrhotic portal hypertension. Liver Transpl. 2005; 11:627-634; discussion 610-611.
23. Dhiman RK, Chawla Y, Vasishta RK, Kakkar N, Dilawari JB, Trehan MS, Puri P, Mitra SK, Suri S. Non-cirrhotic portal fibrosis (idiopathic portal hypertension): Experience with 151 patients and a review of the literature. J Gastroenterol Hepatol. 2002; 17:6-16.
24. Nakanuma Y, Hoso M, Sasaki M, Terada T, Katayanagi K, Nonomura A, Kurumaya H, Harada A, Obata H. Histopathology of the liver in non-cirrhotic portal hypertension of unknown aetiology. Histopathology. 1996; 28:195-204.
25. Ludwig J, Hashimoto E, Obata H, Baldus WP. Idiopathic portal hypertension; A histopathological study of 26 Japanese cases. Histopathology. 1993; 22:227-234.
26. Schouten JN, Nevens F, Hansen B, Laleman W, van den Born M, Komuta M, Roskams T, Verheij J, Janssen HL. Idiopathic noncirrhotic portal hypertension is associated with poor survival: Results of a long-term cohort study. Aliment Pharmacol Ther. 2012; 35:1424-1433.
27. Okuda K, Kono K, Ohnishi K, *et al*. Clinical study of eighty-six cases of idiopathic portal hypertension and comparison with cirrhosis with splenomegaly. Gastroenterology. 1984; 86:600-610.
28. Nevens F, Staessen D, Sciot R, Van Damme B, Desmet V, Fevery J, De Groote J, Van Steenbergen W. Clinical aspects of incomplete septal cirrhosis in comparison with macronodular cirrhosis. Gastroenterology. 1994; 106:459-463.
29. Ichimura S, Sasaki R, Takemura Y, Iwata H, Obata H, Okuda H, Imai F. The prognosis of idiopathic portal hypertension in Japan. Intern Med. 1993; 32:441-444.
30. Eapen CE, Nightingale P, Hubscher SG, Lane PJ, Plant T, Velissaris D, Elias E. Non-cirrhotic intrahepatic portal hypertension: Associated gut diseases and prognostic factors. Dig Dis Sci. 2011; 56:227-235.
31. Cazals-Hatem D, Hillaire S, Rudler M, Plessier A, Paradis V, Condat B, Francoz C, Denninger MH, Durand F, Bedossa P, Valla DC. Obliterative portal venopathy: Portal hypertension is not always present at diagnosis. J Hepatol. 2011; 54:455-461.
32. Nakanuma Y, Tsuneyama K, Ohbu M, Katayanagi K. Pathology and pathogenesis of idiopathic portal hypertension with an emphasis on the liver. Pathol Res Pract. 2001; 197:65-76.
33. Sarin SK, Aggarwal SR. Idiopathic portal hypertension. Digestion. 1998; 59:420-423.
34. Chawla YK, Dilawari JB, Dhiman RK, Goenka MK, Bhasin DK, Kochhar R, Singh K, Kaur U. Sclerotherapy in noncirrhotic portal fibrosis. Dig Dis Sci. 1997; 42:1449-1453.
35. de Franchis R; Baveno V Faculty. Revising consensus in portal hypertension: Report of the Baveno V consensus workshop on methodology of diagnosis and therapy in portal hypertension. J Hepatol. 2010; 53:762-768.
36. Bari K, Garcia-Tsao G. Treatment of portal hypertension. World J Gastroenterol. 2012; 18:1166-1175.
37. Segawa M, Sakaida I. Diagnosis and treatment of portal hypertension. Hepatol Res. 2009; 39:1039-1043.
38. Sarin SK, Wadhawan M, Gupta R, Shahi H. Evaluation of endoscopic variceal ligation (EVL) versus propanolol plus isosorbide mononitrate/nadolol (ISMN) in the prevention of variceal rebleeding: Comparison of cirrhotic and noncirrhotic patients. Dig Dis Sci. 2005; 50:1538-1547.
39. Sarin SK, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: A long-term follow-up study in 568 portal hypertension patients. Hepatology. 1992; 16:1343-1349.
40. Hosking SW, Kennedy HJ, Seddon I, Triger DR. The role of propranolol in congestive gastropathy of portal hypertension. Hepatology. 1987; 7:437-441.
41. Hirota S, Ichikawa S, Matsumoto S, Motohara T, Fukuda T, Yoshikawa T. Interventional radiologic treatment for idiopathic portal hypertension. Cardiovasc Intervent Radiol. 1999; 22:311-314.
42. Yoshida H, Mamada Y, Taniai N, Tajiri T. New trends in surgical treatment for portal hypertension. Hepatol Res. 2009; 39:1044-1051.

(Received July 1 2013; Reviesed July 24, 2013;Accepted August 26, 2013)

Brief Report

DOI: 110.5582/irdr.2013.v2.3.98

In vitro culture and characterization of enteric neural precursor cells from human gut biopsy specimens using polymer scaffold

Janardhanam Krishnamohan[1,2], Venugopal S Senthilnathan[3], Tirunelveli Muthiah Vaikundaraman[4], Thangavelu Srinivasan[5], Madasamy Balamurugan[6], Masaru Iwasaki[7], Senthilkumar Preethy[5,8], Samuel JK Abraham[4,7,*]

[1] *Department of Paediatric Surgery, Madras Medical College, E.V.R Periyar Salai, Park Town, Chennai, India;*
[2] *Department of Paediatric Surgery, Government Kasturba Gandhi Hospital, Triplicane, Chennai, India;*
[3] *Department of Paediatric Surgery, Institute of Child Health & Hospital for Children, Egmore, Chennai, India;*
[4] *The Mary-Yoshio Translational Hexagon (MYTH), Nichi-In Centre for Regenerative Medicine (NCRM), Nungambakkam, Chennai, India;*
[5] *The Fujio–Eiji Academic Terrain (FEAT), Nichi-In Centre for Regenerative Medicine (NCRM), Nungambakkam, Chennai, India;*
[6] *Ruma Biotherapy Research Centre, Alwarpet, Chennai, India;*
[7] *Yamanashi University- School of Medicine, Chuo, Yamanashi, Japan;*
[8] *Hope Foundation (Trust), Choolaimedu, Chennai, India.*

Summary ***In vitro*** **expansion and characterization of neural precursor cells from human gut biopsy specimens with or without Hirschsprung's disease using a novel thermoreversible gelation polymer (TGP) is reported aiming at a possible future treatment. Gut biopsy samples were obtained from five patients undergoing gut resection for Hirschsprung's disease (*n* = 1) or gastrointestinal disorders (*n* = 4). Cells isolated from the smooth muscle layer and the myenteric plexus were cultured in two groups for 18 to 28 days; Group I: conventional culture as earlier reported and Group II: using TGP scaffold. Neurosphere like bodies (NLBs) were observed in the cultures between 8th to 12th day and H & E staining was positive for neural cells in both groups including aganglionic gut portion from the Hirschsprung's disease patient. Immunohistochemistry using S-100 and neuron specific enolase (NSE) was positive in both groups but the TGP group (Group II) showed more number of cells with intense cytoplasmic granular positivity for both NSE and S-100 compared to Group I. TGP supports the *in vitro* expansion of human gut derived neuronal cells with seemingly better quality NLBs. Animal Studies can be tried to validate their functional outcome by transplanting the NLBs with TGP scaffolds to see whether this can enhance the outcome of cell based therapies for Hirschsprung's disease.**

Keywords: Enteric neural precursor cells, Hirschsprung's disease, thermoreversible gelation polymer (TGP)

1. Introduction

The part of the peripheral nervous system (PNS) that controls the peristaltic activity of the gut wall is the enteric nervous system (ENS). This is essential for propulsion of food in the digestive tract. The ENS is composed of a large number of neurons and glial cells, distributed throughout the length of the gut. These ganglion cells develop from the neural crest in the embryo. Hirschsprung's disease or congenital megacolon is the failure or delay of the complete colonization of the gut by these enteric neural crest cells during early development which results in the absence of ganglia or neurons in a portion of the gut, usually the colon, that results in aperistalsis and severe intestinal obstruction. Hirschsprung's disease affects

**Address correspondence to:*
Dr. Samuel JK Abraham, The Mary-Yoshio Translational Hexagon (MYTH), Nichi-In Centre for Regenerative Medicine (NCRM), PB 1262, Nungambakkam, Chennai – 600034, Tamil Nadu. India.
E-mail: drsam@nichimail.jp
Alternate E-mail: drspp@nichimail.jp

1 in 5,000 newborns and affects boys more often than girls at a ratio of 4:1 (*1-3*). It appears either sporadically or has a familial basis and is often associated with other developmental defects. Surgical management continues to be the major treatment approach. The principle involves reconstruction of the intestinal tract by pulling the normal innervated portion of the colon down to the anus preserving the sphincter function. This pull-through surgery's recent variants include total transanal endorectal pull-through (TERPT) and the laparoscopic assisted pull-through (*3*). Inspite of advances in surgical approach, constipation, abdominal distension and enterocolitis are some of the long term obstructive symptoms observed in patients who undergo operative modalities for Hirschsprung's Disease (*3*). The extent of aganglionosis varies between patients and there are reports in the literature in which there are extreme forms of aganglionosis in which even a complete portion of the intestine is devoid of the ganglion cells (*4*). In such cases, surgery is impossible. At this juncture, cell-based therapies to replace the ganglion cells or enteric neuronal cells in the aganglionic portion of the gut aiming at restoring the function of the gut are being considered as a potential solution to Hirschsprung's disease (*5,6*). Earlier studies have reported the isolation of enteric neural precursor cells from normal gut tissues and Hirschsprung's disease affected gut tissues (*5,7*). In this study, we report *in vitro* culture and characterization of enteric neural precursor cells from full thickness gut biopsy samples of patients with or without Hirschsprung's disease in a novel polymer scaffold.

2. Materials and Methods

Postnatal gut full thickness 2-4 mm biopsy samples were obtained from five patients undergoing gut resection surgery after proper informed consent. The work was conducted in accordance with the Declaration of Helsinki (1964). Of the five patients, one had Hirschsprung's disease (Patient I). The remaining four patients (Patient II-V) underwent gut resection surgery for conditions like biliary atresia and exomphalos major with rectal atresia. From patient I, biopsy samples from both the ganglionic portions and the aganglionic portions of the gut were obtained. All samples were washed in phosphate buffered saline (PBS) containing penicillin (100 U/mL), streptomycin (100 µg/mL) and amphotericin (100 U/mL). Using forceps, the outer smooth muscle layers along with the myenteric plexus were peeled off from the underlying tissue as strips. Fibronectin (2 µg/cm^2) (Sigma-Aldrich, USA) was coated on 6-well tissue culture (TC) Plates (Corning Inc., Corning, NY, USA) and the plates were undisturbed for at least two hours. The tissue strips were washed with phosphate buffered saline (PBS) Ca^{2+} and Mg^{2+} free and cut into small pieces for enzymatic digestion using collagenase (1 mg/mL) and dispase (1 mg/mL) and kept in a CO_2 incubator at 37°C for 30 min. Digested tissues were filtered using a 70 µm filter and centrifuged at 1,800 rpm for 8 min at 24°C. Cell count of the pellet was obtained using the Trypan blue dye exclusion method and the cells were divided into two equal portions. The cells thus divided were seeded as two groups, Group I (Gr.I): in DMEM/F12 (Gibco BRL, Gaithersburg, MD, USA) medium supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), L-glutamine (2 mmol/L), and growth factors including basic fibroblast growth factor (bFGF) (20 ng/mL) (Sigma-Aldrich, USA) and epidermal growth factor (EGF) (20 ng/mL) (Sigma-Aldrich, USA) onto the fibronectin coated TC plates according to the protocol reported by Bondurand *et al.* (*2*); Group II (Gr.II): with thermoreversible gelation polymer (TGP). TGP was obtained in a lyophilized vial from Nichi-In Biosciences (P) Ltd, Chennai, India. The Thermo-reversible Gelation Polymer (TGP) used in this study is a copolymer composed of thermo-responsive polymer block [poly(*N*-isopropylacrylamide-co-n-butyl methacrylate) (poly(NIPAAm-co-BMA)] and the hydrophilic polymer block (polyethylene glycol [PEG]) as described by Yoshioka *et al.* (*8*). Because this polymer block is hydrophilic at temperatures below the sol-gel transition temperature (20°C for the TGP used in this study) and hydrophobic at temperatures above this sol-gel transition temperature forming a homogenous three-dimensional (3D) network of the gel in water, cells for culture can be embedded at temperatures lower than 20°C and cultured three dimensionally in the hydrogel state at 37°C. For the present study, the TGP was reconstituted with 10 mL of DMEM/F12 medium and incubated at 4°C overnight. A drop of TGP-DMEM tissue culture (TC) medium mixture was placed at the center of the 6-well Fibronectin coated TC Plates (Corning Inc., Corning, NY) and solidified at 37°C. The cells from the remaining pellet were suspended in the culture medium and placed over this solidified gel-TC mixture after which a drop of the gel-TC medium mixture was again placed to cover the cells. Thus, the cells were embedded within the TGP. Culture medium containing DMEM/F12 medium supplemented with penicillin (100 U/mL), streptomycin (100 µg/mL), L-glutamine (2 mmol/L), bFGF (20 ng/mL) and EGF (20 ng/mL) was overlaid over the TGP. Cells were incubated at 37˚C with 5% CO_2 for 18-28 days. Cells were observed daily and a media change was done every 2-3 days.

3. Results and Discussion

The average cell number obtained from the ganglionic samples was 0.94 million cells. The cell number obtained from the aganglionic portion of Patient I was 0.19 million cells. In all samples (both Gr.I and Gr.II),

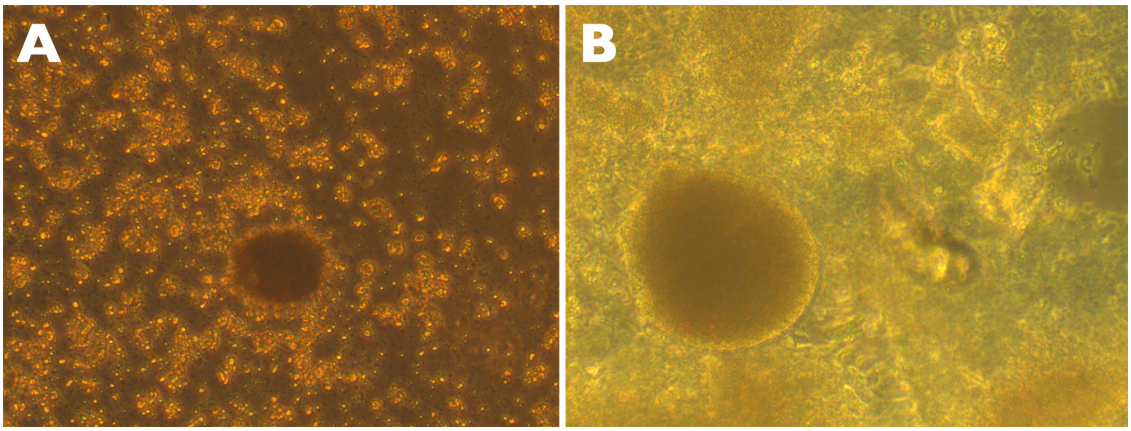


Figure 1. Neurosphere like body (NLB) observed. (A) Group I (Conventional). **(B)** Group II (TGP).

neurosphere like bodies (NLBs) were observed in culture between the 8th day and 12th day (Figure 1) including the aganglionic sample from Patient I with Hirschsprung's disease. The neurosphere like bodies (NLBs) observed in the culture were then subjected to histological and immunohistochemical (IHC) studies for S-100 and neuron specific enolase (NSE), a neuronal specific marker. H&E staining showed that both ganglionic and aganglionic samples in both Gr.I and Gr.II had round to oval cells with a large nucleus and moderate amount of pale cytoplasm. The cell size varied greatly in all the smears. These cells did not form sheets or any other specific pattern. They were mostly loose clusters and vague, short filamentous extensions were seen from the cytoplasmic borders. H&E stains were positive for neural cells (Figure 2). In immunohistochemistry (IHC), morphologically there was no difference in cultured cells from ganglionic and aganglionic segments in Gr.I and Gr.II. However, the cellularity was relatively higher in Gr.II (TGP group). Though both groups showed a cytoplasmic granular appearance, the TGP group (Gr.II) showed more number of cells with intense cytoplasmic granular positivity for both NSE and S-100 (Figure 3B, 4B) compared to Gr.I (Figure 3A, 4A).

Earlier studies have reported the generation of NLBs containing functionally active neural progenitors from gut tissue (*1,9,10*). Almond *et al*. isolated and expanded progenitor/stem cells from the post-coitum embryonic mouse cecum and postnatal human myenteric plexus and successfully transplanted the differentiated neurons and glial cells into aganglionic murine hindgut. The implanted cells colonized postnatal aganglionic bowel and expressed neuronal markers including nitric oxide synthase and vasoactive intestinal polypeptide (*11*). Metzger *et al.* generated NLBs from postnatal human gut mucosal tissue and after transplantation; the cells from NLBs colonized aganglionic chick and human hindgut to generate ganglia-like structures, enteric neurons and glia (*1*). In this study, we have examined the feasibility of culturing NLBs obtained from routine

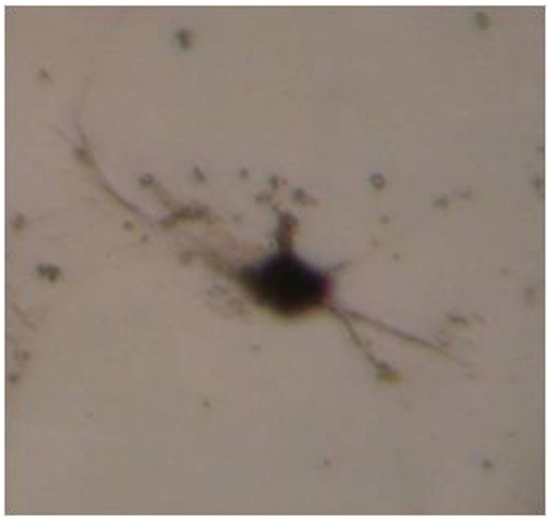


Figure 2. H&E staining of cultured Neurosphere like bodies (NLBs).

gut biopsy samples of patients undergoing surgeries for Hirschsprung's disease or other disorders of the gastrointestinal system in a Thermo-reversible Gelation Polymer (TGP) and compared it to conventional culture techniques. Though ENS progenitors from the ganglionic gut of children diagnosed with and without Hirschsprung's disease have been isolated, characterized and reported earlier (*12*), a study similar to ours using a TGP which has proven to yield an increased number of neural progenitors (*13*), makes this unique. Apart from this, among the various types of thermo-reversible hydrogels reported for cell culture in the literature, the TGP used in this study is novel because it is a purely synthetic hydrogel and does not contain any biological components like proteins such as Hen Egg White Lysozyme (*14*) or poly-saccharides such as Chitosan (*15*) which are used in hydrogels to improve properties like cell-adhesion (*14*). Further, the TGP has been proven to maintain the three-dimensional morphology (*16*) of different kinds of stem cells, pre-cursor cells and adult cells without alteration of their gene expression (*17*) for longer periods of time in contrast to other

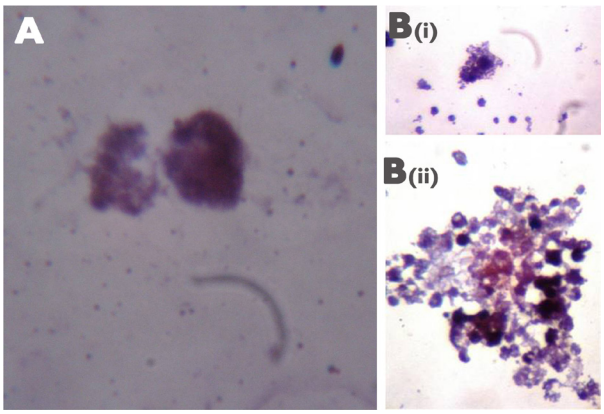


Figure 3. Neuron specific enolase (NSE) positive Neurosphere like bodies (NLBs). (A) Group I (Conventional). **(B(i)** and **B(ii))** Group II (TGP).

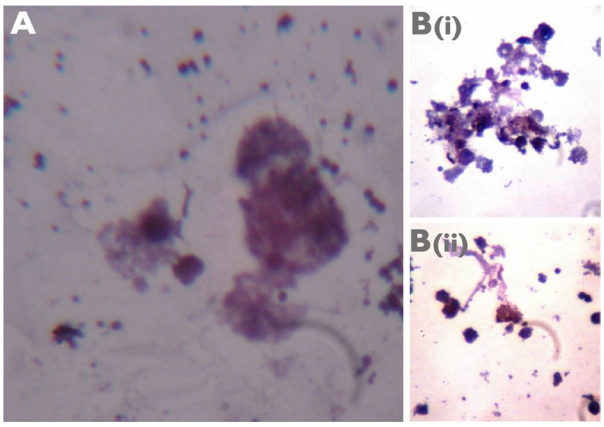


Figure 4. S-100 positive Neurosphere like bodies (NLBs). (A) Group I (Conventional). **(B(i)** and **B(ii))** Group II (TGP).

thermo-reversible hydrogels reported (*14*). Previous studies have suggested that from the 10th day onwards, NLBs can be observed in culture and NLBs have been grown in culture up to 28 days (*2,10*). In our study, the NLBs were observed in culture between the 8th day and 12th day in all samples in both Gr.I and II including that of the aganglionic tissue. Ability of the cells to generate NLBs in the aganglionic sample demonstrates the presence of neural progenitors in that portion of the gut also. However, further characterization and studies are required to identify whether the NLBs from the ganglionic portion and the aganglionic portion have similar characteristics and capability to colonize the aganglionic portion in animal models of Hirschsprung's disease. Furthermore, this would shed light upon the fact that perhaps neural crest progenitors do migrate to the distal colon but fail to proliferate or differentiate due to micro-environmental abnormalities in the distal colon (*3*) and due to *in vitro* culture conditions, they might form NLBs. The relatively higher cellular staining in Gr.II (TGP group) demonstrates that TGP supports growth of NLBs. Because transplantation of neurospheres from fetal and post natal intestine derived neural crest cells has already proven to produce functional neurons in the post natal colon of mice (*18*), further studies asking whether TGP can be used as a carrier for NLB transplantation in aganglionic gut models to help retain the neural progenitors in the region where they should re-colonize and form neural; glial cells are warranted. Suggesting TGP as a carrier for transplantation of NLBs is based upon earlier studies in which TGP was employed for transplantation of stem cells, progenitor cells and adult cells in animal models (*19-21*). The present study is only a preliminary study and extensive studies are warranted before this approach could be standardized and considered for treatment of Hirschsprung's disease.

4. Conclusion

We successfully isolated and expanded human enteric neural precursor cells in the form of NLBs from postnatal gut biopsy samples of patients with Hirschsprung's disease as well as other gastrointestinal disorders. Because the NLBs in the TGP group showed a higher positive in IHC staining compared to the group without TGP, there could be potential to utilize the NLBs cultured in TGP in cell-based therapies for Hirschsprung's disease, after confirming their efficacy in appropriate animal models. The transplantation of NLBs encapsulated in TGP or along with TGP is also another area for further experimentation because it might enhance the outcome, comparing it to earlier published studies.

Acknowledgements

The authors thank *1*) M/S Hope Foundation for funding the study. *2*) Loyola ICAM College of Engineering Technology (LICET) and Loyola Institute of Frontier Energy (LIFE) for their support of our research work. *3*) M/S Department of Clinical Research, Yamanashi University, Japan for their assistance with the publication of the manuscript.

References

1. Metzger M, Caldwell C, Barlow AJ, Burns AJ, Thapar N. Enteric nervous system stem cells derived from human gut mucosa for the treatment of aganglionic gut disorders. Gastroenterology. 2009; 136:2214-2225.e1-3.
2. Bondurand N, Natarajan D, Thapar N, Atkins C, Pachnis V. Neuron and glia generating progenitors of the mammalian enteric nervous system isolated from foetal and postnatal gut cultures. Development. 2003; 130:6387-6400.
3. Gunnarsdóttir A, Wester T. Modern treatment of Hirschsprung's disease. Scand J Surg. 2011; 100:243-249.
4. Berger S, Linke F, Heymanns M, Boor R, Coerdt W, Hofmann-v Kap-herr S. Complex long-segment intestinal dysganglionosis. J Pediatr Surg. 2000; 35:1123-1126.
5. Rauch U, Hänsgen A, Hagl C, Holland-Cunz S, Schäfer

KH. Isolation and cultivation of neuronal precursor cells from the developing human enteric nervous system as a tool for cell therapy in dysganglionosis. Int J Colorectal Dis. 2006; 21:554-559.
6. Gershon MD. Transplanting the enteric nervous system: A step closer to treatment for aganglionosis. Gut. 2007; 56:459-461.
7. Lindley R. Isolation, characterisation and transplantation of human enteric nervous system stem cells (Doctoral thesis). University of Liverpool, Liverpool, UK, 2011. *http://research-archive.liv.ac.uk/4933/* (Accessed July 15, 2013).
8. Yoshioka H, Mikami M, Mori Y, Tsuchida E. A synthetic hydrogel with thermoreversible gelation. I. Preparation and rheological properties. J Macromol Sci Pure Appl Chem. 1994; A31:113-120.
9. Schafer KH, Hagl CI, Rauch U. Differentiation of neurospheres from the enteric nervous system. Pediatr Surg Int. 2003; 19:340-344.
10. Lindley RM, Hawcutt DB, Connell MG, Edgar DH, Kenny SE. Properties of secondary and tertiary human enteric nervous system neurospheres. J Pediatr Surg. 2009; 44:1249-1255.
11. Almond S, Lindley RM, Kenny SE, Connell MG, Edgar DH. Characterisation and transplantation of enteric nervous system progenitor cells. Gut. 2007; 56:489-496.
12. Ruiz-Ferrer M, Torroglosa A, Núñez-Torres R, de Agustín JC, Antiñolo G, Borrego S. Expression of PROKR1 and PROKR2 in human enteric neural precursor cells and identification of sequence variants suggest a role in HSCR. PLoS One. 2011; 6:e23475.
13. Osanai T, Kuroda S, Yasuda H, Chiba Y, Maruichi K, Hokari M, Sugiyama T, Shichinohe H, Iwasaki Y. Noninvasive transplantation of bone marrow stromal cells for ischemic stroke: Preliminary study with a thermoreversible gelation polymer hydrogel. Neurosurgery. 2010; 66:1140-1147.
14. Yan H, Saiani A, Gough JE, Miller AF. Thermoreversible protein hydrogel as cell scaffold. Biomacromolecules. 2006; 7:2776-2782.
15. Chen JP, Cheng TH. Thermo-responsive chitosan-*graft*-poly(*N*-isopropylacrylamide) injectable hydrogel for cultivation of chondrocytes and meniscus cells. Macromol Biosci. 2006; 6:1026-1039.
16. Yasuda A, Kojima K, Tinsley KW, Yoshioka H, Mori Y, Vacanti CA. *In vitro* culture of chondrocytes in a novel thermoreversible gelation polymer scaffold containing growth factors. Tissue Eng. 2006; 12:1237-1245.
17. Kataoka K, Huh N. Application of a Thermo-Reversible Gelation Polymer, Mebiol Gel, for Stem Cell Culture and Regenerative Medicine. J Stem Cells Regen Med. 2010; 6:10-14.
18. Hotta R, Stamp LA, Foong JP, McConnell SN, Bergner AJ, Anderson RB, Enomoto H, Newgreen DF, Obermayr F, Furness JB, Young HM. Transplanted progenitors generate functional enteric neurons in the postnatal colon. J Clin Invest. 2013; 123:1182-1191.
19. Sitalakshmi G, Sudha B, Madhavan HN, Vinay S, Krishnakumar S, Mori Y, Yoshioka H, Abraham S. *Ex vivo* cultivation of corneal limbal epithelial cells in a thermoreversible polymer (Mebiol Gel) and their transplantation in rabbits: An animal model. Tissue Eng Part A. 2009; 15:407-415.
20. Parveen N, Khan AA, Baskar S, Habeeb MA, Babu R, Abraham S, Yoshioka H, Mori Y, Mohammed HC. Intraperitoneal Transplantation of Hepatocytes Embedded in Thermoreversible Gelation Polymer (Mebiol Gel) in Acute Liver Failure Rat Model. Hepatitis Monthly 2008; 8:275-280.
21. William JB, Prabhakaran R, Ayyappan S, Pushkinraj H, Rao D, Manjunath SR, Thamaraikannan P, Dedeepiya VD, Kuroda S, Yoshioka H, Mori Y, Preethy S, Abraham S. Functional recovery of spinal cord injury following application of intralesional bone marrow mononuclear cells embedded in polymer scaffold – Two year follow-up in a canine. J Stem cell Res Ther. 2011; 1:110. doi:10.4172/2157-7633.1000110.

(Received July 19, 2013; Reviesed August 9, 2013; Accepted August 12, 2013)

Commentary

DOI: 110.5582/irdr.2013.v2.3.103

Pelizaeus-Merzbacher disease: Molecular diagnosis and therapy

Jufeng Xia[1], Ling Wang[2,*]

[1] *Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai, China;*
[2] *Laboratory for Reproductive Immunology, Hospital & Institute of Obstetrics and Gynecology, Fudan University Shanghai Medical College, Shanghai, China.*

Summary **Chromosome Xq22.2 contains the entire proteolipid protein 1 gene (*PLP1*), and a genomic duplication in that chromosome is responsible for Pelizaeus–Merzbacher disease (PMD). Duplication can be detected using several molecular diagnostic methods such as comparative multiplex PCR, fluorescent in situ hybridization (FISH), restriction site polymorphism (RSP) analysis, and multiplex ligation-dependant probe amplification (MLPA). The characteristics of these methods should be taken into account when using them. There is currently no treatment for PMD, so a cure is urgently need. Advances in research on stem cell therapies, and especially induced pluripotent stem cell therapy, offer great promise for development of a treatment for PMD.**

Keywords: Pelizaeus-Merzbacher disease, *PLP1* gene, duplication, molecular diagnosis, iPS cell

Pelizaeus-Merzbacher disease (PMD) is an X-linked recessive disorder of the central nervous system (CNS) white matter in which coordination, motor ability, and intellectual function are delayed to a varying extent (*1*). In the US, PMD has an estimated prevalence of about 1/300,000 to 1/500,000, and in Germany PMD has a prevalence of 0.13 of every 10,000 live-born infants (*2*). In China, relevant epidemiological statistics are lacking despite the publication of numerous clinical case reports. In clinical settings, the diagnosis of PMD is often suggested when magnetic resonance imaging (MRI) scans reveal aberrant white matter (high T2 signal intensity, *i.e.* T2 lengthening) throughout the brain. This aberration is typically evident by approximately 1 year of age, but less prominent abnormalities should be evident in infancy. Unless there is a family history of sex-linked inheritance, the condition is often misdiagnosed as cerebral palsy.

PMD is caused by mutations in the proteolipid protein 1 (*PLP1*) gene on Xq22.2. *PLP1* mutations lead to a broad range of clinical syndromes from spastic paraplegia 2 (SPG2), which is characterized primarily by leg spasticity and weakness, to the most severe, connatal, form of PMD (*3*). Mutations, duplication, and deletion can all affect *PLP1* gene expression (*4*) (Figure 1). Small mutations account for about 30% of *PLP1* mutations while *PLP1* duplications account for

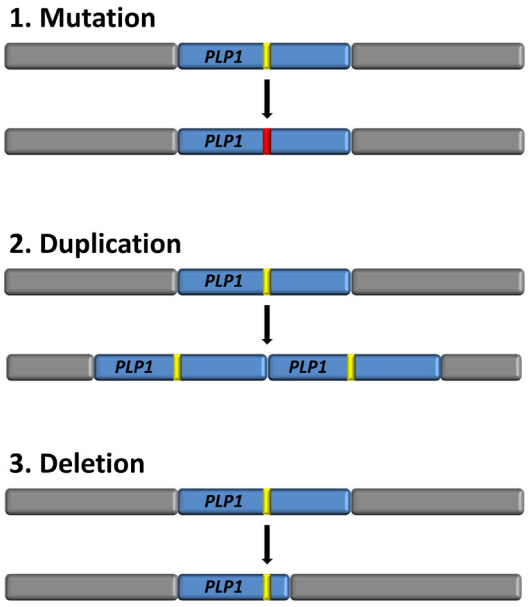


Figure 1. Pathogenetic mechanisms of PMD. There are three major forms of genetic abnormalities in PMD.

**Address correspondence to:*
Dr. Ling Wang, Laboratory for Reproductive Immunology, Hospital & Institute of Obstetrics and Gynecology, Fudan University Shanghai Medical College, 413 Zhaozhou Road, Shanghai 200011, China.
E-mail: dr.wangling@vip.163.com

Table 1. Methods for the detection of *PLP1* duplications

Method	Advantage	Disadvantage	*Ref.*
Comparative/real-time PCR	Duplications can be easily detected.	Results may be ambiguous in detecting duplications.	(*5*)
FISH	Chromosomal translocations in metaphase and duplications in interphase can be detected.	Small duplications cannot be detected even in interphase.	(*6*)
RSP	Inexpensive and easy to perform.	Small duplications cannot be detected.	(*1*)
MLPA	Duplications can be accurately detected.	Signal intensity is not accurately detected.	(*7*)

about 70% of *PLP1* mutations. The duplicated region can range from about 40 Kbp to over 5 Mbp. Since the *PLP1* gene is dosage-sensitive, duplication and regulatory mutations are both likely to cause PMD. Deletion of the *PLP1* locus also leads to PMD but accounts for less than 1% of PMD cases.

Since *PLP1* duplications are noted in about 70% of PMD cases, verifying the presence of duplication is a reasonable approach when PMD is suspected in a clinical setting. Thus far, many different molecular methods have been developed to verify the presence of a *PLP1* duplication, including Southern blotting, a comparative multiplex polymerase chain reaction (PCR) assay (*5*), fluorescent in situ hybridization (FISH) (*6*), restriction site polymorphism (RSP) analysis (1), and multiplex ligation-dependant probe amplification (MLPA) (*7*) (Table 1). At present, these methods have been used in prenatal diagnosis to detect PMD as early as possible. The most common methods of detection are interphase fluorescent in situ hybridization (FISH) and quantitative PCR (Q-PCR). *PLP1* duplications in over 70% of interphase nuclei are diagnosed using FISH. With Q-PCR, multiplex PCR is performed with one or more pairs of primers to screen for *PLP1* duplications using a gene outside the region of duplication as a reference gene. The signal intensity of *PLP1* is compared to that of the reference gene, and a sample with duplication will have a higher signal intensity than a normal sample. These two methods have a high specificity and sensitivity but they require considerable proficiency and expensive equipment. Although RSP is a relatively inexpensive technique, it cannot be used to detect duplications in most male patients because their homozygous alleles are identical. However, these polymorphisms are very helpful when identifying females as carriers. Recently, MLPA has been increasingly used to test for duplications because of its speed and accuracy. However, there is a lack of comparative studies of these diagnostic methods indicating which method is optimal.

There is no cure for PMD nor is there a standard treatment. Treatment, which is symptomatic and supportive, may include medication for seizures and spasticity. The prognosis for individuals with Pelizaeus-Merzbacher disease varies greatly. Children with the most severe form, connatal, usually cannot survive into adolescence, but sometimes survival into the sixties or even seventies is possible, especially with attentive care. Therefore, a cure is urgently needed. In December 2008, StemCells, Inc., a biotech company in Palo Alto, received approval from the U.S. Food and Drug Administration to conduct a Phase I clinical trial to assess the safety of transplanting human neural stem cells as a potential treatment for PMD (*8*). The trial began in November 2009 at the University of California, San Francisco Children's Hospital. In addition, recent studies on using induced pluripotent stem cells (iPSCs) for neural repair have found those iPSCs to show promise as a treatment for PMD. Recently, iPSCs have been used to treat many diseases related to nerve cell damage, such as Parkinson's and Huntington's diseases (*9-11*). In June 2013, a Japanese government panel approved the world's first clinical study using iPSCs for retinal regeneration (*12*). Thus, iPSCs may be able to treat other intractable diseases such as PMD.

References

1. Garbern J, Hobson G. Prenatal diagnosis of Pelizaeus-Merzbacher disease. Prenat Diagn. 2002; 22:1033-1035.
2. Wolf NI, Sistermans EA, Cundall M, Hobson GM, Davis-Williams AP, Palmer R, Stubbs P, Davies S, Endziniene M, Wu Y, Chong WK, Malcolm S, Surtees R, Garbern JY, Woodward KJ. Three or more copies of the proteolipid protein gene *PLP1* cause severe Pelizaeus-Merzbacher disease. Brain. 2005; 128:743-751.
3. Biancheri R, Grossi S, Regis S, Rossi A, Corsolini F, Rossi DP, Cavalli P, Severino M, Filocamo M. Further genotype-phenotype correlation emerging from two families with *PLP1* exon 4 skipping. Clin Genet. 2013; doi: 10.1111/cge.12154.
4. Yamamoto T, Shimojima K. Pelizaeus-Merzbacher disease as a chromosomal disorder. Congenit Anom. 2013; 53:3-8.
5. Regis S, Filocamo M, Mazzotti R, Cusano R, Corsolini F, Bonuccelli G, Stroppiano M, Gatti R. Prenatal diagnosis of Pelizaeus-Merzbacher disease: detection of proteolipid protein gene duplication by quantitative fluorescent multiplex PCR. Prenat Diagn. 2001; 21:668-671.
6. Inoue K, Kanai M, Tanabe Y, Kubota T, Kashork CD, Wakui K, Fukushima Y, Lupski JR, Shaffer LG. Prenatal interphase FISH diagnosis of *PLP1* duplication associated with Pelizaeus-Merzbacher disease. Prenat Diagn. 2001; 21:1133-1136.
7. Kim SJ, Yoon JS, Baek HJ, Suh S, Bae SY, Cho HJ, Ki CS. Identification of proteolipid protein 1 gene

duplication by multiplex ligation-dependent probe amplification: First report of genetically confirmed family of Pelizaeus-Merzbacher disease in Korea. J Korean Med Sci. 2008; 23:328-331.
8. Stemcells, Inc. Clinical Development Programs. *http://www.stemcellsinc.com/therapeutic-programs/clinical-trials.htm* (accessed January, 2013).
9. Daviaud N, Garbayo E, Schiller PC, Perez-Pinzon M, Montero-Menei CN. Organotypic cultures as tools for optimizing central nervous system cell therapies. Exp Neurol. 2013; 248C:429-440.
10. Pan F, Zhang M, Wu G, Lai Y, Greber B, Scholer HR, Chi L. Topographic effect on human induced pluripotent stem cells differentiation towards neuronal lineage. Biomaterials. 2013; 34:8131-9813.
11. Lescaudron L, Naveilhan P, Neveu I. The use of stem cells in regenerative medicine for Parkinson's and Huntington's Diseases. Curr Med Chem. 2012; 19:6018-6035.
12. Riken, Center for Developmental Biology. Information on proposed pilot study of the safety and feasibility of transplantation of autologous hiPSC-derived retinal pigment epithelium (RPE) cell sheets in patients with neovascular age-related macular degeneration. *http://www.cdb.riken.jp/en/02_research/0208_regenerative01.html* (accessed June, 2013).

(Received July 23, 2013; Reviesed August 18, 2013; Accepted August 20, 2013)

IRDR
Intractable & Rare Diseases Research

Guide for Authors

1. Scope of Articles

Intractable & Rare Diseases Research 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.

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 100 references. Mini reviews are also accepted.

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 2,000 words in length (excluding references).

Case Reports should be detailed reports of the symptoms, signs, diagnosis, treatment, and follow-up of an individual patient. Case reports may contain a demographic profile of the patient but usually describe an unusual or novel occurrence. Unreported or unusual side effects or adverse interactions involving medications will also be considered. Case Reports should not exceed 3,000 words in length (excluding references).

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. Letters should not exceed 800 words in length and may contain a maximum of 10 references.

3. Editorial Policies

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.

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 signed 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 http://www.irdrjournal.com/downcentre.php (Download Centre).

Copyright: A signed JOURNAL PUBLISHING AGREEMENT (JPA) form must be provided by post, fax, or as a scanned file before acceptance of the article. Only forms with a hand-written signature are accepted. This copyright will ensure the widest possible dissemination of information. A form facilitating transfer of copyright can be downloaded by clicking the appropriate link and can be returned to the e-mail address or fax number noted on the form (Please visit Download Centre). 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.

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 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 article types including Original Article, Brief Report, Review, Policy Forum, and Case Report, a one-paragraph abstract consisting of no more than 250 words must be included in the manuscript. For News and 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. 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. 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.

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 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. All color figures will be reproduced in full color in the online edition of the journal at no cost to authors.

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 http://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 authord for the publication of their article except for reprints.

(As of February 2013)

Editorial and Head Office:

Pearl City Koishikawa 603
2-4-5 Kasuga, Bunkyo-ku
Tokyo 112-0003, Japan
Tel: +81-3-5840-9968
Fax: +81-3-5840-9969
E-mail: office@irdrjournal.com

IRDR
Intractable & Rare Diseases Research

JOURNAL PUBLISHING AGREEMENT (JPA)

Manuscript No.:

Title:

Corresponding Author:

The International Advancement Center for Medicine & Health Research Co., Ltd. (IACMHR Co., Ltd.) is pleased to accept the above article for publication in Intractable & Rare Diseases Research. The International Research and Cooperation Association for Bio & Socio-Sciences Advancement (IRCA-BSSA) reserves all rights to the published article. Your written acceptance of this JOURNAL PUBLISHING AGREEMENT is required before the article can be published. Please read this form carefully and sign it if you agree to its terms. The signed JOURNAL PUBLISHING AGREEMENT should be sent to the Intractable & Rare Diseases Research office (Pearl City Koishikawa 603, 2-4-5 Kasuga, Bunkyo-ku, Tokyo 112-0003, Japan; E-mail: office@irdrjournal.com; Tel: +81-3-5840-9968; Fax: +81-3-5840-9969).

1. Authorship Criteria

As the corresponding author, I certify on behalf of all of the authors that:

1) The article is an original work and does not involve fraud, fabrication, or plagiarism.
2) The article has not been published previously and is not currently under consideration for publication elsewhere. If accepted by Intractable & Rare Diseases Research, the article will not be submitted for publication to any other journal.
3) The article contains no libelous or other unlawful statements and does not contain any materials that infringes upon individual privacy or proprietary rights or any statutory copyright.
4) I have obtained written permission from copyright owners for any excerpts from copyrighted works that are included and have credited the sources in my article.
5) All authors have made significant contributions to the study including the conception and design of this work, the analysis of the data, and the writing of the manuscript.
6) All authors have reviewed this manuscript and take responsibility for its content and approve its publication.
7) I have informed all of the authors of the terms of this publishing agreement and I am signing on their behalf as their agent.

2. Copyright Transfer Agreement

I hereby assign and transfer to IACMHR Co., Ltd. all exclusive rights of copyright ownership to the above work in the journal Intractable & Rare Diseases Research, including but not limited to the right 1) to publish, republish, derivate, distribute, transmit, sell, and otherwise use the work and other related material worldwide, in whole or in part, in all languages, in electronic, printed, or any other forms of media now known or hereafter developed and the right 2) to authorize or license third parties to do any of the above.

I understand that these exclusive rights will become the property of IACMHR Co., Ltd., from the date the article is accepted for publication in the journal Intractable & Rare Diseases Research. I also understand that IACMHR Co., Ltd. as a copyright owner has sole authority to license and permit reproductions of the article.

I understand that except for copyright, other proprietary rights related to the Work (*e.g.* patent or other rights to any process or procedure) shall be retained by the authors. To reproduce any text, figures, tables, or illustrations from this Work in future works of their own, the authors must obtain written permission from IACMHR Co., Ltd.; such permission cannot be unreasonably withheld by IACMHR Co., Ltd.

3. Conflict of Interest Disclosure

I confirm that all funding sources supporting the work and all institutions or people who contributed to the work but who do not meet the criteria for authors are acknowledged. I also confirm that all commercial affiliations, stock ownership, equity interests, or patent-licensing arrangements that could be considered to pose a financial conflict of interest in connection with the article have been disclosed.

Corresponding Author's Name (Signature):

Date: