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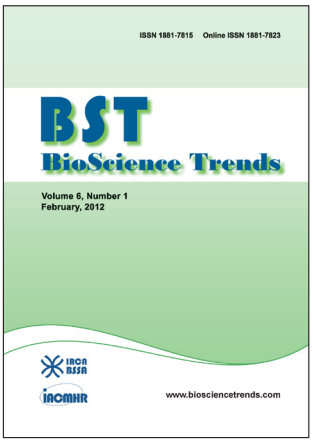

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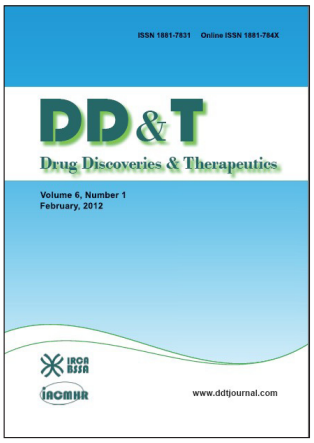

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Editorial

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Intractable and rare diseases research

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Intractable diseases, literally derived from the Japanese word 'nanbyo', mainly refer to rare diseases that have resulted mostly from unidentifiable causes and/or lack of clearly established or curable treatments. Currently, it is estimated that there are 5,000-7,000 distinct rare diseases worldwide, of which 80% have been identified as having genetic origins and 50% occuring in childhood and lasting for a lifetime (*1,2*). It is worth noting that most cancers including all cancers affecting children are within the scope of the concept of rare disease (*2*). Rare diseases bring patients substantial physical suffering and psychological despair due to the lack of therapeutic hope and the absence of practical support for everyday life. In addition, these kinds of diseases require a significant amount of labor for the patient's care, causing a heavy burden on other family members, both financially and mentally. Although each specific disease affects a limited number of patients because of its rarity, the total number of patients with rare diseases represents a striking proportion of the total population. For example, in the European Union (EU) countries approximately 30 million people, which accounts for 6-8% of the total EU population, have various rare diseases (*2*). In the United States (US), it is estimated that 10% of the people suffer from rare diseases (*3*). These facts indicate that the situation of preventing and controlling rare diseases is grim in the world.

Owing to the relentless work of patient and parent organizations, the previously neglected status of these so called 'orphan diseases' has attracted the attention of public health authorities and policy makers in recent decades. With the incentives of the Orphan Drug Act enacted in the US in 1983 and subsequently similar legislation in Japan, Australia and EU, the number of approved orphan drugs has substantially increased. Thus far, approximately 360 and 60 orphan drugs, in which drugs for rare cancers account for 30-40%, are available for rare disease patients in the US and EU (*4,5*). Nevertheless, it is estimated that only ~10% of rare diseases have an available treatment (also including food supplements, devices and nutraceuticals in addition to drugs), and such treatments can often still be improved (*4*). Even for those diseases that have certain therapeutic strategies, many patients still encounter challenges in receiving appropriate care due to low disease awareness and delayed diagnosis. In the long run, strengthening basic and applied research on rare diseases would benefit patients from better diagnosis and more treatment choices.

Early and accurate diagnosis of a rare disease is of critical importance for those particular patients. Take infantile Pompe's disease as an example, the start of treatment after 6 months of age is considered to be too late for those patients. However, the current situation of identification of a specific rare disease is not optimistic. Statistical data demonstrate that the time from appearance of the clinical symptoms to correct diagnosis is within a range of 5-30 years on average (*4*). That is largely because of the lack of scientific knowledge of the pathology of rare diseases for physicians. Moreover, a rare disease is sometimes masked by a host of other conditions, which may lead to misdiagnosis or highly risky delays for accurate diagnosis. Consequently, poor or late diagnosis may lead to multiple medical consultations, inaccurate treatments, inappropriate behavior and inadequate support from family members, and even for other children born with the same disease in the same family. In this regard, increased knowledge of the disease mechanisms and natural courses will potentially allow better diagnosis of rare diseases. In addition, constructing diagnostics and treatment center networks, training physicians in relevant fields, and appropriate screening for rare diseases in a large population especially in children would help identify patients with rare diseases in an early and comprehensive manner.

Despite the growing public awareness of rare diseases in the last several decades, there are still many gaps in knowledge in developing therapeutic tools and defining therapeutic strategy. Biomedical research on rare diseases will provide insights into the pathologies of diseases and illustrate their underlying mechanisms, which may ultimately reveal possible avenues to therapeutics. Once biomedical research identifies suitable drug candidates and becomes more translational, it will be keenly focused by the industry

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with the aim to provide safe and effective orphan drugs. It is interestingly noted that there is a positive relationship between the number of published papers on a particular rare disease and the likelihood of initiation of an orphan drug development program for that disease. Specifically, there have been 154 rare diseases on each of which more than 600 published papers exist that have one or more drug designations, respectively, between 1983 and 2007 in the US and between 2000 and 2007 in the EU. On the other hand, the number of rare diseases with 200-600 published papers and drug designations were only 42 in that same time period (*6*). These figures demonstrated the significance of biomedical research on rare diseases.

The more a rare disease is known, the more likely it is diagnosed rapidly and covered by effective medical intervention. The acquisition and diffusion of scientific knowledge is the vital basis for identification of diseases, and most importantly, for research into new diagnostic and therapeutic procedures. *Intractable & Rare Diseases Research* is being launched in the context of the serious conditions of rare diseases as well as the few such professional journals worldwide covering this topic. It is founded to promote information exchange among researchers active in the field of rare diseases and various difficult and complicated diseases research in the world, and to cultivate and develop a global medical and drug information network. Finally, patients and families together with health professionals including doctors, scientists and healthcare providers are co-producing a knowledge base, which will improve the quality of life of those patients with intractable and rare diseases.

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Rare diseases, orphan drugs, and their regulation in Asia: Current status and future perspectives

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Summary **Rare diseases are an important public health issue and a challenge to medical care. Specific legislation to encourage research of rare diseases and development of orphan drugs has been adopted in the United States (US), the European Union (EU), and elsewhere. In recent years, much progress has been made in some parts of Asia, including Japan, South Korea, and Taiwan, with the enactment of legislation and accompanying regulation of rare diseases and orphan drugs. China is also actively promoting the regulation of rare diseases and orphan drugs. We describe the current status of the regulation of rare diseases and orphan drugs in Asia and we comparatively analyze the regulation of rare diseases and orphan drugs worldwide in order to examine the challenges to and future perspectives on promoting research on rare diseases and development of orphan drugs in China and other Asian countries.**

Keywords: Orphan diseases, orphan drugs, legislation, incentives, reimbursement

1. Introduction

Rare diseases are rare and often debilitating or even life-threatening diseases or conditions with a prevalence of 0.65‰-1‰, as defined by the World Health Organization (WHO). Eighty percent of rare diseases have identified genetic origins, 50% of rare diseases affect children, and 30% of patients with rare diseases die before the age of 5 (*1*). The conventional view is that rare diseases together affect around 10% of individuals worldwide, but the definition and categorization of rare diseases differ slightly by region. The combined number of people suffering from rare diseases in the European Union (EU) and United States (US) is estimated to exceed 55 million and 5,000-7,000 rare diseases are thought to exist, with approximately 250 new diseases being described on an annual basis (*2,3*).

The features of rare diseases and the increasing number of identified rare diseases make these diseases a priority for policymakers, researchers, legislators, and health care professionals (*4*). Currently, orphan drugs – the medicinal products intended for the diagnosis, prevention, or treatment of rare diseases – are a major facet of how rare diseases are dealt with. In the past three decades, many countries have recognized that orphan drugs would not lead to substantial sales under normal market conditions because of the high costs and risks of drug development, insufficient knowledge of pathophysiological mechanisms of rare diseases that the drugs diagnose or treat, and difficulties in conducting clinical trials with small patient populations and a small potential market. Therefore, specific legislation to encourage the discovery and development of orphan drugs was enacted in many countries and regions, including the US in 1983, Japan in 1993, Australia in 1997, the EU in 1999, Taiwan in 2000, and South Korea in 2003 (*5-7*). Incentives include financial subsidies, market exclusivity, tax credits, fee waivers, fast track approval, and protocol assistance, resulting in substantial improvements in the treatment of patients with a range of rare diseases.

In Asia, Japan, South Korea, and Taiwan have established systematic economic and regulatory incentives to encourage the development of drugs for rare diseases. China is also actively promoting the regulation of rare diseases and orphan drugs. Here,

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Japan, South Korea, China, and Taiwan are cited as examples to describe the current status of the regulation of rare diseases and orphan drugs in Asia. The regulation of rare diseases and orphan drugs worldwide has also been comparatively analyzed (Table 1). These two steps should help examine the challenges to and future perspectives on promoting research on rare diseases and development of orphan drugs in China and other Asian countries.

2. The current regulation of rare diseases and orphan drugs in Asian

The regulation of rare diseases and orphan drugs in Japan: i) *Definition of rare diseases*. Originally, rare diseases were known as "intractable diseases (Nambyo)" in Japan. There was no concept of "rareness" until 1995, when the Ministry of Health and Welfare revised the definition of intractable disease (Nambyo) to "a disease of unknown etiology with no effective treatment that presents a major financial and psychological burden and that is rare (fewer than 50,000 total patients)". Currently, rare diseases are termed "rare and intractable diseases" in Japan. ***ii)*** *Incentives for orphan drugs*. Revised orphan drug regulations (amendment of the Pharmaceutical Affairs Act and Drug Fund for Adverse Reaction Relief and Research Promotion Act) were established in 1993 and regulates the incentives, which include financial subsidies for up to 50% of expenses for clinical and non-clinical research during the entire research process, exclusive marketing rights for 10 years (compared to 6 years for other medications), 15% tax credits on research costs excluding financial subsidies and up to a 14% reduction in corporate tax, priority review and fast track approval, free protocol assistance, and user fee waivers. ***iii)*** *Support system*. Support measures include grants-in-aid for research programs (10 billion yen was allocated for the studies of 130 diseases in 2010), price-control policies negotiated by Japanese National Health Insurance (NHI) and pharmaceutical companies, and medical expense reimbursement for 56 diseases. ***iv)*** *Public awareness*. The Intractable Disease Information Center (*http://www.nanbyou.or.jp*) was established in 1997 and provides vast information on rare and intractable diseases, a list of experts, and contact addresses of rare disease patients' support organizations throughout Japan.

The regulation of rare diseases and orphan drugs in South Korea: i) *Definition of rare diseases*. In Korea, rare diseases are defined as diseases that affect fewer than 20,000 people or diseases for which an appropriate treatment or alternative medicine has yet to be developed. ***ii)*** *Incentives for orphan drugs*. The Orphan Drugs Guideline was established in 2003 and stipulates exclusive marketing rights for 6 years to encourage the research and development of orphan drugs. ***iii)*** *Support system*. Support measures include medical expense reimbursement and nationally funded research programs along with support from the Ministry of Family Affairs, Health and Welfare and the Korean Centers for Disease Control and Prevention. ***iv)*** *Public awareness*. The Korean Rare Disease Information Database (*http://helpline.cdc.go.kr*) and Korean Organization for Rare Diseases (*http://www.kord.or.kr*) provide vast information on rare diseases for patients, researchers, pharmaceutical companies, and administrators.

The regulation of rare diseases and orphan drugs in Taiwan: i) *Definition of rare diseases*. In Taiwan, a disease is classified as a rare disease if it is prevalent in fewer than 1/10,000 population, has a genetic origin, and is difficult to diagnose and treat. ***ii)*** *Incentives for orphan drugs*. The Rare Disease Control and Orphan Drug Act was established in 2000, and incentives include financial subsidies, awards to special contributors from the central competent authority, exclusive marketing rights for 10 years (compared to 5 years for other medications), fast track approval, and protocol assistance. ***iii)*** *Support system*. Support measures include a reporting system for patients with rare diseases, "Genetic Counseling Centers" to counsel patients and other individuals, an "Orphan Drug Distribution Center" and a "Special Nutritional Supplement Supply Center" to facilitate the distribution of drugs, and 70% reimbursement for patients with rare diseases (which is expanded to 100% reimbursement for low-income families). ***iv)*** *Public awareness*. The Taiwan Foundation for Rare Disorders (TFRD) (*http://www.tfrd.org.tw*) was established in 1999 and provides general information and support to patients with rare diseases with regard to medication, education, employment, and long-term care.

The regulation of rare diseases and orphan drugs in China: i) *Definition of rare diseases*. Recognition of the concept of rare diseases may date back to the Drug Registration Regulation in 1999, but rare diseases have not been clearly defined by legislation until now. A consensus on the definition of rare disease is emerging according to the Expert Seminar on the Definition of Rare Diseases in China held in 2010, which proposed that a disease be classified as a rare disease if it is prevalent in fewer than 1/500,000 or has a neonatal morbidity of fewer than 1/10,000 (*8*). ***ii)*** *Incentives for orphan drug*. Many regulations – the New Drug Approval Regulation (1999), Drug Registration Regulation (2007), and Special Review and Approval Procedures for Drug Registration (2009) – implemented by the State Food and Drug Administration (SFDA) have set forth general criteria to accelerate the registration and approval of orphan drugs, but detailed rules have yet to be implemented and further incentives have not been proposed until now. ***iii)*** *Support system*. Support measures have been provided mainly from special organizations dealing with rare diseases –

Table 1. Comparison of the regulation of rare diseases and orphan drugs worldwide*

Items	United States	European Union	Australia	Japan	South Korea	Taiwan	China
Criterion for prevalence of rare diseases (‰)	0.75	0.5	0.11	0.4	0.4	0.1	prevalent < 1/500,000, or neonatal morbidity < 1/10,000**
Affected population	25-30 million	27-36 million	1.2 million	*N*	*N*	more than 2,000	16.8 million**
Administrative bodies involved	FDA/OOPD	EMA/COMP	TGA	MHLW	KFDA	DOH	SFDA
Legal framework	Orphan Drug Act (1983), Rare Diseases Act of 2002 (2002)	Regulation (EC) No. 141/2000 (1999)	Orphan Drug Policy (1997)	Revised orphan drug regulations (1993)***	Orphan Drugs Guideline (2003)	Rare Disease Control and Orphan Drug Act (2000)	No
Financial subsidies	government grants for clinical research	framework programs plus national measures	*N*	governmental grants for clinical and non-clinical research	*N*	government grants and awards from the central competent authority	NSFC research grants
Market exclusivity (years)	7	10	5 (similar to other drugs)	10	6	10	*N*
Tax credits	up to 50% for clinical expenses	managed by member states	*N*	15% tax credits, up to 14% corporate tax reduction	*N*	*N*	*N*
Fast track approval	Yes	Yes (centralized approval)	Yes	Yes	*N*	Yes	Yes
Protocol assistance	Yes	Yes	Yes	Yes	*N*	Yes	*N*
Regulatory fee waivers	Yes	Yes	Yes	Yes	*N*	*N*	*N*
Pharmaceutical pricing	market-driven	depending on member states	same as general drugs	price negotiation	*N*	*N*	*N*
Medical expense reimbursement	Yes	Yes	Yes	for 56 diseases	Yes	70% for patients, up to 100% for low-income families	*N*
Public awareness	NORD	EURORDIS	AGSA	IDIC	KRDID, KORD	TFRD	Chinese Rare Disease Net, and others

* Data are from references *2, 3, 5-12, 20, 24-26.*

** Has not being defined by legislation according to the Expert Seminar on the Definition of Rare Diseases in China.

*** Revised orphan drug regulations (amendment of the Pharmaceutical Affairs Act and Drug Fund for Adverse Reaction Relief and Research Promotion Act) (1993).

N, no information; FDA, Food and Drug Administration; OOPD, Office of Orphan Products and Development; EMA, European Medicines Agency; COMP, Committee of Orphan Medicinal Products; TGA, Therapeutic Goods Administration; MHLW, Ministry of Health, Labor and Welfare; KFDA, Korean Food and Drug Administration; DOH, Department of Health; SFDA, State Food and Drug Administration; NSFC, National Natural Science Foundation of China; NORD, National Organization for Rare Disorders; EURORDIS, European Organization for Rare Diseases; AGSA, Association of genetic support of Australasia; IDIC, Intractable Disease Information Center; KRDID, Korean Rare Disease Information Database; KORD, Korean Organization for Rare Diseases; TFRD, Taiwan Foundation for Rare Disorders.

the Committee Specializing in Rare Diseases of the Shenzhen Medical Association, the Committee Specializing in Rare Diseases of the Chinese Medical Association, the Committee Specializing in Rare Diseases of the Shanghai Medical Association, and the Rare Disease Association of Shandong Province – to promote the research on rare diseases and development of orphan drugs. ***iv)*** *Public awareness*. The Rare Disease in China Network (*http://www.hanjianbing.org*), the Chinese Rare Disease Academic Network (*http://www.chinards.com*), and many patients' advocacy groups, such as the China-Dolls Care and Support Association, the Haemophilia Home of China (HHC), the Neuro-Muscular Disease Association of China, and the Chinese Lymphangioleiomyomatosis Organization (LAM-China), are devoted to providing information and improving patients' access to healthcare.

3. Comparative regulatory aspects of rare diseases and orphan drugs worldwide

3.1. *Definition and classification of rare diseases*

There is no internationally accepted definition of rare diseases. In the US, rare diseases are defined as diseases that affect fewer than 200,000 Americans (prevalence of < 0.75‰), while stipulated prevalence rates in other regions were < 0.5‰ in the EU, fewer than 2,000 patients (prevalence of < 0.11‰) in Australia, fewer than 50,000 patients (prevalence of < 0.4‰) in Japan, fewer than 20,000 patients (prevalence of < 0.4‰) in South Korea, and prevalence of < 0.1‰ in Taiwan (*9-11*). The number of patients affected by rare diseases could be about 27-36 million in the EU, 25-30 million in the US, and 1.2 million in Australia (*2,3,12*), but the true burden of rare diseases in the EU and elsewhere is difficult to estimate since epidemiological data for most rare diseases are not available. The primary reasons why such epidemiological data are often lacking could due to *i*) the absence of proper classification and coding for rare diseases and the absence of registration of patients suffering from rare diseases and *ii*) the absence of appropriate biochemical and genetic diagnostic data.

Currently, there is no special coding system for rare diseases. The International Classification of Diseases (ICD) code that is used in most countries is not suitable for rare diseases. The absence of a universally recognized coding system is an obstacle for reliable registration of patients in national or international databases, preventing assessment of the economic and social effects of rare diseases. However, the good news is that the European Rare Disease Task Force of the Health and Consumers Protection Directorate General of the European Commission has set up a working group to collaborate with the WHO on the ICD-10 and is considering all other existing classifications to provide the rare disease community with a uniform system (*13*). Worldwide, major mutation databases include Online Mendelian Inheritance in Man (OMIM), the Human Gene Mutation Database (HGMD), and the Human Genome Variation Society (HGVS). Recently, country-specific databases have also been developed, such as the Singapore Human Mutation/Polymorphism Database (SHMPD) and Korean Mutation Database (KMD) (*14*). Advances in genetic research and establishment of additional databases could facilitate the diagnosis of patients with rare diseases.

Thus, a legislative definition and classification of rare diseases and accurate data on the epidemiology of rare diseases are urgently needed at the national and international levels in order to support health management policies and studies aimed at developing and assessing treatments.

3.2. *Incentives for orphan drug research and development*

Orphan drugs are less likely to be developed by pharmaceutical companies because the market is small and research and development costs are usually too high to make the drugs profitable. Given this context, orphan drug legislation has been adopted in several countries around the world in the past three decades to encourage manufacturers to develop orphan drugs. The primary incentives can be broken down into three types: *i*) marketing exclusivity for the orphan drug, whereby sponsors of this drug are granted a given period of marketing exclusivity during which no other drug will be approved to treat the disease in question; *ii*) the setting up of tax credits and financial subsidies for research; and *iii*) simplification of and preference in drug authorization, including fast track approval, fee waivers, and protocol assistance.

Regulatory systems have both similarities and differences. With regard to financial subsidies, for example, only expenses in clinical research activities are subsidized in the US, while expenses in clinical and non-clinical research during all research can be subsidized in Japan. With regard to market exclusivity, Japan, the EU, and Taiwan offer market exclusivity for 10 years, while the US offers market exclusivity for 7 years. With regard to tax credits, credits can cover up to 50% of clinical expenses in the US, while Japan offered a 6% tax credit for research expenses excluding financial subsidies and up to a 10% reduction in corporate tax (since 1999, Japan has offered a 15% tax credit for research expenses excluding financial subsidies and up to a 14% reduction in corporate tax). Revised orphan drug regulations in Japan also require sponsors to pay a 1% sales tax to offset the subsidies they received from the government when their orphan drug annual profits exceed 100 million yen (*15,16*).

Evidence has shown that all of the incentives have successfully encouraged the development of new pharmaceutical products to treat rare diseases and have

resulted in an increasing number of licensed orphan drugs. Prior to 2010, 352 orphan drugs were approved in the US, helping an estimated 12 million Americans, compared to only 10 such drugs in the decade preceding the Orphan Drug Act (1983). Similarly, 720 drugs had received orphan drug designation from the European Medicines Agency (EMA) and 63 designated orphan medicinal products had received marketing authorization in the EU. Furthermore, data have shown that an average of 15 new orphan drugs are approved annually in the US and 10-12 new orphan drugs are approved annually in the EU (*17-19*).

In Asia, orphan drug legislation has been adopted in Japan, South Korea, and Taiwan, and incentives are playing an important role in encouraging manufacturers to develop orphan drugs. While China is actively preparing to regulate and encourage the development of orphan drugs, but it still lags far behind the US, the EU, Japan, and other countries and regions with orphan drug legislation. The current regulations only set forth general criteria to accelerate the registration and approval of orphan drugs, but detailed rules have not been implemented and further incentives have not been proposed until now. Thus, the pressing issue for China and other Asian countries without orphan drug legislation is to establish domestic legislative regulations and incentives to encourage the development of orphan drugs.

3.3. *Support system for the development of rare disease and orphan drugs*

Special regulatory authority: One of the similarities among the countries and regions with legislation on rare disease and orphan drugs is that they have a special regulatory authority, such as the Office of Orphan Products Development (OOPD) within the Food and Drug Administration (FDA) in the US and the Committee of Orphan Medicinal Products (COMP) within the European Medicines Agency (EMA) in the EU, with the main task of examining applications for orphan drug designation and planning and regulating the development of rare disease and orphan drugs. In Asia, this regulatory authority is the Ministry of Health, Labor, and Welfare (MHLW) in Japan, the Korean Food and Drug Administration (KFDA) in South Korea, and the Department of Health (DOH) in Taiwan. In Taiwan, the Rare Disease Control and Orphan Drug Act was promulgated in 2000, and the DOH has subsequently devised a series of accompanying regulations on the implementation of orphan drug approval, review, registration, supply, and awards (*20*). In China, orphan drugs and other drugs are all currently administered by the SFDA, and a special regulatory authority for rare diseases and orphan drugs has not been established.

Special research support: In the US and EU, special research centers or projects have been established to support research on rare diseases and development of orphan drugs. In the US, the Office of Rare Diseases Research (ORDR) was established in 1993 within the National Institutes of Health (NIH) to coordinate and support rare disease research, explore opportunities to research rare diseases, and provide information on rare diseases. In EU, the Rare Disease Task Force (RDTF) was established in 2004 within the European Commission Public Health Directorate to provide evidence to support policymaking, provision of medical services, and community support for rare diseases and orphan drugs through European coordination. In Asia, the Specified Disease Treatment Research Program was established in Japan in 1972 with the support of MHLW, 130 diseases have been for the subject of special research programs and research grants from government sources expanded to 10 billion yen in 2010 (*21*). Recently, 214 diseases were selected for a second round of special research programs (*22*). In South Korea, the Research Center for Rare Diseases (RCRD) was established in 2008 with the support of the Ministry of Family Affairs, Health and Welfare. The Center oversees 3 collaborative research projects, 9 single-center research projects, and 7 clinical research networks in order to provide a foundation for research on rare diseases and orphan drugs. In China, research support comes mainly from the National Natural Science Foundation of China (NSFC). Data showed that 366 projects (involving 32 rare diseases) were funded by the NSFC from 1999 to 2007 with total funding of 89.358 million RMB and annual funding of about 10 million RMB, accounting for just $1/10^{th}$ of similar funding in the US (*23*).

Access to Orphan Drugs: Pricing and reimbursement are the two major aspects that affect access to orphan drugs for patients with rare diseases. In the US, drug manufacturers negotiate with governmental programs such as Medicaid and Veterans Health Administration and Pharmacy Benefits but remain free to set their own introductory prices and there is little regulation of competition among manufactures in comparison to imposed price restrictions (*24*). In the EU, orphan designation and marketing authorization for orphan drugs are decisions made at the European level according to Regulation (EC) No 141/2000, but pricing and reimbursement decisions are a member state responsibility (*25*): some European countries, such as Belgium, France, Italy, and the Netherlands, compare the price requested by the pharmaceutical company to that in other countries; the United Kingdom has set up a system of profit control to constrain prices and Sweden uses a system of public procurement at the regional level in order to maximize price competition. In 2008, European drug prices averaged 40% less than American prices, with prices in Italy and Germany respectively averaging 55% and 70% of American prices (*19*).

However, most orphan drugs are still expensive due to the high research costs and the small market and most patients will not be able to afford to pay for orphan drugs themselves. Given this context, both the US and EU have established financial support for patients with rare diseases. In Asia, the Japanese NHI negotiates prices with the pharmaceutical companies once a drug is approved for use, allowing a selling price of cost plus 10% for orphan drugs; nearly half of the orphan drugs on the Japanese market originated from the EU or US. Moreover, 56 of 130 designated diseases in Japan are subject to reimbursement of medical expenses, with 30% of expenses paid by insurance companies and the rest paid by national and prefectures governments (*26*). In 2010, reimbursements expanded to 28 billion yen and the number of recipients expanded to approximately 700,000 (*22*). In Taiwan, 77 approved orphan drugs and 40 special nutritional supplements can be imported, and the reimbursement cap is 70% of actual expenses but families that qualify for low-income status can receive reimbursement for up to 100% of drugs and nutritional supplements for patients (*20*). While in China, a sound supply mechanism and reimbursement system have not been established, hampering access to orphan drugs for patients with rare diseases (*27*). However, the good news is that patients with 12 rare diseases in Shanghai recently became eligible for partial reimbursement, and some special orphan drugs for children have been covered by insurance (*28*).

4. Public awareness, research, and global cooperation

In addition to the lengthy development of orphan drugs, the delay in diagnosis is also a huge challenge to cope with. A survey of 18,000 individuals found that 25% of patients waited for 5-30 years before being correctly diagnosed and 40% of patients were diagnosed incorrectly before correctly diagnosed (*29*). The challenge is the lack of quality information and a networking system to facilitate interaction among patients, clinicians, researchers, the pharmaceutical industry, and governmental bodies. In order to change this situation, many patients' advocacy organizations and networks, both nationally and internationally, have been established with or without governmental support. Major organizations in Western countries, such as the National Organization for Rare Disorders (NORD) in the US and the European Organization for Rare Diseases (EURORDIS) in Europe, can provide vast information on rare diseases and improve patients' access to healthcare. The "Rare Disease Day" initiated by EURORDIS in 2008 to raise public awareness started as a European event but has now become a world event, with the US joining in 2009 and patient organizations in 56 other countries participating in 2011(*30*). In Asia, patients' advocacy organizations and networks have also established in Japan, South Korea, China, and Taiwan to provide vast information to patients with rare diseases and to promote research on rare diseases and development of orphan drugs.

In recent years, progress has been made in research on rare diseases and the dissemination of knowledge and information. However, existing research efforts are still scattered and fragmented research is being performed with little coordination between research laboratories. This hampers research on rare diseases and development of orphan drugs, especially with regard to clinical studies on orphan drugs that suffer from the small size of the trial population and the fact that patients are often geographically dispersed. Thus, there is a pressing need to increase international cooperation. In Western countries, some web-based resources have been established. For example, the Rare Diseases Clinical Research Network (RDCRN) was established in the US in order to facilitate collaboration amongst experts from 19 distinctive consortia and to create a platform for collaboration to identify biomarkers for disease risk, disease severity and activity, and clinical outcome (*31*). Orphanet was established in Europe and includes 1,673 organizations affiliated with EURORDIS that gather national expertise on rare diseases together and share reports of member states' assessments of the therapeutic or clinical added value of orphan drugs in order to minimize delays in access to orphan drugs for patients with rare diseases (*32*). Both RDCRN and Orphanet represent a good example for Asian countries to launch their own international collaborative projects to promote research on rare diseases and development of orphan drugs.

5. Conclusion

In conclusion, rare diseases are an important public health issue and a challenge to medical care. In recent years, much progress has been made in some parts of Asia, including Japan, South Korea, and Taiwan, with the enactment of legislation and accompanying regulation of rare diseases and orphan drugs. China is actively promoting the regulation of rare diseases and orphan drugs but still lags far behind the US, EU, Japan, and other countries and regions with orphan drug legislation. Comparative analysis of the regulation of rare diseases and orphan drugs worldwide has shown that public authorities should regard rare diseases as a public health priority and take definite action, including legislation to confirm the definition and classification of rare diseases, assembling accurate epidemiological data on rare diseases, incentives to encourage manufacturers to develop orphan drugs, an appropriate support system to ensure access to orphan drugs, and international cooperation in research on rare diseases and development of orphan drugs. "It is now time for action" (*33*), especially for China and other countries in Asia.

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Rare diseases research in China: Opportunities, challenges, and solutions

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Summary **Rare diseases research in China can be traced back to the 1980s. Currently, control of rare diseases has become a national concern. This paper describes developments concerning rare diseases in China with regard to epidemiology, case registration, basic research, establishment of medical networks, and orphan drugs. A national program for rare disease research is being implemented in China to promote international cooperation in the future.**

Keywords: Rare disease, China, development, epidemiology

1. Introduction

Rare diseases are also known as orphan diseases. Based on WHO criteria, China has rare diseases population of at least 10 million. The diagnosis and treatment of rare diseases has been a long-standing medical problem, yet it also provides a unique perspective to understand the body's physiological and pathological mechanisms. Many breakthroughs in the study of rare diseases have promptly led to new approaches to researching common diseases.

In China, public concern about rare diseases can be traced back to the 1980s, when some radiologists often met to discuss baffling cases. The plight of patients with rare diseases also attracted their attention, and the Chinese version of the concept of rare diseases was proposed by Professor Gui Lin of Fudan University and Professor Chenglin Wang of Peking University Shenzhen Hospital (*1*). The concept was soon confirmed by several well-known Chinese medical experts such as Professor Jieping Wu. With their supports, Professor Wang and his colleagues organized a series of national academic conferences on rare diseases. In 1994, the "Chinese Journal of Rare and Uncommon Diseases," China's first journal of rare diseases, was founded. However, the issue of control of rare diseases had not become a national concern until five years ago. Several academic societies that deal with rare diseases prevention and treatment have been established and many patients' advocacy groups, such as osteogenesis imperfecta organizations, have been created in regions like Shandong Province and the city of Shanghai. Legislation on rare diseases is also encouraged. That said, research on and control of rare diseases in China still faces enormous challenges.

2. Epidemiology

Several epidemiological studies of some rare diseases have been conducted in China. The National Surveillance System for Creutzfeldt-Jakob Disease was established in 2002. Thanks to this system, several organizations, which included the Chinese Center for Disease Control (CDC), obtained epidemiological data on Creutzfeldt-Jakob disease in 2008 (*2*). Continued improvements in China's national system of newborn screening have provided epidemiological data on rare diseases, which include congenital hypothyroidism and phenylketonuria. That said, the lack of important epidemiological data on the overall distribution, definitions and criteria, and types of rare diseases have severely hampered the introduction of national laws dealing with rare diseases.

To determine the distribution of rare diseases, Wang *et al*. (*1*) conducted a statistical study of 117 journals

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in clinical medicine published from 1978 to 1988. Their work covered 19,727 publications and 23,215 reported cases. They found that 10,147 cases (81.8%) were distributed among 12 regions, including Beijing, Shanghai, Hubei, and Zhejiang. Analysis based on the resident population at the time indicated that the rate of rare diseases detection per 100,000 population was 6.77 in Beijing, 4.65 in Shanghai, 1.86 in Hubei, 1.67 in Zhejiang, 1.56 in Jiangsu, 1.41 in Shandong, 1.28 in Hebei, 1.20 in Guangdong, 1.17 in Anhui, 1.10 in Hunan, 1.07 in Henan, and 1.01 in Sichuan.

3. Registration of cases of rare diseases

Forrest *et al*. (*3*) recently proposed the establishment of a global system for patient registration in order to promote epidemiological and basic research and improvement of clinical treatment of rare diseases. In China, a patient organization known as the China-Dolls Care and Support Association started voluntary registration in May 2010. So far it has registered 30 rare diseases and 3,000 cases, about 1,000 of which are osteogenesis imperfecta (*4*).

The current authors and their colleagues from Shandong Academy of Medical Science are proposing a system for registration of rare diseases. An online database for registration of cases of rare diseases has been created (*http://www.chinards.com*) (*1*). Case information is to be collected through a national network of 100 hospitals. Medical specialists will be registering clinical data on around 50 types of rare diseases. The system should be formally implemented in 2012.

4. Genetic identification of rare diseases

Due to the country's large population, there is a large population of patients with rare diseases in China, and the spectrum of diseases reflects regional characteristics. For example, ossification of the ligamentum flavum (OLF) is considered a rare disease in the West, but X-ray screening of a random sample of 1,736 residents of southern China revealed that OLF is not a rare disease in China (*5*). Therefore, China can draw on a large population of patients with rare diseases. Recently, the Beijing Genomics Institute (BGI) launched the "1000 Mendelian Disorders Project". Using exome sequencing, Zhang *et al.* (*6*) confirmed the pathogenic role of *NCSTN* mutations in acne inversa (hidradenitis suppurativa); Wang *et al*. (*7*) identified *TGM6* as a novel gene causing spinocerebellar ataxias; and Yang *et al*. (*8*) identified *ZNF644* gene mutations in high myopia.

These influential developments have greatly stimulated Chinese researchers' interest in genetic studies of the pathogens responsible for rare diseases. So far, the 1,000 Mendelian Disorders Project has initiated genetic studies of the pathogens responsible for over 150 diseases, and more than 50 projects are in the later stages of validation.

China's genetic studies of the pathogens responsible for rare diseases are not confined to new-generation sequencing. Zhang *et al*. (*9,10*) of the Shandong Academy of Medical Sciences conducted a genome-wide association study (GWAS) of leprosy given abundant samples. His team established the world's largest repository of leprosy samples, and they have identified 9 pathogenic genes associated with leprosy thanks to that repository.

5. Establishing networks for treatment of rare diseases

Creating a national network for rare diseases is a medical policy that should significantly reduce misdiagnosis and improve the level of treatment. A number of centers offering counseling on rare diseases have been established in major Chinese cities like Beijing and Shanghai, but a national network has yet to be created. Unlike in countries and regions such as Europe, North America, and Japan, there is a huge gap in terms of medical services in different areas of China. Only several large hospitals can ultimately diagnose rare diseases, so the needs of most Chinese patients are not met. Therefore, the pressing task is to improve China's level of care for rare diseases by creating a national network of diagnosis and treatment and to foster the ability of primary hospitals to recognize rare diseases.

6. Orphan drugs

In 1999, new drugs for rare diseases were included in China's "Regulations for Drug Registration", but orphan drugs have not been clearly defined and no policies for incentives have been adopted. Therefore, there is almost no development of drugs to treat rare diseases in China. Expensive imported drugs are a heavy financial burden on Chinese patients. In recent years, the development of drugs to treat rare diseases has attracted the government's attention. The development of orphan drugs was included for the first time in a national program for innovative new drugs in 2010.

7. Conclusion

In China, the level of care for common diseases such as tumors and cardiovascular disease has significantly improved. Now, the prevention and treatment of rare diseases is also attracting attention. More and more pathogenic genes are being successfully identified, so Chinese researchers have gained greater interest in rare diseases. More importantly, a growing number of clinicians, researchers, and health officials are aware of

the importance of resource allocation, epidemiological study, and clinical study of rare diseases in China. At the moment, a program for collaboration on rare diseases research is being implemented at the national level. This program is committed to promoting the study of rare diseases in China and will encourage international cooperation in this regard.

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Primary sclerosing cholangitis as an intractable disease

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Summary **Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease of unknown origin which eventually results in liver cirrhosis. The disease is reported to be more common among the Western population than in the Asian population. Asian experience remains limited. Diagnosis and treatment standards in the Far East have largely followed those in the West, including liver transplantation. Unlike in the West, however, recent reports from Japan have presented with a higher recurrence rate of PSC after liver transplantation, suggesting the intractable nature of the disease even after the replacement of the entire affected organ.**

Keywords: Primary sclerosing cholangitis (PSC), living donor liver transplantation (LDLT), deceased donor liver transplantation (DDLT), Japan

1. Introduction

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease, characterized by inflammatory and fibrotic bile duct lesions forming multiple strictures and dilatations of the intra- and extrahepatic bile ducts (*1-3*). The disease is more common in men than women, and among Caucasians and Northern Europeans, than in Southern Europeans, Asians, or Africans (*4,5*).

Criteria for diagnosis are; cholangiographic findings of multifocal strictures and beading of the intraand/or extra-hepatic bile ducts, compatible biochemical abnormalities, and exclusion of secondary causes (*1,4,6*). PSC is closely linked to inflammatory bowel disease (IBD), and some cases come to medical attention when patients with IBD are screened for liver disease (*7*). Genetic disposition and disordered immune regulation is considered to have a role in PSC. A recent report suggested that first-degree relatives and siblings are associated with a PSC prevalence approximately 100-fold that of nonrelatives (*8*). However, specific mechanisms remain un-clarified to date (*1*).

Gradual progression of cholestasis results in end-stage liver disease (ESLD). Ursodeoxycholic acid administration, with or without the use of immunosuppressive agents, seems to have a moderate clinical effect on liver function tests, but it is not clear whether there is a beneficial effect on delaying the progression of the disease and improving the overall survival rate (*4,9*). Effective medical treatment remains a matter of debate.

2. PSC and liver transplantation

Liver transplantation is the optimal treatment for patients with PSC presenting with ESLD and associated complications, such as severe manifestations of portal hypertension (*4*). The short-term outcome has been excellent. The impact of a recurrence on long-term survival has been a concern but was not significant in deceased donor liver transplantation (DDLT) based on Western experience (*10*).

In Asia, especially in the Far East, however, organs from deceased donors remain extremely scarce. LDLT has been accepted with enthusiasm and its indications have been extended to adult recipients (*11,12*).

Despite technical difficulties and the ethical dilemma, well planned donor selection protocol and technical innovations have paved the way for

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advancement with satisfactory results (*13,14*). LDLT for adult recipients has become standard practice in the region for various indications including ESLD due to PSC.

3. DDLT and PSC

Although a higher rate of re-transplantation has been reported, PSC is considered a good indication for DDLT with an excellent 5-year graft survival rate of approximately 80%. Evidence of recurrence has been suggested in earlier experience (*15,16*), and is currently an established concept (*17*). Recurrence of PSC after DDLT has been reported at rates between 1% to 33%, depending on the diagnostic criteria and follow-up duration (*4*).

Graziadei and colleagues proposed combined cholangiographic and hepatic histologic criteria with strict exclusion criteria (cases with ABO incompatibility between donor and recipient, nonanastomotic strictures before posttransplantation day 90, anastomotic strictures alone, hepatic artery complications, or ductopenic rejection were excluded) and reported that PSC recurs after DDLT in 20% of cases with typical radiologic manifestations found within a year, and histologic presentation within 3 years after DDLT (*18*).

Factors affecting recurrence have been extensively studied in the Western literature. An earlier retrospective study suggested that the presence of an intact colon before transplantation was significantly associated with recurrence (*17*). Other studies identified different factors as risk: recipient-donor gender mismatch (*19*), use of OKT3 or steroid resistant rejections (*20,21*), persistent ulcerative colitis after transplantation (*22*), human leukocyte antigen DRB1*08 (HLADRB1*08) (*23*). A most recent large series with a longer follow up period supports the findings that colectomy remains a significant risk factor, and further, use of extended donor criteria may also be a significant risk factor (*24*).

4. Recurrence of PSC in LDLT

A case suspected of recurrent PSC following LDLT was first reported by Kita *et al.* (*25*). Since then, there have been sporadic case reports of LDLT for PSC from living related donors (*26-34*) (Figure 1). However, follow-up periods were less than 2 years in most case reports, and recurrence was not reported.

Aside from these case reports, LDLT for PSC can be sporadically identified among moderate to large registries worldwide (*35-38*). From Korea, Moon *et al.* (*35*) reported 2 cases among 580 recipients. From Japan, Soejima *et al.* (*36*) also reported 2 cases of LDLT for PSC in their series of 52 LDLTs. Both, however, lacked a specific description of the long - term outcome. The largest PSC registry analysis by Maheshwari *et al.* (*38*) reported the outcome of 3,309 patients who underwent liver transplantation, among whom 69 cases were LDLT. Only 10 cases presented with a follow-up period longer than a year.

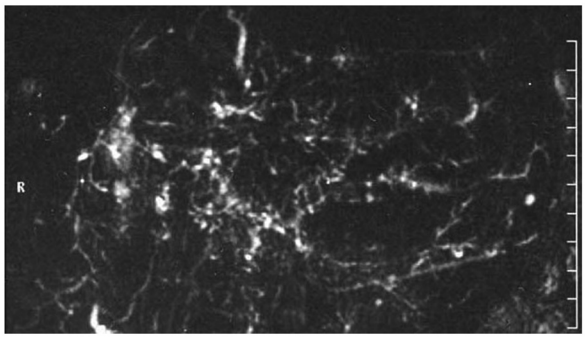

Figure 1. Representative radiologic image of Recurrent Primary Sclerosing Cholangitis following living donor liver transplantation. Drip infusion computed tomography cholangiography obtained from a typical recurrent case. Note the multifocal strictures and beading of the bile ducts within the engrafted partial liver.

An explanation for this lack of information on the long-term outcome of LDLT for PSC, especially regarding recurrence, may be due to the rare incidence of PSC in the Far East where LDLT is far more common than DDLT.

5. PSC in Japan and LDLT

Report on the epidemiology of PSC in Asian countries remains scarce. Currently, large registry analysis or nationwide survey study results are only available from Japan (Figures 2 and 3).

A recent nationwide survey from Japan including 391 patients with PSC with a median follow up period of over five years presented that the estimated median survival of all patients was 13.1 years, with a 5-year survival rate of 74.5%. Thirty-eight patients underwent liver transplantation with a 5-year survival rate of 92.0%. Cholangiocarcinoma was found in 14 (3.6%) patients. Prevalence of IBD was 32%. Interestingly, authors' pointed out two distinctive features recognized among Japanese patients. First, two peaks in age distribution were demonstrated (in the twenties and in the sixties) an uncommon distribution not reported in the Western series. Second, while the presence of IBD was limited to 37%, 7.2% of patients, mainly those older than 50 years of age, experienced autoimmune pancreatitis (*39-41*).

Earlier registry analysis including 66 patients who underwent LDLT for PSC has raised a concern on the impact of recurrence calling for study with well defined diagnostic criteria and a longer follow up period (*42*). Since then, two single center case series of LDLT for PSC have been published from high volume centers (*43,44*).

The Tokyo group described the outcome of 9 adult LDLT cases, of which 8 were living-related. The

median follow-up period was 3.5 years after LDLT. The five-year patient survival rate was 90%. According to Graziadei's criteria (*18*), however, recurrent PSC was diagnosed in four patients. The rate of freedom from recurrent PSC at 5 years was 49%. The mean time to recurrence was 3.3 years. When limited to biologically related donor-recipient cases, recurrent PSC was diagnosed in 50% of cases. None of the patients presented with the HLA haplotypes associated with a higher susceptibility for developing PSC in the Caucasian population (*43*).

The Kyoto group reported 28 patients with PSC who underwent LDLT. Among the 22 patients who survived for more than a year, 13 (59%) presented with PSC recurrence with a mean follow-up period of 31 months, 5 of whom died or required re-transplantation due to graft failure. The HLA haplotypes that may affect recurrence of the hepatic condition remain unclear, although HLA-DR15 is positively associated with ulcerative colitis. The group concluded that unlike PBC, the recurrence of PSC adversely affects the outcome in LDLT (*44*).

Finally, based on the above two reports, a nationwide survey was conducted collecting the data of 132 patients from 29 centers in Japan. Fifty-three (40%) had IBD, and 4 (3%) had cholangiocarcinoma. After excluding patients not undergoing primary standard LDLT, and further, excluding ABO-incompatible cases, those complicated with hepatic artery thrombosis and ductopenic rejection, 96 remained for analysis. Recurrence was diagnosed in 26 patients (27%) after transplantation. Recurrence-free survival was 57% at 5 years after LDLT and graft loss rate was 69% among those with recurrence compared to 23% among those without. Multivariate analysis revealed that high MELD scores, first-degree-relative donors, postoperative CMV infection, and early biliary anastomotic complications were significant risk factors for recurrence (*45*).

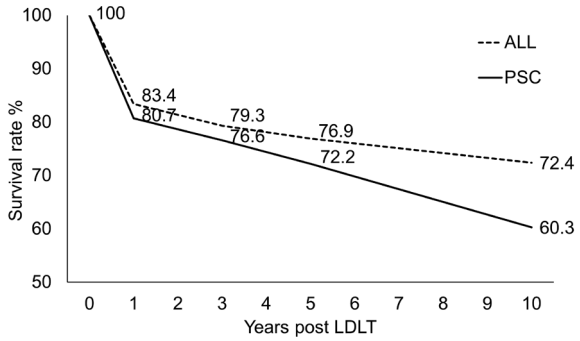

Figure 2. Outcome of living donor liver transplantation in Japan. Data obtained from the annual registry report provided by the Japanese Liver Transplantation Society (*ref. 46*). Abbreviations: ALL, all national cases combined; PSC, primary sclerosing cholangitis; LDLT, living donor liver transplantation.

6. Conclusion

Indeed, presenting with a high rate of recurrence after replacement of the entire affected organ, PSC

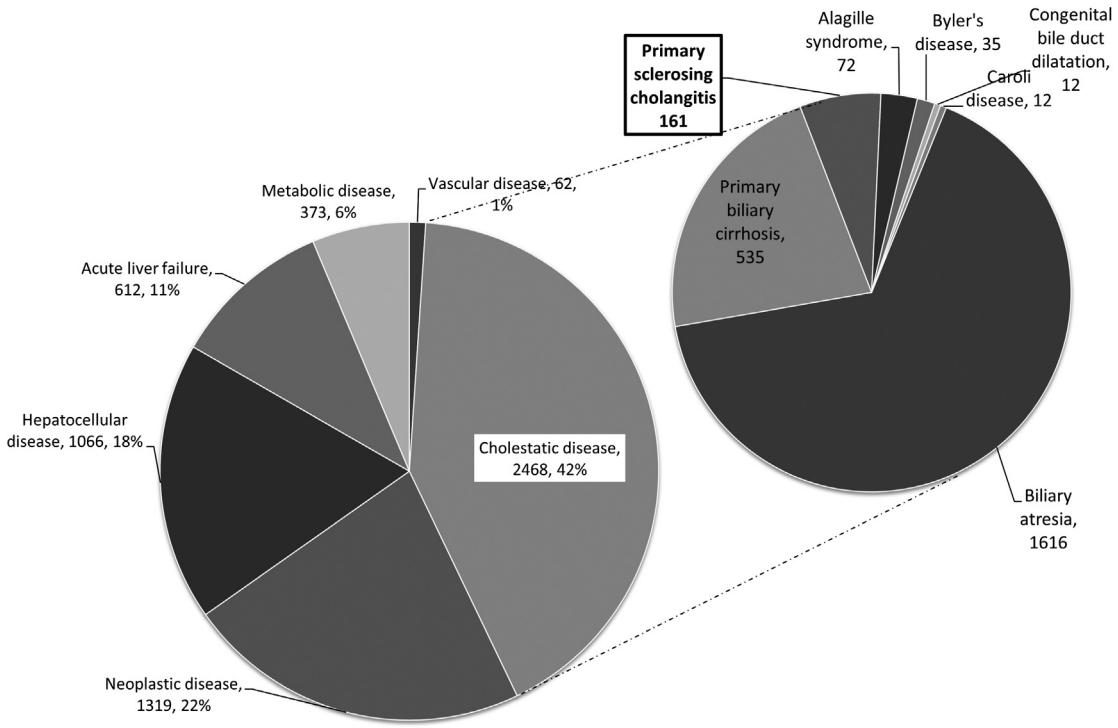

Figure 3. Indication for living donor liver transplantation in Japan. Data obtained from the annual registry report provided by the Japanese Liver Transplantation Society (*ref. 46*). Primary scerosing cholangits remains a rare indication in Japan.

encountered in the LDLT setting seems like an intractable entity. In addition, much remains unknown about the nature of PSC in the Far East where LDLT is predominantly performed in place of DDLT as compared to the West.

Whether the high rate of recurrence following transplantation in Japan may be attributed to the unrecognized genetic background, or to the characteristic organ source found in the region that is often from a biologically related living donor, remains to be elucidated.

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Mini-Review

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Unusual causes of upper gastrointestinal bleeding: Review of Chinese literature

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Summary **Upper gastrointestinal bleeding (UGB) is a life-threatening complication of gastrointestinal diseases. There is a large variety of uncommon reasons which contribute to UGB and might become reasons for misdiagnosis and sometimes lead to fatal consequences. In this review, clinical characteristics of uncommon causes of UGB reported in the Chinese literature are summarized.**

Keywords: Upper gastrointestinal bleeding, unusual cause, China

1. Introduction

Upper gastrointestinal bleeding (UGB) is a life-threatening complication of gastrointestinal diseases. Even though bleeding is the common symptom, different causes of gastrointestinal bleeding are significantly different. Not only the gastrointestinal tract itself, but also lesions in adjacent organs and systemic diseases contribute to bleeding. Therefore, realization of the etiology and characteristics is essential for doctors to diagnose and treat the disease (*1*).

Peptic ulcer remains the most common cause of UGB, representing approximately half of all cases (*2,3*). Esophageal varicose and gastritis (*4,5*), gastric carcinoma (*5*) and benign tumors of the digestive tract (*6*) are also common causes of UGB. There are many case reports of unusual causes of UGB (*7-9*), hence widening our understanding into the etiology of UGB. On the other hand, UGB of rare diseases can also be the reason for misdiagnosis (*10*) and sometimes leads to fatal consequences (*11*).

In this short review, we try to summarize rare causes of UGB from a Chinese doctors' point of view. This will enrich our knowledge to the etiology of UGB and broaden our judgment in the process of diagnosis of UGB, and thus improve the accuracy of diagnosis and efficacy of treatment.

2. Search strategy and data extraction

The electronic databases of Chinese Medicine, Chongqing VIP, from January 1989 to June 2011 were searched to identify all clinical reports related to UGB. The disease-specific search term UGB was used. The abstracts were reviewed and only those reported rare causes of UGB (that is, those other than peptic ulcer, gastric carcinoma, gastritis, varicose rupture and gastric polyps) were included. The full text was read and clinical related data was extracted, summarized and listed as follows.

3. Unusual causes of UGB

3.1. *Crest syndrome*

Crest syndrome is a form of systemic scleroderma associated with antibodies against centromeres that usually spares the kidneys. It is extremely uncommon for Crest syndrome to present with UGB and can be easily misdiagnosed with hemorrhage following variceal rupture. The following features are helpful for the establishment of diagnosis: i) Crest syndrome is a connective tissue disease and multiple organs are involved, ii) rupture of capillaries is the basic pathologic change and both the upper and lower gastrointestinal

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tract can be the source of bleeding, iii) liver damage is slight and coagulation function is normal, thus the bleeding can stop spontaneously and *iv*) laboratory tests show that anticardiolipin antibody is positive, which is the characteristic finding of Crest syndrome (*12*).

3.2. *Dieulafoy's lesion*

Dieulafoy's lesion is a submucosal caliber-persistent artery anomaly. The exact frequency of Dieulafoy's lesion is difficult to estimate. The patients usually have no history of peptic ulcer and liver cirrhosis but the clinical presentation mimics these situations because blood loss is usually significant and rapid. Rahbour *et al*. (*13*) reviewed the English literature and found that 95% of Dieulafoy's lesion occurs in the upper part of the stomach, within 6 cm of the gastroesophageal junction and commonly in the lesser curvature. However, this trend seemed to be not so obvious in the Chinese literature as there are still many cases with a lesion located in the antrum or even the duodenum. Another characteristic feature of Dieulaoy's lesion we summarized after reviewing the Chinese literature is periodic attacks. The bleeding stops temporarily with hypovolemic shock but recurs while resuscitation is achieved. The key point of successful treatment lies in the location of the lesion. But the extent of the lesion is limited and can be missed by endoscopy, and thus sometimes leads to fatal consequences (*14,15*).

3.3. *Mallory-Weiss syndrome*

Mallory-Weiss syndrome refers to a tear or laceration of the mucous membrane, most commonly at the point where the esophagus and the stomach meet (gastroesophageal junction). In the Chinese literature, more than half of the patients had a history of drinking which caused nausea and vomiting. The rapid increase of intra-gastric or intra-abdominal pressure accounted for the tear of mucosa at the gastroesophageal junction which is less flexible. The typical symptom is that vomiting of gastric contents followed by hematemesis, which typically begins with vomiting fresh bright red blood which becomes dark gradually. The diagnosis can be established according to clinical presentation and be confirmed by endoscopic examination (*16*). Atkinson (*17*) believed that the presence of a small hiatal hernia appeared to predispose to mucosal tears. However, there are no reports describing this condition in the Chinese literature.

3.4. *Rupture of aneurysm*

UGB originating from rupture of an aneurysm of the branches of the celiac artery or superior mesenteric artery is rare but the mortality rate could be higher than 75%. Chen *et al*. (*18*) summarized that these patients usually had no specific symptoms before the hemorrhage attack but the following features were valuable for the establishment of diagnosis: i) blood loss was rapid and massive and the patients quickly entered a state of shock, ii) a pulsatile mass could be found on physical examination and iii) endoscopy could not find the source of bleeding but angiography was the most valuable means to locate it.

3.5. *Aortoenteric fistula*

Aortoenteric fistula, a rare cause of gastrointestinal bleeding, is defined as a communication between the aorta and the gastrointestinal tract. It can be divided into: i) primary aortoenteric fistula with no history of aorta surgery but originates from erosion into the gastrointestinal tract (usually the fourth part of duodenum) with expansion of the aneurysm and ii) secondary aortoenteric fistula, which is mostly a complication of surgery, such as rupture of an anastomosis in the aorta, or the erosion of the graft into the duodenum or small intestine. The classic triads of symptoms include abdominal pain, gastrointestinal bleeding and pulsating abdominal mass. Jin *et al*. summarized that more than 90% Chinese patients had slight hematochezia before massive bleeding which could be considered as warning signs of massive hemorrhage (*19*). Early surgical intervention was the only cure for this condition (*19,20*). Apparently, the diagnosis is relatively easy in secondary aortoenteric fistula as the patients have a clear history of aorta surgery. However, because aorta surgery has developed very late in China, the majority of aortoenteric fistulas are primary and not secondary and even a history of an aorta aneurysm can hardly be a question (*19*). This adds to the difficulty of establishing a prompt diagnosis.

3.6. *Ancylostomiasis*

Ancylostomiasis (also anchylostomiasis or ankylostomiasis) is the condition of infestation by ancylostoma hookworms. It has become a less serious public health problem in Western countries but a report showed that the average infestation rate of ancylostoma hookworms was 17.2% in 2000 in China. Jing (*21*) reported 40 cases of ancylostomiasis-related UGB and found that all the patients lived in a rural area and/or presented with hematemesis or hematechezia and chronic anemia. More than 75% of the patients suffered discomfort in the upper abdomen and more than 65% presented with fatigue and bad appetite. Some patients had strange food addictions and hookworm dermatitis. The diagnosis could be established according to the positive result of hookworm ovum and the direct finding of hookworm in the stomach.

3.7. *Small intestine ulcer*

Gao (*22*) reported a case of small intestine ulcer induced by adhesion. In laparotomy, after exclusion of other sources of hemorrhage, a 20 cm long jejunum was found tightly adherent to each other and could not be lysed. Fresh blood accumulated in the lumen of the intestine. After resection of this part of the small intestine, 6 cm × 4 cm ulcers were found on either side of the adhesion. Local ischemia induced by chronic obstruction and long-term distension might have been responsible for the formation of the ulcers.

3.8. *Crohn's disease*

Crohn's disease can be a cause of intestinal bleeding or obstruction. But it is a rare disease in China and an even rarer condition combining bleeding and obstruction if a patient suffers from Crohn's disease. If the surgeon is satisfied with the resolution of obstruction during the first laparotomy, a second operation might become necessary because of post-operative bleeding. Wu *et al*. (*23*) reported a case of UGB whose pre-operative X-ray film also showed signs of ileus. During the operation, after lysis of all adhesions, a short segment of jejunum of 10 cm from Tretiz's ligament was found to be edematous. The pathological examination of this resected intestine showed the typical changes of Crohn's disease with fresh blood inside the lumen. The author believed that UBG was not only the consequence of ileus, but also the co-existing condition. So, resection of the lesion might be a better choice than short-circuit surgery.

3.9. *Ascariasis*

Ascariasis is a very common condition in children. Sangkhathat *et al*. (*24*) reported two cases of ascariasis-related UGB in English. In one case, a roundworm was found adhering to an oozing duodenal ulcer and in another case, numerous ascaris lumbricoides lead to a segmental volvulus of the distal ileum, to gangrene and perforation. Huang *et al*. (*25*) reported another case in which a mass of tapeworms was found piled up in the lumen and mechanical pressure induced mucosal erosion and was the source of bleeding.

3.10. *Rabies*

Wang *et al*. (*26*) reported a case of rabies combined with UGB. The patient was admitted with a history of hematemesis. Conservative therapy was not effective and further inquiry found a history of a dog bite 25 days prior to presentation. The patient presented with typical symptoms of rabies and eventually died of hypovolemic shock and lung dysfunction. The bleeding might have been a type of stress ulcer. Hyperfunction of the sympathetic nerves from the invasion of virus into the brain tissue might be the underlying pathogenesis.

3.11. *Benign tumor of the duodenum*

Benign tumors of the duodenum, such as leiomyoma (*27*), Brunner adenoma (*28*), lymphoceles (*29*) and hemangioma (*30*) are rare conditions. The formation of an ulcer with the growth of tumor can be the source of bleeding. In most circumstances, the hemorrhage is mild. Patients with large tumors present with symptoms of bleeding combined with intestinal obstruction. Endoscopy and a barium meal are the most valuable means to locate the lesion while ultrasonography is not feasible because of interference from intestinal gas. Pre-operative diagnosis is usually difficult because of the difficulty of obtaining a specimen for pathological examination. It can only be established following surgical resection.

3.12. *Rare conditions of the stomach*

There are reports of UGB related to lymphoepithelioma (*31*), lymphoma of mucosa associated lymphoid tissue (*32*), fibrolipoma (*33*) and inflammatory myofibroblastic tumor (*34*) of the stomach in the Chinese literature. The common feature of these uncommon causes of UGB is that the diagnosis can hardly be established before surgery. Fortunately, UGB with an obvious lesion in the stomach itself is a definite indication for surgical intervention and the diagnosis can only be confirmed by pathological examination with the assistance of immunohistochemistry detection of a specific antigen for a particular condition.

3.13. *Pancreas disorders*

Solid pseudopapillary tumor of the pancreas (SPTP), an uncommon neoplasm with low malignant potential often causes few symptoms and may reach a large size before it is detected. It can invade adjacent structures, mostly the duodenum, and cause ulcers and hemorrhage (*35*). Pseudo-cyst of the pancreas shares some common features with SPTP despite the fact that the patients have a clear history of pancreatitis (*36*). Both lesions can be easily located on ultrasonography or CT scans and the final diagnosis needs pathological confirmation. Zhu *et al*. (*37*) summarized their experience of treating pancreatogenic portal hypertension, rupture of varices of gastric fundus complicated with hemorrhage of the splenic artery, pancreatic duct, or pancreatic pseudocyst fistula. They concluded that the characteristic manifestations of the disease were history of chronic pancreatitis, long-term intermittent pain in the epigastric region followed by melena, vomiting of blood, and splenomegaly with normal hepatic function. The key steps of diagnosing the disease were gastroduodenal endoscopy, retrograde pancreatocholangiography and

selective splenic arteriography. Li *et al*. (*38*) reported a case of UGB whose endoscopic examination showed an ulcer in the duodenum. CT scan indicated a mass on the head of the pancreas and the final pathological diagnosis was malignant insulinoma. The author concluded that some neuroendocrine tumors of the pancreas can lead to UGB *via* two approaches: i) direct invasion and ii) influence of hormone levels leading to ulcer formation.

3.14. *Behcet's disease*

Behcet's disease symptoms vary from person to person. Most symptoms of the disease are caused by vasculitis. Inflammation and ulceration throughout the digestive tract caused by Behcet's disease is a less common symptom. Wei *et al*. (*39*) reported a case of UGB with Behcet's disease. Under endoscopy, multiple ulcers in the esophagus and cardia were detected. The patient had a clear history of recurrent mouth and genital sores, a characteristic skin lesion on the face, and the diagnosis of Behcet's disease with UGB was established.

4. Comment

UGB is a common emergency situation needing prompt and accurate diagnosis. Even though the common causes of UGB, such as peptic ulcer and variceal bleeding, account for more than 80 percent of cases, there is still a large variety of uncommon reasons which contribute to UGB with digestive tract bleeding being only part of the symptoms in a certain case.

The spectrum of diseases is different between Western countries and China. We should mention that some rare causes contributing to UGB might be very common in Western countries, for example, Crohn's disease. There were still some really rare causes, for example, UGB derived from small intestine ulcer and rabies, which had not been reported in the English literature. Despite that, what we emphasized in this mini-review was the clinical features of these rare conditions, especially their differences from those reported in the English literature. We hoped to provide evidence that might not be easily accessed by non-Chinese physicians and share our experience with foreign doctors.

A doctor should bear in mind that some rare diseases can cause rapid and massive hemorrhage and that the mortality rate could be higher than 75 percent. That means that not all cases ameliorate with conservative treatment and prompt surgical intervention is the only cure under these circumstances.

Another viewpoint is that not all sources of bleeding can be located by endoscopy. So the rational combination of endoscopy with other imaging detection, such as ultrasonography, CT scan and angiography offers more valuable information for achievement of diagnosis.

There are still some clues for diagnosis of uncommon cases. The doctor's concentration or focus should not only be limited to the digestive tract itself. A detailed history inquiry and thorough physical examination may lead the doctor to the correct diagnosis as even the rare diseases have their own characteristics.

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Mini-Review

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The etiologies of new cases of cerebral venous sinus thrombosis reported in the past year

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Summary **Cerebral venous sinus thrombosis (CVST) is an uncommon but life-threatening stroke subtype with extremely diverse clinical features, predisposing factors, brain imaging findings, and outcomes. Its predominant etiologies were known to be otomastoid, orbit, and central face cutaneous infections, pregnancy and complications associated with the postpartum period, and oral contraceptives. In recent years, however, infections have accounted for fewer cases while oral contraceptives, pregnancy and complications associated with the postpartum period, tumors, and coagulopathies have accounted for more cases of CVST. These conditions have become the predominant risk factors for CVST, but uncommon etiologies have also emerged. This review focuses on the new etiologies of CVST cases reported this year to broaden perspectives on the etiologies of CVST.**

Keywords: Cerebral venous sinus thrombosis, etiology

1. Introduction

Cerebral venous sinus thrombosis (CVST) is an uncommon but life-threatening stroke subtype caused by clotting of blood in cerebral venous or dural sinuses, and, in rare cases, cortical veins, with extremely diverse clinical features, predisposing factors, brain imaging findings, and outcomes (*1,2*). Predisposing clinical conditions usually combine to constitute an underlying etiology. It is a rare but potentially fatal cause of acute neurological deterioration previously related to otomastoid, orbit, and central face cutaneous infections, pregnancy and complications associated with the postpartum period, and oral contraceptives (*2*). With the advent of antibiotics and improved medical care for women, infections and the postpartum period have been controlled, so these conditions have accounted for fewer cases of CVST. In recent years, new risk factors for CVST have emerged as people are living longer. Here, papers published last year and common and uncommon etiologies of CVST at this time have been summarized in order to broaden perspectives on the etiologies of CVST.

2. Search strategy and data extraction

MEDLINE and PUBMED databases were searched for articles in English on etiologies of CVST, including case control, cohort, and case series studies and case reports published in peer-reviewed journals from September 2010 to October 2011.

3. Features and etiologies of CVST

The features and etiologies of CVST in cases reported this year are summarized in Table 1. Patients in these cases were of various ages, from neonates to 56-year-old; most susceptible were younger individuals and

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Table 1. Cerebral venous sinus thrombosis cases reported in the past year

Case number	Etiology	Age	Gender	First author (*Ref. No.*)	Published year
1	Unknown	41	F	Nimjee SM (*3*)	2011
2	Unknown	32	F	Levy M (*6*)	2011
3	Unknown	52	F	David Oehme (*10*)	2011
4	Unknown	18	F	Christopher T. Shah (*30*)	2011
5	Vaginal rings	28	F	Kolacki C (*4*)	2011
6	Vaginal rings	32	F	Fugate JE (*5*)	2011
7	High-voltage electrical burns	10	F	Singh G (*8*)	2011
8	Oral contraceptives	20	F	Min J (*7*)	2011
9	Oral contraceptives	21	F	Slankamenac P (*9*)	2011
10	Oral contraceptives	45	F	Wong VS (*31*)	2011
11	SIH	35	M	Dangra VR (*10*)	2011
12	SIH	26	M	Yoon KW (*13*)	2011
13	The postpartum period	19	F	McCaulley JA (*12*)	2011
14	Paroxysmal nocturnal hemoglobinuria	44	M	van Eimeren VF (*15*)	2011
15	Pregnancy	33	F	Ferreira MM (*16*)	2011
16	Mutations in factor V Leiden and MTHFR A1298C	56	F	Ozkurt S (*17*)	2011
17	Hyperthyroidism	8	F	van Eimeren VF (*15*)	2011
18	Choriocarcinoma	33	F	May T (*18*)	2011
19	Factor V Leiden mutation	12	M	Yilmaz S (*20*)	2011
20	Chemotherapy for acute lymphoblastic leukemia	4	M	Wang TY (*21*)	2011
21	HIV+ protein S deficiency	30	M	Modi M (*22*)	2011
22	Trauma	30	F	Fahim DK (*24*)	2011
23	Ruptured intracavernous carotid artery aneurysm	55	F	Aldea S (*25*)	2011
24	Dural scalp and intracranial hemangiomas	4 months	F	Nahed BV (*26*)	2011
25	Sickle-cell disease	42	F	Vassilopoulou S (*32*)	2011
26	Polycythemia	31	M	Raval M (*27*)	2011
27	Type I antithrombin deficiency	25	F	Sharpe CJ (*28*)	2010
28	Iron deficiency anemia	18 months	M	Habis A (*29*)	2010

SIH means spontaneous intracranial hypotension. Vaginal rings mean contraceptives like NuvaRing (a combined vaginal contraceptive ring).

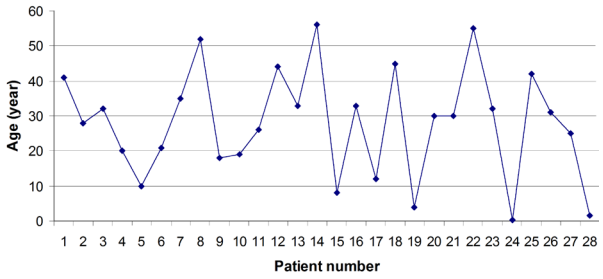

Figure 1. Age of onset in patients with cerebral venous sinus thrombosis.

women. The average age of onset (Figure 1) was 27.99 ± 12.22 years, females accounting for 71.4% of patients and males accounting for 28.6% (Female:Male = 2.5:1). The etiologies of cohort CVST cases reported this year varied. Joining known etiologies were some new risk factors. Contraceptive use accounted for 17.9% of cases and included oral contraceptives in 10.7% and use of a local contraceptive (NuvaRing) in 7.2%. Other common etiologies were hematological diseases (14.3%), a hypocoagulable state (7.2%), systemic cancer and other malignant hematological diseases (7.2%), a hypercoagulable state (type I antithrombin deficiency), and trauma (7.2%). Infection was rare as an etiology in these cohort cases while oral contraceptives were a predominant risk factor. There were, however, 14.3% of cases that had no clear etiology. Uncommon etiologies include spontaneous intracranial hypotension (SIH), hyperthyroidism, paroxysmal nocturnal hemoglobinuria, ruptured intracavernous carotid artery aneurysm, dural scalp and intracranial hemangiomas, sickle cell disease, polycythemia, and iron deficiency anemia (Table 1). Unknown etiologies account for 14.3% of cases of CVST, which is a rather high figure as a percentage of all etiologies, and this finding agrees with the results of a previous study (*1*).

4. Discussion

At this point, the predominant etiologies of CVST are oral contraceptives, hematological disorders, a hypercoagulable state, trauma, cancer, and pregnancy and complications associated with the postpartum period, but some newly emerged risk factors warrant more attention.

NuvaRing, a combined contraceptive vaginal ring, is a contraceptive widely used as an alternative to oral contraceptives with a purported advantage of allowing lower hormonal doses, thus potentially presenting less of a risk for venous thromboembolism (*4*). However, CVST has recently been noted in association with the use of a contraceptive ring, and these cases deserve attention (*4,5*). That is, both oral and local contraceptive use carry a risk of CVST. Emergency physicians should keep in mind that patients using NuvaRing have an increased risk of CVST. That said, there is still a need for large randomized and control studies of the

relationship between local contraceptive use and the onset of CVST.

SIH caused by a cerebrospinal fluid (CSF) leak is another reported risk factor for CVST. SIH may change the velocity of the cerebral blood flow and cause thrombosis (*10,13*).

However, case reports are just individual instances and do not represent general facts. Thus, newly emerging risk factors for CVST need to be studied further. In addition, unknown etiologies also account for a considerable number of cases of CVST (14.3%). Accordingly, the pressing challenge is to identify other potential risk factors for CVST.

In conclusion, the predominant etiologies of new cases of CVST were reported to be oral contraceptives and local contraceptives (*e.g.* NuvaRing), pregnancy and complications associated with the postpartum period, trauma, cancer, hematological disorders, and a hypercoagulable state. Infection is no longer a common etiology of CVST.

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Brief Report

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Identification of a germline mutation in the *HRPT2* gene in a Chinese family with parathyroid carcinomas

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Summary **This study reported a family with primary hyperparathyroidism due to parathyroid carcinoma and investigated the pathological and genetic features of family members. Three members of the family had clinical manifestation of primary hyperparathyroidism and tumors in the neck. All three patients underwent parathyroidectomy, thyroidectomy and level-VI neck dissection and were definitively diagnosed based on pathology. The index case was a patient that was found to have parathyroid carcinoma on the right side and parathyroid adenoma on the left side. The other two patients had local tumor recurrence and metastasis to distant organs. A germline mutation in the *HRPT2* gene (Arg91Pro) was identified in all of the patients in this family. Study of the literature indicated that this is the first report of familial parathyroid carcinomas with an *HRPT2* gene missense mutation. Results also indicated that *HRPT2* may play an important role in the development of parathyroid carcinoma.**

Keywords: Familial, parathyroid tumor, hyperparathyroidism, *HRPT2*

1. Introduction

Parathyroid carcinoma (PTC) is a rare malignant tumor of the endocrine system and accounts for fewer than 1-3% of all cases of primary hyperparathyroidism (*1,2*). Primary hyperparathyroidism usually occurs sporadically but may be inherited as an autosomal dominant trait. However, few previous reports have described familial parathyroid carcinoma. The current study reported a family with three parathyroid carcinoma patients, and a germline mutation in the *HRPT2* gene was identified in these family members.

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2. Materials and Methods

2.1. *Clinical data*

The index case, a 30-year-old man, complained of pain in the lower extremities for 2 years and headache, fatigue, nausea and vomiting for 1 month when seen on November 14, 2008. A computed tomography (CT) scan revealed two tumors with distinct margins (17 × 17 × 25 mm and 15 × 14 × 25 mm) in the posterior and lower portions of both lobes of the thyroid. No significant increase in shadows from neck lymph node shadows on either side was noted (Figure 1A). An emission CT scan revealed nodules in the right lower lobe that had radioactive distribution equal to the surrounding thyroid tissue, and the lower pole of the left lobe had limited radiation uptake (Figure 1B). The serum levels of parathyroid hormone (PTH), alkaline phosphatase (ALP), calcium, phosphorus, and potassium were 1,367 pg/mL, 392 U/L, 4.03 mmol/L, 0.56 mmol/L, and 3.43 mmol/L, respectively. The patient in the index case underwent parathyroidectomy, thyroidectomy, and level-VI neck dissection. Postoperative pathology confirmed parathyroid carcinoma on the right side (Figure 2A) and

parathyroid adenoma on the left side, and no lymph node metastasis was found.

The father and sister of the patient in index case underwent surgery at other hospitals and were confirmed to have parathyroid carcinoma by a pathological examination. Furthermore, both had distant bone metastasis, and the sister had already died of multiple bone metastases.

2.2. *Immunohistochemical staining*

Immunohistochemistry was performed on the tissues of the parathyroid carcinoma and the adenoma from the index case. Immunohistochemical staining was performed with commercially available monoclonal antibodies against human Syn, CgA, Calcitonin, TG, TTF-1 and Ki-67 as described previously (*2*).

2.3. *HRPT2 gene mutation analysis*

Peripheral blood was obtained from all family members (3 patients and 3 asymptomatic relatives) with their informed consent to identify germline *HRPT2* mutations. This was approved by the local ethics committee. Extracted DNA was amplified in 15 segments (from 226 to 599 bp) to allow mutation screening of the 17 translated *HRPT2* exons and their exon-intron boundaries. The primers used for PCR amplification are described in supplemental data. A 20-µL mixture was prepared for each reaction and included 1× HotStarTaq buffer, 2.0 mM Mg^{2+}, 0.2 mM dNTP, 0.2 µM of each primer, 1 U HotStarTaq polymerase (Qiagen Inc. CA, USA), and 1 µL template DNA. The cycling program was 95ºC for 15 min; 11 cycles of 94ºC for 15 sec, 62ºC-0.5ºC per cycle for 40 sec, 72ºC for 1 min; 24 cycles of 94ºC for 15 sec, 56ºC for 30 sec, 72ºC for 1 min; 72ºC for 2 min. PCR products were purified using SAP and Exo I and then sequenced on an ABI 3130XL sequencing system (Applied Biosystems, CA, USA).

3. Results and Discussion

Mutations of the *HRPT2* gene have recently been implicated in the development of parathyroid carcinoma (*3-5*). Familial isolated hyperparathyroidism with the *HRPT2* mutation may represent a risk factor for developing parathyroid carcinoma. However, familial

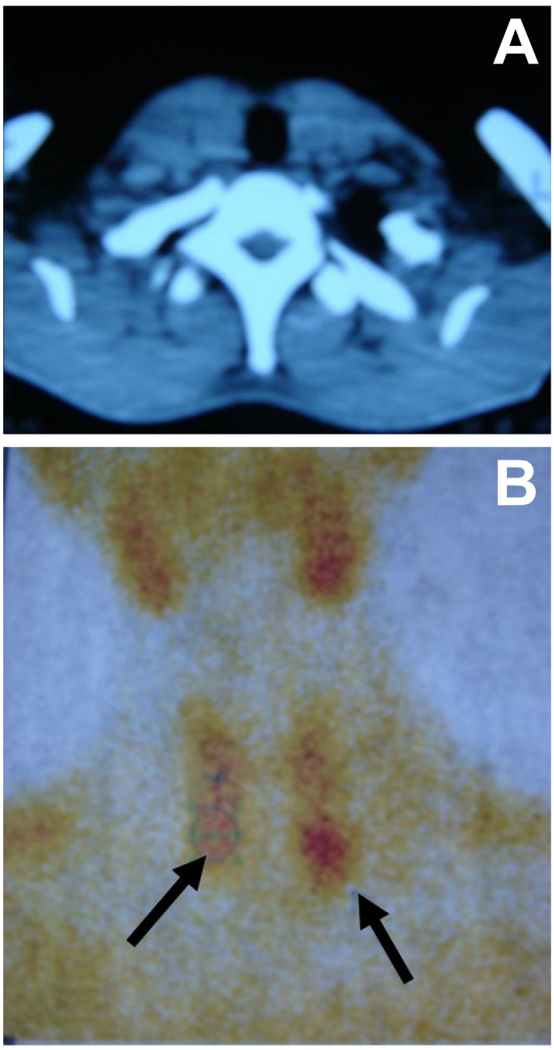

Figure 1. Images of the parathyroid tumors in the index case. (**A**), Computed tomography. (**B**) Emission computed tomography.

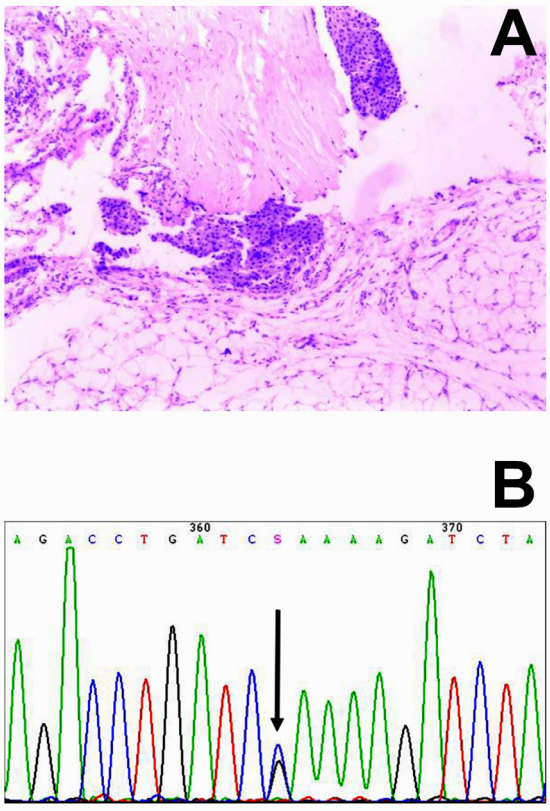

Figure 2. Pathological images of the parathyroid carcinoma in the index case (**A,** HE staining, Original magnification 100×)**, and a *HRPT2* gene missense mutation at exon 3** (Arg91Pro, **B**).

parathyroid with primary hyperparathyroidism has rarely been reported.

The current study is the first to report a family with 3 cases of parathyroid carcinoma with the *HRPT2* gene mutation. A 3-generation pedigree of this family revealed that 3 members had parathyroid carcinoma with a *HRPT2* gene missense mutation at exon 3 (Arg91Pro) (Figure 2B), and asymptomatic relatives were negative for the Arg91Pro germline mutation. Furthermore, the index case had both benign and malignant parathyroid tumors, a finding that has been reported in previous studies (*6*). These findings suggest that the Arg91Pro mutation of *HEPT2* gene might be required for multistage development of parathyroid carcinoma. The molecular characteristics of benign and malignant tumors were also compared based on immunohistochemistry. Immunohistochemistry results for the parathyroid carcinoma tissues (right side) were follows: Syn (weak positive), CgA (weak positive), Calcitonin (-), TG (-), TTF-1 (-), and Ki-67 (positive cells 5-10%). Immunohistochemistry results for the left parathyroid adenoma were as follows: Syn (weak positive), CgA (weak positive), Calcitonin (-), TG (-), TTF-1 (-), and Ki-67 (positive cells < 2%). Molecular characteristics of the parathyroid adenoma and carcinoma were similar, indicating that the parathyroid carcinoma may have originated from the adenoma, which was induced by the *HEPT2* gene mutation at Arg91Pro.

In a previous study, Cetani *et al*. (*7*) identified the Arg91Pro mutation in patients with early onset of primary hyperparathyroidism (PHPT), and they suggested this *HRPT2* mutation may be ascribed to modification of parafibromin conformation and/or stability. The Arg91Pro mutation is also associated with different *HRPT2* somatic alterations in parathyroid tumors, which is consistent with the "two hit" concept of biallelic inactivation of classical tumor suppressor genes.

In conclusion, this report is the first to identify the pedigree of parathyroid carcinomas with the *HRPT2* gene missense mutation. Together with previous findings, the current results suggest that the Arg91Pro mutation of the *HRPT2* gene might be associated with early onset of PHPT and a high risk of developing a parathyroid carcinoma. Results also suggest that *HRPT2* may serve as an important suppressor gene in the development of parathyroid carcinoma.

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Supplemental data

PCR Primers

P2F	GGGGCGCAGTCAGGCGTTCTC
P2R	CCCGAACACCCGTTTTATCCCATCC
P3F	TCATTGTTGTTAGCAAAGTTGTTT
P3R	ATGAATTATTGGCCAGGCACA
P4F	CGCCCAGCCAAGAAAATTA
P4R	CCATAAGGGCAAAGACAGTGC
P5F	TGCAGAGCTGCTTTAAAACTGAA
P5R	CCTTGAGCCAATAGGTTCATCC
P6F	TTGACTCTGGTGAAGGCTTGTC
P6R	CCAATCCCCACACATGTTCTT
P7F	TTGCCATGTAAGTGTTTTTACCAGA
P7R	AACAGGAAAACTGGGCCATTC
P8F	GCCTCCCGAATGTAGCAGTTT
P8R	TTTAATGTTGGGGAGGGCTTT
P9F	TGAGCCATGGTCATGCTACTG
P9R	CCCTTAAACATCAGGCCACAC
P10F	TGCCTTATGACTGAAACTTTGGA
P10R	CCACACAGATCAATCAGCACAA
P11F	GGTTTTTCCAACAGGAGGGTA
P11R	TCGACAGTCTTCAAAGAAACATGA
P12F	TTTCCTTTTGACTACAGATTGTTGAA
P12R	GCCTATAGCACAGAAACCGAAA
P13F	TTGAAACCAGAAGGTGGAGGT
P13R	CAACGTCATCAACGGCAATAA
P14F	AAGGGAACAGAAAGGGCAAAC
P14R	TGTTGTGGGATAGGCAATATTCA
P15F	TTATAATACGGCTTCAGTTGGTGGA
P15R	TTGCAAAAAACACAGGGTTCTC
P16F	CGGATGGTTCACAAAAGGAAA
P16R	CTTGAAGCACAAAGCATCAAA

Original Article

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Mutations in the *RS1* gene in a Chinese family with X-linked juvenile retinoschisis

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Summary **The purpose of our study was to identify the mutations in the retinoschisis 1 (*RS1*) gene, which was associated with X-linked retinoschisis (XLRS) in a four-generation Chinese family, and to provide the theoretical basis for gene diagnosis and gene therapy. Genomic DNA was extracted from peripheral leukocytes. All six exons and flanking intronic regions were amplified by polymerase chain reaction (PCR), followed by direct sequencing. Through our genetic analysis, one frameshift 573delG mutation was identified in the patients of this four-generation pedigree; however, this mutation was absent in normal or non-carrier subjects. In conclusion, this 573delG mutation is reported in the Chinese population for the first time. This mutation widens the mutational spectrum of *RS1* in Asians. Identification of mutations in the *RS1* gene and expanded information on clinical manifestations will facilitate early diagnosis, appropriate early therapy, and genetic counseling regarding the prognosis of XLRS.**

Keywords: X-linked juvenile retinoschisis, polymerase chain reaction, frameshift mutation, foveal schisis

1. Introduction

X-linked retinoschisis (XLRS) is one of the most common causes of juvenile macular degeneration in males, with an incidence of 1 in 15,000 to 1 in 30,000 (*1*). The major clinical characteristics of affected males are visual deterioration, foveal-schisis in almost every patient due to the splitting of the retinal cell layers, and a decrease in the b-wave amplitude of the electroretinogram (ERG). Unfortunately, there is no medication to halt the progression of maculopathy, which may induce complete blindness.

XLRS, a recessively inherited disorder, is caused by mutations in the retinoschisis 1 (*RS1*) gene localized at chromosome Xp22.2. The *RS1* gene consists of six exons, and encodes a 224 amino-acid protein named retinoschisin. This protein, has an N-terminal secretory leader peptide and a conservative discoidin domain encoded in exons four to six, and is expressed and secreted from photoreceptors and bipolar cells, where this protein is anchored to the surface of the secreting cells themselves. This protein is presumed to be responsible for normal adhesion and signaling within the retinal cell layers (*2,3*).

On the basis of previous genetic examinations, a wide spectrum of different mutations in *RS1* have been recorded in the Leiden Open Variation Database, majority of these reported mutations are missense mutations, which primarily exist within exons 4-6 of *RS1* (*4*). Exons 1-3 tend to have mainly translation truncating nonsense mutations (*5*). However, the precise correlation between the phenotype and genotype of XLRS still remains unclear.

In our study, we encountered a large XLRS Chinese family where one family member had already lost his sight completely. In the current study, we aimed to identify the molecular variations of XLRS in this Chinese family. We detected the mutations in the *RS1* gene by direct sequencing.

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2. Materials and Methods

2.1. *Patients and controls*

The study protocol, which was carried out in accordance with the tenets of the Declaration of Helsinki, was approved by the ethical committee of Henan provincial people's Hospital. Twenty-four members of the family with XLRS and 40 eligible volunteers as normal controls were recruited for this research. Family history was collected from the proband. Comprehensive ophthalmic examinations included best-corrected visual acuity (BCVA), ERG, optical coherence tomography (OCT), fluorescein angiogram, slit lamp biomicrocopy, and fundus examinations, in order to confirm the diagnosis. Informed consent was obtained from all participants prior to their evaluations in the course of the study.

2.2. *Blood collection and DNA extraction*

For each subject, venous blood samples (5 mL) were collected into tubes containing EDTA and genomic DNA was extracted using the DNA blood isolation kit. For all probands, exons 1-6 of *RS1* PCR was carried out to amplify the exonic regions of *RS1*, using the primers listed in Table 1. The PCR reaction was performed in a DNA thermocycler (Eppendorf, Hamburg, Germany). The reaction was performed in a total volume of 25 μL containing 2.5 μL 10× buffer (25 mM $MgCl_2$), 0.2 μM each of dNTP, 0.5 μM of each primer, 1 units of Taq DNA polymerase, and 100 ng of genomic DNA. The cycling procedure for the *RS1* gene were as follows: 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 30 sec, annealing for 30 sec at the appropriate temperature for each primer pair, and extension at 72°C for 30 sec. The final extension step was lengthened to 5 min.

2.3. *Direct sequencing*

All amplified fragments were electrophoresed in a 1.5% agarose gel and purified with a DNA extraction kit. Exons and the flanking intronic regions of *RS1* were directly sequenced on an automated sequencer (ABI prism 3130 Genetic Analyzer, Applied Biosystems, CA, USA). The results were compared with the reference sequence of the XLRS sequence variation database.

3. Results

3.1. *Clinical findings*

The pedigree of interest was a four generation family with twenty-four family members, including four affected males and twenty unaffected individuals based on clinical evaluations (Figure 1). An X-linked pattern of inheritance of disease was shown in the family pedigree. However, a high degree of clinical variability is observed among the patients. The mean age at disease onset was 7.5 years, and the initial clinical presentation was poor visual acuity. The clinical characteristic of the proband was mainly bilateral foveal schisis and no peripheral schisis. The grandfather of the proband (I_1, Figure 1), therefore, was identified with a more severe clinical manifestation. The patient had typical foveal and peripheral schisis in both eyes, combined with neovascular glaucoma in the right eye, which required surgical intervention. The age of onset, visual acuity, and peripheral and macular involvements for each affected individual are described in Table 2.

Figure 1 depicts the pedigree of the Chinese family with X-linked juvenile retinoschisis. Squares indicate males, and circles indicate females. Dark-shaded boxes represent affected subjects with X-linked juvenile retinoschisis, while non-shaded boxes and circles mark unaffected family members. Circles with a dot denote

Table 1. Sequence of primers used to amplify the coding regions of the *RS1* gene

Exon	Size of exon (bp)	Size of amplified fragment (bp)	Primer (5'-3')	Annealing temperature (°C)
1	52	216	F-ctcagccaaagacctaag R-gtatgcaatgaatgtcaatgg	58
2	26	175	F-gtgatgctgttggatttctc R-caaagtgatagtcctctatg	56
3	106	177	F-ctgccctgcctctctggttg R-ggtgcttgttgagtattgag	60
4	142	219	F-ggtgcttgttgagtattgag R-aaaatccccgggccctgc	56
5	196	310	F-gagagccgacacctgcgg R-gggtgcgagctgaagttgg	65
6	153	412	F-cccgatgtgatggtgacagg R-ctttgttctgactttctctggc	62

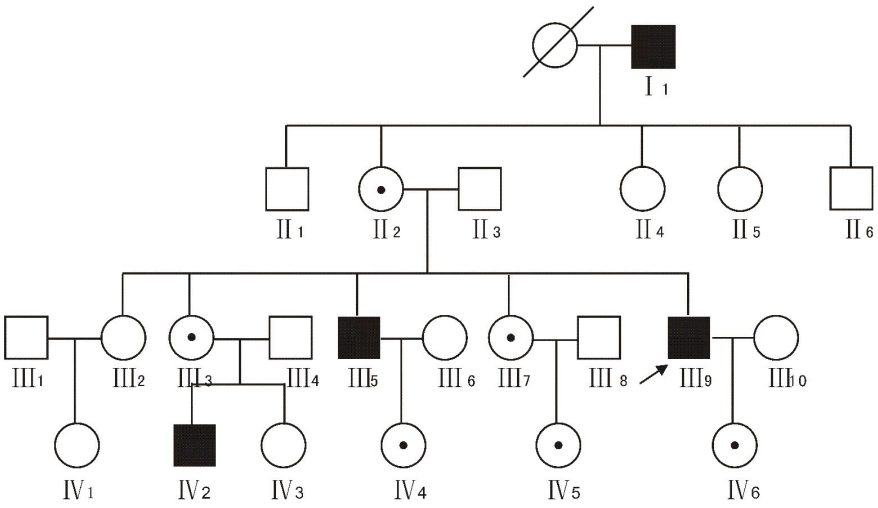

Figure 1. Pedigree of the Chinese family with X-linked juvenile retinoschisis.

Table 2. Clinical features of four affected males in the Chinese pedigree with XLRS

Patient ID	Age at onset (years)	Symptom	Visual acuity		Foveal schisis	Peripheral schisis	Ocular complications
			Right	Left			
I_1	12	Poor VA	Enucleation	LP	Yes	Yes	Neovescular glaucoma
III_5	6	Poor VA	0.15	FC	Yes	No	Not found
III_9	7	Poor VA	0.1	0.3	Yes	No	Not found
IV_2	5	Poor VA	0.2	0.2	No	Yes	Not found

LP, light perception; VA, visual acuity; FC, finger counting.

obligate carriers. A slashed circle indicates a deceased family member. The arrow points to the proband (III_9, Figure 1).

3.2. *Genetic analysis*

All exons and the flanking sequence of the *RS1* gene were screened for mutation detection in the family. A frameshift mutation at exon 6 due to a G deletion at the 573 base position was identified by direct sequencing of the PCR products in the proband (Figure 2B). All other exons were found to be normal. The proband's mother (II_2, Figure 1), who was clinically normal, showed a heterozygous frameshift mutation (Figure 2C). In addition, this mutation was also detected in the other three affected males (I_1, II_5, and IV_2; Figure 1), and the other five female carriers were found to have a heterozygous condition (II_2, III_3, III_7, IV_4, IV_5, and IV_6; Figure 1). All the remaining family members and the matched controls showed a normal sequence (Figure 2A).

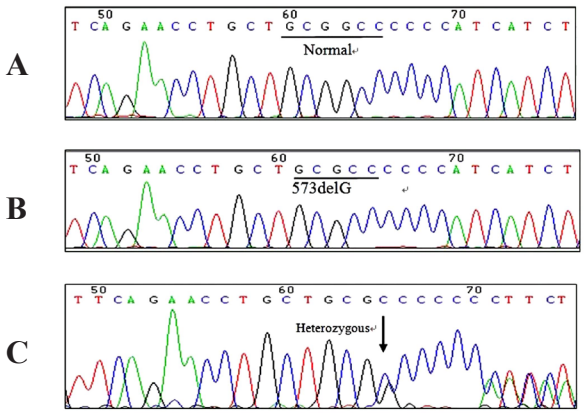

Figure 2. Photograph of DNA sequence of the mutation. (**A**) Normal control; (**B**) DNA sequencing shows a frameshift mutation at exon 6 due to a G deletion at the 573 base position in the proband and the other 6 affected males; (**C**) Heterozygous mutation of the site mentioned above in female carriers.

4. Discussion

XLRS is an X-linked macular disorder caused by mutations in the *RS1* gene. Identification of these mutations is becoming increasingly important in a variety of clinical settings. To the best of our knowledge, over 150 mutations have been recognized (*6*). To gain insight into the genetic mechanisms and pathogenesis of XLRS, we investigated a large Chinese family with XLRS.

Through our thorough clinical examinations and genetic analysis, all four affected males were identified with a single base (G) deletion at the 573

base position (573delG) in exon 6. Moreover, the other three female carriers were found to have a heterozygous condition, while other members of the family and all the healthy controls did not display this mutation. Considering that female carriers are unaffected and the matched controls are not detected to have this mutation, it seems that the absence of a functional *RS1* gene was responsible for retinoschisis in affected males in this family and that the disease was transmitted as an X-linked recessive trait (*7*).

Consequently, this 573delG mutation might shift the open reading frame, resulting in the C-terminal containing a novel set of forty-two amino acids, eleven amino acids longer than the normal protein. It is hypothesized that the activity of this mutant protein may be reduced or completely lost, which would disrupt the signaling pathway and thus lead to destabilization of retinal cell organization and structure (*8*). To date, this is the first report of the 573delG mutation in the Chinese population, which widens the mutational spectrum of *RS1* and is expected to help with diagnosis of this rare genetic disease XLRS in Asians. This identified mutation is present in the hotspot region at amino acid position 192, belonging to the highly conserved discoidin motif of retinoschisin protein, which supports the notion that this domain has functional significance in cell-to-cell adhesion (*9*). Our results are in agreement with previous findings reported in an Australia population by Hewitt *et al*. (*10*).

Although the same frameshift mutation was identified in all four affected males in this family, clinical manifestation of patients within the family exhibited remarkable phenotypic variability, ranging from foveal stellate cystic change to severe bilateral peripheral retinoschisis and highly elevated bullous retinoschisis with vitreous hemorrhage, as well as great variation in age at onset and progression. However, a few studies implied the possibility of a genotype-phenotype relationship. Phenotypes of XLRS were more severe in cases associated with upstream mutations (exons 1-3) in the *RS1* gene. Li *et al*. also reported that the severity of the phenotypes are more likely to relate to the particular mutation types, such as frameshift, splice site, or some missense mutations (*11*). Comparison of our findings with these previously reported patients of other ethnicities, together with the clinical findings within the reported Chinese family in the current study, presumably reflects that there is no simple phenotype-genotype correlation and that disease severity is not mutation dependent only in XLRS. It is therefore presumed that additional factors, perhaps other genetic influences or unique environmental factors might act as contributors to disease severity (*12-14*). More investigations on the possibility of a genotype-phenotype correlation and factors causing phenotypic variation are still needed.

In summary, we identified a frameshift deletion 573delG mutation in the Chinese population for the first time. These findings will provide precise diagnosis when a clinical presentation is uncertain early in the course of the disease, as well as confirmation for genetic counseling and prenatal diagnosis for carriers without any symptoms. Elucidation of the mutation spectrum and phenotypic variability in XLRS is still in progress to better understand the pathogenesis of the disease and to facilitate *RS1* gene replacement therapy.

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Case Report

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Chronic intestinal pseudo-obstruction due to lymphocytic intestinal leiomyositis: Case report and literature review

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Summary **Lymphocytic intestinal leiomyositis is a rare entity, which causes chronic intestinal pseudo-obstruction (CIPO) in children. We present the first case of a boy who had pure red cell anemia 1 year before onset. Prolonged ileus developed after gastroenteritis and the patient was diagnosed using a biopsy of the intestinal wall. Findings from the present case indicate that there are three important factors for accurate diagnosis: history of enteritis, positive serum smooth muscle antibody, and lymphocyte infiltration with muscle destruction in the muscularis propria in the intestinal wall. Earlier diagnosis and induction of immunosuppressive therapy may be essential for a better outcome.**

Keywords: Chronic intestinal pseudo-obstruction (CIPO), pseudo-obstruction, leiomyositis, intestine

1. Introduction

Chronic intestinal pseudo-obstruction (CIPO) is a rare intestinal dysmotility disorder characterized by repetitive or continuous bowel obstruction without mechanical causes (*1-3*). CIPO may be classified either as primary or secondary. Secondary CIPO is classified as a disease of gastrointestinal smooth muscle, nervous system, endocrine system, metabolism, and others (*2*). Smooth muscle fibers of the intestinal wall are affected by connective tissue disorders, muscular dystrophies, infiltrative disease, and mitochondrial myopathy.

Lymphocytic intestinal leiomyositis (LIL) in which lymphocytic infiltration causes muscle degeneration and fibrosis has been rarely reported in the literature (*3-8*). We present a rare case of a boy with CIPO due to T-lymphocytic intestinal leiomyositis (T-LIL). He suffered from pure red cell anemia (PRCA) and T-cell lymphocytosis 1 year before onset of T-LIL. Prolonged ileus developed after a gastroenteritis attack and accurate diagnosis was performed using a histopathological immunostaining study of full-thickness biopsies. We also review T-LIL cases in the literature and discuss the pathogenesis of T-LIL.

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2. Case report

A 2.5-year-old boy was diagnosed with PRCA and T-cell lymphocytosis. A complete response was obtained with steroid therapy. Steroids were ceased 1 year after the initial therapy. He was then admitted to a hospital with diarrhea and abdominal distension with symptoms of acute gastroenteritis. Laboratory data demonstrated leukocytosis (white blood cell count, 42,000/mm^3) and mild elevation of C reactive protein (CRP). Crohn's colitis was suspected and 5-ASA 60 mg/kg/d and prednisone 1 mg/kg/d were started. However, any attempt of oral feeding resulted in severe abdominal distention and vomiting due to paralytic ileus. Complete response was not obtained for 5 months; the patient was given prednisone 2

mg/kg/d with administration of azathioprine 1 mg/kg/d and tacrolimus (target trough: 10 ng/mL) and total parenteral nutrition. Since abdominal symptoms deteriorated after prednisone tapering, prednisone was never discontinued. The patient was transferred to our hospital for further examination. A plain abdominal X ray film demonstrated a huge dilatation of the small intestine with air fluid levels. Small bowel follow through indicated no apparent stricture. No mechanical cause of obstruction and normal mucosal findings were observed by esophagogastroduodenoscopy, colonoscopy, and double balloon enteroscopy. Mucosal biopsy showed mild non-specific inflammation in ileal and colonic mucosa. Laboratory data demonstrated no abnormal findings in blood counts, biochemical studies, CRP, and positive smooth muscle antibody.

We decided to perform laparotomy and a full-thickness biopsy to confirm the suspicion of intestinal disorder related to autoimmune disease because the patient suffered from CIPO with a response to prednisone and immunomodulators, and he had positive smooth muscle antibody A. Laparotomy revealed a huge dilated small intestine without the absence of mechanical obstruction. Enterostomy was created for intestinal decompression and irrigation. Full-thickness biopsies were performed in multiple locations of the small intestine and colon.

Histological findings (Figure 1) in the colon and all small intestine specimens demonstrated massive mononuclear infiltration and muscle fiber degeneration in the muscularis propria and lamina muscularis mucosae in the intestinal wall. Mononuclear cells moderately infiltrated the mucosal and submucosal layers. Ganglion cells in the submucosal and myenteric plexuses were normal. Immunostaining of a small intestine specimen predominantly showed T lymphocytic inflammation consisting of T lymphocytes (CD3, CD4, and CD8), monocytes and macrophages (CD68), and activated white cells (CD45RO). B lymphocytes (CD20, CD30) and NK cells (CD56) were absent. The specimen was also characterized by inflammatory targets that were not smooth muscles of vessels, but they were the muscularis propria and lamina muscularis mucosae in the intestinal wall. Based on the histopathological and immunological findings, the final diagnosis was confirmed as T-LIL.

Postoperatively, the patient began to orally ingest food with regular decompression and irrigation through enterostomy. However, he had intermittent episodes of obstruction associated with intestinal bacterial overgrowth. One year later, the pseudo-obstruction was gradually resistant to treatments and he died from sepsis due to bacterial translocation 1.5 years later.

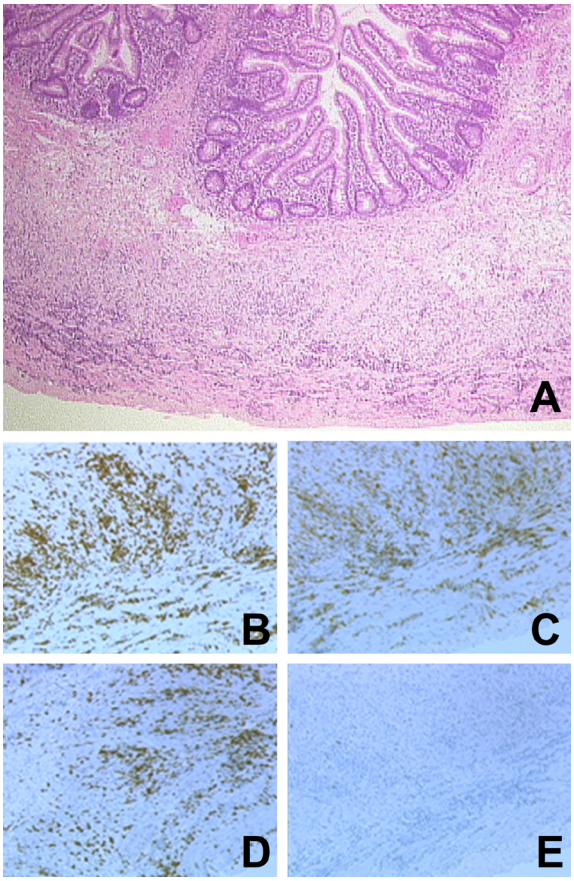

Figure 1. Immunostaining of the biopsy samples. (**A**), Full-thickness biopsy of the small intestine. Histological findings show inflammation in the muscularis propria of the small intestine. Intestinal mucosa and submucosa were mostly normal. Ganglion cells in the submucosal and myenteric plexuses were normal. Immunostaining of a biopsy sample showed predominantly T lymphocytic inflammation consisting of T lymphocytes (**B**, CD3; **C**, CD4; **D**, CD8). B lymphocytes (**E**, CD20) are absent. (Original magnification 100×)

3. Discussion

CIPO is a rare, severe, disabling disorder characterized by repetitive episodes or continuous symptoms and signs of bowel obstruction, including radiographic documentation of a dilated bowel with air-fluid levels, in the absence of a fixed, lumen-occlusive lesion (*2*). CIPO may be classified as either congenital or acquired (*1,2*). Acquired CIPO is classified according to presumed underlying pathogenesis to facilitate an organized approach to evaluation (*9*). Autoimmune reactions to smooth muscle fibers or nerve plexuses have also been reported as very rare causes of acquired CIPO (*7,10*). CIPO due to true T-LIL, such as in the present case, has only been reported in six cases of specific histopathological findings by full-thickness biopsy (*4-8*).

The clinical and histopathological characteristics of this entity are summarized in Tables 1 and 2. It is noteworthy that almost all patients have a preexisting episode of gastroenteritis, and intestinal ileus and abdominal distension occur. Anti-yersinia pseudotuberculosis antibodies were detected in one case (*4*). Molecular mimicry with infectious agents resulting in the initiation of the autoimmune

Table 1. Clinical characteristics in lymphocytic intestinal leiomyositis

Items	Age/Sex	Preexisting disease	Abnormal laboratory findings	Treatment	Progress
Case 1 (*4*)	6 mth./M	*ns*	Anti-*Yersinia pseudotuberculosis*	Steroid	4 yr. follow, death
Case 2 (*5*)	1 yr./M	*ns*	SMA	Steroid	*ns*
Case 3 (*5*)	2.5 yr./F	*ns*	SMA	Steroid	*ns*
Case 4 (*6*)	2 yr./M	AIH, gastroenteritis	SMA, ANCA, ANA	Steroid, AZA, Ciclosporin, enterostomy	3 yr. follow, TPN, relapsing obstruction
Case 5 (*7*)	5 yr./F	Enteritis	SMA	Steroid, AZA, FK 506	1.5 yr. follow, relapsing obstruction after steroid tapering
Case 6 (*8*)	16 yr./F	Enteritis	*ns*	Steroids, AZA, budesonide	2 yr. follow, normal oral diet
our case (2011)	3.5 yr./M	PRCA, TCLC, enteritis	SMA	Steroid, AZA, budesonide, FK 506, enterostomy	1.5 yr. follow, death

mth., month; yr., year; M, male; F, female; AIH, autoimmune hepatitis; *ns*, not specified; PRCA, pure red cell aplasia; TCLC, T cell lymphocytosis and cytopenia; SMA, smooth muscle antibody; ANCA, anti-neutrophil cytoplasmic antibody; ANA, antinuclear antibody; AZA, azathioprine; FK506, tacrolimus; TPN, total parenteral nutrition.

Table 2. Pathological characteristics in lymphocytic intestinal leiomyositis

Items	Affected digestive Organ	Histopathological findings of small intestine			
		MSM	LPM	MP	NP
Case 1 (*4*)	Small intestine	Atrophic	*ns*	Mono infil, degeneration, fibrosis	
Case 2 (*5*)	Small/large intestine	*ns*	*ns*	Severe T-lym infil, degeneration, fibrosis	Intact
Case 3 (*5*)	Small/large intestine	*ns*	*ns*	Severe T-lym infil, degeneration, fibrosis	Intact
Case 4 (*6*)	Ileum, large intestine	Mild inflammation	Moderate T-lym infil	Severe T-lym infil, degeneration	Intact
Case 5 (*7*)	Small/large intestine	Moderate T-lym infil	Moderate T-lym infil	Severe T-lym infil, degeneration	Intact
Case 6 (*8*)	Small intestine	Intact	Intact	T-lym infil, fibrosis, degeneration	Intact
our case (2011)	Small/large intestine	Mild T-lym infil	Moderate T-lym infil, degeneration	Severe T-lym infil, degeneration, fibrosis	Intact

MSM, mucosa and submucosa; LPM, lamina propria mucosae; MP, muscularis propria; NP, nerve plexus; *ns*, not specified; Mono infil, monocyte infiltration; T-Lym infil, T-lymphocytic infiltration.

inflammatory process has been previously suggested for other gastrointestinal autoimmune disorders (*6,11,12*). Myositis is associated with circulating autoantibodies directed against smooth muscle cells with or without nonspecific antibodies to nuclear antigens and neutrophil cytoplasmic antigens.

Diagnosis of LIL was performed by full-thickness biopsy of the small and large intestines. Mucosal and submucosal biopsy through endoscopy never results in a definite diagnosis. Severe T-lymphocyte inflammation is found in the muscularis propria, and there is no significant inflammation in the mucosal and submucosal layers. Although the pathogenesis and mechanism of LIL remain unclear, autoreactive cross-reactivity between pathogens and T-lymphocytes with smooth muscle fibers of the intestinal wall may cause a reaction. However, it is unknown why smooth muscle fibers of vessels are completely intact, while the muscularis propria of the intestinal wall is affected.

In this series, two patients had autoimmune disease as a preexisting disease: autoimmune hepatitis (AIH, case 4) and PRCA (our case). Several diseases such as type I diabetes, Addison's disease, and autoimmune thyroiditis are closely associated with AIH in children. In case 4, autoreactive T-lymphocytes promoted the development of LIL under immunosuppressive therapy for AIH (*6*).

PRCA has been associated with a variety of clinical disorders, and various autoimmune mechanisms have been described to account for red cell suppression because of its frequent association with thymoma and successful responses to thymectomy and immunosuppressive agents (*13*). Generally, the pathogenesis of PRCA is considered to be due to the expansion of B-lymphocytes producing immunoglobulins (IGs), which suppresses erythropoiesis, and IGs are thought to be antibodies against erythropoietin or erythroblasts (*14*). However, another report demonstrated that suppressor/cytotoxic T-lymphocytes can inhibit erythropoiesis (*15*). Recent evidence using gene rearrangement studies has indicated that PRCA with T-lymphocytosis is a clonal chronic T cell lymphoproliferative disorder in which the T cells suppress erythropoiesis (*16*). This disorder has a unique feature of T cell lymphocytosis. The present case had PRCA with T cell lymphocytosis as preexisting disorders of LIL. Additionally, an autoimmune inflammatory reaction, mainly on the muscularis propria in the intestinal wall, was shown by T lymphocytic inflammation using immunostaining. The present case is considered to be the first case of T-LIL with preexisting PRCA. Katabami *et al*.

(*17*) reported an adult female case with polymyositis associated with thymoma who subsequently developed PRCA. They considered that cytotoxic T cells may play an important role in the pathogenesis of polymyositis and PRCA.

Immunosuppressive therapies including steroids and immunomodulators are recommended and they were performed in previous reports. The patient's clinical course is eventful and their quality of life is deteriorated by recurrent relapsing, paralytic ileus, insufficient oral intake, intestinal infections, complications of fluid therapy, and prolonged hospitalizations. Abdominal distension and vomiting recurred after prednisone withdrawal in our case, which is similar to other cases. Oton *et al*. recommended AZA and budesonide while tapering off conventional steroids, if the clinical response continues, to avoid steroid complications (*8*).

Uncontrolled inflammation induces degenerative, atrophic, and fibrotic changes in smooth muscle fibers in the intestinal wall. In case 1, histopathological findings demonstrated a diminished nerve plexus together with mononuclear infiltration, muscle degeneration, and fibrosis proliferation in the muscularis propria. Impairment of the myenteric plexuses is explained as the final histopathological findings (*4*). These seven previous cases and our reports may have consisted of different phenotypes of LIL between the early and end stages. Ruuska *et al*. (*7*) described that disease progress may be prevented resulting in end-stage intestinal motility failure, if immunosuppressive treatments are used aggressively early in the course of illness.

Prognosis of CIPO is generally poor. Generally, liver disease and sepsis due to bacterial overgrowth and complications of TPN are the most common causes of death in CIPO (*18*). Bacterial overgrowth often causes malabsorption and may be associated with increased mucosal permeability and bacterial translocation across the bowel (*19-21*). In the present case, uncontrolled CIPO due to LIL easily caused bacterial overgrowth under immunosuppressive conditions.

Clinicians should be aware of lymphatic intestinal leiomyositis for the differential diagnosis of CIPO. Three important factors for accurate diagnosis are a history of enteritis, positive serum smooth muscle antibody, and T-cell infiltration in the muscularis propria in intestinal full-thickness biopsies. Earlier diagnosis and induction of immunosuppressive therapy may be essential for a better outcome.

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Case Report

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Imaging diagnosis of hepatic ectopic pregnancy: A report of one case

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Summary **This article is about a case of hepatic ectopic pregnancy. A patient suffered from an acute abdomen with 14-day vaginal bleeding. A serum, human chorionic gonadotrophin (HCG) of 8,988 mIU/mL revealed a bit of pelvic effusion. A computed tomography (CT) plain scan displayed a polygonal, moderate density shadow of the left liver lobe. An enhanced CT had no sign of intensification. A magnetic resonance imaging (MRI) plain scan was undertaken. On a T1-weighted imaging (T1WI), the lesion appeared to be a low signal; on a T2-weighted imaging (T2WI), the lesion appeared to be a high signal. With enhanced MRI, the lesion showed an irregular mild plague-like intensification during the venous phase. It was excised by an operation and chorionic tissue was seen under a microscope. The result of pathological diagnosis was hepatic ectopic pregnancy.**

Keywords: Hepatic ectopic pregnancy, ultrasound, computed tomography, magnetic resonance imaging

1. Introduction

Ectopic pregnancy refers to the attachment of fertilized eggs to the extrauterine cavity. It often occurs in fallopian tubes, then in ovaries, broad ligaments and so on, but seldom in the abdominal cavity such as liver, spleen, or peritoneum. Recently a hepatic ectopic pregnancy has been found and accurately diagnosed by a preoperative imaging examination. The details are as follows.

2. Case report

Clinical Manifestations: A 33-year-old woman, accountant, unable to bear children after years of marriage, was admitted to hospital for acute abdominal pain with a 14-day history of vaginal bleeding and a 49-day menolipsis. A T-type contraceptive ring was pulled out of her uterine cavity one year ago presenting symptoms including the last menstruation on November 26, 2006 (the 49th day of menolipsis), no morning sickness, a small bit of vaginal bleeding on December 31 with dark red blood, no evident outflow of granulation tissue and uncomfortable swelling pain in upper abdomen.

Physical examination: Her temperature was 36.8°C, pulse beats 80 times per minute, breath 20 times per minute, and blood pressure 13.3/8.0 kPa. Nutrition was moderate, face painful, and skin and mucous membranes not yellow. The whole body revealed no lymph node enlargement. The trachea was in the middle and breath sounds in two lungs were normal with no cardiac murmurs. The abdomen was flat but a little stiff. Upper left abdominal tenderness was remarkable, and rebound tenderness was obvious. The spleen was not big, and there was no percussion tenderness over the kidney region. Gynecological examination revealed no evident abnormality. There was clear tenderness in the uterine appendages.

Laboratory examination: Total of white blood cells was 10.17×10^9/L, granulocytes 87.6%, and lymphocytes 4.8%; the number of red blood cells was 3.56×10^{12}/L, hemoglobin 114 g/L, packed red cells 33.1% and the content of hemoglobin averaged 32.0 pg. Hemodiastase (AMY) was 38 U/L and within normal limits. Fibrinogen (FIB) was 4.538 g/L, and slightly elevated. Human chorionic gonadotrophin (HCG)

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was 8,988 mIU/mL (normally less than 2.7 mIU/mL) and progestin (Prog) 35.7 nmol/L in serum. Liver and kidney function and routine urine examination showed no abnormalities.

Imaging examination: On January 14, 2007, an ultrasound (US) examination of abdomen displayed the retroposition of uterus of normal size with a 4 mm thick intimate membrane, recto-uterine pouch, no gestational sac, no abnormal echo around bilateral uterine appendages and an approximately 23 mm dark area of fluid in the pelvic cavity. The US diagnosis revealed pelvic effusion (Figures 1A and 1B). On the 15th day, an US of the vagina showed a uterus of normal shape in correct size with the middle endometrial line, and a homogeneous echo. There was no abnormal echo in the uterine cavity. A free opaque dark area of fluid was seen in the right iliac fossa with a maximum of 34 mm in diameter, in which a tiny light echo was visible. The US diagnosis revealed pelvic effusion, but no gestational sac-like echo in the uterine cavity (Figures 1C and 1D). A computed tomography (CT) plain scan performed between the left liver edge and stomach displayed a polygonal, moderate density shadow with a smooth and clear edge and an irregular low-density region in the middle (Figure 2A). Enhanced CT of the focus margin and inside showed no sign of intensification during the

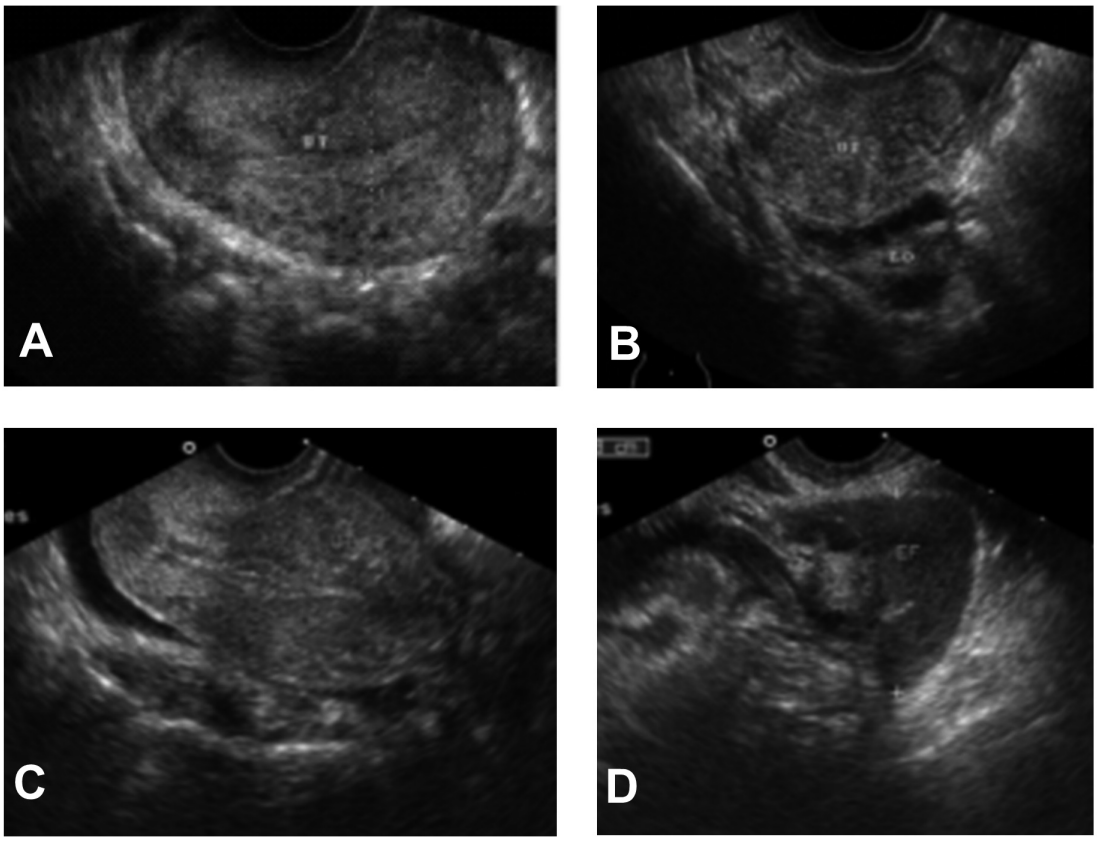

Figure 1. US findings. (**A**, **B**), A routine gynecology US reveals no gestational sac-like echo, no abnormal echo around bilateral uterine appendages and a dark area of fluid in pelvic cavity. (**C**, **D**), US of vagina shows no gestational sac-like echo in uterine cavity, no abnormal echo around bilateral uterine appendages, and a free opaque dark area of fluid in right iliac fossa.

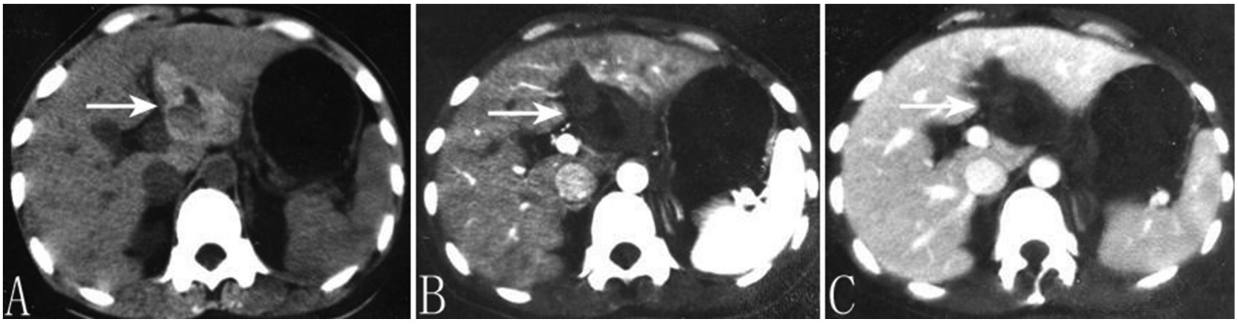

Figure 2. CT findings. (**A**), CT plain scan performed between left liver edge and stomach displayed a polygonal, slight high-density shadow with a smooth and clear edge and irregular low-density region in the middle. (**B**), Enhanced CT of the focus shows no sign of intensification during the arterial phase with a smooth and clear edge and lower-density region in the middle. (**C**), No sign of intensification during the venous phase.

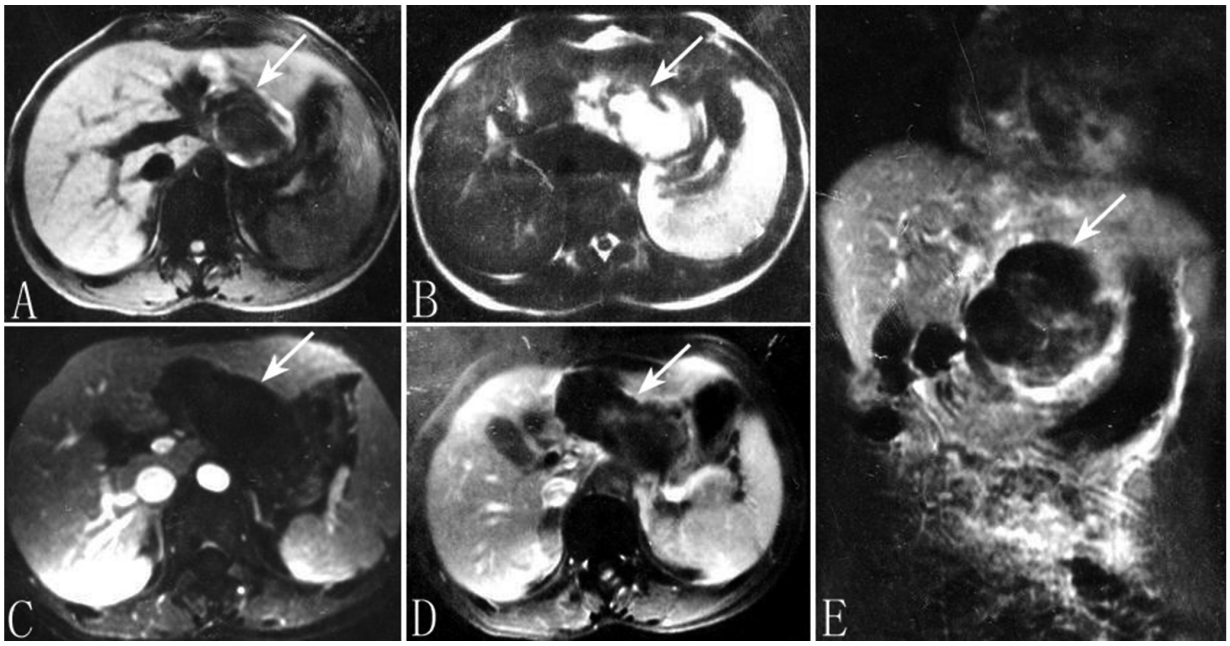

Figure 3. MRI findings. (**A**), T1WI reveals a round low signal focus (about 3 × 4.5 cm in size) between the inferior margin of the left hepatic lobe and lesser curvature of stomach. Most edges of the focus are clear. (**B**), T2WI reveals the focus appears to be obvious high signals, most of which are homogeneous. (**C**), The focus appears to be elliptical low signals with enhanced MRI during the arterial phase and there is no sign of intensification inside the focus and on the edge. (**D**), Slight intensification is displayed in the focus during the venous phase; a bit of ring-shaped intensification may be seen on the edge of the right frontal. (**E**), Coronal scan clearly reveals that the disease takes on a round-like low signal with a slight non continuous ring-like intensification and with slight irregular intensification inside.

arterial and venous phase. The focus appeared to be a high-density area with a clear margin (Figures 2B and 2C). On the 16th day, a magnetic resonance imaging (MRI) plain scan of T1-weighted imaging (T1WI) demonstrated an abnormal round-like low-signal mass between left lobe of the liver and stomach, with irregular ring-shaped high-signal wall on the margin (Figure 3A). The heart of the disease on T2-weighted imaging (T2WI) appeared to be an evidently high signal with an unclear margin. The lesser curvature of stomach was compressed and displaced (Figure 3B). Enhanced MRI of the focus margin and inside showed no intensification during the arterial phase (Figure 3C). An irregular mild plaque-like intensification in the focus was seen during the venous phase, with no distinct boundary from the stomach wall (Figure 3D). A coronal scan clearly revealed that the disease, located between liver and stomach, took on a round-like low signal with no smooth margin, and that there was an irregular mild plaque-like intensification and that the lesser curvature of stomach was clearly compressed and displaced with no distinct boundary (Figure 3E).

Surgical treatment: On the day, a 12 cm incision into abdomen was performed to discover a 1,000 mL hemoperitoneum. There was a ruptured mass with clotted blood and a tissue mixture between the inferior margin of the left hepatic lobe and stomach, closely adherent to the anterior margin of the left hepatic lobe, hepatic ligaments and the membrane of lesser curvature of the stomach. At first, we cleared away the clotted blood and separated chorionic villi. Then, the mass was excised away from the membrane of lesser curvature of the stomach, the lesser omentum, hepatic membrane and small hepatic tissue. Finally, bleeding was arrested with gauze and the abdominal incision was closed layer by layer. The operative process was uneventful.

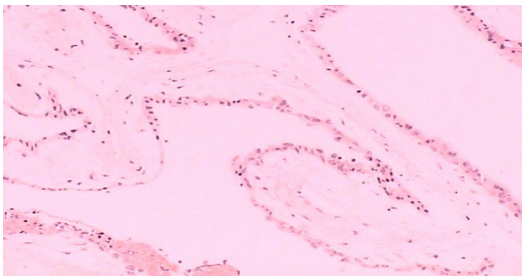

Figure 4. Pathological diagnosis of hepatic ectopic pregnancy (H&E Staining, Original magnification 100×).

Pathological examination: Chorionic tissues, hyperplastic granulation and hepatic tissues were seen under a microscope (Figure 4). The result of pathological diagnosis was hepatic ectopic pregnancy.

3. Discussion

3.1. *Disease incidence*

Ectopic pregnancy in the fallopian tube is a very common form of extrauterine pregnancy. Ectopic

pregnancy in the abdomen is seldom seen, accounting for almost one percent of all ectopic pregnancies (*1*). But ectopic pregnancy in the liver is exceptionally rare. According to the statistics of ERIC and so on, in the past 35 years before November 1999, only 14 cases have been reported in the world, of which a majority was discovered due to postoperative pathological diagnosis of acute abdomen and abdominal hemorrhage and of which a minority, according to medical history (*2-11*), were a preoperative diagnosis of HCG and US. Only one case was discovered with a diagnosis of US and CT (*12*). In the case, hepatic ectopic pregnancy had a complete imaging examination and preoperative diagnosis using US, CT and MRI. No other cases like this have been reported up to now.

3.2. *Clinical manifestations*

The youngest patient with hepatic ectopic pregnancy was aged 25 (*5*) and the oldest was aged 46 (*12*). Their common clinical manifestations were persistent epigastralgia and irregular vaginal bleeding. Once the gestational sac was ruptured, clinical manifestations were acute abdomen such as epigastralgia, rebound tenderness, and right upper abdomen pain, hemorrhage in abdominal cavity, a decrease in blood pressure and shock. Laboratory examination showed a decrease in red cells and hemoglobin, and a rise in partial HCG. The 35-year-old patient in this case was marked with all the clinical characteristics of hepatic ectopic pregnancy, but the only exception was that pain was located in the left upper abdomen, which happened to be the location of the pathological change.

3.3. *Etiology and pathogenesis*

The etiology of hepatic ectopic pregnancy is very complicated. According to document reporting, it may have a relationship with a birth-control operation on the uterus, inflammation of fallopian tube and so on (*7,9*). The pathogenesis of this case is not quite clear. On the basis of document reporting and detailed clinical data of this case, we have inferred the pathogenesis is related to the following factors:

i) *Contraception:* It includes oral contraceptives and contraceptive devices placed inside women's wombs. The cause of the former is unknown. As Duane reported (*2*), a patient who had taken oral contraceptives for 5 years, fell ill after one year of quitting the drug. The latter may connect with the shape of contraceptive devices and the dissection of fallopian tubes. Borlum *et al*. reported one case with a history of using intrauterine devices (*7*).

ii) *Inflammation of fallopian tubes and pelvis:* The research revealed pelvic inflammation easily extended to the upper abdomen and resulted in inflammation of the hepatic periphery (*9*). Most patients with abdominal pregnancy were discovered by operating to have one-sided or bilateral different inflammation of fallopian tubes, pelvic inflammation and pelvic effusion. Some scholars raised the opinion that this case followed tubal pregnancy and that its pathogenesis was that fertilized eggs were displaced from fallopian tubes first, and then entered into the pelvic cavity and contacted the surface of the peritoneum, or were dependent on flow of celiac fluid, then attached to capsula fibrosa in the upper abdomen, grew and developed (*7,9*). The academic opinion won complete support in that the operation of this case was performed, finding wide-ranging inflammation and pelvic effusion in bilateral fallopian tubes and the pelvic cavity.

iii) *Characteristics of pathological dissection of the liver:* Liver is the biggest solid organ of abdominal cavity capsula fibrosa and liver is a favorable site of profuse blood supply suitable for the growth of an embryo. Fertilized eggs were first attached to capsula fibrosa. As the gestational sac developed, chorion infiltrated into the surface of liver to meet constantly enlarged blood supply of the embryo. In general, the gestational sac was ruptured due to the shortage of blood supply in less than 12 weeks (*12*). Shukla *et al*. reported that the longest embryonic development period was 28 weeks (*5*). Surgery was performed finding a normal live fetus. In this case, it was 49 days (7 weeks) from impregnation after menolipsis due to the rupture of the gestational sac.

3.4. *Diagnosis and differentiation*

Hepatic pregnancy occurred on the inferior margin of the right hepatic lobe, approaching the gallbladder and duodenum. The reported cases were all on the inferior margin of the right hepatic lobe and only one case on its superior margin. Due to the main clinical findings of acute abdomen and intra-abdominal hemorrhage, the case is easily misdiagnosed for diseases of acute biliary system and gastroduodenitis (*4*). It is very difficult to diagnose preoperatively. Through this case in combination with document reporting, we think preoperative diagnosis is not difficult according to the following standard only if we make careful observations and analyze patients' clinical data by US, CT and MRI examination.

i) This case is characterized by sudden and quick disease development, acute epigastralgia, a history of menolipsis and an elevated HCG level.

ii) Gestational sacs are invisible in the uterus, fallopian tubes and pelvic cavity by US.

iii) CT and MRI reveal solid tumors, located on the edge of liver and complicated by hemorrhage, appeared to be a disorder of density inside, and have no obvious intensification after injection of contrast-medium. In particular, MRI may be formed in different directions, which has superiority for finding gestational sacs and

localizing the disease.

iv) Ectopic pregnancy, especially in the left liver lobe, is more easily misdiagnosed in the clinic, excluding the possibility of acute abdomen in gallbladder, biliary tract and gastrointestinal tract, subcapsula hematoma, rupture and hemorrhage of hepatic tumor, *etc*.

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