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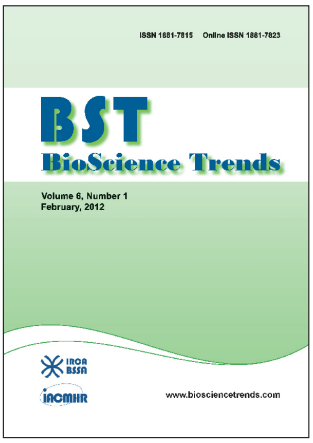

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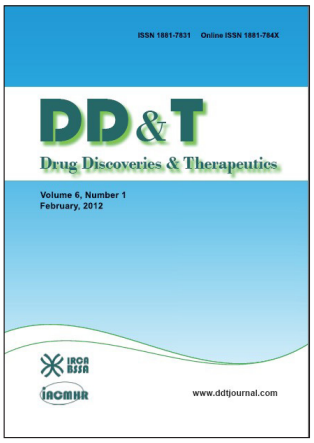

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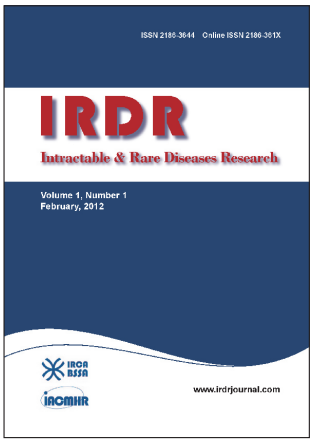

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Review

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Current status of treatment for primary effusion lymphoma

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Summary **Primary effusion lymphoma (PEL) is a rare and aggressive B-cell non-Hodgkin's lymphoma that usually presents with malignant effusions without tumor masses. An extracavitary or solid variant of PEL has also been described. Human herpes virus 8/Kaposi sarcoma-associated herpes virus (HHV-8/KSHV) is universally associated with the pathogenesis of PEL. More than 70% of cases occur with concurrent Epstein-Barr virus infection, but its relation to the pathogenesis is unknown. Patients are found in the context of immunosuppressive states (HIV-1 infection, post-organ transplantation). PEL is usually treated with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone)-like chemotherapy with antiretroviral therapy if HIV-1 is positive. However, it is generally resistant to chemotherapy with a short median survival of less than 6 months. The optimal treatment for PEL has not been established yet. More intensive chemotherapy, such as dose-adjusted EPOCH (DA-EPOCH; etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin) and CDE (cyclophosphamide, doxorubicin, etoposide) are expected to show a favorable prognosis. Recently, the molecular steps in KSHV/HHV-8-driven oncogenesis have begun to be revealed, and molecular targeting therapies such as proteasome, NF-κB, cytokines and surface antigens would provide evidence for their clinical use.**

Keywords: Primary effusion lymphoma (PEL), Human herpes virus-8/Kaposi sarcoma-associated herpes virus (HHV-8/KSHV), HIV-1/AIDS, combination antiretroviral therapy (cART), NF-κB, PEL xenograft mouse model

1. Introduction

Primary effusion lymphoma (PEL) is defined as "a large B-cell neoplasm usually presenting as serious effusions without detectable tumor masses, and is universally associated with human herpes virus 8/Kaposi sarcoma-associated herpes virus (HHV-8/KSHV)" by the WHO Classification of Tumors of Haematopoietic and Lymphoid Tissues (4th edition) (*1*). Rare HHV-8-positive lymphomas indistinguishable from PEL present as solid tumor masses, named extracavitary PEL (*2*).

PEL was first described in 1989 as an AIDS-related lymphoma of uncertain lineage that demonstrated B-cell derivation and included Epstein-Barr virus (EBV) (*3*). In 1995, Cesarman *et al*. identified KSHV DNA sequences within a distinct subtype of AIDS-related lymphoma presenting with lymphomatous effusions (*4*). In 1996, Nador *et al*. designated this lymphoma as "primary effusion lymphoma", which is a distinct entity associated with HHV-8/KSHV (*5*). The majority of cases arise in young and middle-aged homosexual or bisexual men with HIV infection. The disease also occurs in elderly patients and post-transplantation patients (Table 1) (*6-9*). In the majority of PEL cases, co-infection with EBV has been detected. The latency of EBV is type I and the role of EBV in the PEL pathogenesis is still unclear. HIV-infected individuals have a 60-200-times higher relative risk of developing NHL than the HIV-negative population (*10*). Among HIV-associated lymphoma, PEL arises more frequently in the HIV-infected population. PEL accounts for approximately 4% of all HIV-associated NHL cases (*11,12*). PEL is described as a distinct entity and is also included in "lymphomas occurring more specifically

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Table 1. Etiology of primary effusion lymphoma (PEL)

Category	Characteristics
PEL in elderly persons	Especially in endemic areas of HHV-8/KSHV
Post-transplantation PEL	With immunosuppressive therapy
HIV-1-related PEL	Homosexuals have a high prevalence of HHV-8/KSHV

in HIV-positive patients" among HIV-associated lymphoma in the WHO classification (*13*).

In this review article, therapeutic evidence from case series and the potential use of drugs and novel therapeutic approaches from preclinical evaluation of this refractory lymphoma are discussed.

2. Clinical features

PEL is clinically characterized by lymphomatous effusions in body cavities (formerly called body cavity lymphoma) usually without extracavitary tumor masses, and the clinical symptoms depend on the cavities involved. The most common sites are the pleural, peritoneal and pericardical cavities, and joint space and meningeal space are rarely involved (*14*). Patients present with dyspnea from pleural or pericardial effusion, or abdominal distension from ascites, which are the results of mass effects of malignant effusions. Patients with PEL with more than one body cavity involved had a median overall survival (OS) of 4 months compared with 18 months in patients with only one cavity involved (*15*). PEL usually occurs in advanced AIDS patients with a decreased CD4 T-cell count at diagnosis. Approximately half of the patients have pre-existing or develop KS (*16*). HIV-negative patients with PEL are extremely rare but have been described in elderly men from the Mediterranean region (areas with high prevalence for HHV-8 infection) and immunocompromised patients after solid organ transplantation (*17,18*). Recently, rare cases of an extracavitary variant of PEL have been observed in the lymph nodes or extranodal sites, such as the gastrointestinal tract, skin, lung, and CNS without lymphomatous effusions (*2,19*). Since extracavitary PEL has immunoblastic-like and anaplastic features with CD30 expression, it is hard to diagnose without showing the existence of HHV-8/KSHV infection.

3. Laboratory features

Cytologic preparation (Cytospin) of the involved effusion fluid is used for pathological examination and diagnosis. PEL cells show nuclei that are large, round and irregular in shape, with prominent nuclei. The cytoplasm is deeply basophilic with occasional vacuolated cells.

PEL cells typically express a hematolymphoid marker, CD45, but they usually lack expressions of B-cell markers (CD19, CD20, CD79a, surface and cytoplasmic immunoglobulin) (*20*). PEL cells express plasma cell markers, including CD138, VS38c and MUM-1/IRF4. Moreover, the cells generally express various activation markers, such as CD30, CD38, CD71 and epithelial membrane antigen (EMA). They usually lack T-cell markers (CD2, CD3, CD4, CD5, CD7, CD8), although aberrant expression of T cell antigen may occur. *Bcl-6* and *c-myc* are usually absent, and immunoglobulin gene rearrangement shows monoclonality of B-cell origin. Thus, PEL is a post-germinal center tumor at a pre-terminal stage prior to plasma cell differentiation (*21*). Transcript profiling confirmed this genesis (*22*).

The detection of HHV-8 infection in neoplastic cells is needed for definitive diagnosis of PEL (*1*). Immunohistochemistry for latent nuclear antigen-1 (LANA-1) is currently the standard method to detect the presence of HHV-8/KSHV in lymphoma cells (*14*). Typically positive results are characterized by a nuclear dot-like pattern. Polymerase chain reaction (PCR) amplification using a DNA extract from lymphoma cells is also useful to detect HHV-8/KSHV and measure peripheral blood HHV-8/KSHV viral load (*23*) as HHV-8 can be detected in the plasma at the onset of PEL (*24*). Evidence of EBV infection is most reliably detected by in situ hybridization for EBV-encoded small RNA (EBER), while immunohistochemical staining for EBV latent membrane protein-1 (LMP-1) is negative (*25*).

High levels of interleukins (IL-6, IL-10) and soluble forms of antigens such as soluble CD30 might also help in the identification of a clinical marker for treatment (*26,27*). Onset of PEL is mostly related with immunosuppression (*6*) and is associated with HIV load and CD4 cell count in HIV-1 related PEL (*28*).

4. Molecular genetics

The HHV-8/KSHV genome has a 145 kb gene and PEL cells usually contain 40-80 copies of HHV-8/KSHV episomes per cell and express HHV-8/KSHV latent genes (Table 2, Figures 1 and 2). Five latent gene products, which are thought to play significant roles in PEL, are latency-associated nuclear antigen-1 (LANA-1), LANA-2/vIRF-3, viral cyclin (v-Cyclin), viral FLICE inhibitory protein (v-FLIP) and Kaposin (K12). LANA-1 binds to p53 and RB protein, inhibits their function, and impairs the apoptosis of HHV-8/KSHV-infected cells (*29,30*). v-Cyclin (viral homologue of cyclin D), binds to cyclin-dependent kinase 6 (CDK6) and inactivates RB protein (*31*). v-FLIP, a viral homologue of FLICE inhibitory protein (c-FLIP), inhibits apoptosis by blocking Fas-and TNF-mediated caspase activation and activates NF-κB thorough activation of IKKγ (*32,33*). Kaposin A has

Table 2. HHV-8/KSHV-encoded protein implicated in tumorigenesis

HHV-8/KSHV -encoded protein	Host cell homologue	Possible function
LANA-1		Inhibition of p53, Rb and GSK3β Induce hTERT, Id-1 and IL-6
LANA-2/ vIRF-3	Interferon regulatory factor	Inhibition of p53
v-Cyclin	D-Type cyclin	Inactivation of pRB promotes G1 to S phase transition
v-FLIP	FLICE inhibitory protein (c-FLIP)	Activation of NF-κB pathway, Inhibition of CD95L (FasL) and TNF induced apoptosis
Kaposin (K12)		Kaposin A: oncogenic potential Kaposin B: stabilize cytokine expression
K1		Transformation
v-MIPs	CC chemokines	Chemoattraction, angiogenesis
v-IL-6	IL-6	Growth factor
v-Bcl2	Bcl-2 family proteins	Inhibition of apoptosis
v-GPCR	IL-8 GPCR	Cellar growth signal
v-Ox-2	N-CAM family proteins	Cellular adhesion molecule
ORF4	CD21/CR2 complement binding protein	Escape form host immune response

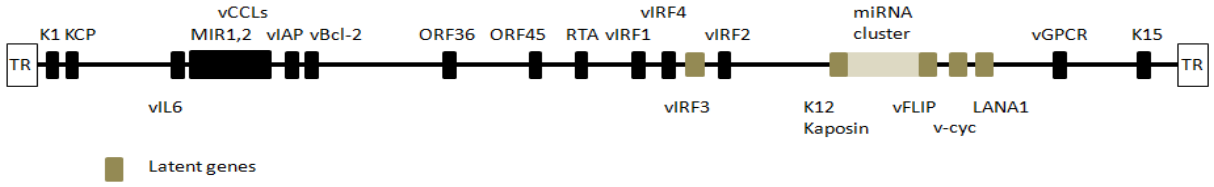

Figure 1. HHV-8/KSHV genome and viral gene expression in PEL. The latent HHV-8 genes LANA, v-cyclin, vFLIP, K12/ Kaposine, and vIRF3 are shown as grey boxes.

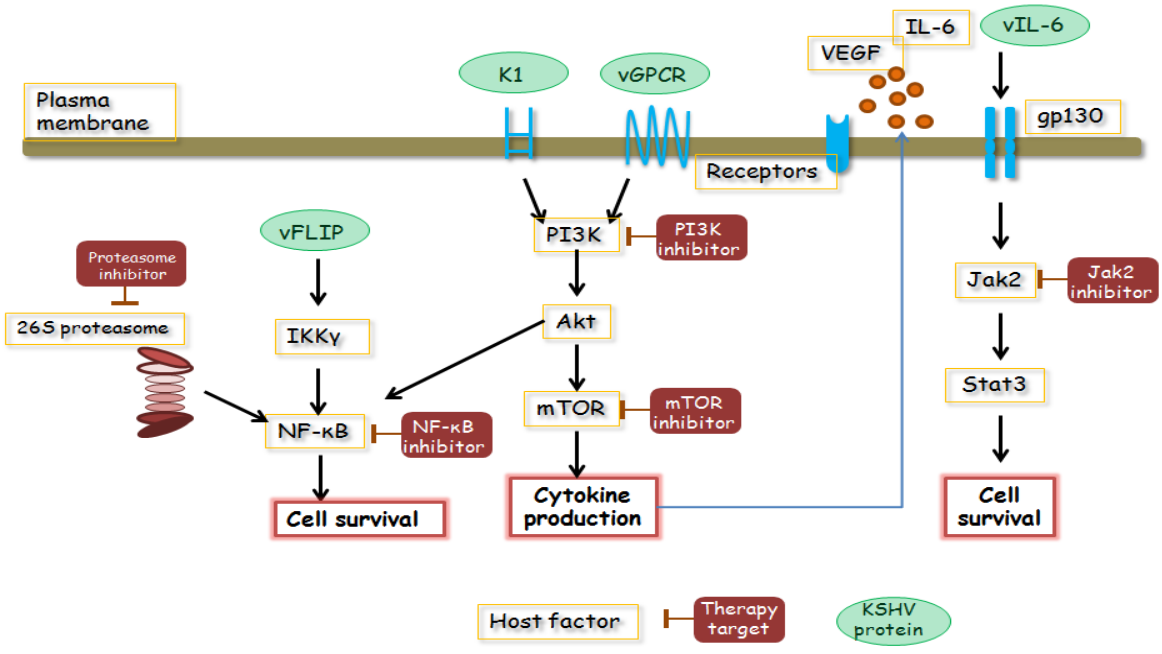

Figure 2. Potential candidate targeting molecules for the treatment of PEL. PEL constitutively activates NF-κB, JAK/STAT and PI3K/AKT/mTOR pathways, which are essential for the survival of PEL cells. These signaling pathways, cytokines and surface antigens are considered as targeting molecules for treatment.

oncogenic potential through cytokesin-1 (*34*). Kaposin B stabilizes cytokine expressions, such as IL-6 and granulocyte-macrophage colony stimulating factor (GM-CSF), by stabilizing cytokine mRNA containing AU-rich elements, which plays a role in latent HHV-8/KSHV infection (*35*). vIL-6 is a homologue of cellular IL-6 (24.6% amino acid sequence identity), directly binds to gp130 without the cooperation of the IL-6 high affinity receptor, and triggers the JAK/STAT (Janus tyrosine kinases/signal transducers and activators of transcription) pathway (*36*). LANA-2/vIRF-3 has a potential role in developing drug resistance by binding to polymerized microtubules, reducing their stability (*37*). Furthermore, HHV-8/KSHV encodes homologous human interferon response factors (IRF), which inhibit interferon-mediated effects (*38*). These viral proteins are essential for the survival of PEL cells and could be a target of PEL treatment. The major latency-associated region of the HHV-8/KSHV genome also encodes 12 micro (mi) RNA genes. Of note, miR-K12-11 is a HHV-8/KSHV miRNA sharing full seed sequence homology with human miRNA, miR-155. Given that miR-155 promotes plasma cell differentiation, miR-K12-11 might contribute to HHV-8/KSHV lymphomagenesis (*39,40*).

Approximately 50%-80% of PEL are co-infected with EBV (*5*). EBV gene expression in dually infected PEL cells is restricted to EBNA-1 and EBER (latency I). Although EBV-positive PEL exhibits a different pattern of gene expression from EBV-negative PEL, there is no evidence that EBV-positive PEL presents with the characteristic clinical manifestation, and the contribution of PEL features is unknown (*13*).

5. Differential diagnosis

The most common differential diagnoses in cases of PEL are other types of non-Hodgkin's lymphoma with lymphomatous effusion, such as diffuse large B cell lymphoma (DLBCL) and Burkitt's lymphoma (BL) with secondary effusion (Table 3). Recently classified HHV-8-negative PEL-like lymphoma shows similar clinical and laboratory features, except for being HHV-8/KSHV negative and CD20 positive (*41*), and the term "HHV-8/KSHV-negative effusion-based lymphoma" was proposed (*42*). This lymphoma also presents with lymphomatous effusion without detectable masses. HHV-8/KSHV is negative in all cases. Hepatitis C virus (HCV) and EBV are positive in nearly 30% of cases, respectively. Patients are generally elderly and have underlying medical conditions, such as cirrhosis or cardiovascular dysfunction. It is also considered to be associated with fluid overload states. Confirmation of the typical morphology and immunophenotype described previously and evidence of HHV-8/KSHV infection are required for the diagnosis of PEL (*1*).

Pyothorax-associated lymphoma (PAL) is a non-Hodgkin's B-cell lymphoma developing in the pleural cavity of patients after a long-term history of pyothorax resulting from an artificial pneumothorax for the treatment of pulmonary tuberculosis or tuberculous pleuritis (*43*). PAL is more common in Japan and usually occurs in elderly men with a history of pulmonary tuberculosis or tuberculous pleuritis. PAL usually shows the diffuse proliferation of large cells of B-cell type (diffuse large B-cell lymphoma; DLBL), and is strongly associated with EBV infection with the expression of EBV latent genes such as EBNA-2, LMP-1, together with EBNA-1 (latency III) and HHV-8/KSHV negative (*44*).

Plasmablastic lymphoma (PBL) is an aggressive non-Hodgkin's B-cell lymphoma that presents at both oral and extra-oral sites (especially the gastrointestinal tract) of chronically HIV-infected immunosuppressed young men. The morphology shows plasmablastic differentiation and plasma cell markers (CD20-, CD38+, CD138+) in all cases. EBV is detected in most cases, but HHV-8/KSHV is negative.

Because of their similar morphology and lack of a B-cell marker, T-cell anaplastic large cell lymphoma is sometimes confused with PEL (*45*). Immunohistochemistry for anaplastic lymphoma kinase (ALK) and the TCR gene rearrangement would be helpful in these cases.

If the morphological findings show large immunoblastic to plasmablastic with anaplastic

Table 3. Classification and differential diagnosis of non-Hodgkin's lymphomas involving the serous body cavities and presenting as effusion lymphomas

Type of lymphoma	Primary effusion lymphoma	HHV-8/KSHV-unrelated PEL-like lymphoma	Extranodal large cell lymphoma	Extranodal Burkitt's lymphoma	Systemic lymphomas or body cavity-based mass-forming lymphomas
effusion	primary	primary	primary	primary	secondary
HHV-8/KSHV	+	-	-	-	-
EBV	+	+/-	+/-	+/-	various
CD20	-	+ (70-80%)	+	+	+
c-*myc*	-	-	-	+	-
Morphology	IBL/ALCL		IBL/DLBCL	BL	Various histoypes

Attenuated from (*13*). IBL: Immunoblastic lymphoma, ALCL: Anaplastic large cell lymphoma, DLBCL: diffuse large B-cell lymphoma, BL: Burkitt's lymphoma.

morphology, virological analysis of HHV-8/KSHV and EBV is essential for diagnosis. HHV-8/KSHV can be demonstrated by PCR, *in situ* hybridization or by immuno-histochemistry against LANA-1, which is consistently expressed in HHV-8/KSHV infected cells.

6. Treatment

The prognosis of PEL is extremely poor with few long-term survivors. Owing to the rarity of the disease, there are very few longitudinal observational series of patients and prospective randomized clinical studies are not feasible; thus, treatment is mostly based on expert consensus opinion and small case series.

6.1. *Chemotherapy*

Traditional chemotherapy with cyclophosphamide, doxorubicin, vincristine and predonisolone (CHOP) is the most common chemotherapy regimen for treating non-Hodgkin's lymphoma (NHL), and has been attempted for the treatment of PEL; however, the prognosis of patients with PEL remains extremely poor. Boulanger *et al*. showed a median survival of 6.2 months and a 1-year overall survival rate of approximately 40% (*46*). Studies using CHOP-like regimens resulted in similar outcomes. Recently, an anti-CD20 monoclonal antibody (Rituximab) -containing regimen became the standard therapy for CD20-positive B cell NHL. Although most PEL cases do not express CD20, Rituximab can be considered for the treatment of rare cases of CD20-positive PEL (*47,48*).

Methotrexate-containing regimens, such as high-dose methotrexate and CHOP with methotrexate, have been studied. However, methotrexate accumulates in effusions, resulting in delayed clearance and an increased risk of systemic toxicity. Infusion therapy such as dose-adjusted EPOCH (DA-EPOCH; etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin) and CDE (cyclophosphamide, doxorubicin, etoposide) has been shown to be well tolerated and effective in the treatment of AIDS-related aggressive B-cell lymphomas, and can be applied for the treatment of PEL (*21*).

6.2. *Stem cell transplantation*

The efficacy of high-dose chemotherapy with autologous stem cell transplantation (ASCT) for chemotherapy-sensitive relapsed disease in HIV-associated lymphoma has been reported (*49,50*). Only two cases have been reported in PEL (*51,52*): one failed to recover from PEL, while the other was successfully treated with high-dose chemotherapy with ASCT following complete remission 12 months post-transplantation. Successful treatment with reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation in second remission has been reported (*53*). This patient remained in complete remission 31 months post-transplantation only on cART with an undetectable HIV viral load.

6.3. *Combination antiretroviral therapy (cART)*

Prior to the administration of cART, the therapeutic results with chemotherapy were unsatisfactory in HIV-1 associated lymphomas. The prognostic impact of cART in combination with chemotherapy has been reported in PEL (*46*), although the impact of cART is lower than for other HIV-1 associated lymphomas such as DLBCL and BL (*54,55*). In addition, complete remission of PEL patients with cART but without chemotherapeutic drugs has been reported (*56-58*). Thus, implementation of cART is recommended when treating PEL patients with HIV-1 infection.

It is important to avoid major drug-drug interactions during chemotherapy (Table 4). Among antiretroviral agents, protease inhibitors modify the metabolism of cytotoxic drugs and potentiate myelotoxicity by inhibiting the CYP3A4 enzyme to various extents (*59*). Thus, anticancer drugs, which rely on Cytochrome P450, should be used carefully with protease inhibitor-based regimens to avoid inadvertent toxicity. Currently, integrase strand transfer inhibitors (INSTI), raltegravir and dolutegravir, are recommended by many experts

Table 4. Adverse effects of anti-HIV-1 reagents during chemotherapy

Agents	Adverse effects
AZT	Bone marrow suppression, contraindication
d4T/ddI	Peripheral nerve disorder/ileus (avoid with VCR) Liver dysfunction (toxic for mitochondria)
Protease inhibitor (PI) RTV > IDV = APV > NFV> = SQV	High blood level of anti-cancer agents (inhibition of CYP450-3A4)
NNRTI Efavirenz (EFV) Nevirapine (NVP)	Reduced function of anti-cancer agents (activate CYP450)
Abacavir (ABC)	Hypersensitivity
Tenofovir (TDF)	Renal dysfunction

Table 5. Anti-HIV-1 treatment during cancer chemotherapy

Recommended therapy	EFV + TDF/FTC RAL + TDF/FTC
Alternative therapy	EFV + ABC/3TC RAL + ABC/3TC

EFV, efavirenz; TDF, tenofovir; FTC, emtricitabine; RAL, raltegravir; ABC, abacavir; 3TC, lamivudine. Summarized from lines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents (*http://aidsinfo.nih.gov/contentfiles/lvguidelines/adultandadolescentgl.pdf*).

to anchor cART regimens in patients receiving chemotherapy (Table 5). Another INSTI, elvitegravir is only available as a component of four-drug combination product, which contains potent CYP3A inhibitor.

6.4. *Treatment of opportunistic infections*

Supportive treatment of opportunistic infections is important in HIV-infected patients and post-organ transplantation patients with PEL. Granulocyte-colony stimulating factor (G-CSF) helps reduce chemotherapy-induced neutropenic complications. All patients need to receive prophylaxis for *Pneumocystis carinii* pneumonia with trimethoprim-sulfamethoxazole, regardless of the CD4 cell count. For patients who have severe neutropenia with chemotherapy, alternation of trimethoprim-sulfamethoxazole for *Pneumocystis carinii* prophylaxis can be considered, including dapsone or aerosolized pentamidine. Infectious complications may be minimized by using prophylactic fluroquinolone antibiotics and azoles during periods of protracted neutropenia. Prophylaxis for *Mycobacterium avium complex* (MAC), *Toxoplasmosis* and other opportunistic infection should be also considered as PEL usually arises in an immunodeficient state and chemotherapy induces myelosuppression. Prophylaxis against infection during chemotherapy may include drugs that interact with cART and anticancer agents. Careful attention must be paid for the adverse effects and drug-drug interaction among these agents (*60*).

6.5. *Clinical trial*

On the basis of recent preclinical data and translational studies, several new targeted therapies are being explored, and several clinical trials have been performed based on expert consensus opinions and evidence in preclinical studies.

A proteasome inhibitor, bortezomib, is expected to show clinical effects against PEL. Despite the promising results of *in vitro* experiments and a mouse model (*61,62*), bortezomib treatment either alone or in combination with chemotherapy showed no clinical improvement (*20,63*). The optimization of treatment protocol and combination therapy with bortezomib may be needed to show the preferable effects of bortezomib.

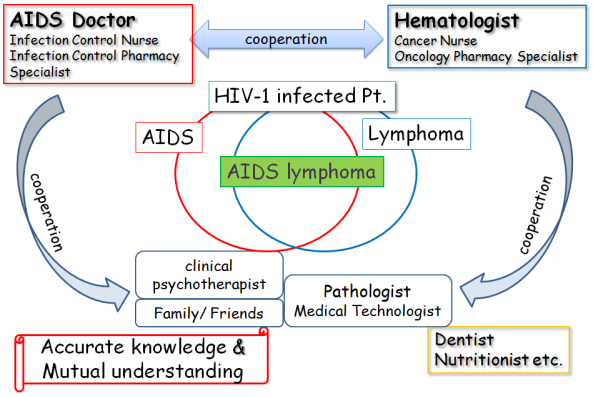

Figure 3. Treatment and support of AIDS-related malignant lymphoma -Team Medical Care-

Lenalidomide is an immunomodulatory drug that is commonly used to treat newly diagnosed and relapsed multiple myeloma as well as a variety of hematological malignancies. It exerts its antitumor action through various mechanisms, such as activation of the immune system, inhibition of angiogenesis and direct antineoplastic effects. Treatment with lenalidomide has never been reported in PEL patients with favorable results (*64*). As lenalidomide was also successfully used to treat three patients with advanced refractory Kaposi sarcoma, this novel agent is expected to be used in prospective studies.

Antiviral treatment can be induced to effect the lytic phase of HHV-8/KSHV viral replication. Complete remission has been reported after the administration of an antiviral nucleotide analogue, cidofovir (*57,65,66*), an antiviral agent with broad activity against multiple DNA viruses, inducing lytic replication of HHV-8/KSHV.

PEL cells are quite sensitive to irradiation in culture and in a xenograft mouse model (*67*). It was reported that chemotherapy-refractory PEL patients achieved remission and survived for more than 12 months with radiation therapy (*68*). Irradiation therapy should be considered as part of the treatment recommendation for patients with chemotherapy-refractory PEL-associated solid masses or localized effusions.

6.6. *Mental support*

There are considerable difficulties in the treatment of AIDS-related lymphoma, including the mental care of patients. The close cooperation of AIDS doctors and hematologists, intensive care by nurse specialists, support from pharmacy specialists, and other co-medical staff is essential. Mental care from a psychiatrist, clinical psychotherapist, and the patient's family and friends are quite supportive for patients. It is especially important to ensure an organic link with the specialist as well as family and friends for treatment (Figure 3).

6.7. *Molecular-targeted preclinical studies*

Since PEL cells display constitutive activity of many signaling pathways and survival, including NF-κB, JAK/STAT and PI3K/AKT pathways, these molecules and HHV-8/KSHV latent proteins are considered ideal for targeted therapy (Figure 2). In particular, vFLIP has the ability to activate the NF-κB pathway by binding to the IκB kinase (IKK) complex (*32,33*), and NF-κB activation is known to be the key player in PEL oncogenesis, so various NF-κB and proteosome inhibitors have been investigated in a preclinical trial. Xenograft PEL mouse models and *in vitro* culture of PEL cell lines were used in preclinical studies, and promising preclinical results were reported with multiple NF-κB inhibitors, such as cepharanthine (*69*), diethyldithiocarbamate (*70*), berberine (*71*), and heat-shock protein 90 (*72,73*). Xenograft mouse models using severe immunodeficient mice are a powerful tool to confirm the effects and adverse effects of candidate reagents in a preclinical study.

The PI3K/AKT pathway, JAK2/STAT3 pathway and mTOR are also activated in PEL cell lines and could be promising targets (74-76). Several inhibitors are currently undergoing clinical trials in patients with hematological malignancies and can be used for the treatment of PEL in the near future (*77*).

Interferon-α and AZT induced TRAIL-mediated apoptosis of PEL (78,79). IFN-α upregulates TRAIL in PEL cells while AZT sensitizes them to TRAIL, resulting in the activation of a suicide program. The efficacy of this approach needs to be validated in clinical trials.

6.8. *Immunotherapy*

Although rituximab, a chimeric anti-CD20 antibody, has provided a significant survival advantage for B-cell NHL in combination with standard chemotherapy, rituximab does not play a significant therapeutic role in PEL because CD20 is not usually expressed on the surface of PEL cells. Rare cases expressing CD20 have been reported to respond to rituximab (*47*).

CD30 is expressed significantly in case of PEL. Brentuximab vedotin (SGN-35) is an antibody–drug conjugate in which a chimeric anti-CD30 antibody is combined with the synthetic microtubule-disrupting agent monomethylauristatin E (MMAE) (*80*). Since treatment with brentuximab vedotin also prolonged the survival of a PEL xenograft mouse model (*81*), brentuximab is expected to be a candidate for the treatment of PEL.

PEL cells secrete vascular endothelial growth factor (VEGF)-A (*82*), and treatment with mouse anti-human VEGF-A monoclonal antibody inhibited the development of ascites in a xenograft mouse model. Because bevacizumab, a humanized VEGF-A monoclonal antibody, is clinically used for the treatment of a variety of human cancers, including colorectal, non-small-cell lung, ovarian and metastatic renal cell carcinoma (*83*), it is also expected to be a novel target of treatment.

7. Conclusion

PEL is a rare but aggressive form of NHL, mostly arising in immunodeficient patients. PEL is commonly resistant to conventional chemotherapy and has a poor prognosis. Currently, more intensive chemotherapy with cART is recommended. The management of opportunistic infection is also needed since PEL arises in immunodeficient states. Drug interaction between anti-cancer reagents and cART, especially protease inhibitors, should be carefully monitored in HIV-1-positive individuals. Close communication among the oncologist, the patient's primary HIV-treating physician, and co-medical staff is needed for the intensive treatment of AIDS-related PEL patients. It is also important to avoid drug interactions in chemotherapy. Several molecular-targeted therapies are in clinical trial and preclinical stages, and their clinical use is anticipated. Since PEL is mostly associated with immunodeficient states, early diagnosis and treatment of HIV-1 may prevent the onset of PEL.

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Review

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Pseudoxanthoma elasticum: A review of 86 cases in China

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Summary **Pseudoxanthoma elasticum (PXE) is a type of rare hereditary disease that affects connective tissue. PXE is found around the world, and its epidemiology in China is still unclear. A database search revealed that 86 patients in total were reported in China from 1985 to 2013. The vast majority of these reports concern single, sporadic cases. This review summarizes the clinical characteristics of PXE and its treatment in China. The hope is to provide a reliable basis for studies on the incidence of PXE and for formulation of relevant policies in the future.**

Keywords: Rare diseases, prevalence, clinical features, literature search

1. Introduction

Pseudoxanthoma elasticum (PXE) is a type of rare hereditary disease that affects connective tissue, causing mineralization of elastic fibers in the skin, eyes, cardiovascular system, and even the digestive system. A typical clinical manifestation of PXE is the appearance of small (one to five millimeters), asymptomatic, soft papules that are yellow/ivory in color on the flexural surfaces (*1*). Skin signs are typically followed by ocular signs, which include angioid streaks, choroidal neovascularization, and subretinal hemorrhaging that result in loss of visual acuity and occasionally lead to blindness. Cardiovascular signs include a pulse that is weak or even absent and intermittent claudication. A few patients may have angina pectoris or hypertension. The disease is often accompanied by gastrointestinal symptoms that may lead to severe joint problems and gastrointestinal bleeding. Statistical analysis has indicated that the incidence of the disease is about one in 50,000 (*2*). The disease affects women more often than men at a ratio of 2:1. The main pathological changes in the skin are the degeneration, swelling, rupturing, and mineralization of elastic fibers. The molecular basis of PXE remains unknown, but the disease is caused by mutations in the *ABCC6* gene encoding an ATP-dependent transmembrane transporter, the substrate and pathophysiological role of which have yet to be elucidated (*3*). In light of its genetic features, inheritance of PXE is usually divided into autosomal dominant inheritance or autosomal recessive inheritance. Based on the severity of the condition, each variant of PXE is divided into type I (typical or severe) or type II (atypical or light) disease. The autosomal recessive form of the disease is often characterized by metabolic abnormalities. Thus far, researchers have yet to identify any specific therapy for the disease, and PXE is often treated symptomatically. Surgery can be performed to remove lax skin hanging in folds, and vitamin E may be used to treat fundus retinopathy (*4*). The current article briefly reviews the current state of PXE epidemiology, diagnosis, and treatment in China.

2. Literature search strategy

The Wanfang Database and China National Knowledge Infrastructure Database were searched using the keyword "Pseudoxanthoma elasticum" along with "diagnosis" or "treatment" or "epidemiology" to identify all relevant literature published in Chinese journals from January 1985 to April 2013. Cases of PXE with a confirmed diagnosis were included. Studies including keywords like angioid streaks were also noted and then examined for mention of PXE. The full text of

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each article was reviewed and the article was selected if relevant. Each patient's medical information was carefully compared and repeated cases were excluded.

3. Prevalence and epidemiology

3.1. *Prevalence and epidemiology*

A search of articles on PXE in the databases indicated that 86 patients in total have been reported in China from 1985 to 2013. In total, 19 men and 67 women were affected by PXE according to reports in China, as shown in Figure 1A. Women were affected more often than men at a ratio of 3.53:1. The patient age at the time of diagnosis ranged from 8 to 72 years; 4 patients were under 10 (4.6%), 18 patients were ages 10-19 (21%), 31 patients were ages 20-29 (36%), and 33 patients were age 30 or older (38.4%) (Figure 1B). PXE was found in 20 provinces and cities in China. Data showed that the incidence was significantly higher in Shanxi Province than in other areas (Figure 1C). This may be related to the local level of medical care. Six of the patients in the literature had a family history of PXE; 3 patients had autosomal dominant type I disease (*5-7*) while 3 patients had autosomal recessive type I disease (*8-10*).

3.2. *Clinical manifestations of PXE in Chinese patients*

All patients present with characteristic skin lesions of yellowish papules that coalesce into plaques. Skin is inelastic and leathery but does not otherwise cause discomfort. The lesions of PXE are typically distributed in intertriginous sites of the body, in other words in the folds of the skin at the sides of the neck, the joints of the limbs, the axillae, the cubital and popliteal fossae, and the creases of the groin and umbilical region. The

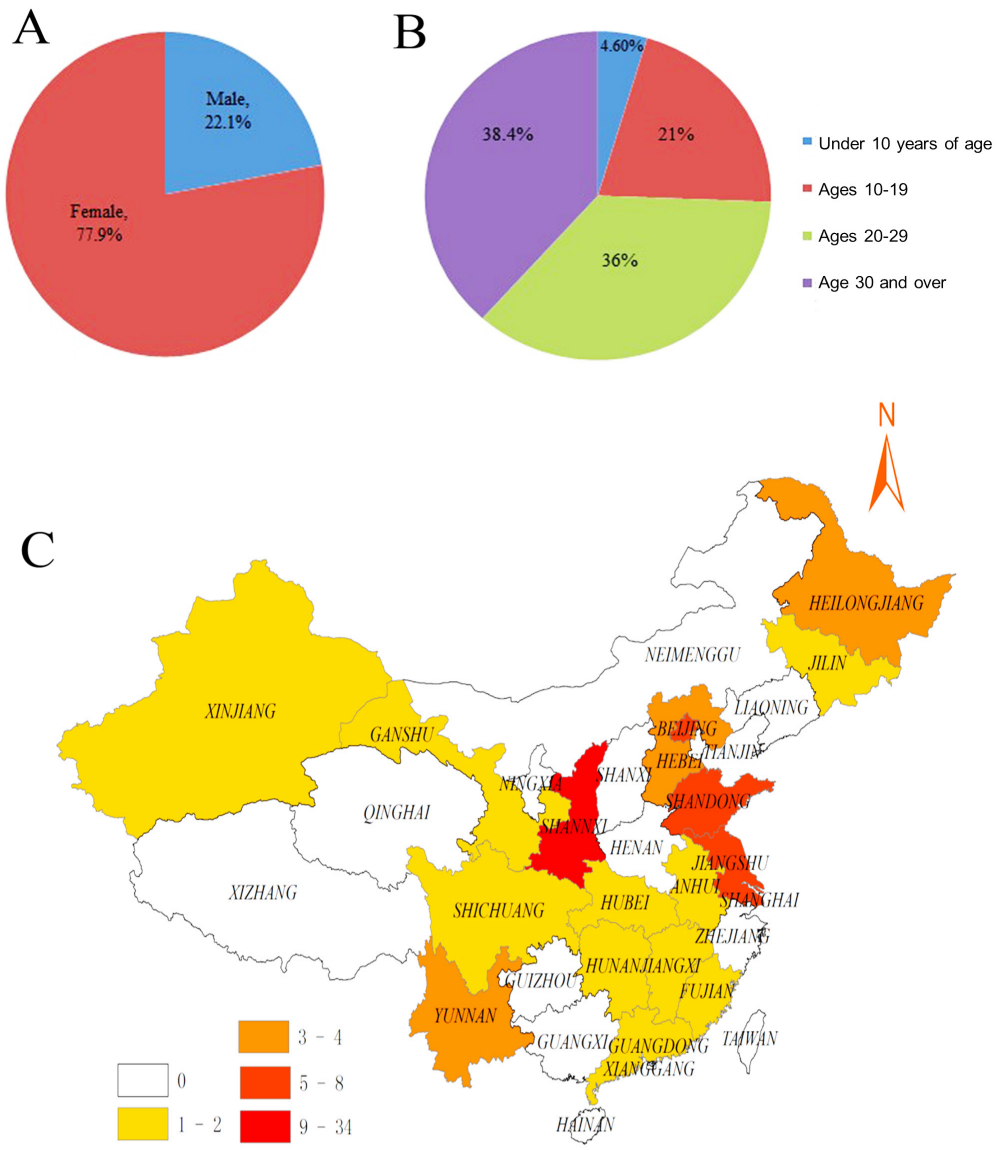

Figure 1. Prevalence and epidemiology according to Chinese case reports. (A), Proportion of men and women affected; **(B)**, Age of patients; **(C)**, Geographical distribution of PXE case reports in China.

onset and duration of the cutaneous lesions of PXE vary. Women noticed abnormal skin at an average age of 22. However, only 5 men were aware of the duration of changes in their skin. This difference in awareness is possibly related to the greater attention that women give to the appearance of the skin on the exposed parts of the body compared to men.

Of 86 patients with PXE, 55 underwent a fundus examination and 21 had angioid streaks originating from the optic disc that radiated outwards as brownish-grey irregular lines. About a fourth of the affected patients had maculopathy while only two patients had a subretinal hemorrhage (*11,12*). Lesions are generally asymptomatic unless they extend into the macula.

Six patients had different degrees of gastrointestinal bleeding. Two had bleeding from the large intestine (*13,14*) and two had involvement of the gastric mucosa (*15,16*). The remaining two patients had internal hemorrhoids that were not serious (*10,17*). Hypertension was present in two of the patients with PXE (*16,17*). Some researchers have noted patients with PXE have a markedly higher incidence of hypertension than normal persons (*18*). Only one of the 86 patients (a 75-year-old woman) had clinically evident myocardial infarction (*19*) and another woman suffered specific cardiomyopathy (*20*). One patient had PXE with hyperthyroidism for three years (*21*). Chen *et al*. reported a 30-year-old female patient with typical pathological changes in the skin and eyes who was at risk of a spontaneous abortion in the first 3 months of both her pregnancies, but they were unable to determine whether the risk of spontaneous abortion was related to PXE (*22*). Various complications noted in the 86 patients with PXE are shown in Figure 2.

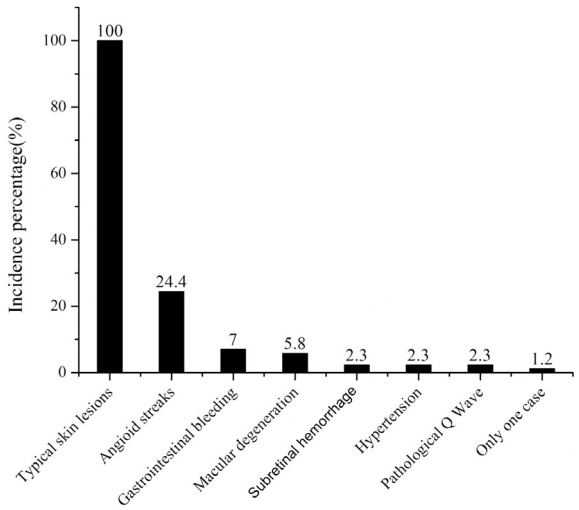

Figure 2. Percentage of various complications. Myocardial infarction, specific cardiomyopathy, aortic fibrosis, right ventricular hypertrophy, coronary artery stenosis, hematuria, premature ventricular contractions, short P-R interval syndrome, mitral inadequacy, hyperthyroidism, and atrial premature contraction were noted in "only one case."

4. Discussion

The main diagnostic criteria for PXE include *i*) small, yellowish, flat papules that typically develop on the skin of the neck, axillae, groin, and flexural creases; *ii*) angioid streaks, which are irregular, reddish-brown, or grey lines that radiate from the optic disc; *iii*) histological characteristics of PXE skin lesions with fragmentation and calcification of mid-dermal elastic fibers on Alizarin red staining. A histological examination of the skin is still the gold standard for diagnosing PXE (*23*). Almost all patients met the first and third criteria. Only 21 patients had angioid streaks since the disease had taken a different course. All patients were diagnosed based on a skin biopsy.

5. Treatment

Based on Chinese reports, doctors symptomatically treat patients with PXE. Limiting dietary calcium intake may reduce the progression of lesions and laser photocoagulation should be performed early on when the macula lutea is affected by angioid streaks. Oral vitamin E can be used to treat ocular symptoms. Only one case report mentioned a 19-year-old female patient with PXE who underwent repair of loose skin of the face and neck, and this is the first such case reported in China (*24*). Some researchers administered oral phosphate binders to 6 patients and they noted marked improvement in clinical symptoms in 3 of those patients (*25*). Moreover, the histopathological changes in lesions gradually disappeared. In a 1-year follow-up, no patients had further eye damage. The current review is key to guiding the treatment of PXE. However, the current findings must be studied further since the sample size was too small.

6. Conclusion

PXE is a type of rare disease, and little is known about its epidemiologic characteristics in China. A literature search revealed that there are more studies and case reports on PXE in Europe and the United States than in China. Researchers conducted a study of 513 patients to determine the effect of gender on PXE, and they found that gender differences were statistically significant (*26*). A literature search revealed that only 86 cases of PXE have been reported in China, and no studies of patients with PXE have been conducted. This greatly hampers an analysis of the clinical features and epidemiological characteristics of patients with PXE in China. In addition, complications of PXE are detected at a higher rate in developed countries than in China. This may relate to the earlier start of PXE research and the establishment of case databases. To the extent known, this is the first systematic review of the incidence of PXE in China. The current state of PXE diagnosis and treatment in China is

unsatisfactory. The low incidence rate does not coincide with the country's large population, indicating that the clinical outlook is bleak. To date, there are no specific pharmacological therapies for PXE. In 2013, China launched its first pilot project covering 20 representative rare diseases in order to improve conditions. PXE is one of the 20 rare diseases (*27*). The current review can help to further elucidate the current state of PXE in China and encourage more statistical research. This review seeks to provide a reliable basis for researchers to diagnose, treat, and study PXE and formulate relevant policies in accordance with conditions in China.

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Review

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An overview of Korean patients with mucopolysaccharidosis and collaboration through the Asia Pacific MPS Network

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Summary **Mucopolysaccharidosis (MPS) is a constellation of disorders characterized by the accumulation of mucopolysaccharides in tissues and organs. This accumulation results in the deterioration and degeneration of multiple organs. This paper describes the general distribution of types of MPS in patients, their clinical characteristics and genotypes, the development of animal studies and preclinical studies, enzyme replacement therapy in South Korea, and the development of idursulfase beta and clinical trials on idursulfase beta in South Korea. In addition, this paper discusses academic collaboration among specialists in MPS care in the Asia-Pacific region, which includes Japan, Taiwan, Malaysia, and South Korea, through an organization called the Asia-Pacific MPS Network (APMN). The Asia-Pacific MPS Registry, an electronic remote data entry system, has been developed by key doctors in the APMN. Rare diseases require international cooperation and collaboration to elucidate their mechanisms and carry out clinical trials; therefore, an organization such as the APMN is required. Furthermore, international collaboration among Asian countries and countries around the world will be of utmost importance in the future.**

Keywords: Mucopolysaccharidosis, Hunter syndrome, enzyme replacement therapy

1. Introduction

Mucopolysaccharidosis (MPS) is a constellation of disorders characterized by the accumulation of mucopolysaccharide in tissues and organs. This accumulation results in the deterioration and degeneration of multiple organs. Research has proven that MPS is caused by genetic defects, and at least 11 genes are causally related to MPS disorders. MPSs are categorized into seven types (I, II, III, IV, VI, VII, and IX) based on which enzyme is affected. These types vary in their prevalence, clinical manifestations, and degree of severity. MPS is inherited in an autosomal recessive manner except for MPS type II (Hunter syndrome), which is transmitted as an X-linked recessive disorder (*1*). Extensive somatic involvement affecting the heart, lungs, bones, joints, and gastrointestinal system is seen in most MPS types, accompanied by central nervous system (CNS) dysfunction in MPS types I, II, III, and VII.

Described here are the general distribution of types of MPS in patients, their clinical characteristics, the development of animal studies, and the clinical trials of enzyme replacement therapy (ERT) in South Korea. Also described is academic collaboration among specialists in MPS care in the Asia-Pacific region, which includes Japan, Taiwan, Malaysia, and South Korea, *via* an organization called the Asia Pacific MPS Network (APMN). *Via* the APMN, doctors and researchers who are interested in the treatment of patients with MPS collaborate and exchange information and they present basic and clinical research related to MPS.

2. Distribution of clinical types and genotypes

Although MPS disorders are distributed worldwide, there are regional differences in their distribution. Almost half of the patients in South Korea with MPS have MPS type II, Hunter syndrome, and the same

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is true for other locations in Asia such as Japan and Taiwan. In contrast, the incidence of MPS type I is higher than that of MPS type II in Western countries (*2*). The distribution of each type is comparable in South Korea and Japan. However, a point of note is that the prevalence of MPS type III may have been underestimated because therapeutic modalities are not available for this type.

The genotype of each type of MPS in South Korea has been reported several times. Because Hunter syndrome is an X-linked recessive disorder, in most cases the mutations causing the disorder are unique mutations, except when there is a shared X chromosome. The current authors reported finding 20 mutations in 25 Korean patients with MPS type II in 2003 (*3*). Thirty-one mutations in 49 Korean patients with MPS type II from 45 families were later reported in 2012 (*4*). IDS-IDS2 recombination mutations were observed most frequently, and all of the patients with this mutation had the severe phenotype. However, most patients (5/7) with the G374G splicing mutation had an attenuated phenotype, except for two siblings with the severe phenotype. Each patient had a unique individual mutation except for a few recurrent mutations, such as G374G, R443X, and L522P, and recombination mutations.

With MPS type I, several mutations are common to both Asian and Western countries. The current authors reported finding 15 mutations from 10 patients with MPS type I in 2004 (*5*). Later, eight more patients with MPS type I were identified. L346R and 704ins5 accounted for approximately one-third of the mutations found in Korean patients with MPS type I. L346R was mostly found in patients with Hurler syndrome.

The current authors reported a total of seven different mutations in six Korean patients with MPS type IVA in 2013 (*6*). Interestingly, the authors identified three patients with MPS type IVA last year. MPS type IVA is relatively rare in South Korea; thus, extensive screening of orthopedic patients for glycosaminoglycans (GAG) in urine may have contributed to the discovery of this type of MPS. Two mutations, c.451C>A and c.1000C>T, accounted for 33% and 19%, respectively, of all mutations in 13 Korean patients with MPS type IVA. A point worth mentioning is that one patient with an unusual presentation was found through next-generation sequencing (*7*). He first presented with hip pain at 11 years of age, and bilateral Perthes-like disease was suspected. Aggravated hip pain led him to see an orthopedic surgeon at age 27. He is of average stature for an adult Korean male, with a height of 169 cm and a weight of 74 kg. Radiographic abnormalities in the spine, pelvis/hips, and knees led to suspected X-linked spondyloepiphyseal dysplasia (SED) tarda, but this was not confirmed by mutation analysis of SEDL (TRAPPC2). Moreover, molecular testing for SEDC (COL2A1) and multiple epiphyseal dysplasia (COMP, MATN3, and COL9A1-3) revealed no deleterious mutations. Whole-exome sequencing identified two novel GALNS mutations, c.317A>G (p.N106S) and c.553delG (p.E185Rfx14), that were confirmed by Sanger sequencing, and reduced GALNS activity confirmed a diagnosis of MPS type IVA. MPS disease may often be radiographically mistaken for multiple epiphyseal dysplasia, SED, or bilateral Perthes-like disease. A full skeletal survey should be performed if MPS or another type of skeletal dysplasia is suspected. Extra-skeletal manifestations, including corneal clouding, specific cardiac abnormalities, and facial dysmorphology, can provide vital clues.

Mutations in the *GLB1* gene, which encodes acid β-galactosidase, can result in two disease phenotypes, namely GM1-gangliosidosis and MPS type IVB disease. The current authors reported the first known case of Morquio B disease in a Korean patient (*8*). This patient had severe skeletal manifestations (dysostosis multiplex) without CNS involvement. The enzyme activity of β-galactosidase in leukocytes was 1.15 nmol/h/mg protein (reference range 78.1-117.7; 1-1.5% of normal). The patient had compound heterozygous mutations of the GLB1 gene, namely c.13_14insA (p.L5HfsX29), as were reported in a patient with infantile GM1 gangliosidosis. The patient also had near-complete absence of enzyme activity and c.367G>A (p.G123R), which is a novel frame-shift mutation.

3. The clinical profiles of MPS patients in South Korea

In 2012, the current authors retrospectively reviewed the medical records of 75 Korean patients with Hunter syndrome (74 males, one female) in order to investigate the frequency of organ involvement and survival at a single center (*9*). The three most common symptoms of organ involvement were hepatosplenomegaly (99%), facial dysmorphism (97%), and frequent otitis media (91%). Cardiovascular involvement was also common, including valvular abnormalities (89%), left ventricular hypertrophy (68%), and hypertension (30%). The 19 patients who died had a median age of 16.8 years at their time of death. Four of them died within one year of the start of ERT; an autopsy revealed myocardial infarction with severe coronary artery disease in one patient. Two other patients died due to pneumonia and sleep apnea, and the cause of death was not investigated in the remaining case. The high incidence of hypertension and the presence of valvular heart disease indicates that close cardiac monitoring is mandatory in all patients with Hunter syndrome, and especially relatively older patients, even if they are being treated with ERT. A point worth mentioning is the high prevalence, even at a young age, of carpal tunnel syndrome in the patients with Hunter syndrome that were studied. This finding should be considered an integral part of the clinical manifestation

of Hunter syndrome (*10*). Short stature is a prominent and consistent feature in MPS type II. The effect of ERT on the growth of 32 Korean patients with Hunter syndrome was evaluated at a single center (*11*); the patients had marked retardation of growth as they grew older. However, ERT may have less of an effect on the growth of patients with the severe form of Hunter syndrome. The height z-scores in patients over six years of age revealed significant differences. Their growth in response to ERT could be an important treatment outcome or endpoint for future study.

The current authors encountered an interesting case of Hunter syndrome in a female patient, a detailed description of which has been given previously (*12*). The patient had mild manifestations of Hunter syndrome and gave birth to a daughter. Both the mother and daughter carried the p.R443X mutation in the *IDS* gene, and Iduronate-2-sulfatase activity in the mother's fibroblasts was as low as that found in male patients with Hunter syndrome, but it was in the low–normal range in the daughter. Unlike her mother, the daughter did not exhibit any physical signs of Hunter syndrome, and her urinary excretion of glycosaminoglycans was within the normal range. However, she had severe pulmonary vein stenosis with pulmonary hypertension and a large atrial septal defect, and she died at 11 months of age. After several years, the patient subsequently gave birth to a healthy daughter who was not a carrier.

The current authors reported the clinical findings, radiological features, and genetic data from 10 Korean patients with MPS type IVA in 2012 (*13*). Together with three other patients who were diagnosed more recently, Eleven patients had the severe clinical phenotype based on their clinical phenotype criteria, one had an intermediate phenotype, and one had an attenuated phenotype. Radiological findings indicated skeletal abnormalities in all patients, and especially in the hips and extremities. Nine patients had odontoid hypoplasia, and one had mild atlantoaxial subluxation and cord myelopathy. Adequate evaluation and therapy in the early stages may improve the quality of life of patients suffering from skeletal abnormalities and it may reduce the life-threatening effects of atlantoaxial subluxation.

4. Diagnosis of MPS in South Korea

The measurement of urinary GAG levels is a useful test to screen for MPS disorders. A positive result is highly suggestive of MPS, but false-negative results are also very common (*14*). False-negative results occur because of the insufficient sensitivity of the various assays or because the samples are too diluted. Thus, a negative urinary GAG analysis does not rule out MPS. Therefore, the urinary GAG test is usually repeated for any patient who is clinically suspected of having MPS but whose urine test is negative. If the urine GAG level is equivocal in spite of the strong suspicion of MPS, exome sequencing is performed to screen for the several genes that are known to be responsible for MPS.

Enzyme activity assays based on cultured fibroblasts, leucocytes, plasma, or serum are definitive for specific MPS disorders and are considered the gold standard for diagnosis. Because gene sequencing follows biochemical diagnosis to identify the mutation(s) present in almost all patients in South Korea, when a sulfatase deficiency is identified the activity of another sulfatase is not usually measured in order to rule out multiple sulfatase deficiencies. If, however, the results of gene sequencing do not definitively confirm the disease, then the activity of another sulfatase should be measured.

The Samsung Medical Center is the main center for diagnosis and treatment of patients with MPS in South Korea. Based on data from the Center, 147 patients had MPS confirmed *via* enzyme assay and molecular analysis from 1994 to 2013. The most common subtype of MPS was Hunter syndrome (54.6%), followed by MPS type III (18.4%). Thirteen patients with MPS type IIIA were noted, thirteen patients with MPS type IIIB were noted, and one patient with MPS type IIIC was noted. No patients with MPS type IIID were noted. Other subtypes included MPS type I (15.3%), MPS type IV (9.5%), and MPS type VI (1.4%), but MPS type VII has yet to be noted.

5. An animal model of MPS

In 2010, the current authors reported producing IDS knock-out (KO) mice and they analyzed the resulting phenotype (*15*). The KO mouse model of Hunter syndrome was produced by replacing part of the IDS gene (1485 bp encompassing exon 2 and exon 3) with the neomycin resistance gene. This animal model contributed both to basic research and to the development of a novel therapeutic approach.

The auditory characteristics of MPS type II and the effect of ERT on hearing were evaluated in IDS-KO mice. At 17 weeks of age, the IDS-KO mice had elevated hearing thresholds, and exudates were found in the middle ear. The hearing thresholds of the IDS-KO mice treated with enzyme (IDS-ERT) were similar to those of wild type (WT) mice at 17 weeks. Hearing deficits in the MPS type II mouse model can be prevented if ERT is started before the onset of hearing impairment (*16*). The changes in myocardial function associated with ERT were evaluated in a mouse model of cardiomyopathy associated with Hunter syndrome (*17*). Thirty nine-week-old IDS-KO mice received either an intravenous injection of human recombinant IDS (ERT group, $N = 15$) or saline (control group, $N = 15$) for five weeks. A significant increase in left ventricular fractional shortening and radial and circumferential strain was observed in only the ERT group. Marked

myocardial fibrosis was observed only in the control group. In the murine model of Hunter syndrome, ERT has a beneficial effect on cardiac function, and this can be evaluated *via* serial echocardiographic evaluation that includes two-dimensional strain analysis.

Current ERT to treat MPS does not ameliorate the CNS or the skeletal system because the current dose of recombinant enzyme administered is not thought to be able to pass the blood-brain barrier. Several studies on overcoming this barrier were conducted using the KO mouse model of Hunter syndrome.

A pseudotyped, recombinant adeno-associated virus 2/8 vector encoding the human IDS gene (rAAV-hIDS) was administered intravenously to adult IDS-KO mice to evaluate the effects of gene therapy in a mouse model of MPS type II (*15*). Gene therapy completely restored IDS activity in the plasma and tissue of the KO mice, and the restored enzymatic activity completely cleared the accumulated GAGs in all of the tissues analyzed. This experiment involving liver-specific gene therapy indicated the effectiveness of high-dose therapy because the high level of enzyme expression in the liver induced a high level of enzymes in the blood.

Early, high-dose ERT was subsequently found to attenuate ventriculomegaly and histologic abnormalities in the brains of IDS-KO mice (*18*). IDS-KO mice received saline or recombinant human IDS (0.5/1.0/2.0 mg/kg) intravenously once a week, starting at four weeks of age, and continued to do so for 20 weeks. ERT with 2.0 mg/kg, but not 0.5 or 1.0 mg/kg, significantly attenuated the enlarged ventricles, as confirmed by *in vivo* 7.0 Tesla brain magnetic resonance imaging (MRI) at 20 weeks. GAG levels were significantly correlated with the ratio (in percent) of the ventricular volume to the total brain volume. These results suggest that high-dose systemic ERT beginning early in life could be a promising therapeutic modality for improving neurological dysfunction, including ventriculomegaly, in children with severe Hunter syndrome. High-dose enzyme treatment attenuated the ventriculomegaly in this animal model and suggested that the adequate therapeutic range for this disorder should be reevaluated (*18*).

Although intermittent intrathecal (IT) injection of a particular enzyme has been cited as a way to overcome the blood–brain barrier, continuous IT infusion of that enzyme would be more physiologically appropriate. Responses in the brains of MPS type II mice to varying doses of continuous IT infusion of recombinant human IDS (rh-IDS) were investigated in MPS type II mice receiving three different doses (2.4, 4.8, and 12 mg/day) of rh-IDS for three weeks *via* osmotic pump (19). Results indicated that mice treated with 12 mg/day had decreased GAG concentrations compared to the untreated KO mouse group ($P < 0.003$). After three weeks of continuous IT ERT, the brain tissues of the KO mice treated with a high dose of IT had reduced vacuolation in the cerebral cortex, thalamus, and cerebellar cortex. The same was not observed in KO mice treated with a low or medium dose. Moreover, anti-NeuN signaling indicative of intact neurons was restored in the cortexes of the mice treated with a high dose. Continuous IT infusion of the deficient enzyme was effective at improving CNS defects in mice with MPS type II and may represent a beneficial therapy to treat neurological deterioration in patients with MPS type II.

6. ERT in South Korea

The treatment of MPS type II was palliative prior to the introduction of ERT. For over 10 years, ERT with recombinant human enzyme for MPS types I, II, and VI (Aldurazyme® for MPS type I, Elaprase® for MPS type II, Naglazyme® for MPS type VI) has been approved in the US, Europe, South Korea, and many other countries worldwide. The current authors have been actively involved in a phase III clinical trial of a recombinant enzyme for Morquio syndrome A. The drug is now permitted in South Korea and is expected to be commercially available within a year. Intravenously-infused enzymes are internalized *via* mannose 6 phosphate receptors located on the cell surface to reach their target site in the lysosomes and replace the defective enzymes (*20*). The earlier ERT is initiated, the better the potential outcome because of the irreversible nature of some of the abnormalities associated with MPS disorders (*21,22*). The benefits of ERT for certain MPS disorders may include improvements in joint mobility, walking ability, and pulmonary and respiratory function; a reduction in liver and spleen volume; and a significant reduction in urinary GAG excretion (*23-27*). When ERT is administered intravenously, the enzyme does not cross the blood–brain barrier at the labelled dose and the therapy has not yielded any neurocognitive benefits. The most common adverse events associated with ERT are infusion-related hypersensitivity reactions that can be characterized by flushing, headache, pyrexia, or urticaria. Such reactions are generally managed by slowing the infusion rate and administering antihistamines and/or steroids (*28*). However, life-threatening anaphylactic reactions can occur in patients receiving ERT. In a previous study (*29*), anaphylaxis associated with the infusion of idursulfase was mediated by anti-idursulfase IgE antibodies, which can be produced by *de novo* synthesis. The skin-prick test was useful at predicting the occurrence of anti-idursulfase IgE–mediated anaphylaxis during infusion. Therefore, the skin-prick test is usually performed before initiating ERT. Several patients with Hunter syndrome developed life-threatening anaphylactic reactions such as hypotension, blurred vision, and angioedema. All of the patients received ERT in accordance with the desensitization protocol over 48 hours a week in the early period, when they exhibited

severe anaphylaxis, and some of the patients were switched to another drug for ERT. Now, all of these patients are receiving ERT in accordance with general protocols and none have exhibited severe anaphylaxis.

7. Development of idursulfase beta and phase I/II clinical trials on idursulfase beta in South Korea

Successful clinical trials (*25,30*) have led to the approval of ERT with human recombinant idursulfase (Elaprase®, Shire Human Genetic Therapies, Lexington, MA) by the US Food and Drug Administration (FDA) in July 2006, and this therapy has been available in South Korea since 2009. Another drug for Hunter syndrome, idursulfase beta (Hunterase®, Green Cross Corp., Yongin, South Korea), was approved by the Korean FDA in January 2012. This drug is a recombinant protein that is produced by genetically engineered Chinese hamster ovary (CHO) cell lines. Idursulfase beta is produced in a serum-free medium *via* suspension cell culture and is purified in several chromatography steps. A number of chromatography and electrophoresis techniques have revealed the purity of the purified protein to be > 99.9%. Preclinical studies using a KO mouse model of MPS type II suggested that a course of 24 weeks of ERT with idursulfase beta (Hunterase) was effective at reducing urinary GAG excretion and GAGs stored in several tissues, including the liver, spleen, heart, lungs, and kidneys (unpublished data). A 24-week randomized, single-blinded, active comparator–controlled, phase I/II clinical trial of idursulfase beta was conducted to evaluate its efficacy and safety in the treatment of MPS type II patients. This study (*31*) was the first active comparator–controlled clinical trial of idursulfase beta for Korean male patients with MPS type II. The idursulfase beta treatment was well tolerated by Korean patients with MPS type II and resulted in a significant reduction in urinary GAG excretion as well as an improvement in the distance on the six-minute walking test when compared to the active comparator. The effect of the treatment on pulmonary function, cardiac function, and joint mobility was similar to that of the active comparator. A long-term clinical trial to establish the long-term efficacy and safety of idursulfase beta for the treatment of MPS type II is currently underway. However, further experiments will be needed, particularly in patients who have never received ERT. A considerable number of patients in South Korea are now being treated with Hunterase®. Hunterase has recently been exported to other countries in Asia and to the Middle East. In addition, a global clinical trial of Hunterase will soon begin.

8. The Asia-Pacific MPS Network (APMN)

Although individually rare, MPS disorders as a whole are highly prevalent, with an overall incidence of 1:22,000-52,000. Asia is the world's most populous continent, with approximately 4.3 billion people. Many patients may have gone undiagnosed or may be receiving minimal care in this region. In most countries, there is a lack of specialized healthcare personnel to provide adequate, comprehensive care for patients with MPS. Although the management of some clinical problems associated with the disease may seem routine, management is usually complicated and requires physicians' awareness of issues specific to the disease. Therefore, a multidisciplinary approach and the coordination of efforts by the professional, social, and governmental sectors are required. An important step is to establish an infrastructure of experts in each country and promote cooperation within the Asia-Pacific region in order to improve specialist training and communication. Given the need for a system of cooperation, the APMN was established by several MPS experts in South Korea, Japan, and Taiwan in January 2013. The four main objectives of the APMN are as follows: *1*) to organize an Asia-Pacific research network for MPS (establishing a registry and standard treatment guidelines); *2*) to understand the current state of the disease and exchange information on it; *3*) to provide support for preclinical studies related to MPS and patients with MPS/parents in the Asia-Pacific region; and *4*) to encourage and engage in an international exchange of younger doctors who are treating MPS patients. A survey to assess the current patterns of MPS diagnosis and treatment in the Asia-Pacific region should be conducted to identify gaps in knowledge and unmet needs that must be addressed and to provide opportunities for research on particularly relevant topics in the region. The professional knowledge and experience of MPS experts should be shared through international cooperation in order to provide better treatment to patients with MPS in Asia, in the Pacific region, and around the world. All of these efforts should be aimed at providing effective care for MPS patients. Recently, the APMN has been expanding through the participation of other Asian experts. The APMN developed an MPS-specific registry a year after its establishment.

9. The Asia-Pacific MPS Registry (APMR)

One of the obstacles to the identification, understanding, and treatment of MPS is the relative paucity of information. Therefore, regional and national disease registries are needed to facilitate the better understanding of the natural history of this clinically heterogeneous disease and to generate long-term data to evaluate existing and new therapies. One way that patients can take part in ongoing research efforts is through their enrolment in disease-specific registries. Observations based on these international registries will provide insights into the natural history of the disease and

Table 1. Recommended schedule of assessments in South Korea

Assessments	Initial	Every 3 mo	Every 6 mo	Every 12 mo
General				
Medical photo (if needed)	√		√ (1st)	
Height/weight/head circumference	√	√		
V/S(BP,RR,HR)	√	√ (weekly)		
P/Ex (including Tanner stage)	√	√ (weekly)		
N/Ex	√	√ (weekly)		
Laboratory test	√		√	
Urine GAG	√	√		
Current medication	√	√		
ERT				
Drug		√		
Dose		√		
Frequency		√		
Adverse reactions		√		
Premedication		√		
Clinical assessments				
Gastrointestinal system				
Abd US (hepatosplenomegaly)	√			√
Feeding problem (gastrostomy tube/L-tube)	√	√		
Umbilical/inguinal hernia	√	√ (weekly)		
Airway				
Pulmonary function test (if possible)	√			√
Respiratory assistance	√	√ (weekly)	√	
Number of bouts of pneumonia	√			
Sleep study (if needed)	√			√ (if needed)
Eye, Nose, & Throat			√ (or yearly)	
Audiometry	√		√ (or yearly)	
Airway patency	√		√	
Frequency of ear infections/surgery	√		√	
Placement and/or replacement of grommets	√			
Neurologic system			√	
Epilepsy medication	√			
MRI of brain	√			√ (if needed)
MRI of spine (if needed)	√			√ (if needed)
Cognitive testing (IQ/developmental milestone)	√			√
Bones & Joints				
Skeletal survey by X-ray	√		√	√ (if needed)
Surgical history	√		√ (or yearly)	
Range of motion of shoulder joint	√			
Median nerve conduction velocity (if needed)	√			√
6 min walking test	√			√
Cardiovascular system				
Echocardiogram/ECG	√			√
NT proBNP	√			√
Chest X-ray	√			√
Eye				
Visual acuity/ Visual field	√			√
Fundoscopy/ Retinoscopy (if needed)	√			√
Quality of life			√ (or yearly)	
Activities of Daily Living (FIM)	√			
Dental exam	√			√

Mo, month; V/S, vital sign; BP, blood pressure; HR, heart rate; P/Ex, physical examination; N/Ex, neurologic examination; ERT, enzyme replacement therapy; US, ultrasonography; IQ, intelligence quotient; ECG, electrocardiogram; FIM, functional independence measure

the long-term effects of various therapies. Such data can be used to help identify unmet patient needs and encourage further research. Registries have been established for MPS type I (*www.mpsiregistry.com*), MPS type II (*www.elaprase.com/patients_families/about_hunter/outcomes/*), MPS type IVA, and MPS type VI (*http://www.naglazyme.com/en/Clinical-resources/surveillance-program.aspx*), and the Morquio A Clinical Assessment Program has also been initiated. However, these registries are managed by pharmaceutical companies that make drugs for MPS patients. Moreover, they mainly include patients from Europe and the US, and most Asian patients with MPS have not been enrolled in these registries. The registration of patients and subsequent epidemiological research should be independent of any pharmaceutical

company in order to objectively evaluate the efficacy and side effects of ERT drugs. The APMR, an electronic remote data system, was established by key doctors in the APMN.

The healthcare systems in individual Asian-Pacific countries differ greatly in terms of their structure and access to diagnostic tools and treatment. Furthermore, there are large variations in insurance coverage in different countries and even in regions within the same country. In countries where ERT is not currently available, symptomatic management remains the primary treatment option. There is also substantial national variation in patterns of MPS monitoring. The APMR has been structured to accommodate different clinical practices in different countries/regions because data related to procedures that are not routinely performed in a particular country are sometimes requested. The APMR also includes patients who have not received ERT. The APMR will include patients with all types of MPS and it will act as a hub in global clinical trials of any new drugs for patients with MPS. The registration of patients with Hunter syndrome began in July 2014, and the APMR will be expanded into a global MPS registry (GMR) as the number of nations participating in the APMN increases.

10. The Korean Mucopolysaccharidosis Expert Council (KMEC)

Clinical and genetic characteristics and medical conditions differ in each country. A standard guideline for Korean patients with MPS was required for the appropriate evaluation of treatments and to guarantee the safety of patients. In addition, evidence of the need for treatment needed to be compiled. In light of these requirements, the KMEC was established by several MPS experts in South Korea in January 2013. The Korean guideline for Hunter syndrome was published in May 2014. Individual guidelines for other subtypes of MPS will be published regularly, and the recommended follow-up schedule for assessment of MPS is shown in Table 1.

11. The Korean MPS Symposium

An annual Korean MPS Symposium is held in South Korea with the support of the Korean Society of Inherited Metabolic Disease. The first Korean MPS Symposium was held in May 2002, while the most recent, the 13th Korean MPS Symposium, was held in May 2014. Initially, the symposium was more of a domestic conference. However, the scale of the symposium has expanded, and speakers and attendees now come from around the world. The attendees of the 13th Korean MPS Symposium not only came from Asia-Pacific countries but also from North and South America, Europe, and Africa. The symposium consisted of two sessions – a scientific session and a session for patients and families. In the academic session, many global experts on MPS presented clinical studies and up-to-date results from laboratory research. The 13th symposium, as an example, dealt with clinical issues, including early diagnosis, newborn screening, the long-term outcomes of ERT and hematopoietic stem cell transplantation, and brand new approaches being explored to address "hard-to-treat" organs such as the brain and bones. At the symposium, attendees were able to engage in detailed discussions and they had the opportunity to share their experiences with global experts. The family session provided time for family support in various areas and for Korean families of patients with MPS to become acquainted. This assembly of patients and family groups empowers offers support and advocacy.

In conclusion, the Korean MPS population has been discussed here both from the patient's perspective and also in terms of medical care and research. An organization like APMN is necessary because international cooperation and collaboration are needed to elucidate the mechanisms of rare diseases and to conduct clinical trials on those diseases. The collaboration between Japan, South Korea, and the other Asian countries will be of the utmost importance in the future.

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Commentary

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Noninvasive prenatal testing in China: Future detection of rare genetic diseases?

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Summary **Noninvasive prenatal testing (NIPT) provides an innovative method to detect genetic conditions in fetuses using a maternal blood sample, thus avoiding the risk of miscarriage associated with traditional invasive procedures. Since 80% of rare diseases are genetic diseases, NIPT has the potential to detect rare genetic diseases early on and it has been used in many countries and regions. Since China has the world's largest population of patients with rare diseases, NIPT has been implemented in China since 2010. However, the regulations governing NIPT in China are weak and NIPT oversight and research are still lacking. Strict registration is needed to ensure the quality of NIPT, additional certification can help a developer/manufacturer of an NIPT test to compile clinical data and to improve innovation, and academic societies can provide committee opinions that are suited to the current situation in China. These efforts may improve regulations governing NIPT and NIPT oversight and research in China. With these improvements, NIPT may offer promise in terms of the early detection of rare diseases.**

Keywords: Premarket notification, Laboratory Developed Tests (LDTs), Clinical Laboratory Improvement Amendments (CLIA), Committee opinion

On February 12, 2014, Ariosa Diagnostics, Inc. obtained licensure approval from New York for Harmony™ noninvasive prenatal testing (NIPT) (*1*). This testing uses fetal genetic material obtained from a maternal blood sample to carry out genetic sequencing in order to detect certain genetic conditions during pregnancy based on the discovery of free fetal DNA in maternal plasma (*2*). NIPT offers advantages compared to traditional invasive procedures. NIPT can be performed with a maternal blood sample, which means that it is not associated with a risk of miscarriage. This aspect also means that NIPT is better suited to screening (*3*). Therefore, NIPT is likely to serve as a tool to screen for genetic defects in fetuses without risking a miscarriage.

Since 80% of rare diseases are genetic diseases (*4*), any method that can help to detect genetic conditions can be seen as a possible way to respond to rare diseases. Earlier diagnosis and treatment of diseases like phenylketonuria can mean a better prognosis (*5-7*). Therefore, prenatal testing is more likely to help improve the treatment of rare diseases than newborn testing, and this is especially true for rare diseases (*5,6,8*). As mentioned earlier, NIPT avoids the risk of miscarriage associated with traditional invasive procedures, and this aspect means that NIPT is more likely to detect rare genetic diseases early on. Currently, NIPT is focused on detecting genetic diseases such as Trisomy 21, 18, and 13, Turner syndrome, 22q11.2 deletion syndrome, and 1p36 deletion syndrome, all of which are rare genetic diseases.

China is a country with the world's largest population of patients with rare diseases (*7*), and China faces huge challenges in responding to rare diseases. Faced with these challenges, China has adopted several strategies to improve the healthcare of patients with rare diseases, including reimbursement of medical expenses for children with congenital heart disease and leukemia (*9*) and a new born screening program (*5,10*). As NIPT has matured, this technology has been marketed

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(*11,12*) (Table 1). NIPT has been implemented in China since May 2010, and most NIPT technology is from US companies (*22*). In China, NIPT can test for Trisomy 21, 18, and 13 just as NIPT in Europe can, but NIPT in the US can test for 4 more conditions (Turner syndrome, Triploidy detection, 22q 11.2 deletion syndrome, and 1p36 deletion syndrome). China has the largest number of tested samples, approximately twice the number in the US and 33 times that in Europe, which shows that China has a large market for NIPT.

However, regulations governing NIPT in China are still weak and NIPT management is still lacking. Since the first NIPT was carried out in China in 2010, approximately 210,000 samples have been tested without oversight because of the lack of regulations. Under these circumstances, the China Food and Drug Administration (CFDA) and National Health and Family Planning Commission of the People's Republic of China (NHFPC) jointly issued a "Notice on the enhanced clinical use of products related to genetic sequencing and increased oversight of genetic sequencing" on February 9, 2014 (*23*). This move indicates that the Chinese government has begun overseeing the market for NIPT-related products. On March 3, 2014, the NHFPC issued a "Notice regarding approval procedures for a pilot project involving clinical use of high-throughput genetic sequencing" (*24*) to improve the system for registration of genetic sequencing in China. These notices are mainly focused on criteria and authority for registration. Regulations on how NIPT should be performed and maintaining its quality are still lacking.

The US government has experience overseeing NIPT that may be helpful: *i*) Strict registration is needed to ensure the quality of NIPT. In the US, a developer/manufacturer of an NIPT test must first obtain a 510(k) premarket notification (510k) from the US Food and Drug Administration (FDA) (*25*). The 510k requires an *in vitro* diagnostic test yield a certain number of positive results from among tested samples. Using Trisomy 21 as an example, Trisomy 21 has an incidence of 1/1000. Given a target of 100 positive results, at least 100,000 samples would have to be tested to meet the requirements of a 510k. This requirement ensures the quality of any NIPT test approved for a 510k. *ii*) Additional certification can help a test developer/manufacturer to compile clinical data and to improve innovation. Since obtaining the number of positive results required by a 510k is difficult, only one NIPT device has been approved by the FDA. Applying for Laboratory Developed Tests (LDTs) certification and Clinical Laboratory Improvement Amendments (CLIA) certification from the Centers for Medicare & Medicaid Services (CMS) (*26*) provide another way for a developer/manufacturer to certify an NIPT test without clinical data. LDTs and CLIA certification are focused more on the developer/manufacturer's knowledge, training and experience, reagents and materials

Table 1. Current state of NIPT in the United States, Europe, and China

Region	Company	Service	Diseases tested for	Date started	Number of samples tested	*Ref.*
United States	Sequenom	MaterniT21™ PLUS	Trisomy 21/ Trisomy 18/ Trisomy 13/ Turner syndrome/ 22q 11.2 deletion syndrome/ 1p36 deletion syndrome	October 2011	100,000 (as of April 16, 2013)	(*13*)
	Natera	Panorama™	Trisomy 21/ Trisomy 18/ Trisomy 13/ Turner syndrome/ Triploidy detection / 22q 11.2 deletion syndrome/ 1p36 deletion syndrome	Unknown	Unknown	(*14*)
	Verifi	Verifi®	Trisomy 21/ Trisomy 18/ Trisomy 13/ Turner syndrome	March 2012	Unknown	(*15,16*)
	Ariosa	Harmony Prenatal Test™	Trisomy 21/ Trisomy 18/ Trisomy 13/ Turner syndrome	May 2012	40,000 (as of November 13, 2013)	(*17*)
Europe	LifeCodexx	PrenaTest®	Trisomy 21/ Trisomy 18/ Trisomy 13	August 2012	Nearly 6,000 (as of August 22, 2013)	(*18,19*)
China	BGI	NIFTY	Trisomy 21/ Trisomy 18/ Trisomy 13	May 2010	109,582 (as of April 2, 2013)	(*12,20*)
	Berry genomics	BambniTest	Trisomy 21/ Trisomy 18/ Trisomy 13	April 2011	Over 100,000	(*11,21*)

NIPT: noninvasive prenatal testing

preparation, characteristics of operational steps, calibration, quality control, and proficiency testing materials, test system troubleshooting and equipment maintenance, and interpretation and judgment (*26*). This certification can help a developer/manufacturer that does not have enough tested samples to market a NIPT test, and most manufacturers of NIPT tests in the US currently have their NIPT products certified based on LDTs and CLIA. After the developer/manufacturer obtains CLIA certification, the Centers for Medicare & Medicaid Services (CMS) will conduct subsequent inspections on a biennial basis or with such frequency as necessary to ensure compliance, thus requiring the developer/manufacturer to maintain the quality of its test (*27*). And study has also indicated that LDTs and CLIA certification can improve innovation (*28*).

Research into NIPT in China is still lacking. With a new technology like NIPT, recommendations or official opinions should be provided by academic societies to give guidance on how NIPT should be used and where the cutting edge of research lies. As of this writing, however, there are no such recommendations or opinions in China been reviewed. Several opinions on NIPT have been issued by academic committees in places like the U.S., Europe, Canada, and Japan from December 2012 to May 2013 (*29,30*). These opinions include statements on the clinical use of NIPT, the limitations of current NIPT, who is eligible to undergo NIPT, and guidelines for medical staff. In order to enhance research into NIPT in China, academic societies of geneticists, obstetricians, gynecologists and specialists in maternal-fetal medicine should provide committee opinions that are suited to the current situation in China.

NIPT has potential in terms of detecting rare genetic diseases and it has been used in many countries and regions. China is a country with the world's largest population of patients with rare diseases patients, and China has implemented NIPT since 2010. However, NIPT oversight and research in China are still lacking. With the improvement of these aspects, NIPT may offer promise in terms of the early detection of rare diseases.

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Commentary

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Current research on pycnodysostosis

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Summary **Pycnodysostosis is a rare autosomal recessive disorder caused by an inactivating mutation in *cathepsin K* (*CTSK*) and characterized by dysmorphic facial features, a short stature, acroosteolysis, osteosclerosis with increased bone fragility, and delayed closure of cranial sutures. Patients usually present with short stature or dysmorphic features the Pediatric Endocrinology or Genetics clinics, with atypical fractures to the orthopedics clinics or hematological abnormalities to the hematology clinics. However, under-diagnosis or misdiagnosis of this condition is a major issue. Pycnodysostosis is not a life threatening condition, but craniosynostosis, frequent fractures, respiratory-sleep problems, and dental problems may cause significant morbidity. Although no specific treatment for this disorder has been described, patients should be followed for complications and treated accordingly. A specific treatment for the disorder must be established in the future to prevent complications and improve quality of life for patients in the current era of advanced molecular research.**

Keywords: Pycnodysostosis, *cathepsin K*, osteopetrosis

Pycnodysostosis is a rare autosomal recessive disorder with an estimated prevalence of 1 to 1.7 per million. The disorder is caused by a homozygous or compound heterozygous mutation in *cathepsin K* (*CTSK*), which is a lysosomal cysteine protease that is highly expressed in osteoclasts. *CTSK* is involved in the degradation of bone matrix proteins, type I and type II collagen, osteopontin, and osteonectin at a low pH (*1-7*). To date, forty-five different *CTSK* mutations have been reported, including nonsense, missense, frameshift, and splice site mutations as well as small deletions, small and big insertions (Alu sequence) (Figure 1).

The condition is also known as Maroteaux-Lamy syndrome and is characterized by a short stature, acroosteolysis of the distal phalanges, dysplasia of the clavicle, osteosclerosis with increased bone fragility, and delayed closure of sutures (*1-5*). French artist Henri de Toulouse Lautrec (1864-1901) was suggested to have this condition since he exhibited several phenotypic features of the disorder such as a short stature, parental consanguinity, facial dysmorphism, frequent bone fractures, and large fontanels (*8*). In 1994, Julia Frey claimed that Toulouse Lautrec might have had a disorder other than pycnodysostosis (*9-11*), though Maroteaux rebutted that assertion and Frey in turn defended it (*10-12*). In fact, the artist had facial features quite typical to the disorder, and confusion could be due to the evaluation of the artist's features at different ages by the two authors. In affected patients, the facial features become more prominent with age, which is most probably due to progressive acroosteolysis of the facial bones (based on the current authors' experience).

Patients usually present with short stature or dysmorphic features to the Pediatric Endocrinology

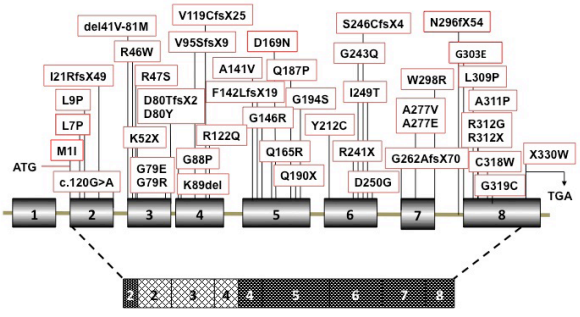

Figure 1. A diagram of the CTSK gene. The genomic structure of *CTSK* with 8 exons and a total of 45 reported mutations are shown.

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Table 1. Typical clinical features of pycnodysostosis

Skeleton
• Short stature
• Increase in bone density
• Fractures
• Stubby hands and feet with osteolysis of the distal phalanges
• Dysplastic nails
• Clavicular dysplasia, congenital pseudarthrosis of the clavicle
• Spondylolysis
Head and Neck
Face:
• Frontal and parietal bossing
• Beaked nose
• Prominent eyes with bluish sclerae
• Hypoplasia of the maxilla and mandible
Cranium:
• Open fontanels and sutures
• Craniosynostosis
• Non-pneumatized paranasal sinuses
• Arnold-Chiari malformation
Mouth and Teeth:
• Delayed eruption of permanent teeth
• Persistence of deciduous teeth, dental crowding
• Malocclusion
• Obtuse mandibular angle
• Grooved palate

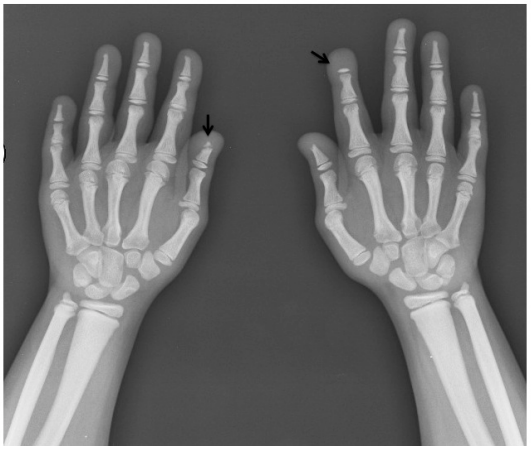

Figure 2. A typical finding of pycnodysostosis. Osteosclerosis and acroosteolysis of distal phalanges on X-rays (arrows).

or Genetics clinics and, with atypical fractures to the orthopedics clinics. A summary of the clinical features of the disorder is shown in Table 1. Pycnodysostosis is a specific form of osteopetrosis and affected patients have osteosclerosis related to decreased bone resoption. Atypical facial features are usually suggestive of the disorder, but the presence of osteosclerosis and acroosteolysis of distal phalanges provides more of a definitive diagnosis (Figure 2). In a cohort of 16 patients with clinical manifestations suggesting pycnodysostosis, molecular genetic testing resulted positive for CTSK mutation in all (*13*). At this point, recognizing the disorder's clinical manifestations is important, but recognizing its dysmorphic features is sometimes difficult. Misdiagnosis can occur, especially in the absence of acroosteolysis. Absence of acroosteolysis can be misleading, and in such instances osteopetrosis is often the diagnosis (erroneously) made. This was true in several patients studied by Panrazio *et al*. (*14*), who performed exome sequencing for patients with a pedigree suggesting autosomal recessive osteopetrosis and classical features of mild to moderate osteopetrosis, like blindness, anemia or bicytopenia, and splenomegaly. Panrazio *et al*. detected *CTSK* mutations in these patients they studied. Unlike patients with osteopetrosis, patients with pycnodysostosis rarely have hematological abnormalities, and the phenotypic features of pycnodysostosis allow the disorder to be distinguished from osteopetrosis. However, facial dysmorphism may not be readily evident, and this is especially true at young ages and in different ethnic groups. However, the presence of acroosteolysis together with osteosclerosis is a highly indicative feature (*5,13*). If a patient is misdiagnosed as having osteopetrosis, this could lead to additional misdecisions such as performing a bone marrow transplant to treat cytopenia and optic atrophy (*14*). However the optic atrophy seen in pycnodysostosis is usually a consequence of craniosynostosis, so its primary treatment is neurosurgery and not a bone marrow transplant (*13,15,16*).

Pycnodysostosis is not a life threatening condition, but frequent fractures, craniosynostosis, respiratory-sleep problems, and dental problems and their treatments may cause significant suffer to the patients (*5,13-18*). In addition, the severity of the disorder in terms of height, frequency of fractures, or additional anomalies like craniosynostosis and an Arnold-Chiari malformation can vary from patient to patient even if they have the same mutation (*5,13-16*). However, patients with a more severe genotype appear to suffer fractures at a younger age (*13*), and the youngest patient with such fractures in the literature is a 10-month-old who had two siblings that died from the disorder, suggesting that the family had a more severe phenotype and/or genotype (*15*).

Although no specific treatment for the disorder has been described, patients should be followed for complications and treated accordingly by Neurosurgery, Orthopedics and Orthodontics, Respiratory Medicine, Sleep Medicine, and Rehabilitation (*5,13-18*). Maintenance of oral hygiene and regular dental care are key to preventing oral complications. Postextraction osteomyelitis can appear due to increased bone density, and risk factors should be carefully addressed while planning tooth extraction and other surgeries (*5*).

Furthermore, short stature is a significant complaint and documented final heights of patients are below 150 cm for boys and 130-134 cm for girls. Recently, growth hormone therapy has resulted in a significant improvement in height velocity and final height in pycnodysostosis (*19,20*). Almost half of affected patients have a growth hormone deficiency but all

have low IGF-1 levels, and administration of growth hormone results in a satisfactory elevation in IGF-1 (*21,22*). Patients with a growth hormone deficiency also have pituitary hypoplasia, but no abnormalities in other pituitary hormones and pubertal development have been detected (*21*).

Pycnodysostosis is a rare clinically distinct entity with a number of different clinical signs and is usually under-diagnosed. Geneticists as well as orthopedists, hematologists, endocrinologists, and even neurosurgeons should aware of this condition. In addition, a specific treatment for the disorder must be established in the future to prevent complications and improve quality of life for patients in the current era of advanced molecular research.

Acknowledgements

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Commentary

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The Ice Bucket Challenge: The public sector should get ready to promptly promote the sustained development of a system of medical care for and research into rare diseases

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Summary **In order to promote public awareness and raise charitable donations for patients with amyotrophic lateral sclerosis (ALS), a charity activity known as the "Ice Bucket Challenge" went "viral" from social media in the US to the rest of the world in the summer of 2014. The Challenge had an obvious impact with a large number of participants and increasing charity donations. However, the effort has also garnered criticism for wasting water, possible safety concerns, and its status as a publicity stunt or grandstanding. A system of medical care for and research into rare diseases has been established in some countries in order to protect the rights and interests of patients with rare diseases, but such systems have yet to be established in other countries. An activity like the "Ice Bucket Challenge" is clearly not enough to improve the plight of patients with ALS or other rare diseases. However, the public awareness attracted by this challenge may provide the impetus for those countries that lack a system of medical care for and research into rare diseases to establish such a system. The public sector should bear the responsibility for taking on the important task of promoting the sustained development of a system of medical care for and research into rare diseases.**

Keywords: Amyotrophic lateral sclerosis, rare diseases, orphan drugs, health insurance system

What is the hottest event on the Internet this summer? The answer would be the "Ice Bucket Challenge." Over the past two months, social networking platforms have been saturated with videos of people dumping buckets of ice water on their heads in order to promote public awareness and raising charitable donations for patients with amyotrophic lateral sclerosis (ALS). After it first started in the US, the challenge has gone "viral" through social media to become the most successful and influential fund-raising event, and the challenge has even spread as far as countries like China, South Korea, Japan, Germany, and France.

The simple rule of the challenge is to "either donate $100 to a given cause or douse yourself with ice water, film it, and pass the challenge on to 3 people *via* social media." Participants like celebrities, political figures, and business leaders have experienced the "freezing" water, and the challenge is still spreading. Actually, this challenge had nothing to do with ALS originally, it came from a dare that was circulating among a group of professional athletes with the rule of whoever refused to take an ice bath had to give $100 to a charity of the challenger's choosing. Currently, however, the challenge has provided a windfall of charitable donations for ALS patients.

Why would a public charity activity catch the attention of people worldwide? The activity features low barriers to entry, a strong sense of participation, and self-aggrandizement as more and more people post videos of themselves on social networking platforms taking the "Ice Bucket Challenge". The activity has also been helped by celebrity appeal. Furthermore, being drenched with ice is an alternative to contributing actual money, so the lack of a required donation encourages the vast spread of the challenge, expressing shared values and communicating personal information about the participants. The challenge has had a profound impact.

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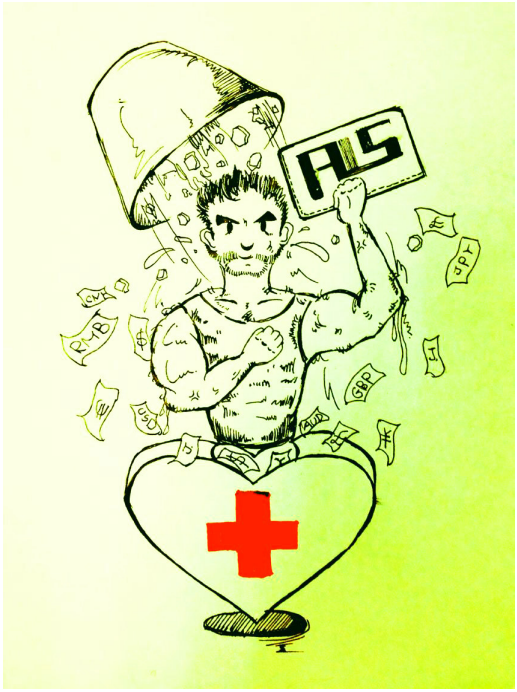
As of August 25, the ALS Association in the US had received $79.7 million in donations compared to $2.5 million during the same time period last year (July 29 to August 25) (*1*).

However, the "Ice Bucket Challenge" has received mixed reviews as it has spread rapidly around the world. Though there are many people who support the challenge, others criticize it for wasting water, possible safety concerns, and its status as a publicity stunt or grandstanding. The challenge in China is a good example. The challenge resulted in charitable donations of RMB 6.94 million yuan for ALS patients, 4.01 billion pageviews, and 3.72 million discussions prior to August 25 (*2,3*). However, few of the videos appear to contain any substantive information about ALS, such as why the money is needed or how it will be used. So to what extent could this activity help patients with ALS? More importantly, when the "Ice Bucket Challenge" fever eventually dies down, who will fill the "vacancy" and maintain attention on and support patients with ALS or other rare diseases? These are thought-provoking questions.

The ALS Association has stated that raising public awareness and improving research into ALS are much more important than donations in the long term (*4*). Researchers of rare disease have indicated that countries that that lack a system of medical care for and research into rare diseases need to promptly institute the following changes: *i*) a specific definition and classification of rare diseases; *ii*) specific legislation to encourage discovery and development of orphan drugs; *iii*) government-funded special biomedical research programs to enhance basic and applied research on rare diseases; and *iv*) patients' advocacy organizations and disease registry networks to provide vast information on rare diseases (*5-7*).

Although an amusing charity activity may be enjoyable, rare diseases including ALS pose a serious challenge in China. To date, there is still no official definition of rare diseases is in China and little official information about rare diseases is available. According to the World Health Organization (WHO), rare diseases are rare and often debilitating or even life-threatening diseases or conditions with a prevalence of 0.65-1‰ (*7*). Based on this standard, there are about 5,000-6,000 rare diseases in China, and the number of Chinese patients with ALS in particular may be as high as 20,000. Over the past decades, many nonprofit patients' advocacy organizations have thoroughly publicized rare diseases, but most Chinese have paid little attention to rare diseases. China has not been home to a noticeable public event like the Ice Bucket Challenge for ALS patients. The lack of a specific definition of rare diseases, the lack of legislation concerning rare diseases, and the lack of a system of medical care for and research into rare diseases have hampered protection of the rights and interests of patients suffering from rare diseases.



(Illustration by Jing Fan)

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. Worldwide, the orphan drug legislation and supporting policy measures have been formulated and implemented in some countries and regions, such as the US, Europe, Japan, and Australia. The incentives of financial subsidies, market exclusivity, tax credits, fee waivers, fast track approval, and protocol assistance have resulted in substantial improvements in the treatment of patients with a range of rare diseases (*6,7*). China, however, lacks systematic economic and regulatory incentives to encourage the development of drugs for rare diseases, so few orphan drugs have been discovered by pharmaceutical companies. Orphan drugs are highly expensive and most are not covered by the health insurance system. Moreover, strict rules on importing orphan drugs and the relative delay of research and development of orphan drugs have resulted in patients with rare diseases becoming a neglected vulnerable group in China.

Over the past few years, calls to draft legislation on rare diseases and protect the rights and interests of patients with rare diseases have increased in China. Who should be responsible for taking on this important task? Based on the experience of those advanced countries with a system of medical care for and research into rare diseases, the public sector should get ready to promptly promote the sustained development of a system of medical care for and research into rare diseases. The public sector should formulate a specific definition of rare diseases, draft specific legislation on orphan drugs,

and improve the health insurance system. The public sector should also provide sufficient support in terms of basic medical assistance, education, employment, and facility improvements to help and care for patients with rare diseases.

In conclusion, an activity like the "Ice Bucket Challenge" is clearly not enough to improve the plight of patients with ALS or other rare diseases. However, the public awareness attracted by this challenge may provide the impetus for those countries that lack a system of medical care for and research into rare diseases to establish such a system. The public sector should bear the responsibility for taking on the important task of promoting the sustained development of a system of medical care for and research into rare diseases.

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Commentary

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Amniotic Fluid Embolism (AFE) in China: Are maternal mortality and morbidity preventable?

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Summary **A case of hospital-patient conflict has occurred in China that has lifted billows in the public and highlighted the lethality of amniotic fluid embolism (AFE). AFE is a rare but severe obstetric complication with high maternal mortality and morbidity. Globally, the incidence of AFE is estimated to be approximately 2 to 6 per 100,000 deliveries. The maternal mortality rate (MMR) attributable to AFE ranges between 0.5 to 1.7 deaths per 100,000 deliveries in the developed world and 1.9 to 5.9 deaths per 100,000 deliveries in the developing world. In developed countries, AFE often accounts for a leading cause of maternal mortality; whereas the proportion of maternal death caused by AFE tends to be not as dominant compared to common perinatal complications in developing countries. With the mechanism remaining to be elucidated, AFE can neither be predicted nor prevented even in developed countries. Treatment requires a set of highly intensive advanced emergency obstetric care, challenging obstetric care in developing countries. Although this complication is currently far from preventable, China has potential to improve the prognosis of AFE by strengthening the emergency obstetric care system.**

Keywords: Amniotic Fluid Embolism (AFE), maternal mortality, obstetric complication, China

In August of this year in China, a case of hospital-patient conflict occurred in Xiangtan County Maternal and Child Hospital which highlighted lethality of amniotic fluid embolism (AFE), a rare but severe obstetric complication, characterized by sudden cardiovascular collapse, altered mental status, and disseminated intravascular coagulation (*1*). When an expectant mother died from AFE during her delivery, her family was enraged and the mass media sensationally directed its spearhead against the hospital, underlying which is the unprecedentedly intensified contradiction between hospital and patients in China. For most non-professional people, it was probably the first time to hear the new word "amniotic fluid embolism". They never knew that high maternal mortality and morbidity from such a fatal condition is far from preventable. Even for a large number of medical professionals, the relevantly rare incidence of less than 10 per 100,000 deliveries makes AFE only appear as a term in their textbooks but not a real experience in their clinical practices. Such a fatal case has lifted awareness in the public.

Globally, the incidence of AFE is estimated to be approximately 2 to 6 per 100,000 deliveries (*1-4*). The maternal mortality rate (MMR) attributable to AFE ranges between 0.5 to 1.7 deaths per 100,000 deliveries in the developed world and 1.9 to 5.9 deaths per 100,000 deliveries in the developing world. Based on the reported data, risk factors associated with an increased risk of AFE include advanced maternal age (older than 35 years), placental abnormalities, cesarean/instrumental vaginal delivery, placenta previa, eclampsia, polyhydramnios, cervical lacerations and uterine rupture (*1-7*). The estimation is based on large-scale epidemiological data in North American and European developed countries and the figure varies according to different studies. In most developed countries, AFE often accounts for a leading cause of

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maternal mortality: for example, in Japan, where MMR is the lowest in the world, it is the highest cause of maternal death and accounts for as much as 24.3% of all fatal cases (*8*). In contrast, the pattern of causes of maternal mortality varies by regions in the world and the proportion of maternal death caused by AFE tends to be not dominant compared to common perinatal complications such as hemorrhage, hypertensive disorders and sepsis in most developing countries (*9,10*). The priority of reduction of MMR to achieve the Millennium Development Goals in the developing world remains in measures tackling common perinatal complications contributing to maternal mortality and morbidity, which are preventable and avoidable by effective facility-based and population-based interventions with good cost-effectiveness.

Compared to common perinatal complications worldwide, AFE can neither be predicted nor prevented as cases occur sporadically with a broad spectrum of clinical manifestations that vary widely (*11*). The pathological mechanism of onset of AFE remains unclear. Globally, without reliance on laboratory markers, the current diagnosis is based on one or more of four key typical symptoms: cardiovascular collapse, respiratory distress, coagulopathy, and/or coma/seizures. The accurate laboratory test is only operated at forensic autopsy (after death of the mother) to detect fetal materials in the maternal pulmonary circulation. The definition of AFE isn't clarified. Current ongoing clinical research on the pathological mechanism has shifted from embolism toward a maternal immune response to the fetus, amniotic fluid-dependent anaphylactic reaction and complement activation, with the hypothesis raising from pregnant women's immune tolerance on the presence of foreign antigen within both their uterus and their circulation; whereas no related theory based on robust evidence has been widely accepted nor have amniotic fluid-specific markers been developed, so far (*12,13*). With the mechanism remaining to be elucidated, it is difficult to identify effective practices and actions. No study has proved the effectiveness of typical interventions such as an antenatal care package and high-risk pregnancy management on reduction of mortality and morbidity caused by AFE, though they are effective to reduce MMR. Therefore, even in developed countries, AFE remains a difficult clinical problem and a higher level of evidence rather than case reports or case series is necessary. An inclusive hospital record database worldwide with uniform diagnostic criteria should be created for addressing numerous unanswered questions (*1*).

Based on suggestions of case series, survival of AFE cases crucially relies on early identification and quick clinical operation response (*14*). The management of AFE is supportive and directed towards maintenance of oxygenation, cardiac output and blood pressure, and correction of the coagulopathy, and the initial goal of the treatment is the rapid correction of maternal hemodynamic instability (*1*). For a good prognosis outcome, treatment needs to ideally take place in an intensive care unit (ICU) by a multidisciplinary team, and the necessary rescue healthcare includes a series of technique-intensive therapeutic measures, such as cardiopulmonary resuscitation, uterine evacuation, continuous cardiac telemetry/respiratory/blood pressure monitoring, pulmonary artery catheter, transesophageal echocardiography, administration of oxygen, fluid therapy, recombinant activated factor VIIa, transfusion, *etc* (Table 1). Such highly intensive advanced emergency obstetric care remains a tremendous challenge in developing countries and the situation can be explained

Table 1. Major treatment of AFE in ICU

Measures of treatment	Purposes
Cardiac telemetry monitoring, Respiratory monitoring, Blood pressure monitoring, Pulmonary artery catheter Transesophageal echocardiography	*Evaluation of cardiac and respiratory function*
Cardiopulmonary resuscitation Uterine evacuation when resuscitation failed Cardiopulmonary bypass	*Maintenance of cardiac and respiratory function*
Oxygen administration Optimization of preload Fluid therapy Vasopressors	*Correction of hemodynamic instability*
Transfusion of blood products Recombinant activated factor VIIa Intravenous oxytocin Serine proteinase inhibitor FOY Hysterectomy Heparin therapy	*Correction of coagulopathy and disseminate intravascular coagulation (DIC)*

by a much higher MMR due to AFE there compared to the figure in developed countries.

Although this complication is currently far from preventable, China has the potential to improve the prognosis of AFE by strengthening the emergency obstetric care system. As China has been characterized by a huge geographical diversity in health resources and health outcomes, AFE occurring in diversified regions probably has different treatment outcomes and opportunities to survive. With advancement of maternal healthcare, maternal mortality caused by AFE has largely declined (*15-17*). Most successful survival cases of AFE have been reported in top-level tertiary comprehensive hospitals of large capital cities, which represent the highest level of medical technology and treatment outcomes of the country. For the latest reported case successfully rescued in Shanghai, it's reported that more than 10,000 cc packed blood products were assembled from all blood centers of the metropolitan city with a population of over twenty million. The success of the resuscitation definitely was attributed to a well-functioning emergency obstetric care system and social infrastructure, whereas the fatal case which occurred in Xiangtan County Maternal and Child Hospital seemed not be favored with these resources. Moreover, as the onset of AFE cannot be avoided and the prognosis depends on health professional's quick response to the emergency, a training program and simulation experience needs to be conducted for improving the knowledge, attitude and skill of obstetric health professionals.

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