ISSN 2186-3644 Online ISSN 2186-361X

IRDR

Intractable & Rare Diseases Research

Volume 4, Number 3
August, 2015

IRCA BSSA

IACMHR

www.irdrjournal.com

IRDR
Intractable & Rare Diseases Research

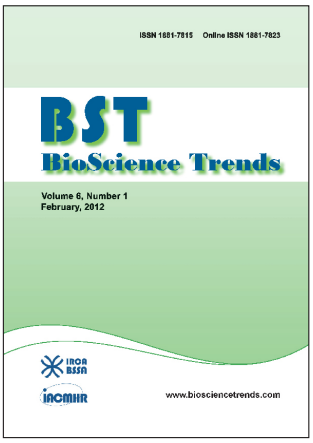
ISSN: 2186-3644
Online ISSN: 2186-361X
CODEN: IRDRA3
Issues/Year: 4
Language: English
Publisher: IACMHR Co., Ltd.

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

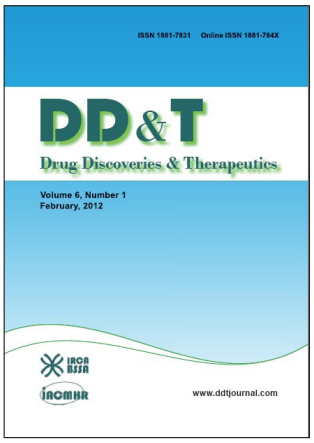
Intractable & Rare Diseases Research devotes to publishing the latest and most significant research in intractable and rare diseases. Articles cover all aspects of intractable and rare diseases research such as molecular biology, genetics, clinical diagnosis, prevention and treatment, epidemiology, health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

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

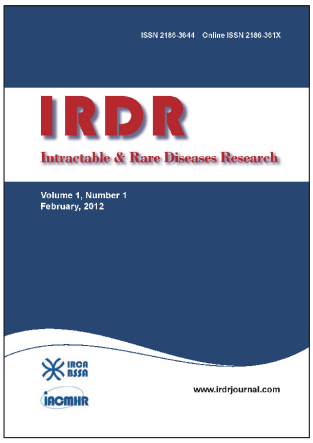
IRCA-BSSA Group Journals



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



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



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

Intractable & Rare Diseases Research

Editorial and Head Office

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

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

Editorial Board

Editor-in-Chief:

Masatoshi MAKUUCHI
Japanese Red Cross Medical Center, Tokyo, Japan

Chief Director & Executive Editor:

Wei TANG
The University of Tokyo, Tokyo, Japan

Co-Editors-in-Chief:

Jinxiang HAN
Shandong Academy of Medical Sciences, Jinan, China

Jose-Alain SAHEL
Pierre and Marie Curie University, Paris, France

Editorial Board Members

Tetsuya ASAKAWA
(*Hamamatsu, Japan*)
Karen BRØNDUM-NIELSEN
(*Glostrup, Denmark*)
Yazhou CUI
(*Jinan, China*)
John DART
(*Crowthorne, UK*)
Masahito EBINA
(*Sendai, Japan*)
Clodoveo FERRI
(*Modena, Italy*)
Toshiyuki FUKAO
(*Gifu, Japan*)
Ruoyan GAI
(*Jinan, China*)
Shiwei GONG
(*Wuhan, China*)
Jeff GUO
(*Cincinnati, OH, USA*)
Toshiro HARA
(*Fukuoka, Japan*)
Reiko HORIKAWA
(*Tokyo, Japan*)
Takahiko HORIUCHI
(*Fukuoka, Japan*)
Yoshinori INAGAKI
(*Tokyo, Japan*)
Masaru IWASAKI
(*Yamanashi, Japan*)
Baoan JI
(*Houston, TX, USA*)
Xunming JI
(*Beijing, China*)
Guosheng JIANG
(*Jinan, China*)
Si JIN
(*Wuhan, China*)
Yasuhiro KANATANI
(*Saitama, Japan*)
Mureo KASAHARA
(*Tokyo, Japan*)
Jun-ichi KIRA
(*Fukuoka, Japan*)
Toshiro KONISHI
(*Tokyo, Japan*)
Masato KUSUNOKI
(*Mie, Japan*)
Shixiu LIAO
(*Zhengzhou, China*)
Zhibin LIN
(*Beijing, China*)
Reymundo LOZANO
(*NewYork, NY, USA*)
Kuansheng MA
(*Chongqing, China*)
Katia MARAZOVA
(*Paris, France*)
Chikao MORIMOTO
(*Tokyo, Japan*)
Noboru MOTOMURA
(*Tokyo, Japan*)
Masanori NAKAGAWA
(*Kyoto, Japan*)
Jun NAKAJIMA
(*Tokyo, Japan*)
Takashi NAKAJIMA
(*Kashiwazaki, Japan*)
Ming QIU
(*Shanghai, China*)
Phillips ROBBINS
(*Boston, MA, USA*)
Hironobu SASANO
(*Sendai, Japan*)
Shinichi SATO
(*Tokyo, Japan*)
Yasuyuki SETO
(*Tokyo, Japan*)
Qingfang SUN
(*Shanghai, China*)
Samia TEMTAMY
(*Cairo, Egypt*)
Yisha TONG
(*Heidelberg, Australia*)
Hisanori UMEHARA
(*Ishikawa, Japan*)
Chenglin WANG
(*Shenzhen, China*)
Haibo WANG
(*Hong Kong, China*)
Huijun WANG
(*Shanghai, China*)
Qinghe XING
(*Shanghai, China*)
Zhenggang XIONG
(*New Orleans, LA, USA*)
Toshiyuki YAMAMOTO
(*Tokyo, Japan*)
Huijun YUAN
(*Beijing, China*)
Wenhong ZHANG
(*Shanghai, China*)
Xianqin ZHANG
(*Wuhan, China*)
Yanjun ZHANG
(*Cincinnati, OH, USA*)
Yumin ZHANG
(*Bethesda, MD, USA*)
Yuesi ZHONG
(*Guangzhou, China*)
Jiayi ZHOU
(*Boston, MA, USA*)
Wenxia ZHOU
(*Beijing, China*)

Web Editor:

Yu CHEN
(*Tokyo, Japan*)

Proofreaders:

Curtis BENTLEY
(*Roswell, GA, USA*)
Thomas R. LEBON
(*Los Angeles, CA, USA*)

Office Staff:

Apolline SONG
(*Tokyo, Japan*)

Editorial and Head Office:

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

(*As of July 2015*)

CONTENTS

Volume 4, Number 3, 2015

Review

Original Articles

Case Reports

Commentary

CONTENTS (*Continued*)

Letter

Guide for Authors

Copyright

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

Review

DOI: 10.5582/irdr.2015.01014

Pseudoxanthoma elasticum and skin: Clinical manifestations, histopathology, pathomechanism, perspectives of treatment

Barbara Marconi[1,*], Ivan Bobyr[1,*], Anna Campanati [1,], Elisa Molinelli[1], Veronica Consales[1], Valerio Brisigotti[1], Marina Scarpelli[2], Stefano Racchini[2], Annamaria Offidani[1]**

[1] *Dermatological Unit, Department of Clinical and Molecular Sciences, Polytehnic Marche University, Ancona, Italty;*
[2] *Institute of Pathological Anatomy and Histopathology, Polytechnic University Marche, Ancona, Italty.*

Summary **Pseudoxantoma elasticum (PXE), also known as Groenblad-Strandberg syndrome, is a rare heritable disease with an estimated prevalence of 1:50,000 in the general population. PXE is considered a prototype of multisystem ectopic mineralization disorders and it is characterized by aberrant mineralization of soft connective tissue with degeneration of the elastic fibers, involving primarily the eyes, the cardiovascular system, and the skin. Cutaneous lesions consist of small, asymptomatic, yellowish papules or larger coalescent plaques, typically located on the neck and the flexural areas. PXE is caused by mutations in the *ABCC6* (ATP-binding cassette subfamily C member 6) gene that encodes a transmembrane ATP binding efflux transporter, normally expressed in the liver and the kidney; however, the exact mechanism of ectopic mineralization remains largely unknown. The histological examination of cutaneous lesions, revealing accumulation of pleomorphic elastic structures in middermis, is essential for the definitive diagnosis of PXE, excluding PXE-like conditions. PXE is currently an intractable disease; although the cutaneous findings primarily present a cosmetic problem, they signify the risk for development of ocular and cardiovascular complications associated with considerable morbidity and mortality. The purpose of this review is to present a comprehensive overview of this rare form of hereditary connective tissue disorders, focus on the pathogenesis, the clinical manifestation, and the differential diagnosis of PXE. Emphasis is also placed on the management of cutaneous lesions and treatment perspectives of PXE.**

Keywords: Pseudoxanthoma elasticum, skin, orphan disease

1. Introduction

Pseudoxanthoma elasticum (PXE), also known as Gröenblad-Strandberg syndrome, is an heritable multi-system disorder, characterized by aberrant mineralization of soft connective tissue resulting in fragmentation of elastic fibers, involving primarily the skin, eyes and cardiovascular system (*1*). Notably, PXE is caused by mutations in the *ABCC6* (ATP-binding cassette subfamily C member 6) gene, located on short-arm of human chromosome 16, encoding a transmembrane ATP binding driven anion transporter, normally expressed in the liver and the kidney. However, the pathophysiology, particularly the mechanism of ectopic mineralization remains largely unknown (*2,3*). PXE, as other genodermatoses (*4*), is currently an intractable disease, associated with considerable morbidity and occasional mortality due to cardiovascular complications (*5*). In this review, we discuss the clinical and histological features of PXE, focusing on cutaneous manifestations of the disease. In addition, we summarize the recent evidence concerning molecular genetics and pathomechanisms underlying PXE, and finally we present a comprehensive overview of treatment perspectives.

Released online in J-STAGE as advance publication July 6, 2015.

*These authors contributed equally to this works.
***Address correspondence to:*
Dr. Anna Campanati, Dermatological Unit, Department of Clinical and Molecular Sciences, Polytechnic Marche University, Via Conca 71, Ancona 60020, Italty.
E-mail: anna.campanati@gmail.com

2. Epidemiology, historical background and terminology

PXE is a rare disease, with an estimated prevalence of 1:50,000, which affects approximately 150,000 people in the world, assuming the same global prevalence (*1*). Females are more commonly affected than males (2:1); the clinical manifestations are rarely present at birth and usually become evident during the second or third decade of life (*6,7*).

The disease was previously described by the French dermatologist Rigal in 1881, whereas Ferdinand-Jean Darier coined the term pseudoxanthoma elasticum in 1896, delineating the connective disorder as a clinical entity, distinct from xanthomas (hence pseudoxanthoma) (*8,9*). Angioid streaks of the retina were initially described by Robert W. Doyne and by Otto Pflange in 1889 and 1892, respectively (*10,11*). In 1929 two Swedish physicians, ophthalmologist Ester Gröenblad and dermatologist James Strandberg, first defined the association between angioid streaks and pseudoxanthoma elasticum and coined the term Gröenblad-Strandberg syndrome, currently used synonymously with PXE (*12,13*).

3. Molecular genetics

PXE is considered as a paradigm of heritable connective tissue disorders, characterized by ectopic mineralization and consequently fragmentation of elastic fibers in the extracellular matrix (*14*). PXE is a multisystem orphan disease with autosomal recessive patterns of inheritance. Currently, no molecular evidence of autosomal dominant inheritance has been reported; the recurrence of PXE in successive generations could be explained through a pseudo-dominant pedigree pattern, due to familial consanguinity (*15*). In addition, a relatively small proportion of cases occurs sporadically (*16*).

PXE is characterized by considerable intra- and inter-familial heterogeneity, with respect to the age of onset, the entity of tissue mineralization, and the severity of clinical manifestations, suggesting a putative role of genetic and environmental modifying factors in PXE phenotypic expression (*17*). Specifically, genetic polymorphisms in the promoter of the *SPP1* gene (also known as osteopontin) may represent a genetic risk factor contributing to PXE susceptibility (*18*). Moreover, dietary factors, a high intake of dairy products rich in calcium and phosphate during childhood and adolescence or intake of aluminium hydroxide, a phosphate binder, may play a role in the pathologic mineralization process in PXE (*19,20*).

Notably, PXE is caused by mutations of the *ABCC6* gene transporter protein, also known as multidrug resistance-associated protein 6 (MRP6), a member of adenosine triphosphate-binding cassette proteins, predominantly expressed in the liver and in minor amounts in the proximal tubules of kidneys and intestine (*12*). To date, over 300 mutations in the *ABCC6* gene, including missense and nonsense mutations, intronic mutations, small deletions and insertions, have been described in PXE patients (*12,13-21*). The most recurrent loss-of-function mutations are p.R1141X and g.del23-29, which account for up to approximately 45% of all pathogenic PXE mutations (*21*).

Although the substrate specificity of ABCC6 is currently unknown; recent evidence suggests that it functions as a transmembrane transporter of polyanionic glutathione-conjugated molecules (*22*).

4. Pathomechanism

The pathophysiology of elastic fiber mineralization, including the exact correlation with the defective ABCC6 transporter, is still unclear (*23*). A systematic experimental study confirmed that targeted ablation of the mouse *ABCC6* gene results in progressive mineralization of connective tissue, representing a cardinal feature of PXE phenotype. In addition, it has been demonstrated that mineral deposits in mice models consist of calcium and phosphate forming hydroxyapatite crystals, as in the human affected tissue (*24*).

To explain the potential relationship between defective ABCC6 transporter and pathological mineralization, two theories have been proposed. The first hypothesis (metabolic hypothesis) postulates that the absence of ABCC6 activity in the liver results in deficiency of circulating anti-mineralization factors which are necessary to prevent precipitation of calcium/phosphate complexes and aberrant mineralization in homeostatic conditions (*25*). The second hypothesis (cellular hypothesis) states that the accumulation of minerals in soft connective tissues may be associated with the absence of ABCC6 expression in resident cells of the affected organs, primarily fibroblasts, resulting in cell perturbation (changes of biosynthetic expression profile, proliferative capacity, and cell-cell and cell-matrix interactions) and consequent local mineralization and elastic fiber alterations (*26,27*).

Moreover, growing evidence appears to indicate that the ABCC6 mutations may contribute to alter redox potential restoration following oxidative stress, leading to soft tissue calcification in PXE patients (*28,29*). Defects in additional genes, including the *GGCX* and the *ENPP1* genes, have recently been implicated in the development of PXE-like cutaneous findings, associated with unusual phenotypes (*30-32*). The *GGCX* gene encodes a vitamin k-dependent enzyme responsible for γ-glutamyl carboxylation of Gla-proteins, including several vitamin K-dependent coagulation factors and matrix-Gla proteins (MGP) (*33*).

MGP in fully carboxylated form is a potent anti-mineralization factor expressed in peripheral connective tissues. The inactivating missense mutations in both

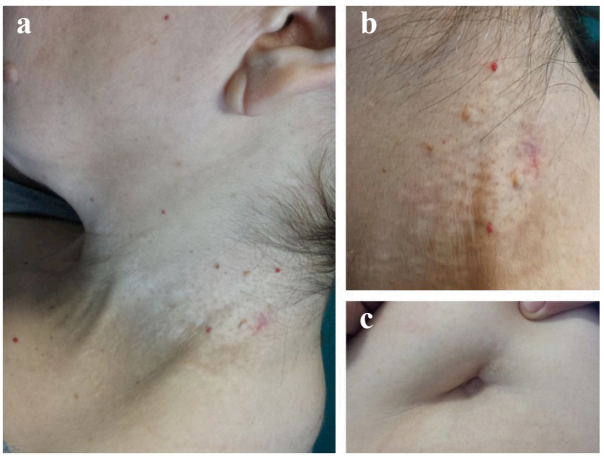


Figure 1. Cutaneous manifestations of PXE. Small yellowish papules coalescing into larger plaques in the neck (a,b), and periumbilical region (c).

alleles of *GGCX* gene generally result in cutaneous lesions consistent with PXE and bleeding disorders (*34*). Recently, it has been demonstrated that MGP in its uncarboxylated form has high expression in calcified tissues of PXE patients as well as in the ABCC6$^{-/-}$ mouse model (*35,36*).

Based on these observations, it has been proposed that ectopic mineralization in PXE could partially reflect deficiency of vitamin K, leading to reduced levels of fully carboxylated active MGP (*37*). The hypothesis has been tested in the animal model, indicating that vitamin K supplementation does not prevent the deposition of minerals in the ABCC6$^{-/-}$ mouse model (*38,39*).

Generalized arterial calcification of infancy (GACI) is a life-threatening, autosomal recessive disease, characterized by extensive arterial calcification, manifesting within the first months of life. It is associated with inactivating mutations in the *ENPP1* (ectonucleotide pyrophosphatase/phosphodiesterase 1) gene, which leads to decreased levels of inorganic pyrophosphate, a potent physiological inhibitor of hydroxyapatite-crystal deposition and extracellular matrix calcification (*40*). Interestingly, cutaneous manifestations consistent with PXE have been described in patients with GACI (*32,41*).

Lastly, a lower expression of fetuin-A, an important negative regulator of mineralization, has been demonstrated in serum of patients with PXE, suggesting fetuin-A as a contributing factor of PXE pathogenesis (*42*).

5. Clinical phenotype

5.1. *Cutaneous manifestations*

Cutaneous features are usually the first sign of pseudoxanthoma elasticum and consist of small (1 to 5 millimetres), asymptomatic, yellowish or skin-colored papules, presenting in a reticular pattern, that progressively coalesce into larger plaques (Figure 1). The affected skin typically becomes lax, wrinkled, and redundant (*23*).

Skin alterations commonly appear during childhood or adolescence and progress slowly and unpredictably during adulthood. They are initially located on the lateral and posterior regions of the neck. Flexural areas, including axillae, inguinal region, antecubital and popliteal fossae, and periumbilical area, are frequently involved during the progression of the disease (*43*). Mucosal lesions of the oral cavity, especially the inner lower lip, and genital area, can also be detected and resemble cutaneous changes (*44*). Although the cutaneous lesions principally represent a cosmetic problem, they predict the risk for development of ocular and cardiovascular manifestations, with a considerable morbidity and occasional mortality (*41*).

5.2. *Ocular manifestations*

Ophthalmological features of PXE include primarily peau d'orange, comet lesions, angioid streaks, choroidal neovascularization (CNV), hemorrhages, and scar formation (*41*).

Peau d'orange is the earliest funduscopically visible alteration in patients with PXE, preceding the development of angioid streaks. It consists of pigment small dark spots resulting in a mottled aspect prominently of the temporal retinal midperiphery (*45*). The pathogenesis of peau d'orange remains unclear. Notably, the ocular phenotype is the consequence of the progressive calcification of Bruch's membrane (BM) which is composed primarily of elastic fibers. It has been hypothesized that peau d'orange represents a visible transition zone of BM calcification (*46*).

Comet lesions are characterized by chorioretinal atrophic spots, preferably with peripheral localization; occasionally they present a tail pointing toward the optic nerve head, leading to the descriptive term comet tail lesions (*47*). It has been suggested that comet and comet tail lesions are the only ocular pathognomonic features of PXE (*47*).

Angioid streaks are the most obvious and consistent features of PXE fundus abnormalities. They present as irregular and jagged brownish-grey lines that radiate from a concentric peripapillary ring into the periphery. The streaks are most pronounced at the posterior pole of the eye and typically taper and fade toward the equator of the eye, usually dividing into smaller branches (*48*). Histopathologically angioid streaks represent breaks of the calcified and thickened Bruch's membrane. It has been postulated that calcification of BM increases the vulnerability of the membrane, inducing ruptures in calcifying BM and resulting in angioid streak formation (*16,49*).

CNV of the macular region is a frequent complication in patients with PXE; it usually occurs in association with angioid streaks leading to subretinal hemorrhages,

exudation and fibrovascular scar formation, with consequent visual acuity loss (*50*). Pattern dystrophy-like changes and chorioretinal atrophy, originating secondary to CNV or developing in the context of areas of pattern dystrophy, are recognized features in PXE patients (*51*).

Additionally, PXE patients have an increased risk of developing optic nerve head (ONH) drusen. The exact mechanism is incompletely understood but it is probably related to abnormal mineralization of the lamina cribrosa (*48,51*).

5.3. *Cardiovascular manifestations*

As in many other cutaneous diseases (*52,53*), the cardiovascular manifestations in PXE patients are numerous and include reduced peripheral pulse, hypertension, angina pectoris, and intermittent claudication. Gastrointestinal hemorrhages, manifesting as hematemesis and melena, are frequently observed. PXE patients can also develop premature atherosclerosis with early acute myocardial infarcts and cerebrovascular accidents (*54,55*).

Specifically, cardiovascular changes in PXE patients are mainly caused by mineralization and fragmentation of elastic fibers of the internal elastic lamina, medial and adventitial layers of medium-sized arteries and aorta, as well as of the endocardium, pericardium, connective tissue in the myocardium and intramyocardial arterioles and epicardial coronary arteries (*56*).

In addition, alterations in lipoprotein composition with lowered plasma HDL cholesterol levels and hypertriglyceridemia were found in plasma samples of PXE patients (*57*).

6. Histopathology

The histological examination of cutaneous lesions is essential for the definitive diagnosis of PXE. The primary histological feature of PXE is progressive mineralization and fragmentation of mid-dermal elastic fibers, resulting in a histological image pattern known as elastorrhexis (Figure 1) (*58*).

In particular, light microscopy (LM) using Verhoeff-Van Gieson (VVG) stain, specific for the elastic fibers, or von Kossa or Alizarin Red calcium stains, showing respectively fragmented elastic fibers and mid-dermal calcified, is crucial for the diagnosis of PXE (*59*) (Figure 2). Calcification mostly affects the elastic fibers core; electron microscopy (EM) observation can reveal two types of mineralization: fine deposits in the center of the fibers and bulky precipitates deforming the fibers (*60*).

Mineral precipitates are usually composed of hydroxyapatite and calcium biphosphate. Other mineral precipitates, as iron, phosphate and carbonate, have also been detected in altered connective tissue. Rarely, dermal mineralized areas evolve to ossification (*27,61*).

Additionally, deposits of abnormal collagen fibrils, as collagen flowers, and abnormal amounts of proteoglycans in the context of mineralized elastic fibers can be observed (*61,62*). Fibroblasts are usually numerous and characterized by hypertrophy of endoplasmic reticulum. Macrophages are also abundant within the calcified deposits (*58*). LM alterations in non-lesional skin are generally absent. Conversely, ultrastructural elastic tissue degeneration can be observed in both lesional and clinically non-involved skin (*63*). Dermoscopy examination may reveal multiple

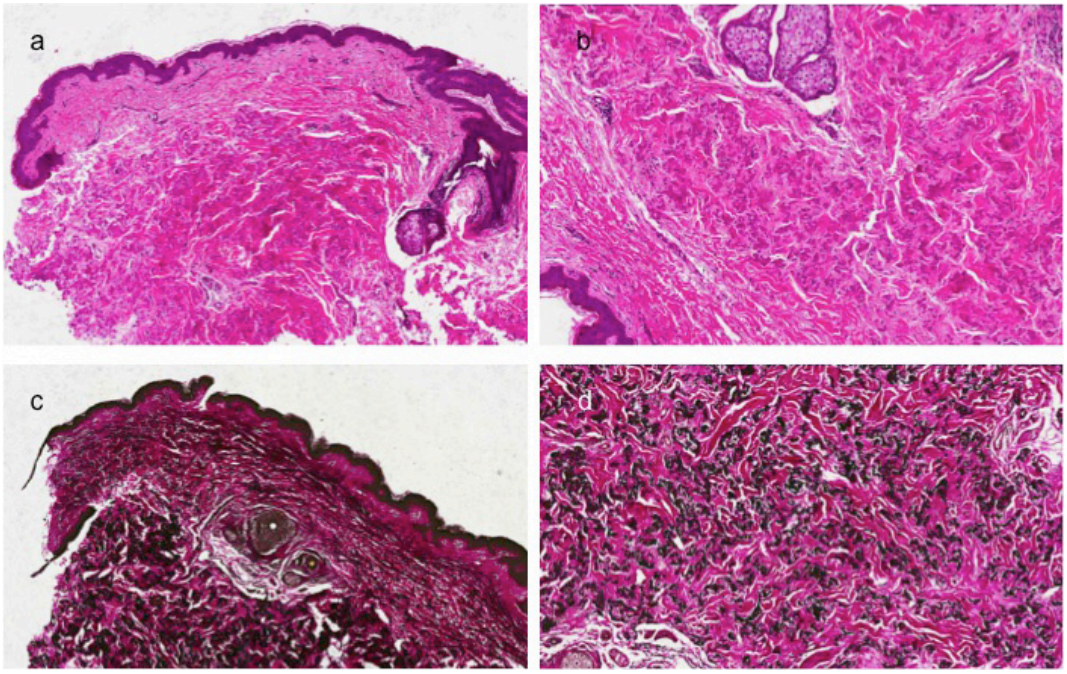


Figure 2. Histological characteristics of PXE skin biopsy of the neck with fragmentation and calcification of middermal elastic fibers on hematoxylin and eosin staining (a,b) and Verhoeff-Van Gieson staining (c,d).

Table 1. Differential diagnosis of PXE

Items	PXE	PXE-like	Cutis laxa	GACI
Generalized skin folds/laxity	Not present	Always present	Often present	Not present
Positive Von Kossa stain of reticular dermis	Present	Present	Not present	Not present
Electron microscopy of elastic fibers	Mineralized in the core of fibers	Mineralized in marginal areas of fibers	Scarce and mottled elastic fibers	Calcification of the arterial internal elastic lamina
Retinopathy	Present and often severe	Present but mild	Not present	Not present
Decreased visual acuity	60%	Not present	Infrequent	Not present
Clotting deficiency	Not described	Always present	Not described	Not described
Atherosclerosis	(Sub)clinical in 55%	Subclinical in 50%	Infrequent	Present and often severe
Hypertension	Present but mild	Infrequent	Not present	Severe
Arterial calcification	Present but mild	Infrequent	Not present	Present and often severe
Cerebral aneurysms	Infrequent	Present	Infrequent	Present
Abnormal bleeding tendency	Infrequent (10%)	Present (50%)	Not described	Not described
Mutations	ABCC6 (96%)	GGCX	ELN,ATP6V0A2, FBLN4-5, PYCR1	ENPP1 (75%)
First manifestations	Childhood or adolescence	Adult	At birth	In utero or the first 6 months of life
Prognosis	Good	Good	Variable	Infaust

yellowish-colored nonfollicular papules arranged in cobbelstone-pattern. No specific dermoscopic features of PXE have been described in literature.

7. Differential diagnosis

Numerous systemic and dermatologic disorders could manifest clinical and histological features resembling classic PXE (*43,59*) (Table 1). Moreover, the absence of skin alterations does not exclude a diagnosis of PXE (*64*).

The term PXE-like syndrome has been used to describe cutaneous, ocular, and cardiovascular alterations characteristic of PXE that occur in association with other systemic or dermatologic diseases or secondary to genetic mutations different from *ABCC6* (*56*). Cutaneous lesions of PXE-like phenotype have been described in association with vitamin-K dependent coagulation factor deficiency (factor II, VII, IX, and X) (*65*).

Cutaneous peau d'orange lesions distributed symmetrically on the neck and flexural regions may be the first sign of PXE-like; skin alterations tend to progress towards thick and leathery redundant folds, especially in the flexural areas. The ocular and cardiovascular manifestations are usually mild or absent (*30,59*).

As mentioned above, the PXE-like is caused by loss-of-function mutations in the *GGCX* gene, encoding a gamma-carboxylase that mediates the activation of vitamin K-dependent coagulation factors and several inhibitors of mineralization (as MGP) in the liver and peripheral tissue, respectively (*66*).

The histological finding is indistinguishable from classic PXE on light microscopy. Conversely, electron microscopy reveals mineralized aggregates confined to the periphery of the elastic fibers, while PXE usually shows deposits in the core of the fibers (*59*).

Clinical features closely resembling PXE are also reported in association with inherited hemoglobinopathies, such as thalassemia and sickle cell disease. Specifically, yellowish papular eruption and ocular changes are comparable to PXE manifestations, except for the elderly onset of the symptoms. In this circumstance, hemoglobin electrophoresis should be performed in patients exhibiting clinical and/or histological findings mimicking PXE (*67*).

Other dermatologic disorders resembling PXE are cutis laxa, fibroelastolytic papulosis, PXE-like papillary dermal elastolysis, late-onset focal dermal elastosis, and perforating calcific elastosis (*68-73*). Lastly, several dermatologic diseases, including elastosis perforans serpiginosa, upper and middermal elastolysis, papular elastorrhexis and linear focal elastosis, can manifest similar histological phenotypes as observed in PXE (*70,74-77*).

8. Treatment of cutaneous manifestations

No specific or effective treatment is currently available for the systemic mineralization and fragmentation of elastic fibres in the skin, eyes and arterial blood vessels caused by PXE. Based on literature reports, we propose a review of therapeutic options for this intractable disease. However, significant progress has been made in the

therapy of ocular complications. Treatment options for choroidal neovascularisation (CNV) of Retinal Pigment Epithelium (RPE) secondary to pseudoxanthoma elasticum include laser photocoagulation, transpupillary thermotherapy and photodynamic therapy, macular translocation surgery and anti-vascular endothelial growth factor (anti-VEGF) treatments (*51*). It is beyond the scope of this paper to examine the myriad of therapeutic possibilities for ocular complications.

8.1. *Surgical treatment*

Rare reports of the surgical management of PXE for cosmetic improvement of skin manifestations have been described in the literature as one of the therapeutic options available (*78*). Few cases with surgical implication (presence of redundant, lax and indurated skin of the neck, axillae and groin region with a typical "hound dog" appearance) have been treated by cutaneous rhytidectomy with SMAS (superficial musculoaponeurotic system) (*79*).

Cosmetic surgery usually consists of lower subcutaneuos rhytidectomy and neck skin lift performed through a standard preauricular facelift incision with postauricular extension and transverse extension into the hairline with excellent results and minimal complications. Furthermore, revision surgery using a vertical elliptical skin excision, incorporating a Z-plasty after a standard rhytidectomy, which produced minimal improvement, resulted in a satisfactory outcome (*80*). A characteristic horizontal "mental" crease connecting deep rhytides of the lower jaw was successfully treated with injectable collagen, providing a temporary but immediately visible improvement (*81*).

8.2. *Systemic treatment*

Some investigators have noted an association of idiopathic hyperphosphatemia and PXE (*82*). Moreover, it was found that the imposition of low-calcium diet could produce clinical, histopathologic, and electron-microscopic improvement in the number of abnormal calcified elastic fibers in the dermis (*83*). These observations suggest a possibility of clinical use of oral phosphate binders in the treatment of PXE. Administration of aluminium hydroxide in 6 patients has produced marked improvement of skin lesions in 3 of those patients (*19*). Moreover, all 3 patients had reduced von Kossa's staining of histopathological changes in their target lesions. In a 1-year follow-up, there was no clinically significant deterioration of eye damage.

Subsequent studies examined the efficacy of sevelamer hydrocloride (aluminium-free phosphate binder) in the normalization of the serum calcium-phosphate products and clinical outcome in patients with PXE (*84*). In the randomized, double blind, placebo-controlled study, sevelamer hydrochloride produced an improvement in clinical scores and in calcification during the first year of treatment. However, the difference was not statistically significant compared with placebo, because of the addition of magnesium stearate (an agent that has been implicated in reducing calcium levels) in the composition of each of the pills used in control and study groups. The authors discovered that sevelamer hydrochloride's phosphate-binding capacity is not as strong as aluminium hydroxide, and did not prove to be as efficacious. Furthermore, a diet addition of sevelamer hydrochloride did not improve mineralization as compared with Abcc6$^{-/-}$ mice fed a normal diet (*20*). The drug caused a compensatory increase in serum phosphorous concentration produced by impaired intestinal absorption of phosphate. Within these results, has been an option to use lanthanum carbonate, an alternate phosphate binder, which has a similar phosphate-binding capacity as aluminium hydroxide. Experiments with diet supplementation using lanthanum carbonate did not interfere with the mineralization process in Abcc6$^{(-/-)}$ mice (*20*).

A potential way of preventing ectopic mineralization revolves around supplementation with fetuin-A, a major systemic inhibitor of calcification. A set of experiments has suggested that concentration of fetuin-A in PXE patients, as well as Abcc6$^{-/-}$ mice, were lower than in unaffected first-degree relatives and controls (*42*). Overexpression of fetuin-A in Abcc6$^{(-/-)}$ mice due to construct containing full-length mouse fetuin-A complementary DNA (cDNA), linked to a His-tag, resulted in elevated serum levels of this protein. These results suggest that normalization of serum fetuin-A can reduce soft tissue mineralization by approximately 70% at 12 weeks, but its effect is transient (*85*). Studies of mouse Abcc6$^{-/-}$ models suggest that increasing magnesium content of the diet (fivefold) may be useful to prevent the ectopic mineralization in these animals (*86*).

Furthermore, treatment of mice with a magnesium carbonate-enriched diet (magnesium concentration being 5-fold higher than in the control diet) completely prevents mineralization of the vibrissae up to 6 months of age. The magnesium carbonate-enriched diet also prevents the progression of mineralization when mice were placed on that experimental diet at 3 months of age and followed up to 6 months of age. These results suggest that magnesium carbonate may offer a potential treatment modality for PXE (*31*).

As confirmation of the above conclusions, recent studies have demonstrated that the magnesium poor diet accelerates the connective tissue mineralization in PXE mice (*87*). Considering the results of the preclinical studies, the research team of Dr. Mark Lebwohl initiated in 2013 a study to test the efficacy of magnesium-enriched diet (900 mg daily) in a double-blind 2 years long clinical trial to evaluate the progress of the mineralization in a cohort of patients with PXE.

In this context, it should be noted that until now

standardized methodologies are not available to monitor progress of mineralization in PXE except for clinical follow-up and skin biopsy. However, recent studies have demonstrated that measurement of carotid intima-media thickness (CIMT), a risk factor for cardiovascular events and stroke, might provide a predictive biomarker of clinical response in PXE patients in future clinical trials. This type of assessment has been used in Abcc6$^{-/-}$ mice fed standard rodent diet with or without magnesium oxide supplementation. Baseline CIMT was significantly higher in Abcc6$^{-/-}$ that in Abcc6$^{+/+}$ mice and CIMT was significantly lower in the magnesium-treated Abcc6$^{-/-}$ mice group than in untreated Abcc6$^{-/-}$ mice (*88*).

9. Treatment perspectives

Novel potential treatments of PXE have been explored by a number of molecular and cell-based approaches. For example, transplantation of bone marrow derived mesenchymal stem cells (MSCs) has demonstrated homing of cells to the liver and their ability to contribute to liver regeneration. These data suggest that purified MSCs have the capability of differentiating into hepatic lineages with an aim for partial correction of the PXE phenotype in Abcc6$^{-/-}$ knockout mice (*38*).

As confirmation of the importance of liver cells in the pathway of PXE, 3 cases of pseudoxanthoma elasticum have been reported that occurred after deceased donor liver (and in one case, subsequent kidney) transplantation from a donor with unrecognized PXE (*89*).

Furthermore, a possible correction of nonsense ABCC6 mutation by a read-through mechanism through PTC124 (a non-aminoglycoside nonsense mutation suppressor molecule) has been evaluated (*90*). Considering the redundancy of the genetic code, it was postulated that in the case of the most common recurrent nonsense mutation, p.R1141X, the read-through may result in substitution of arginine 1,141 by glycine, tryptophan, or cysteine. In a recently developed zebrafish messenger RNA (mRNA) rescue assay it was demonstrated that all three mRNA transcripts were able to rescue the ABCC6a morpholino-induced phenotype of zebrafish. Thus, the results suggest that read-through of nonsense mutations in ABCC6 by PTC124 may provide a novel means to treat PXE patients.

A recent study suggests that allele-specific therapy with 4-phenylbutyrate (4-PBA), a drug that has already been approved by FDA for clinical use, can be useful for PXE patients as well as for GACI (generalized arterial calcification of infancy) patients (*91*). Efficacy of pharmacological correction of the plasma membrane localization of four ABCC6 mutants (R1114P, S1121W, Q1347H, and R1314W) could be studied in upcoming clinical trials.

Other studies suggest that the factor that normally prevents PXE is pyrophosphate, which is provided to the circulation in the form of nucleoside triphosphates *via* an as-yet unidentified but ABCC6-dependent mechanism (*92*). This finding provides leads for the treatment of this intractable disease.

10. Conclusions

There is no effective and specific treatment for the systemic manifestations of PXE until now, but effective therapies for the ocular complications are currently available (*93*). All clinical manifestations in the skin, eyes and arterial blood vessels are consequence of calcium phosphate deposition in elastic fibers. A number of observations have indicated different potential treatment modalities for PXE. Specifically, studies of mouse Abcc6$^{-/-}$ models suggested that the mineral composition of diet, particularly supplementation with magnesium, could prevent deposition of minerals in connective tissue and can influence the severity of the mineralization phenotype (*12,86,87*).

Another potential way for prevention of mineralization processes is possible through introduction of anti-mineralization factors to the circulation. Several molecules (aluminium hydroxide (*19*), sevelamer hydrochloride (*81*), and fetuin-A (*82*)) have proven to be effective in mouse Abcc6$^{-/-}$ models and in some patients with PXE.

Modern molecular approaches for correction of nonsense ABCC6 mutation read-through of translation by PTC124 (*90*) and chaperon-assisted corrections of the cellular localization of the mutant protein by allele-specific therapy with 4-PBA (*88*) would be expected to be useful in the treatment of patients with PXE. A further aim of restoring functional ABCC6 transporter activity by cell-based approaches is possible. For example, transplantation of allogenic mesenchymal stem cells (MSCs) has demonstrated homing of cells to the liver and their ability to contribute to liver regeneration (*66*). In addition to this strategy, liver transplantation or a partial lobe replacement would be a way to safeguard ABCC6 activity (*82,94*). Furthermore, correction of the anaesthetic skin manifestations could be performed by plastic cosmetic surgery (*78*). Early identification of ABCC6 mutation can be used for confirmation of the clinical diagnosis, carrier detection, and presymptomatic recognition of affected individuals. Furthermore, early diagnosis of the disease could be helpful for increased surveillance of the clinical complications, allowing prevention and timely therapy. These observations suggest that appropriate dietary interventions, oral phosphate binders, allele-specific, molecular and cell-based approaches, coupled with lifestyle modifications, including smoking cessation, might alleviate the symptoms and improve the quality of life of affected individuals.

Meanwhile, continued progress in understanding the pathomechanisms, genetic and epigenetic factors of the severity of phenotype is required for development

of effective, pathophysiology-related therapy of this currently intractable clinical syndrome (*17*).

References

1. Uitto J. Rare heritable skin diseases: Targets for regenerative medicine. J Invest Dermatol. 2012; 132:2485-2488.
2. Le Saux O, Urban Z, Tschuch C, Csiszar K, Bacchelli B, Quaglino D, Pasquali-Ronchetti I, Pope FM, Richards A, Terry S, Bercovitch L, de Paepe A, Boyd CD. Mutations in a gene encoding an ABC transporter cause pseudoxanthoma elasticum. Nat Genet. 2000; 25:223-227.
3. Matsuzaki Y, Nakano A, Jiang QJ, Pulkkinen L, Uitto J. Tissue-specific expression of the *ABCC6* gene. J Invest Dermatol. 2005; 125:900-905.
4. Campanati A, Marconi B, Penna L, Paolinelli M, Offidani A. Pronounced and early acne in Apert's syndrome: A case successfully treated with oral isotretinoin. Eur J Dermatol. 2002; 12:496-498.
5. Trip MD, Smulders YM, Wegman JJ, Hu X, Boer JM, ten Brink JB, Zwinderman AH, Kastelein JJ, Feskens EJ, Bergen AA. Frequent mutation in the *ABCC6* gene (R1141X) is associated with a strong increase in the prevalence of coronary artery disease. Circulation. 2002; 106:773-775.
6. Li Q, Jiang Q, Pfendner E, Váradi A, Uitto J. Pseudoxanthoma elasticum: Clinical phenotypes, molecular genetics and putative pathomechanisms. Exp Dermatol. 2009; 18:1-11.
7. Chassaing N, Martin L, Calvas P, Le Bert M, Hovnanian A. Pseudoxanthoma elasticum: A clinical, pathophysiological and genetic update including 11 novel *ABCC6* mutations. J Med Genet. 2005; 42:881-892.
8. Rigal D. Observation pour servir a l'histoire de la chéloïde diffuse xantelasmique. Annal Dermatol Syphiligraphie. 1881: 2:491-501.
9. Darier J. Pseudoxanthoma elasticum. Monatshefte Praktische Dermatologie. 1896:609-617.
10. Doyne R. Choroidal and retinal changes the result of blows on the eyes. Trans Ophthalmol Soc UK. 1889; 9:128.
11. Pflange O. Pigmentary striae with secondary changes in the retina after haemorrhage. Arch Ophthalmol. 1892; 21:282.
12. Strandberg J. Pseudoxanthoma elasticum. Zeitschrift fur Haut- und Geschlechtskrankheiten. 1929; 31:689.
13. Groenblad E. Angioid streaks-pseudoxanthoma elasticum. Acta Ophtal. 1929; 7:329.
14. Neldner KH. Pseudoxanthoma elasticum. Clin Dermatol. 1988; 6:1-159.
15. Ringpfeil F, McGuigan K, Fuchsel L, Kozic H, Larralde M, Lebwohl M, Uitto J. Pseudoxanthoma elasticum is a recessive disease characterized by compound heterozygosity. J Invest Dermatol. 2006; 126: 782-786.
16. Georgalas I, Papaconstantinou D, Koutsandrea C, Kalantzis G, Karagiannis D, Georgopoulos G, Ladas I. Angioid streaks, clinical course, complications, and current therapeutic management. Ther Clin Risk Manag. 2009; 5:81-89.
17. Uitto J, Varadi A, Bercovitch L, Terry PF, Terry SF. Pseudoxanthoma elasticum: Progress in research toward treatment: Summary of the 2012 PXE international research meeting. J Invest Dermatol. 2013; 133:1444-1449.
18. Hendig D, Arndt M, Szliska C, Kleesiek K, Götting C. SPP1 promoter polymorphisms: Identification of the first modifier gene for pseudoxanthoma elasticum. Clin Chem. 2007; 53:829-836.
19. Sherer DW, Singer G, Uribarri J, Phelps RG, Sapadin AN, Freund KB, Yanuzzi L, Fuchs W, Lebwohl M. Oral phosphate binders in the treatment of pseudoxanthoma elasticum. J Am Acad Dermatol. 2005; 53:610-615.
20. LaRusso J, Jiang Q, Li Q, Uitto J. Ectopic mineralization of connective tissue in Abcc6$^{-/-}$ mice: Effects of dietary modifications and a phosphate binder--a preliminary study. Exp Dermatol. 2008; 17:203-207.
21. Pfendner EG, Vanakker OM, Terry SF, *et al*. Mutation detection in the *ABCC6* gene and genotype-phenotype analysis in a large international case series affected by pseudoxanthoma elasticum. J Med Genet. 2007; 44:621-628.
22. Hosen MJ, Coucke PJ, Le Saux O, De Paepe A, Vanakker OM. Perturbation of specific pro-mineralizing signalling pathways in human and murine pseudoxanthoma elasticum. Orphanet J Rare Dis. 2014; 9:66.
23. Uitto J, Li Q, Jiang Q. Pseudoxanthoma elasticum: Molecular genetics and putative pathomechanisms. J Invest Dermatol. 2010; 130:661-670.
24. Kavukcuoglu NB, Li Q, Pleshko N, Uitto J. Connective tissue mineralization in Abcc6$^{-/-}$ mice, a model for pseudoxanthoma elasticum. Matrix Biol. 2012; 31:246-252.
25. Jiang Q, Li Q, Uitto J. Aberrant mineralization of connective tissues in a mouse model of pseudoxanthoma elasticum: Systemic and local regulatory factors. J Invest Dermatol. 2007; 127:1392-1402.
26. Passi A, Albertini R, Baccarani M, de Luca G, de Paepe A, Pallavicini G, Pasquali Ronchetti I, Tiozzo R. Proteoglycan alterations in skin fibroblast cultures from patients affected with pseudoxanthoma elasticum. Cell Biochem Funct. 1996; 14:111-120.
27. Quaglino D, Boraldi F, Barbieri D, Croce A, Tiozzo R, Pasquali Ronchetti I. Abnormal phenotype of *in vitro* dermal fibroblasts from patients with Pseudoxanthoma elasticum (PXE). Biochim Biophys Acta. 2000; 1501:51-62.
28. Mueller CF, Widder JD, McNally JS, McCann L, Jones DP, Harrison DG. The role of the multidrug resistance protein-1 in modulation of endothelial cell oxidative stress. Circ Res. 2005; 97:637-644.
29. Cole SP, Deeley RG. Transport of glutathione and glutathione conjugates by MRP1. Trends Pharmacol Sci. 2006; 27:438-446.
30. Vanakker OM, Martin L, Gheduzzi D, Leroy BP, Loeys BL, Guerci VI, Matthys D, Terry SF, Coucke PJ, Pasquali-Ronchetti I, De Paepe A. Pseudoxanthoma elasticum-like phenotype with cutis laxa and multiple coagulation factor deficiency represents a separate genetic entity. J Invest Dermatol. 2007; 127:581-587.
31. Li Q, Larusso J, Grand-Pierre AE, Uitto J. Magnesium carbonate-containing phosphate binder prevents connective tissue mineralization in Abcc6$^{(-/-)}$ mice-potential for treatment of pseudoxanthoma elasticum. Clin Transl Sci. 2009; 2:398-404.
32. Nitschke Y, Baujat G, Botschen U, *et al*. Generalized arterial calcification of infancy and pseudoxanthoma elasticum can be caused by mutations in either *ENPP1* or *ABCC6*. Am J Hum Genet. 2012; 90:25-39.
33. Stafford DW. The vitamin K cycle. J Thromb Haemost.

2005; 3:1873-1878.
34. Li Q, Grange DK, Armstrong NL, Whelan AJ, Hurley MY, Rishavy MA, Hallgren KW, Berkner KL, Schurgers LJ, Jiang Q, Uitto J. Mutations in the *GGCX* and *ABCC6* genes in a family with pseudoxanthoma elasticum-like phenotypes. J Invest Dermatol. 2009; 129:553-563.
35. Li Q, Jiang Q, Schurgers LJ, Uitto J. Pseudoxanthoma elasticum: Reduced gamma-glutamyl carboxylation of matrix gla protein in a mouse model (Abcc6$^{-/-}$). Biochem Biophys Res Commun. 2007; 364:208-213.
36. Gheduzzi D, Boraldi F, Annovi G, DeVincenzi CP, Schurgers LJ, Vermeer C, Quaglino D, Ronchetti IP. Matrix Gla protein is involved in elastic fiber calcification in the dermis of pseudoxanthoma elasticum patients. Lab Invest. 2007; 87:998-1008.
37. Vanakker OM, Martin L, Schurgers LJ, Quaglino D, Costrop L, Vermeer C, Pasquali-Ronchetti I, Coucke PJ, De Paepe A. Low serum vitamin K in PXE results in defective carboxylation of mineralization inhibitors similar to the GGCX mutations in the PXE-like syndrome. Lab Invest. 2010; 90:895-905.
38. Jiang Q, Li Q, Grand-Pierre AE, Schurgers LJ, Uitto J. Administration of vitamin K does not counteract the ectopic mineralization of connective tissues in Abcc6 $^{(-/-)}$ mice, a model for pseudoxanthoma elasticum. Cell Cycle. 2011; 10:701-707.
39. Gorgels TG, Waarsing JH, Herfs M, Versteeg D, Schoensiegel F, Sato T, Schlingemann RO, Ivandic B, Vermeer C, Schurgers LJ, Bergen AA. Vitamin K supplementation increases vitamin K tissue levels but fails to counteract ectopic calcification in a mouse model for pseudoxanthoma elasticum. J Mol Med. 2011; 89:1125-1135.
40. Rutsch F, Ruf N, Vaingankar S, *et al*. Mutations in *ENPP1* are associated with 'idiopathic' infantile arterial calcification. Nat Genet. 2003; 34:379-381.
41. Li Q, Schumacher W, Jablonski D, Siegel D, Uitto J. Cutaneous features of pseudoxanthoma elasticum in a patient with generalized arterial calcification of infancy due to a homozygous missense mutation in the *ENPP1* gene. Br J Dermatol. 2012; 166:1107-1111.
42. Hendig D, Schulz V, Arndt M, Szliska C, Kleesiek K, Götting C. Role of serum fetuin-A, a major inhibitor of systemic calcification, in pseudoxanthoma elasticum. Clin Chem. 2006; 52:227-234.
43. Varadi A, Szabo Z, Pomozi V, de Boussac H, Fülöp K, Arányi T. *ABCC6* as a target in pseudoxanthoma elasticum. Curr Drug Targets. 2011; 12:671-682.
44. Hu X, Plomp AS, van Soest S, Wijnholds J, de Jong PT, Bergen AA. Pseudoxanthoma elasticum: A clinical, histopathological, and molecular update. Surv Ophthalmol. 2003; 48:424-438.
45. Naouri M, Boisseau C, Bonicel P, Daudon P, Bonneau D, Chassaing N, Martin L. Manifestations of pseudoxanthoma elasticum in childhood. Br J Dermatol. 2009; 161:635-639.
46. Charbel Issa P, Finger RP, Gotting C, Hendig D, Holz FG, Scholl HP. Centrifugal fundus abnormalities in pseudoxanthoma elasticum. Ophthalmology. 2010; 117:1406-1414.
47. Gass JD. "Comet" lesion: An ocular sign of pseudoxanthoma elasticum. Retina. 2003; 23:729-730.
48. De Zaeytijd J, Vanakker OM, Coucke PJ, De Paepe A, De Laey JJ, Leroy BP. Added value of infrared, red-free and autofluorescence fundus imaging in pseudoxanthoma elasticum. Br J Ophthalmol. 2010; 94:479-486.
49. Georgalas I, Tservakis I, Papaconstaninou D, Kardara M, Koutsandrea C, Ladas I. Pseudoxanthoma elasticum, ocular manifestations, complications and treatment. Clin Exp Optom. 2011; 94:169-180.
50. Gliem M, Finger RP, Fimmers R, Brinkmann CK, Holz FG, Charbel Issa P. Treatment of choroidal neovascularization due to angioid streaks: A comprehensive review. Retina. 2013; 33:1300-1314.
51. Finger RP, Charbel Issa P, Ladewig M, Götting C, Holz FG, Scholl HP. Fundus autofluorescence in Pseudoxanthoma elasticum. Retina. 2009; 29:1496-1505.
52. Campanati A, Giuliodori K, Ganzetti G, Liberati G, Offidani AM. A patient with psoriasis and vitiligo treated with etanercept. Am J Clin Dermatol. 2010; 11(Suppl 1):46-48.
53. Ganzetti G, Campanati A, Offidani A. Alopecia Areata: A possible extraintestinal manifestation of Crohn's disease. J Crohns Colitis. 2012; 6:962-963.
54. Jiang Q, Endo M, Dibra F, Wang K, Uitto J. Pseudoxanthoma elasticum is a metabolic disease. J Invest Dermatol. 2009; 129:348-354.
55. Braun SA, Finis D, Helbig D. Pseudoxanthoma elasticum. More than a skin problem. Hautarzt. 2013; 64:222, 224-225. (in German)
56. Campens L, Vanakker OM, Trachet B, Segers P, Leroy BP, De Zaeytijd J, Voet D, De Paepe A, De Backer T, De Backer J. Characterization of cardiovascular involvement in pseudoxanthoma elasticum families. Arterioscler Thromb Vasc Biol. 2013; 33:2646-2652.
57. Kuzaj P, Kuhn J, Michalek RD, Karoly ED, Faust I, Dabisch-Ruthe M, Knabbe C, Hendig D. *et al*. Large-scaled metabolic profiling of human dermal fibroblasts derived from pseudoxanthoma elasticum patients and healthy controls. PLoS One. 2014; 9:e108336.
58. Gheduzzi D, Sammarco R, Quaglino D, Bercovitch L, Terry S, Taylor W, Ronchetti IP. Extracutaneous ultrastructural alterations in pseudoxanthoma elasticum. Ultrastruct Pathol. 2003; 27:375-384.
59. Hosen MJ, Lamoen A, De Paepe A, Vanakker OM. Histopathology of pseudoxanthoma elasticum and related disorders: Histological hallmarks and diagnostic clues. Scientifica (Cairo). 2012; 2012:598262.
60. Maccari F, Gheduzzi D, Volpi N. Anomalous structure of urinary glycosaminoglycans in patients with pseudoxanthoma elasticum. Clin Chem. 2003; 49:380-388.
61. Walker ER, Frederickson RG, Mayes MD. The mineralization of elastic fibers and alterations of extracellular matrix in pseudoxanthoma elasticum. Ultrastructure, immunocytochemistry, and X-ray analysis. Arch Dermatol. 1989; 125:70-76.
62. Baccarani-Contri M, Vincenzi D, Cicchetti F, Mori G, Pasquali-Ronchetti I. Immunochemical identification of abnormal constituents in the dermis of pseudoxanthoma elasticum patients. Eur J Histochem. 1994; 38:111-123.
63. Passi A, Albertini R, Baccarani Contri M, de Luca G, de Paepe A, Pallavicini G, Pasquali Ronchetti I, Tiozzo R. Proteoglycan alterations in skin fibroblast cultures from patients affected with pseudoxanthoma elasticum. Cell Biochem Funct. 1996; 14:111-120.
64. Limas C. Late onset focal dermal elastosis: A distinct clinicopathologic entity? Am J Dermatopathol. 1999; 21:381-383.
65. Le Corvaisier-Pieto C, Joly P, Thomine E, Lair G, Lauret

P. Generalized pseudoxanthoma elasticum combined with vitamin K dependent clotting factors deficiency. Ann Dermatol Venereol. 1996; 123:555-558. (in French)
66. Berkner KL. The vitamin K-dependent carboxylase. Annu Rev Nutr. 2005; 25:127-149.
67. Aessopos A, Farmakis D, Loukopoulos D. Elastic tissue abnormalities resembling pseudoxanthoma elasticum in beta thalassemia and the sickling syndromes. Blood. 2002; 99:30-35.
68. Song YC, Oh BH, Ko JH, Kim JY, Hwang YJ, Lee YW, Choe YB, Ahn KJ, Song KY. A case of fibroelastolytic papulosis on the neck of a young man. Ann Dermatol. 2011; 23:193-197.
69. Alves R, Ferreira L, Vale E, Bordalo O. Pseudoxanthoma elasticum papillary dermal elastolysis: A case report. Dermatol Res Pract. 2010; 2010.
70. Akagi A, Tajima S, Kawada A, Ishibashi A. Coexistence of pseudoxanthoma elasticum-like papillary dermal elastolysis and linear focal dermal elastosis. J Am Acad Dermatol. 2002; 47:S189-S192.
71. Kossard S. Pseudoxanthoma-like late-onset focal dermal elastosis. Australas J Dermatol. 2005; 46:47-50.
72. Camacho D, Machan S, Pielasinski U, Revelles JM, del Carmen Fariña M, Santonja C, Requena L. Familial acral localized late-onset focal dermal elastosis. Am J Dermatopathol. 2012; 34:310-314.
73. Kocaturk E, Kavala M, Zindanci I, Koç M. Periumbilical perforating pseudoxanthoma elasticum. Indian J Dermatol Venereol Leprol. 2009; 75:329.
74. Mehta RK, Burrows NP, Payne CM, Mendelsohn SS, Pope FM, Rytina E. Elastosis perforans serpiginosa and associated disorders. Clin Exp Dermatol. 2001; 26:521-524.
75. Choi Y, Jin SY, Lee JH, Kwon HB, Lee AY, Lee SH. Papular elastorrhexis: A case and differential diagnosis. Ann Dermatol. 2011; 23(Suppl 1):53-56.
76. Lewis KG, Bercovitch L, Dill SW, Robinson-Bostom L. Acquired disorders of elastic tissue: Part II. decreased elastic tissue. J Am Acad Dermatol. 2004; 51:165-185.
77. Patroi I, Annessi G, Girolomoni G. Mid-dermal elastolysis: A clinical, histologic, and immunohistochemical study of 11 patients. J Am Acad Dermatol. 2003; 48:846-851.
78. Viljoen DL, Bloch C, Beighton P. Plastic surgery in pseudoxanthoma elasticum: Experience in nine patients. Plast Reconstr Surg. 1990; 85:233-238.
79. Ng AB, O'Sullivan ST, Sharpe DT. Plastic surgery and pseudoxanthoma elasticum. Br J Plast Surg. 1999; 52:594-596.
80. Akali AU, Sharpe DT. Cervical midline Z-plasty revision surgery for pseudoxanthoma elasticum. Br J Plast Surg. 2003; 56:289-291.
81. Galadari H, Lebwohl M. Pseudoxanthoma elasticum: Temporary treatment of chin folds and lines with injectable collagen. J Am Acad Dermatol. 2003; 49:S265-S266.
82. Mallette LE, Mechanick JI. Heritable syndrome of pseudoxanthoma elasticum with abnormal phosphorus and vitamin D metabolism. Am J Med. 1987; 83:1157-1162.
83. Martinez-Hernandez A, Huffer WE, Neldner K, Gordon S, Reeve EB. Resolution and repair of elastic tissue calcification in pseudoxanthoma elasticum. Arch Pathol Lab Med. 1978; 102:303-305.
84. Yoo JY, Blum RR, Singer GK, Stern DK, Emanuel PO, Fuchs W, Phelps RG, Terry SF, Lebwohl MG. A randomized controlled trial of oral phosphate binders in the treatment of pseudoxanthoma elasticum. J Am Acad Dermatol. 2011; 65:341-348.
85. Jiang Q, Dibra F, Lee MD, Oldenburg R, Uitto J. Overexpression of fetuin-a counteracts ectopic mineralization in a mouse model of pseudoxanthoma elasticum (abcc6$^{(-/-)}$). J Invest Dermatol. 2010; 130:1288-1296.
86. LaRusso J, Li Q, Jiang Q, Uitto J. Elevated dietary magnesium prevents connective tissue mineralization in a mouse model of pseudoxanthoma elasticum (Abcc6$^{(-/-)}$). J Invest Dermatol. 2009; 129:1388-1394.
87. Jiang Q, Uitto J. Restricting dietary magnesium accelerates ectopic connective tissue mineralization in a mouse model of pseudoxanthoma elasticum (Abcc6$^{(-/-)}$). Exp Dermatol. 2012; 21:694-699.
88. Kupetsky EA, Rincon F, Uitto J. Rate of change of carotid intima-media thickness with magnesium administration in Abcc6$^{(-)/(-)}$ mice. Clin Transl Sci. 2013; 6:485-486.
89. Bercovitch L, Martin L, Chassaing N, Hefferon TW, Bessis D, Vanakker O, Terry SF. Acquired pseudoxanthoma elasticum presenting after liver transplantation. J Am Acad Dermatol. 2011; 64:873-878.
90. Zhou Y, Jiang Q, Takahagi S, Shao C, Uitto J. Premature termination codon read-through in the *ABCC6* gene: Potential treatment for pseudoxanthoma elasticum. J Invest Dermatol. 2013; 133:2672-2677.
91. Pomozi V, Brampton C, Fulop K, Chen LH, Apana A, Li Q, Uitto J, Le Saux O, Váradi A. Analysis of pseudoxanthoma elasticum-causing missense mutants of **ABCC6** *in vivo*; pharmacological correction of the mislocalized proteins. J Invest Dermatol. 2014; 134:946-953.
92. Jansen RS, Kucukosmanoglu A, de Haas M, Sapthu S, Otero JA, Hegman IE, Bergen AA, Gorgels TG, Borst P, van de Wetering K. *ABCC6* prevents ectopic mineralization seen in pseudoxanthoma elasticum by inducing cellular nucleotide release. Proc Natl Acad Sci U S A. 2013; 110:20206-20211.
93. Uitto J, Jiang Q, Varadi A, Bercovitch LG, Terry SF. Pseudoxanthoma elasticum: Diagnostic features, classification, and treatment options. Expert Opin Orphan Drugs 2014; 2:567-577.
94. Li Q, Uitto J. Heritable ectopic mineralization disorders: The paradigm of pseudoxanthoma elasticum. J Invest Dermatol. 2012; 132:E15-E19.

(Received April 16, 2015; Revised June 9, 2015; Accepted June 10, 2015)

Original Article

DOI: 10.5582/irdr.2015.01029

Anxiety disorders in fragile X premutation carriers: Preliminary characterization of probands and non-probands

Lisa Cordeiro[1], Floridette Abucayan[2], Randi Hagerman[2,3], Flora Tassone[2,4], David Hessl[2,5,*]

[1] *Department of Pediatrics, University of Colorado, Denver, USA;*
[2] *MIND Institute, University of California Davis, Sacramento, USA;*
[3] *Department of Pediatrics, University of California Davis, Sacramento, USA;*
[4] *Department of Biochemistry and Molecular Medicine, University of California Davis, Sacramento, USA;*
[5] *Department of Psychiatry and Behavioral Sciences, University of California Davis, Sacramento, USA.*

Summary **A very high proportion of individuals with fragile X syndrome (FXS) (*FMR1* full mutation, > 200 CGG repeats) experience clinically significant anxiety. Recent evidence suggests that adult fragile X premutation carriers (55-200 CGG repeats) also are at risk for anxiety disorders, and they demonstrate limbic system alterations mediated by FMRP and/or elevated *FMR1* mRNA that may explain this heightened risk. However, less is known about psychiatric symptoms including anxiety among children and adolescents with the premutation. We completed structured DSM-IV based diagnostic interviews focused on current anxiety in 35 children, adolescents or young adults with the premutation (ages 5-23 years, M = 11.3 ± 4.3; 27 male; 20 probands and 15 non-probands) and 31 controls (ages 5-18 years, M = 9.9 ± 3.6; 22 males). Among premutation carriers, 70.6% met criteria for at least one anxiety disorder (most frequently generalized anxiety disorder, specific phobia, social phobia, or obsessive compulsive disorder), compared to 22.6% of controls and 9.8% of the general population in this age range. Premutation carriers with intellectual disability, male gender, and proband status were associated with the highest rates of anxiety disorders. However, non-probands did have higher rates of having any anxiety disorder (40.0%) compared to general population norms. Although the results implicate anxiety as a target of screening and intervention among youth with the premutation, larger studies of unselected samples from the population of premutation carriers are needed to confirm and specify the degree and extent of psychiatric disorders in this condition.**

Keywords: Premutation carriers, proband, fragile X syndrome (FXS), anxiety, social phobia, specific phobia, intellectual disability

1. Introduction

Fragile X syndrome (FXS) is the most common inherited form of intellectual disability (ID) and the most common known genetic cause of autism. It is caused by a trinucleotide expansion (CGG) of greater than 200 CGG repeats in the 5' untranslated region of the fragile X mental retardation 1 gene (*FMR1*) located on the X chromosome and occurs in approximately 1 per 5,000 males and 1 in 2,500-8,000 females (*1-6*). The inheritance pattern of fragile X is based on progressive generational expansion of the repeat size passed down from mother to child. Individuals are normally categorized based on the size of the CGG repeat expansion, in which normal alleles have 5-44 CGG repeats, while full mutation alleles have > 200 CGG repeats (*7*). Premutation carriers have a molecular phenotype characterized by abnormally elevated *FMR1* mRNA, which positively tracks with CGG repeat size within the premutation range. The fragile X premutation has an expansion of between 55 and 200 repeats, contributing to risk for expansion to a full mutation on transmission from mother to offspring in a single generation. The prevalence of the fragile X premutation in the general population is approximately 1 in 260-815

**Address correspondence to:*
Dr. David Hessl, MIND Institute, University of California Davis, 2825 50th Street, Sacramento, CA 95817, USA.
E-mail: drhessl@ucdavis.edu

males and 1 in 130-290 females, which in comparison to FXS is relatively high (*8*).

Individuals with the *FMR1* premutation are at risk for the two well-established phenotypes, the fragile X-associated primary ovarian insufficiency (FXPOI) and fragile X-associated tremor/ataxia syndrome (FXTAS). FXPOI occurs in approximately 20% of females with the premutation compared to 1% of the general population and is defined by cessation of menses before age 40 (*9,10*). FXTAS is a neurodegenerative disease seen in a significant proportion of older males and smaller number of older females with the premutation. Symptoms of FXTAS include intention tremor, cerebellar ataxia, neuropathy, autonomic dysfunction, cognitive decline, and brain atrophy with white matter disease (*11-15*).

Individuals with full FXS demonstrate high rates of anxiety disorders, which are regularly observed in clinical practice and through detailed research-based psychiatric interviews with parents (*16*). Though it was previously believed that fragile X premutation carriers develop normally through childhood, and young and middle adulthood, there is evidence to suggest that perhaps at least a subgroup is affected (*17-19*). For example, there is evidence of significant executive function (EF) deficits in carriers, particularly males (*20,21*), although this has not been consistently found in all studies (*22*). Females, on the other hand, are less likely to demonstrate EF deficits (*23,24*), but may be prone to mood and anxiety disorders, notably major depressive disorder, panic disorder without agoraphobia, agoraphobia without panic disorder, and social phobia (*25,26*). Although these symptoms were previously attributed to stresses on mothers with the premutation raising children with FXS, the participants in the study by Roberts and colleagues (*25*) retrospectively reported significant distress before the children were born. Franke and colleagues (*27*) carried out a remarkable study of mothers with the premutation to determine whether psychological problems were related to the premutation itself or to the stress of raising a developmentally impaired child. This study compared 13 mothers with the full mutation, 61 mothers with the premutation, 17 women with the premutation who were siblings of the first two groups but did not have children with FXS, and 18 women siblings without the *FMR1* mutation and without children, and 42 mothers without the *FMR1* mutation who had children with autism. The study used a psychiatric interview to obtain DSM-IV diagnoses and to assess personality disorders. Mothers with a premutation, as well as their siblings with the premutation but without affected children, were more likely to be diagnosed with social phobia than a control group of mothers of children with autism. A recent large family survey of children with the full mutation and premutation, in which parents were simply asked whether their child had been diagnosed with or been treated for a range of conditions, showed that 33.3% of 57 males and 35.6% of 119 females with the premutation were identified as having significant anxiety, compared to 8.8% of males and 15.3% of females with normal *FMR1* alleles matched for age and family income (*28*). However, it should be noted that Hunter and colleagues (*29*) examined mood and anxiety in 119 males and 446 females age 18-50 ascertained from families with a history of FXS and from the general population. Repeat length was not associated with anxiety, but was marginally associated with depression and negative affect in males and negative affect only in females. Thus the authors concluded that phenotypic differences were subtle and had a small effect size. However, elevated mRNA or reduced FMRP play a more important role in clinical outcomes among carriers than CGG size alone. For example, psychological symptoms, such as anxiety and obsessive-compulsive features are associated with abnormal elevation of *FMR1* mRNA in adult male premutation carriers with and without FXTAS (*15*). Also, both reduced FMRP and elevated mRNA contribute to alterations in limbic function and symptom expression in young adult carriers (*30,31*), providing a gene-brain-behavior basis for an understanding of emergence of these difficulties.

Anxiety disorders are among the most common psychiatric disorders in the general population, occurring in 2.4-10.7% of children (*32-34*). In a large epidemiological study funded by the National Institutes of Mental Health (Center for the Study of Emotion and Attention; CSEA-NIMH, 35), 9.8% of children met criteria for an anxiety disorder (n = 1,289, 9-17 years) (*36*). The most common anxiety disorders in the NIMH study were social phobia (4.5%), overanxious disorder (3.1%) and separation anxiety (2.3%).

The goal of the present study was to assess the frequency of anxiety disorders among children, adolescents and young adults who carry the *FMR1* premutation. Further, we sought to determine whether clinical features such as autism, intellectual disability and proband status, might be associated with the presence of anxiety disorders in premutation carriers.

2. Materials and Methods

2.1. *Participants*

Participants included 35 individuals with the *FMR1* premutation [27 males, ages 5.20-20.14, M = 11.04 (3.85); 8 females, ages 4.98-22.96, M = 12.13 (5.79)], and 31 healthy controls with normal *FMR1* alleles [22 males, ages 5.07-17.83, M = 9.73 (3.37); 9 females, ages 5.74-17.40, M = 10.26 (4.33)] (Table 1). Fifty-seven percent of the participants with the *FMR1* premutation were the first in their family pedigree to come to the attention of a clinician (probands), leading to fragile X DNA testing (17 males, 3 females). The remainder of premutation carriers (42.9%) was identified by cascade DNA testing

Table 1. Participant descriptive data

Items	Premutation Carriers			Controls			*t*-test
	Total	Males	Females	Total	Males	Females	
Age N	35	27	8	31	22	9	
M (SD)	11.29 (4.30)	11.04 (3.85)	12.13 (5.79)	9.88 (3.61)	9.73 (3.37)	10.26 (4.33)	t (64) = 1.43,
Range	4.98-22.96	5.20-20.14	4.98-22.96	5.07-17.83	5.07-17.83	5.74-17.4	p = 0.158
FSIQ* N	35	27	8	31	22	9	
M (SD)	93.49 (25.68)	90.30 (23.72)	104.25 (30.66)	114.16 (14.15)	113.59 (13.21)	115.56 (17.00)	t (54) = -4.11,
Range	36-141	36-141	40-126	85-143	88-136	85-143	p < 0.000
Proband Status N (%)							
Proband	20 (57.1)	17 (63.0)	3 (37.5)	0 (0%)	0 (0)	0 (0)	
Non-proband	15 (42.9)	10 (37.0)	5 (62.5)	31 (100%)	22 (100)	9 (100)	
Intellectual Disability N (%)							
IQ Below 80	11 (31.4)	9 (33.3)	2 (25.0)	0 (0)	0 (0)	0 (0)	
IQ Above 80	24 (68.6)	18 (66.7)	6 (75.0)	31 (100)	22 (100)	9 (100)	
ADOS Category N (%)							
No ASD	25 (73.5)	19 (70.4)	6 (85.7)	31 (100)	22 (100)	9 (100)	
ASD	5 (14.7)	5 (18.5)	0 (0.0)	0 (0)	0 (0)	0 (0)	
Autism	4 (11.8)	3 (11.1)	1 (14.3)	0 (0)	0 (0)	0 (0)	

*Full Scale IQ (IQ tests included WASI, WISC-IV and DAS-II). ADOS, Autism Diagnostic Observation Schedule; ASD, Autism Spectrum Disorder.

(10 males, 5 females). Controls were recruited through announcements and flyers in the community and local school districts. Race and ethnicity data were collected in accordance with NIMH funded project requirements. The majority of the sample was Caucasian (86.4%) and not Hispanic or Latino (62.1%). Twelve (34.3%) premutation carriers and 2 (6.5%) controls were taking psychoactive medications at the time of assessment. For carriers, medications included: SSRI/antidepressant (n = 3), antianxiety/sedative (n = 1), antipsychotic (n = 3), stimulant (n = 3), and anticonvulsant (n = 3). For controls, medications included: SSRI/antidepressant (n = 1), antipsychotic (n = 1), and stimulant (n = 1). Ten of the 35 premutation carriers had a sibling with the *FMR1* full mutation.

2.2. *Measures*

The Anxiety Disorders Interview Schedule for DSM-IV: Parent Report Version (ADIS-IV; *37*) is a structured interview designed to assess and diagnose the presence of anxiety disorders according to DSM-IV criteria. ADIS-IV was used specifically to measure the severity and occurrence of anxiety disorders through parent ratings of disorder features and symptomology. The parent ratings indicate either the severity of distress or the amount of interference the item has on the person's overall functioning (0 = none to 8 = very severe). ADIS-IV has demonstrated good test-retest reliability (k = 0.73) and excellent inter-rater reliability (k = 0.80-1.0) between the parent- and child- report version of the ADIS for both principal diagnosis and individual anxiety disorders (*38,39*). Administration of the ADIS takes approximately two hours and was completed with the primary caregiver, usually the mother. ADIS has been used extensively in published studies of anxiety across many settings and populations (*40-44*). It has also been used in validation studies (*45-48*) and a federally-funded pediatric anxiety treatment trial (*49*). Finally, our group has validated the use of the ADIS in a population of children, adolescents and young adults with FXS, with and without ID (*16*).

Intelligence testing was conducted by a trained clinician. Due to the wide age range of participants, several measures were used, including the Wechsler Intelligence Scales: WASI (43.9%), WPPSI-III (6.1%), WISC-IV (30.3%), WAIS-III (6.1%). In addition, the Stanford-Binet Intelligence Scale, Fifth Edition (12.1%), and Leiter International Performance Scale-Revised (1.5%) were also used. Those with an IQ score below 80 were classified as having an ID (borderline range inclusive).

The presence of a possible autism spectrum disorder (ASD) was screened using the Social Communication Questionnaire (SCQ; *50*) and confirmed as necessary with the Autism Diagnostic-Observation Scale (ADOS-G; *51*). All diagnostic assessments used to determine ASD status were administered by a trained clinician. The control group did not include any participants with a previously-diagnosed disorder and all had an SCQ score within the normal range.

CGG repeat size and methylation status were determined for all participants on genomic DNA isolated from peripheral blood mononucleated cells (*PBMC*) using PCR and Southern Blot analysis as previously described (*52,53*). qRT-PCR by Taqman assay was used to measure *FMR1* mRNA expression levels as reported in Tassone and colleagues (*54*).

2.3. *Procedures*

All participants (and parents, if applicable) signed either a consent or assent to participate in the study under the

approval of the institutional review board (IRB). As part of a larger study, participants were seen for 1 to 3 days examining physiological correlates of anxiety in individuals with neurodevelopmental disorders. A list of current medications was reported by the parents. The ADIS was administered by an experienced licensed clinical psychologist (D.H.) or graduate level student (L.C., L.A., A.C.) who had passed reliability training on the instrument, as described previously (*16*). Any discrepancies or disagreements of diagnosis were handled by case discussion and reviewed by a licensed clinical psychologist for final diagnosis (D.H.). Administration was standardized to collect specific information to aid in the differential diagnoses of intellectual disability (ID) and autism (AUT), as described previously (*16*). DSM-IV adaptations for children were used for children with or without ID, as well as for adults with ID. As for adults without ID, standard DSM-IV criteria were used.

During the ADIS, interviewees were asked to provide specific examples regarding symptom description in order to ensure comprehension and proper symptom endorsement. For example, if fear of spiders was endorsed in the specific phobia section, the interviewee was asked to describe the reaction, the last time it occurred, the consistency of the fear, and the severity and type of interference in daily functioning. The interviewee was also asked whether the participant "reported" fear or whether they had "observed" a reaction indicating distress or a fearful response. The information collected provided the interviewer with enough data to make any diagnostic adjustments, if necessary.

2.4. *Statistical analysis*

Proportion tests (*z*-tests) (*55,56*) were carried out using the SPSS Custom Tables module to determine if the prevalence of anxiety disorders in the current study groups were significantly different from the prevalence in previous studies of the general population. General population prevalence rates were taken from the largest published NIMH epidemiological study of psychiatric disorders in children and adolescents (n = 1,285) using DSM-III-R criteria (*36*). Alpha values were adjusted using a Bonferroni correction. Given the potential impact of having a sibling with FXS on expression of anxiety, we conducted Chi-Square analyses to examine the association between having an affected sibling and the presence or absence of each anxiety disorder type.

Correlations were used to assess the association between the number of anxiety disorders and both mRNA and number of CGG repeats.

3. Results

3.1. *Rates of anxiety disorders*

Among all premutation carriers, 70.6% (n = 25) met criteria for at least one anxiety disorder, while 22.6% (n = 8) of the control group met criteria for at least one anxiety disorder (Table 2). The most common anxiety disorders in the premutation carrier group were generalized anxiety disorder (37.1%; n = 13), specific phobia (31.4%; n = 11), social phobia (28.6%; n = 10) and obsessive-compulsive disorder (22.9%; n = 8). The most common anxiety disorders in the control group were social and specific phobia (each 12.9%; n = 4). To further characterize anxiety among key clinical aspects of the premutation, rates were examined by gender, among those with and without an intellectual disability (ID), and by proband status (Table 3). Overall, more males (76.9%) than females (50.0%), those with ID (81.8%) than without ID (62.5%), and probands (94.7%) than non-probands (40.0%) met criteria for at least one disorder. Both males and females had a similar pattern of anxiety although twice as many females met criteria for separation anxiety. The pattern among those with an ID was somewhat different compared to those without an ID. Separation anxiety and selective mutism were more common among those with ID, while those without an ID had higher rates of GAD and OCD. Probands and non-probands had similar patterns of anxiety disorders, although many more probands met criteria for social phobia (40.0%) and specific phobia (50.0%) compared to non-probands (13.3% and 6.7%, respectively). Chi-Square analyses showed that there was no association between having a sibling with FXS and presence of any anxiety disorder (all $p > 0.25$).

3.2. *Comparison of anxiety disorder rates with the general population*

Both the premutation, and to a lesser extent the control group participants with average IQ had higher rates of anxiety compared to the general population (Table 4). Premutation carriers had significantly higher rates of social, specific and GAD compared to the general population, as well as a rate of having any anxiety disorder (all $p < 0.0083$ after controlling for multiple

Table 2. Percentage of premutation carriers and control group meeting criteria for DSM-IV anxiety disorders

Anxiety Type	Premutation carriers (%) (n = 35)	Controls (%) (n = 31)
Any disorder	70.6	22.6
Separation anxiety	8.6	6.5
Social phobia	28.6	12.9
Specific phobia	31.4	12.9
Panic disorder	0	0
Agoraphobia	0	0
GAD	37.1	3.2
OCD	22.9	3.2
PTSD	8.6	6.5
Selective mutism	8.6	0

GAD, generalized anxiety disorder; OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder.

Table 3. Percentage of premutation carriers meeting criteria for clinical anxiety disorders

Anxiety type	Gender		ID status		Proband status	
	Male	Female	ID	Non-ID	Proband	Non-proband
Any disorder	76.9%	50.0%	81.8%	62.5%	94.7%	40.0%
Separation anxiety	7.4%	12.5%	18.2%	4.2%	10.0%	6.7%
Social phobia	29.6%	25.0%	27.3%	29.2%	40.0%	13.3%
Specific phobia	29.6%	37.5%	54.5%	20.8%	50.0%	6.7%
Panic disorder	0	0	0	0	0	0
Agoraphobia	0	0	0	0	0	0
GAD	37.0%	37.5%	27.3%	41.7%	45.0%	26.7%
OCD	22.2%	25.0%	0	33.3%	25.0%	20.0%
PTSD	11.1%	0	9.1%	8.3%	15.0%	0
Selective Mutism	11.1%	0	18.2%	4.2%	10.0%	6.7%

GAD, generalized anxiety disorder; OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder.

Table 4. *Z*-test of proportions comparing non-proband premutation carriers to control group and general population rates of clinical anxiety disorders

Anxiety Type	Non-proband $n = 15$	Controls[a] $n = 31$	General population rates[b]
Any disorder	40.0%	24.1%	9.8%*
Separation anxiety	6.7%	6.5%	2.3%
Social phobia	13.3%	12.9%	4.5%
Specific phobia	6.7%	12.9%	1.3%
Panic disorder	0	0	N/A
Agoraphobia	0.	0	1.4%
GAD	26.7%	3.2%^	3.1%**
OCD	20.0%	3.2%	N/A
PTSD	0	6.9%	N/A
Selective mutism	6.7%	0	N/A

[a] Control for multiple comparisons used, significant differences are $p < 0.01$. [b] Control for multiple comparisons used, significant differences are $p < 0.007$. * Difference is significant at the corrected *p*-value. ** Difference was not significant after control for multiple comparisons. GAD, generalized anxiety disorder; OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder.

Table 5. *Z*-test of proportions comparing rates of anxiety disorders in control and premutation groups to general population rates

Anxiety Type	General population	Premutation[a] $n = 35$	Controls[a] $n = 31$
Any disorder	9.8%	70.6%*	24.1%
Separation anxiety	2.3%	8.6%	6.5%
Social phobia	4.5%	28.6%*	12.9%
Specific phobia	1.3%	31.4%*	12.9%**
Panic disorder	N/A	0	0
Agoraphobia	1.4%	0*	0*
GAD	3.1%	37.1%*	3.2%
OCD	N/A	22.9%	3.2%
PTSD	N/A	8.6%	6.9%
Selective Mutism	N/A	8.6%	0

[a] Control for multiple comparisons used, significant differences are $p < 0.0083$. * Difference is significant at the corrected *p*-value. ** Difference was not significant after control for multiple comparisons. GAD, generalized anxiety disorder; OCD, obsessive compulsive disorder; PTSD, post-traumatic stress disorder.

comparisons) (Table 4). The control group had significantly higher rates of specific phobia compared to the general population, but this was no longer significant after controlling for multiple comparisons. Both premutation carriers and controls had significantly lower rates of agoraphobia compared to the general population.

The rates of anxiety disorders among non-probands were not significantly different from the rates among the control group or the general population, with the exception of the rate of *any anxiety disorder* compared to the general population (non-probands = 40.0%; general population = 9.8%; $p = 0.0172$, after control for multiple comparisons) (Table 5). Both the premutation and control group participants with average IQ had higher rates compared to the general population rates.

3.3. *Relationship to molecular variables*

No significant correlations and no trends were found between molecular variables (highest CGG repeat, mRNA) and the number of anxiety disorders among premutation carriers.

4. Discussion

The results of the study showed that an overall rate of anxiety disorders among a sample of children, adolescents and young adults with the premutation was significantly higher than controls and the general population. After examining the premutation carrier group by gender, proband status and presence of intellectual disability, the rates of having any anxiety disorder were highest among probands (94.7%), and remarkably high among those with an intellectual disability (81.8%) and males (70.6%). With regards to the premutation group, further analysis revealed that the significantly higher rates of many of the anxiety disorders compared to the general population were driven by the rates among the probands. The control group had the same rate of GAD as has been reported in the general population (3.2%), suggesting that the significantly higher rate among the premutation group (37.1%) may not be an artifact of the measure used or study design. The higher rate of any anxiety disorder

among non-probands compared to controls and the general population may be the most important result of the study, and provides perhaps better evidence of increased risk for anxiety among carriers. Clinic referral bias among probands is likely to inflate and overestimate the true rate of anxiety disorders among premutation carriers. For example, a child with developmental problems and anxiety may be referred for *FMR1* testing for FXS, and he/she may be found to carry a premutation allele that is not causally related to the symptoms.

There was no relationship between molecular measures (CGG repeat number or mRNA) and anxiety in our study. The lack of such correlations can be interpreted in a number of ways. First, as has been shown in prior work (*30*), correlations between CGG expansion size or mRNA and behavior may not be evident unless measurement of brain function underlying such behavior is accounted for in providing a key link between genetics and behavior. Second, molecular measures in blood may not be reflected similarly in brain tissue, making it more difficult to establish links between genetic variables and behavior. Third, reduced FMRP that occurs in some carriers, often with higher CGG alleles, could underlie anxiety in the premutation; unfortunately we did not have these measures available for this study. And finally, it is possible that the lack of association with the molecular measures is an indication that the *FMR1* premutation does not actually contribute risk for anxiety disorder. Psychosocial factors may also play a role. For example, an individual's knowledge that he/she has the premutation may contribute to anxiety, as this condition clearly confers risk for ovarian insufficiency and FXTAS, and the simple awareness of having a genetic mutation could be anxiogenic. The anxiety disorders observed in this younger sample of carriers may be consistent with previously published studies of older adults with the premutation who reported an increased rate of some types of lifetime anxiety and mood disorders (*25,26*).

There are several notable limitations of this study. First, interviewers were usually but not always blind to the *FMR1* status of the participants. Second, it is possible that the higher rates of anxiety among probands is at least partially a result of self-selection bias in that parents of probands enrolled in the study often sought assistance for developmental concerns, including related behavioral or emotional symptoms. However, enrollment in this study was continuous enrollment of premutation carriers coming to the center – some being clinic referred, typically probands, and others were siblings of probands or selected from pedigrees for research only. Third, this study assessed the presence of current anxiety disorders and we therefore cannot report the lifetime incidence in this sample. Fourth, the small sample size and the higher proportion of males *vs*. females with the premutation in the study limits the generalizability of findings to the larger population of premutation carriers, and to females, who are more likely to inherent the mutation. Fifth, the premutation group included more individuals with ID and had lower IQs overall than the control group. Ideally we would have chosen to more precisely match IQs. We included those in the borderline range (*70-79*) in the premutation group mainly to improve the sample size, which would have otherwise been too small for analysis. Finally, our results are based on parental report and may not reflect the exact internal states that participants are experiencing. However, the measure used in this study (ADIS-R) has been validated for parental report of symptomology.

A larger study that balances sample characteristics (*i.e*. proband status, autism diagnosis, *etc*.) and recruitment methods to reduce bias, would help validate the findings of this preliminary report. Given our finding of significant anxiety, treatment of these problems needs to be considered by the clinician who cares for these individuals as we have recommended previously (*57,58*).

In conclusion, this study provides evidence that anxiety disorders may be relatively common among children and adolescents with the premutation, especially probands. These findings warrant a thorough clinical assessment and potentially treatment of anxiety symptoms in these individuals.

Acknowledgements

The authors thank Susan Harris, Elizabeth Ballinger and Alyssa Chavez for their contribution to this research. We are especially grateful to the participants and their families for their contribution to the understanding of premutation carriers. This study was supported by NIH grants MH77554, MH080025, HD036071, and HD02274.

References

1. Coffee B, Keith K, Albizua I, Malone T, Mowrey J, Sherman SL, Warren ST. Incidence of fragile X syndrome by newborn screening for methylated FMR1 DNA. Am J Hum Genet. 2009; 85:503-514.
2. Crawford DC, Meadows KL, Newman JL, Taft LF, Scott E, Leslie M, Shubek L, Holmgreen P, Yeargin-Allsopp M, Boyle C, Sherman SL. Prevalence of the fragile X syndrome in African-Americans. Am J Med Genet. 2002; 110:226-233.
3. Morton JE, Bundey S, Webb TP, MacDonald F, Rindl PM, Bullock S. Fragile X syndrome is less common than previously estimated. J Med Genet. 1997; 34:1-5.
4. Pesso R, Berkenstadt M, Cuckle H, Gak E, Peleg L, Frydman M, Barkai G. Screening for fragile X syndrome in women of reproductive age. Prenat Diagn. 2000; 20:611-614.
5. Song FJ, Barton P, Sleightholme V, Yao GL, Fry-Smith A. Screening for fragile X syndrome: A literature review and modelling study. Health Technol Assess. 2003; 7:1-106.
6. Turner G, Webb T, Wake S, Robinson H. Prevalence of

fragile X syndrome. Am J Med Genet. 1996; 64:196-197.
7. Maddalena A, Richards CS, McGinniss MJ, Brothman A, Desnick RJ, Grier RE, Hirsch B, Jacky P, McDowell GA, Popovich B, Watson M, Wolff DJ. Technical standards and guidelines for fragile X: The first of a series of disease-specific supplements to the Standards and Guidelines for Clinical Genetics Laboratories of the American College of Medical Genetics. Quality Assurance Subcommittee of the Laboratory Practice Committee. Genet Med. 2001; 3:200-205.
8. Tassone F, Iong KP, Tong T-H, Lo J, Gane LW, Berry-Kravis E, Nguyen D, Mu LY, Laffin J, Bailey DB, Hagerman RJ. FMR1 CGG allele size and prevalence ascertained through newborn screening in the United States. Genome Med. 2012; 4:100.
9. Allingham-Hawkins DJ, Babul-Hirji R, Chitayat D, *et al*. Fragile X premutation is a significant risk factor for premature ovarian failure: The international collaborative POF in fragile X study- preliminary data. Am J Med Genet. 1999; 83:322-325.
10. Sullivan AK, Marcus M, Epstein MP, Allen EG, Anido AE, Paquin JJ, Yadav-Shah M, Sherman SL. Association of FMR1 repeat size with ovarian dysfunction. Hum Reprod. 2005; 20:402-412.
11. Hagerman R, Hagerman P. Advances in clinical and molecular understanding of the FMR1 premutation and fragile X-associated tremor/ataxia syndrome. Lancet Neurol. 2013; 12:786-798.
12. Berry-Kravis E, Abrams L, Coffey SM, *et al*. Fragile X-associated tremor/ataxia syndrome: Clinical features, genetics, and testing guidelines. Mov Disord. 2007; 22:2018-2030.
13. Jacquemont S, Hagerman RJ, Leehey M, *et al*. Fragile X premutation tremor/ataxia syndrome: Molecular, clinical, and neuroimaging correlates. Am J Hum Genet. 2003; 72:869-878.
14. Jacquemont S, Hagerman RJ, Leehey MA, *et al*. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. JAMA. 2004; 291:460-469.
15. Hessl D, Tassone F, Loesch DZ, *et al*. Abnormal elevation of FMR1 mRNA is associated with psychological symptoms in individuals with the fragile X premutation. Am J Med Genet B Neuropsychiatr Genet. 2005; 139B:115-121.
16. Cordeiro L, Ballinger E, Hagerman RJ, Hessl D. Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: Prevalence and characterization. J Neurodev Disord. 2011; 3:57-67.
17. Farzin F, Perry H, Hessl D, Loesch D, Cohen J, Bacalman S, Gane L, Tassone F, Hagerman P, Hagerman R. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. J Dev Behav Pediatr. 2006; 27(2 Suppl):S137-S144.
18. Chonchaiya W, Au J, Schneider A, Hessl D, Harris SW, Laird M, Mu Y, Tassone F, Nguyen DV, Hagerman RJ. Increased prevalence of seizures in boys who were probands with the FMR1 premutation and co-morbid autism spectrum disorder. Hum Genet. 2012; 131:581-589.
19. Hagerman P. Fragile X-associated tremor/ataxia syndrome (FXTAS): Pathology and mechanisms. Acta Neuropathol. 2013; 126(1):1-19.
20. Cornish K, Turk J, Levitas A. Fragile X syndrome & autism: Common developmental pathways? Current Pediatric Reviews. 2007; 3:61-68.
21. Grigsby J, Cornish K, Hocking D, Kraan C, Olichney JM, Rivera SM, Schneider A, Sherman S,Wang JY, Yang JC. The cognitive neuropsychological phenotype of carriers of the FMR1 premutation. J Neurodev Disord. 2014; 6:28.
22. Hunter JE, Sherman S, Grigsby J, Kogan C, Cornish K. Capturing the fragile X premutation phenotypes: A collaborative effort across multiple cohorts. Neuropsychology. 2012; 26:156-164.
23. Bennetto L, Pennington BF, Taylor A, Hagerman RJ. Profile of cognitive functioning in women with the fragile X mutation. Neuropsychology. 2001 ;15:290-299.
24. Franke P, Leboyer M, Hardt J, Sohne E, Weiffenbach O, Biancalana VV, Cornillet-Lefebre P, Delobel B,Froster U, Schwab SG, Poustka F, Hautzinger M, Maier W. Neuropsychological profiles of FMR-1 premutation and full-mutation carrier females. Psychiatry Res. 1999; 87:223-231.
25. Roberts JE, Bailey, DB, Mankowski J, Ford, A, Sideris J, Weisenfeld LA, Heath TM, Golden RN. Mood and anxiety disorders in females with the FMR1 premutation. Am J Med Genet B Neuropsychiatr Genet. 2009; 150B:130-139.
26. Bourgeois JA, Seritan AL, Casillas EM, Hessl D, Schneider A, Yang Y, Kaur I, Cogswell JB, Nguyen DV,Hagerman RJ. Lifetime prevalence of mood and anxiety disorders in fragile X premutation carriers. The Journal of Clinical Psychiatry. 2011; 72:175-182.
27. Franke P, Leboyer M, Gänsickea M, Weiffenbach O, Biancalana V, Cornillet-Lefebre P, Croquette MF, Froster U, Schwab SG, Poustka F, Hautzinger M, Maier W. Genotype-phenotype relationship in female carriers of the premutation and full mutation of FMR-1. Psychiatry Res. 1998; 80:113-127.
28. Bailey DB Jr, Raspa M, Olmsted M, Holiday DB. Co-occurring conditions associated with FMR1 gene variations: Findings from a national parent survey. Am J Med Genet A. 2008; 146A:2060-2069.
29. Hunter JE, Allen EG, Abramowitz A, Rusin N, Leslie M, Novak G, Hamilton D, Shubeck L, Charen K,Sherman SL. Investigation of phenotypes associated with mood and anxiety among male and female fragile X premutation carriers. Behav Genet. 2008; 38:493-502.
30. Hessl D, Wang JM, Schneider A, Koldewyn K, Le L, Iwahashi C, Cheung K, Tassone F, Hagerman PJ,Rivera SM. Decreased fragile X mental retardation protein expression underlies amygdala dysfunction in carriers of the fragile X premutation. Biol Psychiatry. 2011; 70:859-865.
31. Hessl D, Rivera SM, Koldewyn K, Cordeiro L, Adams J, Tassone F, Hagerman PJ, Hagerman RJ. Amygdala dysfunction in men with the fragile X premutation. Brain. 2007; 130:404-416.
32. Fergusson DM, Horwood LJ, Lynskey MT. Prevalence and Comorbidity of DSM-III-R Diagnoses in a Birth Cohort of 15 Year Olds. J Am Acad Child Adolesc Psychiatry. 1993; 32:1127-1134.
33. McGee ROB, Feehan M, Williams S, Partridge F, Silva PA, Kelly J. DSM-III Disorders in a Large Sample of Adolescents. J Am Acad Child Adolesc Psychiatry. 1990; 29:611-619.
34. Costello E, Egger H, Angold A. The Developmental Epidemiology of Anxiety Disorders: Phenomenology,

prevalence, and comorbidity. Child Adolesc Psychiatr Clin N Am. 2005; 14:631 -648.
35. Center for the Study of Emotion and Attention (CSEA-NIMH). The International Affective Picture System. University of Florida, Gainsville, FL, USA, 1998.
36. Shaffer D, Fisher P, Dulcan MK, Davies M, Piacentini J, Schwab-Stone ME, Lahey BB, Bourdon K, Jensen PS, Bird HR, Canino G, Regier DA. The NIMH Diagnostic Interview Schedule for Children Version 2.3 (DISC-2.3): Description, acceptability, prevalence rates, and performance in the MECAStudy. Methods for the Epidemiology of Child and Adolescent Mental Disorders Study. J Am Acad Child Adolesc Psychiatry. 1996; 35:865-877.
37. Silverman WK, Albino AM. The Anxiety Disorders Interview Schedule for Children for DSM-IV: Child and Parent Versions. Psychological Corporation, San Antonio, TX, USA, 1996.
38. Silverman WK, Saavedra LM, Pina AA. Test-retest reliability of anxiety symptoms and diagnoses with the Anxiety Disorders Interview Schedule for DSM-IV: Child and parent versions. J Am Acad Child Adolesc Psychiatry. 2001; 40:937-944.
39. Lyneham HJ, Abbott MJ, Rapee RM. Interrater reliability of the Anxiety Disorders Interview Schedule for DSM-IV: Child and parent version. J Am Acad Child Adolesc Psychiatry. 2007; 46:731-736.
40. Ross CJ, Davis TM, Hogg DY. Screening and assessing adolescent asthmatics for anxiety disorders. Clin Nurs Res. 2007; 16:5-24; discussion 25-28.
41. Panichelli-Mindel SM, Flannery-Schroeder E, Kendall PC, Angelosante AG. Disclosure of distress among anxiety-disordered youth: Differences in treatment outcome. J Anxiety Disord. 2005; 19:403-422.
42. Leyfer O, Woodruff-Borden J, Mervis C. Anxiety disorders in children with Williams syndrome, their mothers, and their siblings: Implications for the etiology of anxiety disorders. J Neurodev Disord. 2009; 1:4-14.
43. Bodden DHM, Bögels SM, Nauta MH, De Haan E, Ringrose J, Appelboom C, Brinkman AG, Appelboom-Geerts KC. Child versus family cognitive-behavioral therapy in clinically anxious youth: An efficacy and partial effectiveness study. J Am Acad Child Adolesc Psychiatry. 2008; 47:1384-1394.
44. Waters AM, Mogg K, Bradley BP, Pine DS. Attentional bias for emotional faces in children with generalized anxiety disorder. J Am Acad Child Adolesc Psychiatry. 2008; 47:435-442.
45. Thaler NS, Kazemi E, Wood JJ. Measuring anxiety in youth with learning disabilities: Reliability and validity of the Multidimensional Anxiety Scale for Children (MASC). Child Psychiatry Hum Dev. 2010; 41:501-514.
46. Simon E, Bögels SM. Screening for anxiety disorders in children. Eur Child Adolesc Psychiatry. 2009; 18:625-634.
47. Bodden DHM, Bögels SM, Muris P. The diagnostic utility of the Screen for Child Anxiety Related Emotional Disorders-71 (SCARED-71). Behav Res Ther. 2009; 47:418-425.
48. Leyfer OT, Ruberg JL, Woodruff-Borden J. Examination of the utility of the Beck Anxiety Inventory and its factors as a screener for anxiety disorders. J Anxiety Disord. 2006; 20:444-458.
49. Compton SN, Walkup JT, Albano AM, Piacentini JC, Birmaher B, Sherrill JT, Ginsburg GS, Rynn MA,McCracken JT, Waslick BD, Iyengar S, Kendall PC, March JS. Child/Adolescent Anxiety Multimodal Study (CAMS): Rationale, design, and methods. Child Adolesc Psychiatry Ment Health. 2010; 4:1.
50. Rutter M, Bailey A, Berument SK, Lord C, Pickles A. Social Communication Questionnaire (SCQ). Western Psychological Services, Los Angeles, CA, USA, 2003.
51. Lord C, Risi S, Lambrecht L, Cook EH, Jr., Leventhal BL, DiLavore PC, Pickles A, Rutter M. The autism diagnostic observation schedule-generic: A standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord. 2000; 30:205-223.
52. Tassone F, Pan R, Amiri K, Taylor AK, Hagerman PJ. A rapid polymerase chain reaction-based screening method for identification of all expanded alleles of the fragile X (FMR1) gene in newborn and high-risk populations. J Mol Diagn. 2008; 10:43-49.
53. Filipovic-Sadic S, Sah S, Chen L, Krosting J, Sekinger E, Zhang W, Hagerman PJ, Stenzel TT, Hadd AG,Latham GJ, Tassone F. A novel FMR1 PCR method for the routine detection of low abundance expanded alleles and full mutations in fragile X syndrome. Clin Chem. 2010; 56:399-408.
54. Tassone F, Hagerman RJ, Chamberlain WD, Hagerman PJ. Transcription of the FMR1 gene in individuals with fragile X syndrome. Am J Med Genet. 2000; 97:195-203.
55. Newcombe RG. Interval estimation for the difference between independent proportions: Comparison of eleven methods. Stat Med. 1998; 17:873-890.
56. Newcombe RG. Two-sided confidence intervals for the single proportion: Comparison of seven methods. Stat Med. 1998; 17:857-872.
57. Besterman AD, Wilke SA, Mulligan TE, Allison SC, Hagerman R, Seritan AL, Bourgeois JA. Towards an understanding of neuropsychiatric manifestations in fragile X premutation carriers. Future Neurol. 2014; 9:227-239.
58. Polussa J, Schneider A, Hagerman R. Molecular advances leading to treatment implications for fragile X premutation carriers. Brain Disord Ther. 2014; 3.

(Received July 17, 2015; Revised August 5, 2015; Accepted August 8, 2015)

Original Article

DOI: 10.5582/irdr.2015.01018

A study of deafness-related genetic mutations as a basis for strategies to prevent hereditary hearing loss in Hebei, China

Junzhen Zhu[1,*], Qinying Cao[2,*], Ning Zhang[1], Jun Ge[2], Donglan Sun[2,], Qingqi Feng[3]**

[1] *Center for Exceptional Care in 3 Areas, The People's Hospital of Hebei Province, Shijiazhuang, Hebei Province, China;*
[2] *The Fourth Hospital of Shijiazhuang, Shijiazhuang, Hebei Province, China;*
[3] *Xinji Maternal and Child Health Hospital, Xinji, Hebei Province, China.*

Summary **Hearing loss is the most common sensory disorder, and at least 50% of cases are due to a genetic etiology. Two-thirds of individuals with congenital deafness are nonsyndromic. Among the nonsyndromic forms, the large majority are monogenic autosomal recessive traits. The current work summarizes mutations in the *GJB2*, *SLC26A4*, *12SrRNA*, and *GJB3* and their prevalence in 318 students with autosomal recessive nonsyndromic hearing loss at schools for the deaf or special needs schools in 9 cities in Hebei Province, China. Deafness gene mutations were identified in 137 students *via* a gene chip, time-of-flight mass spectrometry, fluorescence quantitative PCR, and gene sequencing. Mutations were detected at a rate of 43.08%. A homozygous mutation of the *GJB2* gene was found in 16 students (5.03%), a heterozygous mutation of that gene was found in 38 (11.95%), a homozygous mutation of the *SLC26A4* gene was found in 22 (6.92%), a heterozygous mutation of that gene was found in 59 (18.55%), and a heterozygous mutation of the mitochondrial *12SrRNA* gene was found in 2 (0.63%). In addition, there were 15 families in which a student's parents had normal hearing. Compound heterozygous mutations of the *GJB2* gene were found in 3 families (20%) and mutations of the *SLC26A4* gene were found in 9 (60%). Thus, this study has provided a molecular diagnostic basis for the causes of deafness, and this study has also provided a scientific basis for the early prevention of and intervention in deafness.**

Keywords: Hereditary hearing loss; gene mutation; gene chip; time-of-flight mass spectrometry; sequencing

1. Introduction

Congenital deafness is an irreversible condition due to intrauterine dysplasia or genetic factors. An estimated 30,000 babies are born with congenital hearing impairment per 20 million live births every year in China, and this impairment seriously affects their quality of life. Worldwide, the incidence of congenital deafness, including deafness caused by many genetic and environmental factors, is about 1/1000 (*1*). An estimated 80,000 new patients appear each year because of the clinical use of a large number of antibiotics. People with disabilities in Hebei number about 519.5 million, accounting for 1.86% of the province's population. People with disabilities include 126.0 million with a hearing disability and 7.7 million with a speech disability, and these 2 types of disabilities account for 25.74% of all disabilities.

Gap junction protein beta-2 (*GJB2*) (MIM 220290) was the first gene in which mutations were reported to cause autosomal recessive nonsyndromic hearing loss (ARNSHL) in 1997 (*2*). Although mutations in *GJB3* (MIM 603324) and *GJB6* (MIM604418) were subsequently discovered, *GJB2* remains the most common cause of hereditary deafness in many populations. Mutations in *GJB2* were discovered and were shown to cause up to 50% of ARNSHL in Caucasian populations, but their frequency is much lower in other parts of the world (*3-5*). The c.235delC

*These authors contributed equally to this works.

***Address correspondence to:*
Dr. Donglan Sun, The Fourth Hospitals of Shijiazhuang, No. 212, Zhong Shan Dong Lu, Chang-an Qu, Shijiazhuang, Hebei Province, China.
E-mail: s-d-l2007@163.com

mutation is the most frequent pathogenic variant in Japanese (*6*), while the c.35delG mutation is the most frequent pathogenic variant in the majority of Caucasian populations (70%) (*7*), and the c.167delT mutation is the most common in Ashkenazi Jews (*8*). Indeed, mutations in other connexin genes, such as *GJB6* for Cx30 and *GJB3* for Cx31, have been identified and shown to cause hearing impairment (*9,10*).

The *SLC26A4* gene encodes pendrin, which is a transmembrane anion exchanger that belongs to the solute carrier 26 family and that exchanges chloride, iodide, bicarbonate, and formate. Pendrin is expressed in different tissues, including the thyroid, the kidneys, and the inner ear. In the cochlea, pendrin is found in the apical membrane of the outer sulcus and spiral prominence epithelial cells that border the endolymph, in the spiral ganglion, and in supporting cells (*11*). DNA sequencing has identified more than 100 different mutations in *SLC26A4* (*12-15*). *SLC26A4* mutations may account for as much as 10% of the hereditary deafness in diverse populations (*16*). Four of these mutations, IVS7-2A>G, 2168A>G, 84C>A, 1975G>C, 754 T>C, and IVS 9+1G>A, were previously reported in patients with hearing loss (*17-19*), and IVS7-2A >G is the most prevalent mutation in China (*20*).

Although most cases of hereditary hearing loss are caused by nuclear gene defects, a study has shown that mutations in mitochondrial DNA (mtDNA) can also cause nonsyndromic hearing loss (*21,22*). The 1555A>G mutation is the best studied of these mutations in the mitochondrial *12S rRNA* gene. The second mutation identified in the mitochondrial *12S rRNA* gene is the 1494C>T in the conserved stem structure of *12SrRNA* (*23*). Other nucleotide changes at positions 961 and 1095 in the *12S rRNA* gene have been shown to be associated with hearing loss, but their pathogenic mechanisms of action in the predisposition of carriers to aminoglycoside toxicity are much less clear (*24,25*). The mtDNA 1555A>G mutation accounts for a small fraction of nonsyndromic hearing loss, with a prevalence of 3.43% in China, 3% in Japan, and 3.43% in Indonesia (*26-28*); this mutation is less evident in Caucasian populations, with a prevalence between 0.6% and 2.5% (*29-31*).

The present study comprehensively analyzed 4 prominent deafness-related genes, *GJB2*, *GJB3*, *SLC26A4*, and mtDNA *12SrRNA*, in 318 students at schools for the deaf or special needs schools and their parents in 9 cities in Hebei Province, China.

2. Subjects and Methods

Potential subjects were students at schools for the deaf or special needs schools in 9 cities in Hebei Province. Subjects were 318 students with non-syndromic deafness (with 30-48 subjects per city). Subjects consisted of 64 males and 154 females ranging in age from 2 months to 58 years, with an average age of 10.48 years. Once parental consent was obtained, 15 families were studied (Figure 1). After subjects and their guardians agreed to voluntarily participate in genetic testing, they provided informed consent in writing. Subjects were asked to provide basic personal information, including marriage information, family history, pregnancy history, gestation history, medication history, history of infection, whether abnormalities occurred during pregnancy, whether birth

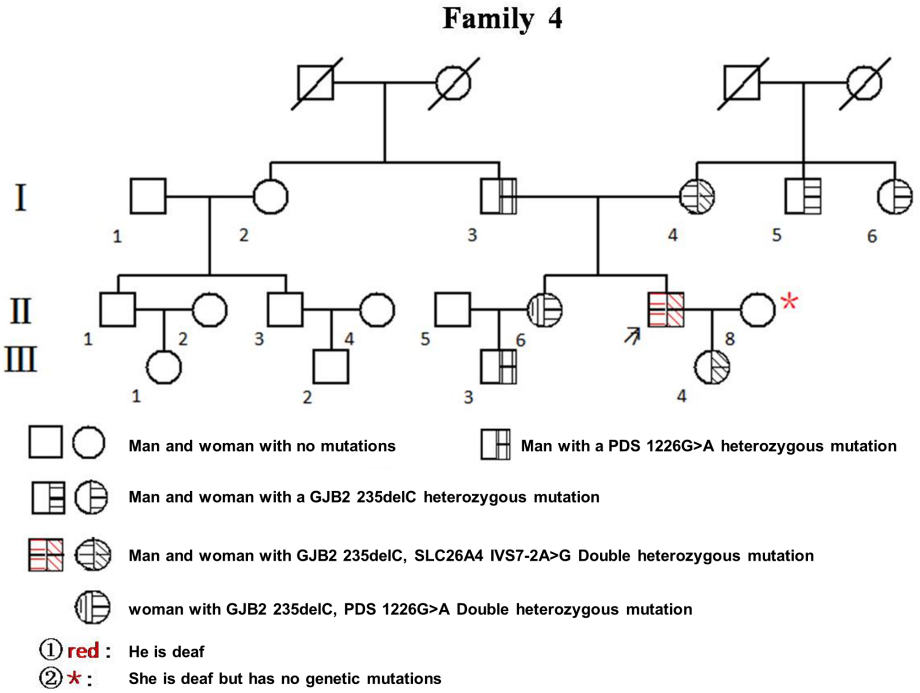


Figure 1. Family 4 was studied.

Table 1. Mutations and the number of students with those mutations

Gene name	Mutation	Type of mutation (no. students)		
		Homozygous mutant	Heterozygous mutation	Homogeneous mutation
GJB2	35delG		1	
	235delC	16	18	
	299-300delAT		13	
	176del 16bp		6	
GJB3	0	0	0	
SLC26A4	IVS7-2A>G	18	33	
	2168 A>G	4	13	
	589G>A		1	
	1174A>T		1	
	1226G>A		3	
	1229C>T		7	
	2027T>A		1	
12SrRNA	1555A>G			2

was premature, whether the neonate had a low birth weight, whether there was an obvious history of head injury before deafness, the use of ototoxic drugs, and detailed medical records. Specimens were then collected.

Three to 5 mL of peripheral blood was collected with a vacuum blood collection tube. Specimens were numbered and blood was dropped on filter paper. DNA was extracted from 2 specimens per subject. UV spectrophotometry was used to quantify and test the purity of the specimen. Specimens were recorded and stored separately by city. Nine gene loci - *GJB2* 35delG, 176del16, 235delC, 299-300delAT, *GJB3* 538, *SLC26A4* 2168A>G, and IVS7-2A>G, *12SrRNA* 1494C>T, and 1555A>G - were detected in 4 common genes (*GJB2*, *GJB3*, *SLC26A4*, and mtDNA *12SrRNA*) using the GeneChip (Beijing Boao Bio Co., Ltd.). Twenty other gene loci - *GJB2* 167delT, *GJB3* 547G>A, *SLC26A4* 281C>T, 589G>A, 1174A>T, 1226 G>A, 1229C>T, IVS15+5 G>A, 1975G>C, 2027T>A, and 2162C>T - were detected with time-of-flight mass spectrometry. Fourteen gene loci - *GJB2* 35delG, 176del16, 235delC, 299-300delAT, 155delTCTG, 512insAACG, *SLC26A4* 2168A>G, IVS7-2A>G 1229C>T, and 1174A>T, *GJB3* 538C>G, 547G>A, *12SrRNA* 1494C>T, and 1555A>G - in the 4 genes were analyzed in 36 of the 318 students using fluorescence quantitative PCR (Jinan Yingsheng Biology). Exons of *GJB2* were analyzed in 3 students using gene sequencing.

All of the members of 15 families underwent acoustic immittance testing, and 13 of the 20 family members with mutations or variants in *SLC26A4* underwent a temporal bone computed tomography (CT) scan for diagnosis of enlarged vestibular aqueducts or inner ear malformation.

3. Results

Gene mutations were detected in 137 (43.08%) of 318 students. *GJB2* mutations were detected in 54 students (16.98%), *SLC26A4* gene mutations were detected in 81 (25.47%), and mitochondrial *12SrRNA* gene mutations were detected in 2 (0.63%). No *GJB3* mutations were detected. A homozygous mutation was detected in 40 students (12.58%). A homozygous mutation in *GJB2* was detected in 16 students (5.03%), a homozygous mutation in *SLC26A4* was detected in 22 (6.92%), and a homogeneous mutation in mtDNA *12rRNA* was detected in 2 (0.63%). Heterozygous mutations were detected in 97 students (30.50%). Heterozygous mutations in *GJB2* were detected in 38 students (11.95%), heterozygous mutations in *SLC26A4* were detected in 59 (18.55%) (Table 1), compound heterozygous mutations were detected in 23 (7.23%), and double heterozygous mutations were detected in 23 (7.23%). A compound heterozygous mutation was detected in 19 students (*GJB2* in 11 students and *SLC26A4* in 8 students) (5.97%), and a double heterozygous mutation was detected in 3 (1.26%) (Tables 2 and 3).

Table 2. Compound heterozygous mutations and the number of students with those mutations

Gene name	Mutation		No. students
GJB2	235delC	299-300delAT	7
	235delC	176-191del16	3
	299-300delAT	176-191del16	1
SLC26A4	IVS7-2A>G	2168 A>G	4[#]
	IVS7-2A>G	589G>A	1
	IVS7-2A>G	2027T>A	1
	IVS7-2A>G	2162C>T	1
	1975G>C	2168 A>G	1
	1226G>A	2168 A>G	1
	IVS7-2A>G	235delC	2
	1226G>A	235delC	1

[#] 1 case was *SLC26A4* IVS7-2A>G/2168 A>G and *GJB2* 235delC compound heterozygous mutation

Table 3. Double heterozygous mutations and the number of students with those mutations

Gene name	Mutation		No. students
SLC26A4/GJB2	IVS7-2A>G	235delC	2
	1226G>A	235delC	1

Heterozygous mutations in the form of exon

Table 4. Twenty-six new polymorphic *GJB2* mutations detected in 36 students

Gene name	Mutation			No. students
GJB2	79G>A Heterozygote			6
	79G>A Heterozygote	341A>G Heterozygote		11
	79G>A Heterozygote	109 G>A Heterozygote	341A>G Heterozygote	1
	79G>A Homozygote	341A>G Heterozygote		3
	79G>A Homozygote	341A>G Homozygote		1
	608T>C Heterozygote			4

polymorphisms in the *GJB2* gene were detected in 36 students. Gene sequencing indicated that 26 students had mosaic or compound heterozygous mutations (66.67%), with 1 student exhibiting a 79G>A homozygous mutation and 1 exhibiting a heterozygous mutation in the form of 341A>G polymorphism. A 79G>A homozygous mutation was detected in 5 students (13.89%), a 608T>C heterozygous mutation was detected in 4 (11.11%), and a complex polymorphic mutation was detected in 15 (41.67%) (Table 4).

A family study found that the proband's parents had normal hearing in 15 pedigrees (Table 5). Pedigrees 1, 2, and 14 exhibited *GJB2* mutations, with pedigree 2 exhibiting a heterozygous mutation in *GJB2* and pedigrees 1 and 14 exhibiting a compound heterozygous mutation in *GJB2*. These 3 pedigrees accounted for 20% of the 15 pedigrees. *SLC26A4* gene mutations were found in 9 pedigrees: 3, 6, 7, 8, 9, 10, 11, 12, and 13 (including pedigrees 6, 7, 8, 9, and 13 with a homozygous mutation and pedigrees 3, 10, 11, and 12 with a heterozygous mutation). These 9 pedigrees accounted for 4.5% of 60 pedigrees in total. Pedigrees with a double *GJB2*/*SLC26A4* heterozygous mutation (including a *GJB2*/235delC homozygous mutation in pedigree 5 and a double heterozygous *SLC2/6A4* mutation and double heterozygous mutations of *GJB2* 235delC and IVS7-2 A>G in pedigree 4) accounted for 13.33% of the pedigrees. Pedigree 15 had a *SLC26A4* 1229C>T and 2168A>G compound heterozygous mutation.

4. Discussion

Congenital deafness is one of the most common birth defects in humans, with an incidence of about 1 ‰ to 3 ‰ (*32*). This condition seriously affects an individual's quality of life. About 50-60% of these cases have a genetic cause. The cause is an autosomal recessive condition in 80% and an autosomal dominant condition in 10% to 20%. These conditions are sex-linked at a rate between 1% and 2%. Thus far, at least 44 deafness-related genes have been identified. The most common gene is *GJB2*, which is located in 13q11-12. In 1998, Xia *et al*. (*33*) first reported *GJB3*, a gene located in 1p33-35. A mutation in *SLC26A4*, which is located in q22-31.1, has been found to be associated with large vestibular conduit syndrome. A mutation in *12SrRNA* in mtDNA has been proven to be associated with drug-induced deafness. At present, strategies to prevent deafness universally include newborn hearing screening, which is one of the keys to the early detection and diagnosis of hearing impairment (*34*), and these strategies have achieved remarkable developments. Although genetic testing enables an estimation of the chance of reoccurrence, there are many other reasons why children with congenital hearing loss should undergo genetic evaluation and receive genetic counseling (*32*). Providers of genetic testing and counseling services have an important role to play in reducing hearing loss in newborns and young children.

Genetic screening detected mutations in 137 (43.08%) of 318 students. Genetic mutations were identified in 13 of 51 deaf children (25.49%) in Qianghu, Anhui (*35*). Gene mutations at 9 sites of 4 genes, including *GJB2*, *GJB3*, *SLC26A4*, and *12SrRNA*, were found in a total of 22 (34.4%) of 64 patients with nonsyndromic hearing impairment in Henan (*36*). Furthermore, patients in Shanxi and Liaoning provinces tested positive for mutations in *SLC26A4*, *GJB2*, or *12SrRNA* 1555 A>G/1494C>T at a rate of 33.3% and 42.5%, respectively (*37,38*). Thus, research has shown that the rate of mutations in deafness genes is higher in Hebei than in other regions. In the current study, mutations in *SLC26A4* were identified in 25.47% of students (81/318) with hearing impairment in Hebei of China. Thirty of the 81 students had 2 mutant alleles while 51 had 1 mutant allele. The most common mutation was IVS7-2A>G. The spectrum of *SLC26A4* mutations in Hebei is similar to that reported in the overall Chinese population, with IVS7-2A>G being a hotspot mutation. In Japan, H723R is the most prevalent mutation (*16*). In South Korea, IVS7-2A>G and H723R are the two most prevalent (*39*). A recent study of 109 unrelated probands with enlarged vestibular aqueducts in a Danish population found that the most frequent mutation was 1246A>C. This implies that Danish and Chinese populations are of different ancestry (*40*). In the current study, *GJB2* gene mutations were detected in 54 students (16.98%). 35delG is most common mutation in Caucasians. Yuan *et al*. (*41*) reported that 235delC accounted for 71.64% of *GJB2* mutant alleles in China, and this figure agrees with the findings of the current study (71.43%, 50/70 students). This mutation is detected at the highest rates in Asian populations, with a prevalence of approximately 41% and 57%

Table 5. The relationship between 50 family mutations and deafness

Family	Patient	Family member	Gene	Mutational bits	Mutational types	Items
1	II_2		*GJB2*	235delC, 299-300delAT	Heterozygous mutation	Some remaining hearing
		I_1		235delC	Heterozygous mutation	Normal hearing
		I_2		299-300delAT	Heterozygous mutation	Normal hearing
2	III_1		*GJB2*	299-300delAT	Heterozygous mutation	Passed
		I_1		235delC, 299-300delAT	Heterozygous mutation	Normal hearing
		I_2		235delC	Heterozygous mutation	Normal hearing
		I_3		299-300delAT	Heterozygous mutation	Normal hearing
		II_1		235delC, 299-300delAT	Heterozygous mutation	Some remaining hearing
		II_2		299-300delAT	Heterozygous mutation	serious hearing
3	II_1		*SLC26A4*	IVS7-2A>G	Heterozygous mutation	Nerve deafness
		II_2		IVS7-2A>G	Heterozygous mutation	Nerve deafness
		II_3		IVS7-2A>G	Heterozygous mutation	Nerve deafness
		I_1		Negative		Normal hearing
		I_2		IVS7-2A>G	Heterozygous mutation	Nerve deafness
4	II_7		*GJB2/SLC26A4*	235delC, IVS7-2A>G	Heterozygous mutation	serious hearing
		I_4	*GJB2/SLC26A4*	235delC, IVS7-2A>G	Heterozygous mutation	Normal hearing
		I_3	*SLC26A4*	1226G>A	Heterozygous mutation	Normal hearing
		I_5	*GJB2*	235delC	Heterozygous mutation	Normal hearing
		I_6	*SLC26A4*	IVS7-2A>G	Heterozygous mutation	Normal hearing
		II_6	*GJB2/SLC26A4*	235delC, 1226G>A	Heterozygous mutation	Normal hearing
		II_8		Negative		serious hearing
		III_3	*SLC26A4*	1226G>A	Heterozygous mutation	Normal hearing
		III_4		IVS7-2A>G	Heterozygous mutation	Normal hearing
5	II_2		*GJB2/*	235delC	Homozygous mutation	Mid-serious deafness
		$I_{1,2}$	*SLC26A4*	IVS7-2A>G	Heterozygous mutation	Mid-serious deafness
		II_1		Negative		Normal hearing
		III_1		Negative		Some remaining hearing
			GJB2	235delC	Heterozygous mutation	Normal hearing
6-7	II_1		*SLC26A4*	IVS7-2A>G	Homozygous mutant	Severe deafness
		$I_{1,2}$		IVS7-2A>G	Heterozygous mutation	Normal hearing
8	II_1			IVS7-2A>G	Homozygous mutant	Severe deafness
		I_1		IVS7-2A>G	Heterozygous mutation	Normal hearing
		I_2		Negative		Normal hearing
9	II_1			IVS7-2A>G	Homozygous mutant	serious hearing
		$I_{1,2}$		IVS7-2A>G	Heterozygous mutation	Normal hearing
10	II_1			IVS7-2A>G	Heterozygous mutation	Nerve deafness
		I_1		Negative		Normal hearing
		I_2		IVS7-2A>G	Heterozygous mutation	Normal hearing
11	II_1			IVS7-2A>G	Heterozygous mutation	Nerve deafness but ability to hear speech
		I_1		1229C>T	Heterozygous mutation	Normal hearing
		I_2		IVS7-2A>G	Heterozygous mutation	Normal hearing
12	II_1			IVS7-2A>G	Heterozygous mutation	Severe nerve deafness
		$I_{1,2}$		Negative		Normal hearing
13	II_1			IVS7-2A>G	Homozygous mutant	Severe nerve deafness
		I_1		IVS7-2A>G	Heterozygous mutation	Normal hearing
		I_2		IVS7-2A>G	Heterozygous mutation	Normal hearing
14	II_1		*GJB2*	235delC 299-300delAT	Heterozygous mutation	Severe nerve deafness
		I_1		299-300delAT	Heterozygous mutation	Normal hearing
		I_2		235delC	Heterozygous mutation	Normal hearing
15	II_1		*SLC26A4*	1229C>T and 2168A>G	Heterozygous mutation	Very severe hearing loss
		I_1		2168A>G	Heterozygous mutation	Normal hearing
		I_2		1229C>T	Heterozygous mutation	Normal hearing

according to 2 Japanese studies, 67% according to 1 Taiwanese study, and 73% according to 1 South Korean study (*41-46*). *12SrRNA* mutations were detected in 2 students (0.63%), although this rate is lower than their prevalence nationally (2.83%) (*47*). No *GJB3* mutations were noted in students.

Studies have shown that the *SLC26A4* gene, the *GJB2* gene, and the mitochondrial *12SrRNA* gene are prominent mutations that lead to most of the hereditary deafness in Asia. Screening for mutations in these 3

genes is crucial to identifying nonsyndromic hereditary hearing loss and drug-induced deafness. The current study identified 15 pedigrees of mutations. Genetic testing can provide a scientific basis for guiding and advising deaf patients and their family members. One example is the members of pedigree 4. Two individuals were lovers who sought consultation before marriage (*48*). The first question they asked was whether they could get married. The second question was whether their child would be deaf like them. They consented to undergo genetic testing. The woman's test results were negative for mutations while the man's *GJB2* genetic testing revealed double heterozygous mutations of 235delc and *SLC26A4* IVS7-2 A>G. Given a scientific estimate regarding the potential for them to have a hearing child, they decided to marry and gave birth to a baby girl with normal hearing. When the gene chip was used to detect 9 loci of 4 genes, it only found *GJB2* and *SLC26A4* gene mutations, but subsequent time-of-flight mass spectrometry found 20 mutations (4 gene loci) in 11 students (3.46%). The S*LC26A4* gene mutation of 1226 G> A was detected in 3 students, the gene mutation of 1229 C>T was detected in 7, and the gene mutation of 2027 T>A was detected in 1. Genetic information can provide more comprehensive information for genetic counseling. Thus, this study suggests that high-risk families should choose 2 methods of genetic testing to avoid a false negative for mutations.

This study detected polymorphisms of the *GJB2* gene. Four polymorphisms were detected in 36 students, including heterozygous mutations in 109 G>A in 1 (2.78%) and in 79 G>A in 22 (61.11%). Homozygous mutations were detected in 1 student. Heterozygous and homozygous mutations in 341A>G were detected in 14 students (38.89%). A heterozygous mutation in 608 T>C was detected in 4 students (11.11%). A study by Liu *et al*. (*49*) found that 79G>A, 341A>G, 109G>A, and 608T>C were common polymorphisms in the Dai and Han ethnic groups. A study by Li *et al*. (*50*) detected *GJB2* mutations in neonates and identified the 4 types of mutation they considered to be polymorphisms. Other studies have shown that 79G>A, 341A>G, and 608T>C are found in the general population but do not cause deafness (*51-53*). The current study found that these changes are common *GJB2* gene polymorphisms. A change in genetic polymorphism means that the structure of DNA molecules changes in an individual in a population, but the aspects of gene expression and gene function remain the same. A change in polymorphism is a normal phenomenon, spontaneously occurring at a rate of around 1% (*54*). Currently, the 109 G>A mutation is assumed to be a mutation resulting in substitution of G for A. A point mutation will result in substitution of the encoded amino acid (valine replaced with isoleucine). However, the issue of whether a mutation substituting G for A at locus 109 of the *GJB2* gene can directly lead to hereditary deafness remains controversial in domestic and foreign literature. Kelly *et al*. (*55*) has detected the 109 G>A mutation in normal populations, implying that the polymorphic change does not lead directly to hereditary deafness. However, a study by Abe *et al*. (*45*) in Japan reached the opposite conclusion. There is no clear consensus in academic circles on whether a mutation at a specific locus leads to hereditary deafness. Thus, whether the 109 G>A mutation in *GJB2* leads to deafness must be studied further.

Hereditary deafness is a common form of severe hearing loss. Genetic testing is a useful way to dispel misinformation or alleviate concerns that parents have about what may have caused hearing loss. Although gene screening plays an important role in decreasing the birth rate of deaf infants, many problems still need to be solved, such as the widespread shortage of technical personnel in genetic testing laboratories. Another problem is the lack of guidelines indicating whether genetic testing for deafness should be performed prior to marriage, prior to pregnancy, prior to birth, or after birth. The lack of solutions constrains the development of methods of genetic screening for deafness. Under current conditions, genetic screening still has a long way to go to facilitate the detection of deafness genes. In other words, the development of prenatal diagnosis and genetic counseling will greatly reduce the birth rate of deaf children, decrease the number of deaf patients, improve the quality of births, and reduce the social and family burden of deafness.

References

1. Corlin RJ, Toriello HV, Cohen MM. Hereditary hearing loss and its syndrome. New York: Oxford University Press, NY, USA, 1995.
2. Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, Mueller RF, Leigh IM. Connexin 26 mutations in hereditary nonsyndromic sensorineural deafness. Nature. 1997; 387:80-83.
3. Seeman P, Bendova O, Raskova D, Malikova M, Groh D, Kabelka Z. Double heterozygosity with mutations involving both the *GJB2* and *GJB6* genes is a possible, but very rare, cause of congenital deafness in the Czech population. Ann Hum Genet. 2005; 69:9-14.
4. Oguchi T, Ohtsuka A, Hashimoto S, Oshima A, Abe S, Kobayashi Y, Nagai K, Matsunaga T, Iwasaki S, Nakagawa T, Usami S. Clinical features of patients with *GJB2* (connexin 26) mutations: Severity of hearing loss is correlated with genotypes and protein expression patterns. J Hum Genet. 2005; 50:76-83.
5. Tekin M, Xia XJ, Erdenetungalag R, Cengiz FB, White TW, Radnaabazar J, Dangaasuren B, Tastan H, Nance WE, Pandya A. *GJB2* mutations in Mongolia: Complex alleles, low frequency, and reduced fitness of the deaf. Ann Hum Genet. 2010; 74:155-164.
6. Ohtsuka A, Yuge I, Kimura S, Namba A, Abe S, Van Laer L, Van Camp G, Usami S. *GJB2* deafness gene shows a specific spectrum of mutations in Japan, including a

frequent founder mutation. Hum Genet. 2003; 112:329-333.
7. Snoeckx RL, Huygen PL, Feldmann D, *et al*. *GJB2* mutations and degree of hearing loss: A multicenter study. Am J Hum Genet. 2005; 77:945-957.
8. Morell RJ, Kim HJ, Hood LJ, Goforth L, Friderici K, Fisher R, Van Camp G, Berlin CI, Oddoux C, Ostrer H, Keats B, Friedman TB. Mutations in the connexin 26 gene (*GJB2*) among Ashkenazi Jews with nonsyndromic recessive deafness. N Engl J Med. 1998; 339:1500-1505.
9. Yu F, Han DY, Dai P, Kang DY, Zhang X, Liu X, Zhu QW, Yuan YY, Sun Q, Xue DD, Li M, Liu J, Yuan HJ, Yang WY. Mutation of the *GJB2* gene in Chinese patients with nonsyndromic hearing impairment: Analysis of 1,190 cases. Natl Med J China. 2007; 87:2814-2819. (in Chinese)
10. Grifa A, Wagner CA, D'Ambrosio L, Melchionda S, Bernardi F, Lopez-Bigas N, Rabionet R, Arbones M, Monica MD, Estivill X, Zelante L, Lang F, Gasparini P. Mutations in *GJB6* cause nonsyndromic autosomal dominant deafness at DFNA3 locus. Nat Genet. 1999; 23:16-18.
11. Yoshino T, Sato E, Nakashima T, Teranishi M, Yamamoto H, Otake H, Mizuno T. Distribution of pendrin in the organ of corti of mice observed by electron immunomicroscopy. Eur Arch Otorhinolaryngol. 2006; 263:699-704.
12. Wilcox SA, Saunders K, Osborn AH, Arnold A, Wunderlich J, Kelly T, Collins V, Wilcox LJ, McKinlay Gardner RJ, Kamarinos M, Cone-Wesson B, Williamson R, Dahl HH. High frequency hearing loss correlated with mutations in the *GJB2* gene. Hum Genet. 2000; 106:399-405.
13. Prasad S, Kolln KA, Cucci RA, Trembath RC, Van Camp G, Smith RJ. Pendred syndrome and DFNB4-mutation screening of *SLC26A4* by denaturing high-performance liquid chromatography and the identification of eleven novel mutations. Am J Med Genet A. 2004; 124:1-9.
14. Albert S, Blons H, Jonard L, Feldmann D, *et al*. *SLC26A4* gene is frequently involved in nonsyndromic hearing impairment with enlarged vestibular aqueduct in Caucasian populations. Eur J Hum Genet. 2006; 14:773-779.
15. Wang QJ, Zhao YL, Rao SQ, Guo YF, Yuan H, Zong L, Guan J, Xu BC, Wang DY, Han MK, Lan L, Zhai SQ, Shen Y. A distinct spectrum of *SLC26A4* mutations in patients with enlarged vestibular aqueduct in China. Clin Genet. 2007; 72:245-54.
16. Park HJ, Shaukat S, Liu XZ, *et al*. Origins and frequencies of *SLC26A4* (PDS) mutations in East and South Asians: Global implications for the epidemiology of deafness. J Med Genet. 2003; 40:242-248.
17. Yao G, Li S, Chen D, *et al*. Compound heterozygous mutations of *SLC26A4* in 4 Chinese families with enlarged vestibular aqueduct. Int J Pediatr Otorhinolaryngol. 2013; 77:544-549.
18. Jiang L, Feng Y, Chen H, He C, Mei L. An investigation of *SLC26A4* gene mutation in nonsydromic hearing impairment in Hunan province of China. Journal Clinical Otorhinolaryngology and Head and Neck Surg. 2010; 24:587-591. (in Chinese)
19. Huang S, Han D, Yuan Y, Wang G, Kang D, Zhang X, Yan X, Meng X, Dong M, Dai P. Extremely discrepant mutation spectrum of *SLC26A4* between Chinese patients with isolated Mondini deformity and enlarged vestibular aqueduct. J Transl Med. 2011; 9:167.
20. Xin F, Yuan Y, Deng X, Han M, Wang G, Zhao J, Gao X, Liu J, Yu F, Han D, Dai P. Genetic mutations in patients of Chinese minority groups and the Han ethnic group with nonsyndromic deafness in Yunnan, China. J Transl Med. 2013; 11:312. (in Chinese)
21. Fischel-Ghodsian N. Mitochondrial genetics and hearing loss: The missing link between genotype and phenotype. Proc Soc Exp Biol Med. 1998; 218:1-6.
22. Hutchin TP, Cortopassi GA. Mitochondrial defects and hearing loss. Cell Mol Life Sci. 2000; 57:1927-1937.
23. Zhao H, Li R, Wang Q, Yan Q, Deng JH, Han D, Bai Y, Young WY, Guan MX. Maternally inherited aminoglycoside-induced and nonsyndromic deafness is associated with the novel C1494T mutation in the mitochondrial *12S rRNA* gene in a large Chinese family. Am J Hum Genet. 2004; 74:139-152.
24. Castillo FJ, Rodriguez-Ballesteros M, Martin Y, Arellano B, Gallo-Teran J, Morales-Angulo C, Ramirez-Camacho R, Cruz Tapia M, Solanellas J, Martinez-Conde A, Villamar M, Moreno-Pelayo MA, Moreno F, Del Castillo I. Heteroplasmy for the 1555A>G mutation in the mitochondrial *12S rRNA* gene in six Spanish families with non-syndromic hearing loss. J Med Genet. 2003; 40:632-636.
25. Li R, Xing G, Yan M, Cao X, Liu XZ, Bu X, Guan MX. Cosegregation of C-insertion at position 961 with the A1555G mutation of the mitochondrial *12S rRNA* gene in a large Chinese family with maternally inherited hearing loss. Am J Med Genet A. 2004; 124:113-117.
26. Liu X, Dai P, Huang DL, Yuan HJ, Li WM, Cao JY, Yu F, Zhang RN, Lin HY, Zhu XH, He Y, Yu YJ, Yao K. Large-scale screening of mtDNA A1555G mutation in China and its significance in prevention of aminoglycoside antibiotic induced deafness. Zhong hua Yi Xue Za Zhi. 2006; 86:1318-22. (in Chineset)
27. Usami S, Abe S, Akita J, Namba A, Shinkawa H, Ishii M, Iwasaki S, Hoshino T, Ito J, Doi K, Kubo T, Nakagawa T, Komiyama S, Tono T, Komune S. Prevalence of mitochondrial gene mutations among hearing impaired patients. J Med Genet. 2000; 37:38-40.
28. Malik SG, Pieter N, Sudoyo H, Kadir A, Marzuki S. Prevalence of the mitochondrial DNA A1555G mutation in sensorineural deafness patients in island Southeast Asia. J Hum Genet. 2003; 48:480-483.
29. Kupka S, Toth T, Wrobel M, Zeissler U, Szyfter W, Szyfter K, Niedzielska G, Bal J, Zenner HP, Sziklai I, Blin N, Pfister M. Mutation A1555G in the *12S rRNA* gene and its epidemiological importance in German, Hungarian, and Polish patients. Hum Mutat. 2002; 19:308-309.
30. Ostergaard E, Montserrat-Sentis B, Gronskov K, Brondum-Nielsen K. The A1555G mtDNA mutation in Danish hearing-impaired patients: Frequency and clinical signs. Clin Genet. 2002; 62:303-305.
31. Tekin M, Duman T, Bogoclu G, Incesulu A, Comak E, Fitoz S, Yilmaz E, Ilhan I, Akar N. Frequency of mtDNA A1555G and A7445G mutations among children with prelingual deafness in Turkey. Eur J Pediatr. 2003; 162:154-158.
32. White KR. Early hearing detection and intervention programs: Opportunities for genetic services. AJMG. 2004; 130A:29-36.
33. Xia JH, Liu CY, Tang BS, *et al*. Mutations in the gene encoding gap junction Protein beta-3 associated with

autosomal dominant hearing impairment. Nat Genet. 1998; 4:370-373.
34. Burton SK, Withrow K, Arnos KS, Kalfoglou AL, Pandya A. A focus group study of consumer attitudes toward genetic testing and newborn screening for deafness. Genet Med. 2006; 8:779-783.
35. Liu SF, Bu LN, Wu LJ, Wang CX, Feng G. Analysis of common genetic mutations in 51 patients with profound deafness. Anhui Medical Journal. 2014; 35:566-568. (in Chinese)
36. Zhao HR, Liao SX, Ding XB, Wang L, Yang K. Use of a gene chip in the genetic analysis of 64 patients of the Han ethnic group with nonsyndromic hearing impairment (NSHI) in Henan Province. Journal Shanxi Medical University. 2012; 43:649-651. (in Chinese)
37. Hu ZP, Li J, Li XZ, Zhang Y, Liang F, Wei W. Genetic testing of 108 deaf children at a school in Changzhi. Chinese J Birth Health & Heredity. 2014; 22:42-44.
38. Qu CH, Zhang N, Cao DH, Hao DM. Screening for common deafness genes in 240 deaf patients. Acad J Chin PLA Med Sch. 2014; 35:1019-1021. (in Chinese)
39. Tsukamoto K, Suzuki H, Harada D, Namba A, Abe S, Usami S. Distribution and frequencies of PDS (*SLC26A4*) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: A unique spectrum of mutations in Japanese. Eur J Hum Genet. 2003; 11:916-922.
40. Rendtorff N, Schrijver I, Lodahl M, Rodriguez-Paris J, Johnsen T, Hansen E, Nickelsen L, Tumer Z, Fagerheim T, Wetke R, Tranebjaerg L. *SLC26A4* mutation frequency and spectrum in 109 Danish Pendred syndrome/DFNB4 probands and a report of nine novel mutations. Clin Genet. 2013; 84:388-391.
41. Yuan YY, You YW, Huang DL, Cui JH, Wang Y, Wang Q, Yu F, Kang DY, Yuan HJ, Han DY, Dai P. Comprehensive molecular etiology analysis of nonsyndromic hearing impairment from typical areas in China. Journal of Translational Medicine. 2009; 7:79.
42. Park HJ, Hahn SH, Chun YM, Park K, Kim HN. Connexin26 mutations associated with nonsyndromic hearing loss. Laryngoscope. 2000; 110:1535-1538.
43. Ohtsuka A, Yuge I, Kimura S, Namba A, Abe S, Van Laer L, Van Camp G, Usami S. *GJB2* deafness gene shows a specific spectrum of mutations in Japan, including a frequent founder mutation. Hum Genet. 2003; 112:329-333.
44. Hwa HL, Ko TM, Hsu CJ, Huang CH, Chiang YL, Oong JL, Chen CC, Hsu CK. Mutation spectrum of the connexin 26 (*GJB2*) gene in Taiwanese patients with prelingual deafness. Genet Med. 2003; 5:161-165.
45. Abe S, Usami S, Shinkawa H, Kelley PM, Kimberling WJ. Prevalent connexin 26 gene (GJB2) mutations in Japanese. J Med Genet. 2000; 37:41-43.
46. Wang YC, Kung CY, Su MC, Su CC, Hsu HM, Tsai CC, Lin CC, Li SY. Mutations of Cx26 gene (*GJB2*) for prelingual deafness in Taiwan. Eur J Hum Genet. 2002; 10:495-498.
47. Dai P, Liu X, Yu F, Zhu QW, Yuan YY, Yang SZ, Sun Q, Yuan HJ, Yang WY, Huang DL, Han DY. Molecular etiology of patients with nonsyndromic hearing loss from deaf-mute schools in 18 provinces of China. Chin J Otol. 2006; 1:1-5. (in Chinese)
48. Cao QY, Ge J, Zhang WX, Zhang N, Ma HQ, Guo SH, Zhu JZ. An analysis of premarital genetic counseling for and the fertility of the deaf. Chinese Journal Birth Health & Heredity. 2013; 11:79-88. (in Chinese)
49. Liu DS, Wang JL, Sa YL, Yan XM, Wang XJ, Shen T, Peng ZG. Analysis of the *GJB2* gene in non-syndromic deaf patients of the Dai and Han ethnic groups. Journal of Kunming Medical University, 2012; 10:49-52. (in Chinese)
50. Li LQ, Tan JJ, Zhou Y, Yu JL. Does congenital cytomegalovirus infection lead to hearing loss by inducing mutation of the *GJB2* gene? Pediatr Res. 2013; 74:121-126.
51. Dai P, Yu F, Han B, *et al*. *GJB2* mutation spectrum in 2,063 Chinese patients with nonsyndromic hearing impairment. J Transl Med. 2009; 7:26
52. Hilgert N, Smith RJ, Van Camp G. Forty-six genes causing nonsyndromic hearing impairment: Which ones should be analyzed in DNA diagnostics? Mutat Res. 2009; 681:189-96.
53. Wang C, Cai X, Zou G, Tie L, Li Y. Genetic mutations of *GJB2* in non-syndromic deaf patients of the Kazak ethnic group in Xinjiang. Journal Clinical Otorhinolaryngology and Head and Neck Surg. 2014; 28:676-679. (in Chinese)
54. Fei Yu, Pu Dai, Dongyi Han. *GJB2* gene mutations and hereditary non-syndromic deafness prior to speech. Foreign Medical Sciences: Otolaryngology. 2005; 6:359-361. (in Chinese)
55. Kelly PM, Harris DJ, Comer BC, Askew JW, Fowler T, Smith SD, Kimberling WJ. Novel mutations in the connexin 26 gene (*GJB2*) that cause autosomal recessive (DFNBI) hearing loss. Am J Hum Genet. 1998; 62:792-799.

(Received May 19, 2015; Revised July 26, 2015; Accepted August 5, 2015)

Case Report

DOI: 10.5582/irdr.2015.01028

Psychosis and catatonia in fragile X: Case report and literature review

Tri Indah Winarni[1,2], Andrea Schneider[1,3], Neera Ghaziuddin[4], Andreea Seritan[5], Randi J Hagerman[1,3,*]

[1] *MIND Institute, University of California Davis, Medical Center, Sacramento, USA;*
[2] *Center for Biomedical Research (CEBIOR), Faculty of Medicine Diponegoro University, Semarang, Indonesia;*
[3] *Department of Pediatrics, University of California Davis, Medical Center, Sacramento, USA;*
[4] *University of Michigan Hospitals and Health Center, Ann Arbor, USA;*
[5] *Department of Psychiatry and Behavioral Sciences, University of California Davis, Medical Center, Sacramento, USA.*

Summary **Fragile X mental retardation 1 (*FMR1*) premutation associated phenotypes have been explored extensively since the molecular mechanism emerged involving elevated *FMR1* messenger ribonucleic acid (mRNA) levels. Lowered fragile X mental retardation protein (FMRP) can also occur which may have an additive effect to the high levels of mRNA leading to neurodevelopmental problems and psychopathology. This paper was aimed to review psychosis and catatonia in premutation carriers, express the role of elevated *FMR1* mRNA and lowered FMRP in the phenotype of carriers and present a case of psychosis and catatonia in a carrier. This case also demonstrates additional genetic and environmental factors which may also affect the phenotype. We review the literature and report an exemplary case of a 25 year old male premutation carrier with elevated *FMR1* mRNA, low FMRP, a cytochrome P450 family 2 subfamily D polypeptide 6 (*CYP2D6*)*2xN* mutation and a perinatal insult. This patient developed an autism spectrum disorder, psychosis, catatonia with subsequent cognitive decline after electro-convulsive therapy (ECT) for his catatonia. He had a premutation of 72 CGG repeat in *FMR1*, *FMR1* mRNA level that was over 2.4 times normal and FMRP level at 18% of normal, and additionally, a CYP2D6 allelic variant which leads to ultrarapid metabolism (UM) of medication. There is an overlapping pathophysiological mechanism of catatonia and fragile X-associated premutation phenotypes including autism and psychosis. This case demonstrates the shared phenotype and the overlap of the pathophysiological mechanisms that can influence the intervention. Multiple genetic and environmental hits can lead to more significant involvement in premutation carriers.**

Keywords: Catatonia, fragile X syndrome, premutation, psychosis

1. Introduction

Fragile X-associated premutation disorders represent a wide spectrum of clinical manifestations including neurodevelopmental disorder, autism spectrum disorder (ASD), attention deficit hyperactivity disorder (ADHD); a neurodegenerative disorder, fragile X-associated tremor ataxia syndrome (FXTAS); neuropsychiatric disorders (depression, anxiety); and reproductive disorders (fragile X-associated premature ovarian insufficiency (FXPOI)) (*1-5*). The prevalence of the premutation is high in the general population and estimated approximately at 1:200 in females and 1:450 in males (*6,7*). In premutation carriers, in whom the expansion of CGG repeats in the promoter region of *FMR1* gene is 55-200, the *FMR1* gene remains active and demonstrates an increase in transcriptional activity, thus leading to increased *FMR1* mRNA levels up to 8-fold higher than in the normal range with 5-44 CGG repeats (*8-10*). The elevated *FMR1* mRNA causes toxicity because the hairpin formation in the CGG

**Address correspondence to:*
Dr. Randi J. Hagerman, MIND Institute, UC Davis Health System, 2825 50th Street, Sacramento, CA 95817, USA.
E-mail: rjhagerman@ucdavis.edu

expansion sequesters important proteins needed for normal cellular function (*11*). In those with FXTAS there is inclusion formation in both neurons and astroglial cells in the brain and in the peripheral nervous system and these inclusions have the excess *FMR1* mRNA, fragile X mental retardation protein (FMRP) and many other proteins and neurofilaments (*11-13*).

Decreased levels of FMRP are observed in some premutation carriers due to reduced translational efficiency of *FMR1* mRNA containing the expanded CGG repeat (*14-16*). Lowered FMRP in the premutation range will often lead to neurodevelopmental problems including intellectual disability (ID), ASD and ADHD (*1*), although additional environmental or medical problems such as seizures, trauma, toxins or additional genetic mutations may also cause further developmental problems in these carriers (*17-19*).

FMRP is a mRNA-binding protein that regulates hundreds of mRNA targets at the synapse and it inhibits protein translation that is stimulated by metabotropic glutamate receptors (mGluRs) (*20,21*). The lack of FMRP will cause down-regulation of the gamma-amino butyric acid (GABA) A and B receptors and up-regulation of metabotropic glutamate receptor 5 (mGluR5) throughout the brain (*22-24*). FMRP is highly expressed in neurons and FMRP is critical for synaptic plasticity (*25,26*). Accordingly, the absence of this key protein in the synapse irreversibly alters neuronal connectivity to produce significant behavioral disorders including ID, ASD schizophrenia, bipolar disorder, and major depression (*27-29*). Therefore, the role of FMRP in the synapse affects not only fragile X-associated disorders but other neuropsychiatric disorders such as schizophrenia in those without an *FMR1* mutation (*30,31*). Moreover, Aziz *et al*. (*32*) reported that *FMR1* expanded CGG repeat of premutation and gray zone alleles (45-54 repeats), may demonstrate some clinical features of fragile X syndrome (FXS) in those who presented clinically. It is uncertain if the gray zone allele has FMRP deficits but the premutation demonstrates FMRP deficits that increase according to the CGG repeat number increases (*33*).

Catatonia is a neuropsychiatric syndrome characterized by abnormalities of movement, speech, functional skills and behavior and most commonly associated with mood disorders, psychotic disorders, ASD or other medical conditions, in the absence of psychiatric illness (*34-37*). Its historical association with schizophrenia is now widely regarded as an erroneous tradition, and has been revised in the most recent version of the Diagnostic and Statistical Manual (DSM) for psychiatric disorders, 5th edition (*38*). The central symptom of catatonia is disturbance of motor activity (overall increased activity, reduced activity or mixed) and a variety of other abnormal movements (*e.g*. reduced eye blink rate, grimacing, sudden cessation of movements or immobility) (*39*). Clinical diagnosis of catatonia requires at least 3 of the following symptoms: stupor (no psychomotor activity), catalepsy (maintaining a passively induced posture), waxy flexibility (slight, even resistance to positioning by the examiner), mutism, negativism (opposition or no response to instructions or external stimuli), posturing (spontaneous and active maintenance of a posture against gravity), mannerisms, stereotypies, agitation, grimacing, echolalia or echopraxia (*38*).

There are three neurochemical alterations that basically underlie the mechanism of catatonia *i.e*. dopamine hypoactivity, GABA hypoactivity, and glutamate hyperactivity (*40,41*). The second and the third alteration are similar to the FMRP deficient phenotype that is seen in the majority of FXS and the minority of fragile X-associated disorders individuals. However, dopamine dysfunction also occurs in those with FXS leading to ADHD in childhood (*42*). Aging individuals with FXS and those with FXTAS often have Parkinsonian symptoms related to dopamine dysfunction (*43,44*). Catatonia is a heterogeneous condition with discriminate subtypes of pathophysiological mechanisms, consequently, multiple agents may be required to treat acute catatonia, maintain or prevent the reoccurrence of chronic catatonia (*41*). However, at this stage benzodiazepines and electroconvulsive therapy (ECT) remain the most effective treatments of catatonia (*45*).

Psychiatric spectrum disorders have been discovered associated with fragile X-associated disorders include FXS and fragile X premutation (FXPM) since the 1990s ranging from mild to severe, such as hypersensitivity to stimuli, hyperarousal, inattention, hyperactivity, explosive and aggressive behavior, ASD, social anxiety, depression, mood/bipolar disorders, and psychosis (*46-49*). The neurobiology of fragile X syndrome is relatively well defined, while scientists have struggled to understand the consistent neurobiology of ASD, the most common neurodevelopmental psychiatric disorder. In FXS, decreased levels of FMRP will result in the FXS phenotype due to impaired synaptic plasticity leading to the cognitive impairment, relatively constant behavioral abnormalities and ASD in the majority of patients (*50,51*). Psychosis is seen in less than 10% of those with FXS (*49*) and some cases suggest that those with mosaicism or a lack of methylation so that there is both lowered FMRP and elevated mRNA, often called a double hit, have a higher rate of psychosis (*52-57*). Psychosis combined with catatonia has not previously been described in those with FXS or in those with the premutation. Below is a case of a premutation male with both psychosis and catatonia.

2. Case Report

2.1. *Subject and setting*

A 25-year old male with the fragile X premutation,

who had been diagnosed with ADHD, ASD, bipolar disorder, obsessive compulsive disorder (OCD) and Tourette syndrome presented initially at age 20 years old to the Medical Investigation of Neurodevelopmental Disorders (MIND) Institute at UC Davis Medical Center. His CGG repeat size was 72, *FMR1* mRNA was 2.4 (± 0.23) times normal and his FMRP level was 18.3 (± 0.2) which is severely deficient. He was born at 32.5 weeks gestation (premature) and delivered by C-section. He was a second twin born; he weighed 4lb 8oz whereas his fraternal twin sister (fragile X negative) weighed 4lbs 2oz. He had respiratory distress, was intubated and was in the intensive care unit for 40 days. He suffered an intraventricular hemorrhage (IVH) that affected his frontal lobes, although subsequent MRIs in childhood were read as normal.

Although he was a good eater, he was developmentally delayed with sitting at 13.5 months, crawling at 14 months, walking at 16 months, saying words at 12 months and phrases at about 30 months. He received speech and language therapy at age 2 because of language delay. He had a variety of autistic features such as memorizing names, phone numbers, neighborhood license plate numbers, poor eye contact, stereotypies and was diagnosed with a pervasive developmental disorder not other specified (PDD NOS). His psychiatrist diagnosed Tourette syndrome and obsessive compulsive disorder (OCD) because verbal and motor tics and obsessive symptoms developed when he was approximately 6 years old. He was also diagnosed with Bipolar Disorder when he was 10 years old. He had staring spells from 19 months through the ninth grade that were initially thought to be seizures. His electroencephalography (EEG) showed mild abnormalities and he was treated with valproate for both possible seizures and mood stabilization. His EEG was normal at age 18. Because of mood instability he was treated with lithium beginning in mid-adolescence, although he had an episode of lithium toxicity related to dehydration. He was also tried on multiple antipsychotics, risperidone, olanzapine, ziprasidone, asenapine, and eventually clozapine with very little benefit and many side-effects so they were all discontinued.

He had multiple psychiatric hospitalizations during his teenage and young adult life mainly related to behavior and emotional problems including mood instability, aggression, agitation and subsequently catatonia diagnosed at age 21 years old. At the time of his presentation with catatonia, some symptoms had been present for the past one year and included markedly increased motor activity with incessant pacing up to 5 or 6 hours each day , other abnormal movements (stereotyped finger movements, change in posture, episodic cessation of motor activity/freezing and grimacing), reduced speech, sudden and relatively unprovoked physical aggression, increased anxiety and obsessional preoccupations, diminished awareness of surroundings for personal safety, decline in skill level including inability to perform previously attained skills, and a delusional belief that his "father was John Lennon". There were no hallucinations noted and the delusion was reported to be associated with starting an antipsychotic agent. There was progressive weight loss with his catatonia with a total loss of 50 lbs over a one year period.

Pharmacogenetics examination found that he had a *CYP2D6*2xN* (duplication) indicating he was an ultrarapid metabolizer (UM) of medications, and developed akathisia (inner restlessness secondary to antipsychotics) and then he became mute. He received high doses of benzodiazepines (lorazepam) with partial improvement, followed by weekly ECT beginning at age 20, leading to gradual improvement of his catatonic symptoms. He received 21 bilateral treatments using the Monitored Electro Convulsive Therapy (MECTA) device (MECTA Corporation, Tualatin, Ore). After this treatment and improvement of his catatonia his ECT was gradually decreased and stopped for a year and restarted when his catatonic symptoms gradually reoccurred.

2.2. *Assessments, follow-up, and interventions*

He was examined at the MIND Institute, UC Davis Medical Center at age 20 and then subsequently at age 25 years old. He is a tall young man with a long face but his ears are not prominent, although his palate is high arched and his jaw is mildly prominent. He also has large testicles (40 to 45 ml bilaterally). He does not have tremor nor ataxia but he appears mildly sedated on his medications. He demonstrates poor eye contact and he speaks in a slow monotone voice. The Autism Diagnostic Observation Scale (ADOS) module 4 score falls in the autism range at age 25 with a significant worsening of his autism score since age 20. The patient's mother provided previous cognitive test results from outside assessments, which are included in Figure 1. Figure 1 gives an overview of the trajectory of his cognitive results.

The patient shows a significant decline in both

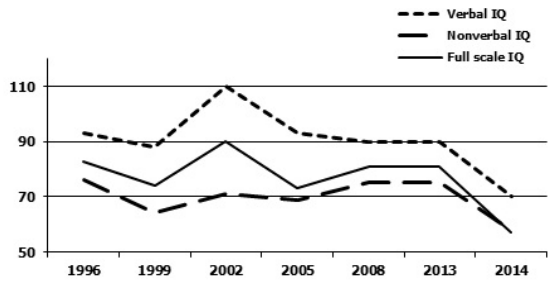


Figure 1. Overview of cognitive testing results. Trajectory of IQ testing 1996-2014, X-Axis shows the year the testing was done, the Y-axis shows the IQ score range (IQ scale: mean 100 and SD 15).

Table 1. Comparison of cognitive assessment sub-domains from 2005 and 2014

Items	2005 WISC-IV	2014 WAIS-IV
Verbal Comprehension[a]	93	70
Vocabulary	10	6
Similarities	9	3
Arithmetic	5	4[b]
Information	6	5
Comprehension	11	6
Perceptual Organization[a]	69	58
Picture completion	8	4
Block Design	6	6
Matrix Reasoning	7	1
Picture Arrangement	5	N/A
Visual Puzzles	N/A	2
Working Memory[a]	99	66
Digit span	9	4
Letter-Number Sequencing	Not reported	2
Processing Speed[a]	50	59
Digit Symbol Coding	4	1
Symbol search	Not reported	4

[a] Results given in Standard Score (Mean 100, SD 15), all other scores given in Scaled Scores (Mean 10, SD 3), [b] part of working memory in Wechsler Intelligence Scale for Children-IV (WAIS-IV).

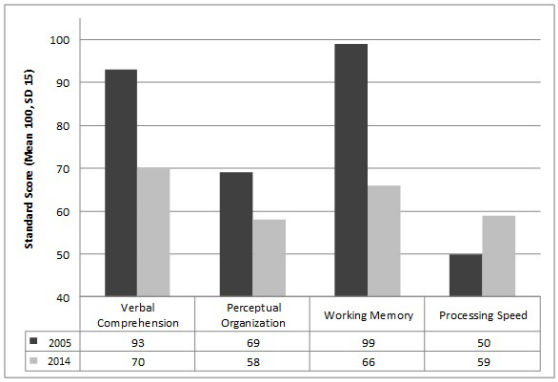


Figure 2. Comparison of IQ sub-domains from 2005 and 2014. The decreasing of three IQ sub-domains (verbal comprehension, perceptual organization, working memory) trajectory in 9 years.

verbal and nonverbal intellectual quotient (IQ) domains since age 20 (full scale IQ 81) compared to his current testing (full scale IQ 57) at age 25 (Figure 1). There is a recent significant drop in the full scale IQ score that is related to a decline in working memory capacity. From the literature, generally a decline in processing speed is reported during ECT (*58*), which could not be seen in our patient. The cognitive decline may or may not be directly related to the ECT, but his high level of benzodiazepine medication and sedation are likely contributing factors. Table 1 and Figure 2 give a more detailed overview of the different sub domains in the cognitive assessments. The patient was assessed with the Wechsler Intelligence Scale for Children-IV (WISC-IV) at age 15, and the WAIS-IV at age 25, both age-appropriate cognitive assessments with similar test-structure.

The psychiatric evaluation was based on the Structured Clinical Interview (SCI) for Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) (SCID) (*59*) and conducted with the patient and his mother. The SCID confirmed the previously established diagnoses of Bipolar Disorder type II (age of onset 10 years old), psychotic symptoms and OCD. Per mother, the patient's obsessions and compulsions started at age 2 (as previously described), with an exacerbation around age 14, when he exhibited contamination fears, excessive hand washing, and watching favorite movies over and over. His psychotic symptoms became apparent around age 14 and consisted of misidentification delusions (believing that familiar people are famous persons), grandiose delusions (believing he will win a large amount of money or that he will become famous), and nihilistic delusions (thinking that everybody in the world will die, with him being the sole survivor). His magnetic resonance imaging (MRI) is normal except that his corpus callosum is somewhat narrowed.

He is currently receiving maintenance ECT treatment at age 25, with the goal of decreasing frequency as tolerated. Lorazepam was switched to clonazepam (1 mg three times a day and 0.5 mg at 8 pm). He is currently also taking lithium 300 mg three times daily and thioridazine 42.5 mg three times a day (because of his psychotic thinking and he has not tolerated all other antipsychotics), in addition to melatonin 3 mg at bedtime.

3. Discussion

The patient presented here is an individual with the fragile X premutation with both elevated *FMR1* mRNA levels and a significant deficit of FMRP, termed a double hit. He has features of FXS including a long face, high narrow palate and macroorchidism which are seen with more significant developmental problems or cognitive deficits in carriers (*60*). He had a history of questionable seizures which is also associated with ASD in our previous studies of carriers (*17*). His birth history included hypoxia which lowers FMRP levels and an IVH with damage to the frontal lobes that could add additional problems including executive dysfunction to the premutation condition. In addition, he has significant psychosis which is occasionally seen in those with low FMRP and high mRNA, a double hit FMRP (*52*).

FMRP deficits are not only associated with the severity of ID in FXS (*29,61-64*), but are also seen in other neuropsychiatric disorders without an *FMR1* mutation including schizophrenia, ASD, OCD, mood disorders, major depressive disorder and bipolar disorder (*27,30,31*). There may be many proteins/micro

RNAs (miRNAs) that regulate the expression of FMRP in those without an *FMR1* mutation. Kovacs *et al*. (31) found that the age of onset of schizophrenia and the IQ correlates with the level of FMRP in blood in psychotic patients that do not have an *FMR1* mutation. It is likely that those with the premutation may be even more vulnerable to the effects of lowered FMRP since their neurons already die earlier in culture related to the RNA toxicity of elevated *FMR1* mRNA (*65*). Further studies of psychotic thinking in those with the premutation and in those with the full mutation are warranted especially when it is associated with catatonia.

Individuals with autism are at an increased risk to develop catatonia, which occurs in 17% of adolescents and young adults with ASD (*36,66*). Age appears to be the risk factor of catatonia in addition to stressful events, passivity in social situations, and impairment of expressive language skills (*66*). Catatonia is a severe neuropsychiatric syndrome with a 2.5% risk of developing malignant catatonia (label used when no documented exposure to antipsychotic agents) and Neuroleptic Malignant Syndrome (NMS; label used when known exposure to antipsychotics). There is a considerable risk of mortality in individuals who develop malignant catatonia/NMS which is known to present with severe functional impairment, autonomic and cardiovascular instability, reduced food and fluid intake resulting in dehydration, weight loss, multi-organ failure and other medical complications (*41*). The neurochemical pathology of catatonia includes decreased GABA activity and up-regulation of the glutamate system, both of which occur in those with a premutation and a full mutation (*22,67,68*).

His treatment history was complicated by the pharmacogenetic result of *CYP2D6*2P/2P* variant which cause UM of *CYP2D6* metabolized drugs. Prevalence rates of the UM phenotype in American Caucasians is reported to be low at 4.3% compared to those in Ethiopians (30%) and in Saudi Arabian (20%) (*69-71*). Acknowledged UM allelic variants are *CYP2D6*1,*2,*35*, and **41* duplicated and multiduplicated, among those, *CYP2D6*2* and **41* are the most frequent variants of *CYP2D6* gene that cause extremely increased enzyme activity, wherein the lack of drug response/treatment failure is the most common clinical consequences (*72*). He has homozygous *CYP2D6*2P/2P* (*CYP2D*2xN*) promoter polymorphism (two copies of the gene), which may explain why he had failed multiple drug treatments. The failure of treatment may also be associated with various behavioral and psychiatric problems including catatonia, Bipolar Disorder type II with severe mood lability, aggression and psychotic thinking.

The patient's catatonia did not respond completely to benzodiazepines alone but he had a robust response to ECT. ECT is a well-established treatment for catatonia across the age span including children and adolescents (*73,74*). This is the first report of catatonia and ECT therapy in a premutation carrier, although the neurochemical changes that occur in both FXS and in premutation carriers (lower GABA and elevated glutamate, specifically mGluR5 up-regulation because of an FMRP deficit) is likely to predispose to catatonia (*40*). We would suggest testing for the *FMR1* mutation or at least checking FMRP levels when they become clinically available for those who experience catatonia.

Although this patient responded well to ECT therapy it is of great concern that his IQ declined over time. The etiology of his cognitive decline is unclear. Generally a decline in processing speed reported during ECT (*58*), but not decline in IQ. Cognitive functions are known to recover once ECT is completed and the recovery appears to be irrespective of the age of the patient (*75*). A recent review by the Food Drug Administration (FDA) found that cognitive function recovery following ECT may take up to six months after the completion of ECT (*76*). Therefore, measuring cognitive functions during ongoing ECT is likely to identify deficits, which are expected to recover upon completion of the treatment while a global score such as intellectual functioning will likely be influenced by deficits in language, fluency, spatial orientation and memory. However, other causes of cognitive decline, such as chronic catatonia should also be considered as contributing factors (*77*). We know that seizures can worsen cognitive and behavioral aspects of FXS and seizures are associated with ASD in premutation carriers (*17*). In addition his relatively high dose of benzodiazepines and thioridazine may have deleterious cognitive effects and could lead to intermittent sedation and a lack of stimulation in his environment especially since he is not in school currently. We have recommended cognitive stimulation with digital programs and vocational rehabilitation. His history of IVH might contribute to his cognitive deficits, although it would not explain the recent decline.

Recent work by the Benke laboratory at the University of Colorado has demonstrated that seizures in early life in rats without an *FMR1* mutation will disrupt the FMRP/Akt complex causing FMRP to pull away from the dendrites and move to the cell body, thereby disrupting the development of synaptic plasticity (*78*). Of concern is what ECT therapy will do to FMRP levels in those at risk for lowered FMRP levels particularly those with an *FMR1* mutation, such as the patient presented here (*77*). Studies of animal models that undergo ECT will help to evaluate this concern. In addition further work is needed to understand the relationship between premutation involvement, psychosis, ASD and catatonia and the most optimal treatments for these problems.

Acknowledgements

This work was supported by National Institute of Health funding HD 036071 and the MIND Institute

Intellectual and Developmental Disabilities Research Center (U54 HD079125).We thank Prof. Flora Tassone from Department of Biochemistry and Molecular Medicine, School of Medicine, UC Davis, Davis, CA, for providing the molecular data and the family who participated to this study.

References

1. Farzin F, Perry H, Hessl D, Loesch D, Cohen J, Bacalman S, Gane L, Tassone F, Hagerman P, Hagerman R. Autism spectrum disorders and attention-deficit/hyperactivity disorder in boys with the fragile X premutation. J Dev Behav Pediatr. 2006; 27(2 Suppl):S137-S144.
2. Hagerman RJ, Greco C, Chudley A, Leehey M, Tassone F, Grigsby J, Hills J, Wilson R, Harris SW, Hagerman PJ. Neuropathology and neurodegenerative features in some older male premutation carriers of fragile X syndrome. Am J Hum Genet. 2001; A8 (Suppl 69):177.
3. Hunter JE, Leslie M, Novak G, Hamilton D, Shubeck L, Charen K, Abramowitz A, Epstein MP, Lori A, Binder E, Cubells JF, Sherman SL. Depression and anxiety symptoms among women who carry the *FMR1* premutation: Impact of raising a child with fragile X syndrome is moderated by *CRHR1* polymorphisms. Am J Med Genet B Neuropsychiatr Genet. 2012; 159B:549-559.
4. Sherman SL. Premature ovarian failure in the fragile X syndrome. Am J Med Genet. 2000; 97:189-194.
5. Hagerman R, Hagerman P. Advances in clinical and molecular understanding of the *FMR1* premutation and fragile X-associated tremor/ataxia syndrome. Lancet Neurol. 2013; 12:786-798.
6. Hantash FM, Goos DM, Crossley B, Anderson B, Zhang K, Sun W, Strom CM. *FMR1* premutation carrier frequency in patients undergoing routine population-based carrier screening: Insights into the prevalence of fragile X syndrome, fragile X-associated tremor/ataxia syndrome, and fragile X-associated primary ovarian insufficiency in the United States. Genet Med. 2011; 13:39-45.
7. Tassone F, Iong KP, Tong TH, Lo J, Gane LW, Berry-Kravis E, Nguyen D, Mu LY, Laffin J, Bailey DB, Hagerman RJ. *FMR1* CGG allele size and prevalence ascertained through newborn screening in the United States. Genome Med. 2012; 4:100.
8. Tassone F, Hagerman RJ, Taylor AK, Gane LW, Godfrey TE, Hagerman PJ. Elevated levels of *FMR1* mRNA in carrier males: A new mechanism of involvement in the fragile-X syndrome. Am J Hum Genet. 2000; 66:6-15.
9. Tassone F, Beilina A, Carosi C, Albertosi S, Bagni C, Li L, Glover K, Bentley D, Hagerman PJ. Elevated *FMR1* mRNA in premutation carriers is due to increased transcription. RNA. 2007; 13:555-562.
10. Tassone F, Adams J, Berry-Kravis EM, Cohen SS, Brusco A, Leehey MA, Li L, Hagerman RJ, Hagerman PJ. CGG repeat length correlates with age of onset of motor signs of the fragile X-associated tremor/ataxia syndrome (FXTAS). Am J Med Genet B Neuropsychiatr Genet. 2007; 144B:566-569.
11. Hagerman P. Fragile X-associated tremor/ataxia syndrome (FXTAS): Pathology and mechanisms. Acta Neuropathol. 2013; 126:1-19.
12. Greco CM, Hagerman RJ, Tassone F, Chudley AE, Del Bigio MR, Jacquemont S, Leehey M, Hagerman PJ. Neuronal intranuclear inclusions in a new cerebellar tremor/ataxia syndrome among fragile X carriers. Brain. 2002; 125:1760-1771.
13. Hunsaker MR, Greco CM, Spath MA, Smits AP, Navarro CS, Tassone F, Kros JM, Severijnen LA, Berry-Kravis EM, Berman RF, Hagerman PJ, Willemsen R, Hagerman RJ, Hukema RK. Widespread non-central nervous system organ pathology in fragile X premutation carriers with fragile X-associated tremor/ataxia syndrome and CGG knock-in mice. Acta Neuropathol. 2011; 122:467-479.
14. Kenneson A, Zhang F, Hagedorn CH, Warren ST. Reduced FMRP and increased *FMR1* transcription is proportionally associated with CGG repeat number in intermediate-length and premutation carriers. Hum Mol Genet. 2001; 10:1449-1454.
15. Primerano B, Tassone F, Hagerman RJ, Hagerman PJ, Amaldi F, Bagni C. Reduced *FMR1* mRNA translation efficiency in Fragile X patients with premutations. RNA. 2002; 8:1482-1488.
16. Tassone F, De Rubeis S, Carosi C, La Fata G, Serpa G, Raske C, Willemsen R, Hagerman PJ, Bagni C. Differential usage of transcriptional start sites and polyadenylation sites in *FMR1* premutation alleles. Nucleic Acids Res. 2011; 39:6172-6185.
17. Chonchaiya W, Au J, Schneider A, Hessl D, Harris SW, Laird M, Mu Y, Tassone F, Nguyen DV, Hagerman RJ. Increased prevalence of seizures in boys who were probands with the *FMR1* premutation and co-morbid autism spectrum disorder. Hum Genet. 2012; 131:581-589.
18. Paul R, Pessah IN, Gane L, Ono M, Hagerman PJ, Brunberg JA, Tassone F, Bourgeois JA, Adams PE,Nguyen DV, Hagerman R. Early onset of neurological symptoms in fragile X premutation carriers exposed to neurotoxins. Neurotoxicology. 2010; 31:399-402.
19. Lozano R, Hagerman RJ, Duyzend M, Budiminovic DB, Eichler EE, Tassone F. Genomic Studies in Fragile X Premutation Carriers. J Neurodev Disord. 2014; 6:27.
20. Li Z, Zhang Y, Ku L, Wilkinson KD, Warren ST, Feng Y. The fragile X mental retardation protein inhibits translation via interacting with mRNA. Nucleic Acids Res. 2001; 29:2276-2283.
21. Laggerbauer B, Ostareck D, Keidel EM, Ostareck-Lederer A, Fisher U. Evidence that fragile X mental retardation protein is a negative regulator of translation. Hum Mol Genet. 2001; 10:329-338.
22. Bear MF, Huber KM, Warren ST. The mGluR theory of fragile X mental retardation. Trends Neurosci. 2004; 27:370-377.
23. Paluszkiewicz SM, Martin BS, Huntsman MM. Fragile X syndrome: The GABAergic system and circuit dysfunction. Dev Neurosci. 2011; 33:349-364.
24. Heulens I, D'Hulst C, Van Dam D, De Deyn PP, Kooy RF. Pharmacological treatment of fragile X syndrome with GABAergic drugs in a knockout mouse model. Behav Brain Res. 2012; 229:244-249.
25. Huber KM, Gallagher SM, Warren ST, Bear MF. Altered synaptic plasticity in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A. 2002; 99:7746-7750.
26. Sidorov MS, Auerbach BD, Bear MF. Fragile X mental retardation protein and synaptic plasticity. Mol Brain. 2013; 6:15.

27. Fernandez E, Rajan N, Bagni C. The FMRP regulon: From targets to disease convergence. Front Neurosci. 2013; 7:191.
28. Fatemi SH, Folsom TD, Kneeland RE, Liesch SB. Metabotropic glutamate receptor 5 upregulation in children with autism is associated with underexpression of both Fragile X mental retardation protein and GABAA receptor beta 3 in adults with autism. Anat Rec (Hoboken). 2011; 294:1635-1645.
29. Loesch DZ, Huggins RM, Bui QM, Epstein JL, Taylor AK, Hagerman RJ. Effect of the deficits of fragile x mental retardation protein on cognitive status of fragile x males and females assessed by robust pedigree analysis. J Dev Behav Pediatr. 2002; 23:416-423.
30. Fatemi SH, Folsom TD. The role of fragile X mental retardation protein in major mental disorders. Neuropharmacology. 2011; 60:1221-1226.
31. Kovacs T, Kelemen O, Keri S. Decreased fragile X mental retardation protein (FMRP) is associated with lower IQ and earlier illness onset in patients with schizophrenia. Psychiatry Res. 2013; 210:690-693.
32. Aziz M, Stathopulu E, Callias M, Taylor C, Turk J, Oostra B, Willemsen R, Patton M. Clinical features of boys with fragile X premutations and intermediate alleles. Am J Med Genet B Neuropsychiatr Genet. 2003; 121B:119-127.
33. Pretto DI, Mendoza-Morales G, Lo J, Cao R, Hadd A, Latham GJ, Durbin-Johnson B, Hagerman R, Tassone F. CGG allele size somatic mosaicism and methylation in *FMR1* premutation alleles. J Med Genet. 2014; 51:309-318.
34. Dhossche DM, Shah A, Wing L. Blueprints for the assessment, treatment, and future study of catatonia in autism spectrum disorders. Int Rev Neurobiol. 2006; 72:267-284.
35. Chalasani P, Healy D, Morriss R. Presentation and frequency of catatonia in new admissions to two acute psychiatric admission units in India and Wales. Psychol Med. 2005; 35:1667-1675.
36. Mazzone L, Postorino V, Valeri G, Vicari S. Catatonia in patients with autism: Prevalence and management. CNS drugs. 2014; 28:205-215.
37. Jaimes-Albornoz W, Serra-Mestres J. Prevalence and clinical correlations of catatonia in older adults referred to a liaison psychiatry service in a general hospital. Gen Hosp Psychiatry. 2013; 35:512-516.
38. American Psychiatric Association. Desk reference to the diagnostic criteria from DSM-5. American Psychiatric Publishing, Washington DC, USA, 2013; pp.18-45.
39. Ghaziuddin N, Dhossche D, Marcotte K. Retrospective chart review of catatonia in child and adolescent psychiatric patients. Acta Psychiatr Scand. 2012; 125:33-38.
40. Northoff G. What catatonia can tell us about "top-down modulation": A neuropsychiatric hypothesis. Behav Brain Sci. 2002; 25:555-577.
41. Carroll BT, Lee JWY, Appiani F, Thomas C. The pharmacotherapy of catatonia. Prim Psychiatry. 2010; 17:41-47.
42. Wang H, Kim SS, Zhuo M. Roles of Fragile X Mental Retardation Protein in Dopaminergic Stimulation-induced Synapse-associated Protein Synthesis and Subsequent α-Amino-3-hydroxyl-5-methyl-4-isoxazole-4-propionate (AMPA) Receptor Internalization. J Biol Chem. 2010; 285:21888-21901.
43. Utari A, Adams E, Berry-Kravis E, Chavez A, Scaggs F, Ngotran L, Boyd A, Hessl D, Gane LW, Tassone F,Tartaglia N, Leehey MA, Hagerman RJ. Aging in fragile X syndrome. J Neurodev Disord. 2010; 2:70-76.
44. Jacquemont S, Hagerman RJ, Leehey M, et al. Fragile X premutation tremor/ataxia syndrome: Molecular, clinical, and neuroimaging correlates. Am J Hum Genet. 2003; 72:869-878.
45. Rosebush PI, Mazurek MF. Catatonia and its treatment. Schizophr Bull. 2010; 36:239-242.
46. Tsiouris JA, Brown WT. Neuropsychiatric symptoms of fragile X syndrome: Pathophysiology and pharmacotherapy. CNS drugs. 2004; 18:687-703.
47. Jeffries FM, Reiss AL, Brown WT, Meyers DA, Glicksman AC, Bandyopadhyay S. Bipolar spectrum disorder and fragile X syndrome: A family study. Biol Psychiatry. 1993; 33:213-216.
48. Reiss AL, Hagerman RJ, Vinogradov S, Abrams M, King RJ. Psychiatric disability in female carriers of the fragile X chromosome. Arch Gen Psychiatry. 1988; 45:25-30.
49. Hagerman RJ. Physical and behavioral phenotype. In: Fragile X Syndrome: Diagnosis, Treatment, and Research (Hagerman RJ, Hagerman PJ, eds.). Johns Hopkins University Press, Baltimore, Maryland, 2002; pp. 3-109.
50. Zalfa F, Giorgi M, Primerano B, Moro A, Di Penta A, Reis S, Oostra B, Bagni C. The fragile X syndrome protein FMRP associates with BC1 RNA and regulates the translation of specific mRNAs at synapses. Cell. 2003; 112:317-327.
51. McKinney RA. Physiological roles of spine motility: Development, plasticity and disorders. Biochem Soc Trans. 2005; 33:1299-1302.
52. Schneider A, Seritan A, Tassone F, Rivera SM, Hagerman R, Hessl D. Psychiatric features in high-functioning adult brothers with fragile x spectrum disorders. Prim Care Companion CNS Disord. 2013; 15.
53. Bourgeois JA, Coffey SM, Rivera SM, Hessl D, Gane LW, Tassone F, Greco C, Finucane B, Nelson L, Berry-Kravis E, Grigsby J, Hagerman PJ, Hagerman RJ. A review of fragile X premutation disorders: Expanding the psychiatric perspective. J Clin Psychiatry. 2009; 70:852-862.
54. Wheeler AC, Bailey DB Jr, Berry-Kravis E, Greenberg J, Losh M, Mailick M, Milà M, Olichney JM,Rodriguez-Revenga L, Sherman S, Smith L, Summers S, Yang JC, Hagerman R. Associated features in females with an FMR1 premutation. J Neurodev Disord. 2014; 6:30.
55. Hessl D, Tassone F, Loesch DZ, et al. Abnormal elevation of *FMR1* mRNA is associated with psychological symptoms in individuals with the fragile X premutation. Am J Med Genet B Neuropsychiatr Genet. 2005; 139B:115-121.
56. Bourgeois JA, Seritan AL, Casillas EM, Hessl D, Schneider A, Yang Y, Kaur I, Cogswell JB, Nguyen DV,Hagerman RJ. Lifetime prevalence of mood and anxiety disorders in fragile X premutation carriers. J Clin Psychiatry . 2011; 72:175-182.
57. Hessl D, Wang JM, Schneider A, Koldewyn K, Le L, Iwahashi C, Cheung K, Tassone F, Hagerman PJ, Rivera SM. Decreased fragile X mental retardation protein expression underlies amygdala dysfunction in carriers of the fragile X premutation. Biol Psychiatry. 2011; 70:859-865.
58. Tsourtos G, Spong J, Stough C. The effects of electro-convulsive therapy on the speed of information processing

in major depression. J Affect Disord. 2007; 103:263-266.
59. First MB, Spitzer RL, Gibbon M, Williams JBW. Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Patient Edition (SCID-I/P). New York: Biometrics Research, New York State Psychiatric Institute, New York, USA, 2002.
60. Lozano R, Summers S, Lozano C, Mu Y, Hessl D, Nguyen D, Tassone F, Hagerman R. Association Between Macroorchidism and Intelligence in *FMR1* Premutation Carriers. Am J Med Genet A. 2014; 164A:2206-2211.
61. Hatton DD, Sideris J, Skinner M, Mankowski J, Bailey DB Jr, Roberts J, Mirrett P. Autistic behavior in children with fragile X syndrome: Prevalence, stability, and the impact of FMRP. Am J Med Genet A. 2006; 140A:1804-1813.
62. Kaufmann WE, Abrams MT, Chen W, Reiss AL. Genotype, molecular phenotype, and cognitive phenotype: Correlations in fragile X syndrome. Am J Med Genet. 1999; 83:286-295.
63. Loesch DZ, Huggins RM, Hagerman RJ. Phenotypic variation and FMRP levels in fragile X. Ment Retard Dev Disabil Res Rev. 2004; 10:31-41.
64. Tassone F, Hagerman RJ, Taylor AK, Mills JB, Harris SW, Gane LW, Hagerman PJ. Clinical involvement and protein expression in individuals with the *FMR1* premutation. Am J Med Genet. 2000; 91:144-152.
65. Chen Y, Tassone F, Berman RF, Hagerman PJ, Hagerman RJ, Willemsen R, Pessah IN. Murine hippocampal neurons expressing *Fmr1* gene premutations show early developmental deficits and late degeneration. Hum Mol Genet. 2010; 19:196-208.
66. Wing L, Shah A. Catatonia in autistic spectrum disorders. Br J Psychiatry. 2000; 176:357-362.
67. D'Hulst C, Kooy RF. The GABAA receptor: A novel target for treatment of fragile X? Trends Neurosci. 2007; 30:425-431.
68. Braat S, D'Hulst C, Heulens I, De Rubeis S, Mientjes E, Nelson DL, Willemsen R, Bagni C, Van Dam D, De Deyn PP, Kooy RF. The GABAA receptor is an FMRP target with therapeutic potential in fragile X syndrome. Cell Cycle. 2015. (DOI:10.4161/15384101.2014.989114)
69. London SJ, Daly AK, Leathart JB, Navidi WC, Carpenter CC, Idle JR. Genetic polymorphism of CYP2D6 and lung cancer risk in African-Americans and Caucasians in Los Angeles County. Carcinogenesis. 1997; 18:1203-1214.
70. Aklillu E, Persson I, Bertilsson L, Johansson I, Rodrigues F, Ingelman-Sundberg M. Frequent distribution of ultrarapid metabolizers of debrisoquine in an ethiopian population carrying duplicated and multiduplicated functional *CYP2D6* alleles. J Pharmacol Exp Ther. 1996; 278:441-446.
71. McLellan RA, Oscarson M, Seidegaård J, Price Evans DA, Ingelman-Sundberg M. Frequent occurrence of *CYP2D6* gene duplication in Saudi Arabians. Pharmacogenetics. 1997; 7:187-191.
72. Zanger UM, Raimundo S, Eichelbaum M. Cytochrome P450 2D6: Overview and update on pharmacology, genetics, biochemistry. Naunyn Schmiedebergs Arch Pharmacol. 2004; 369:23-37.
73. Consoli A, Benmiloud M, Wachtel L, Dhossche D, Cohen D, Bonnot O. Electroconvulsive therapy in adolescents with the catatonia syndrome: Efficacy and ethics. J ECT. 2010; 26:259-265.
74. Bush G, Fink M, Petrides G, Dowling F, Francis A. Catatonia. II. Treatment with lorazepam and electroconvulsive therapy. Acta Psychiatr Scand. 1996; 93:137-143.
75. Cohen D, Taieb O, Flament M, Benoit N, Chevret S, Corcos M, Fossati P, Jeammet P, Allilaire JF, Basquin M. Absence of cognitive impairment at long-term follow-up in adolescents treated with ECT for severe mood disorder. Am J Psychiatry. 2000; 157:460-462.
76. Jenkins JK. CDER New Drug Review: 2011 update. *http://www.fda.gov/ downloads/AboutFDA/CentersOffices/CDER/UCM282984.pdf* (accessed July 25, 2015).
77. Baker IW, Jackson M, Bass C. Catatonia causing permanent cognitive impairment: A case study. Cogn Behav Neurol. 2005; 18:141-143.
78. Bernard PB, Castano AM, O'Leary H, Simpson K, Browning MD, Benke TA. Phosphorylation of FMRP and alterations of FMRP complex underlie enhanced mLTD in adult rats triggered by early life seizures. Neurobiol Dis. 2013; 59:1-17.

(Received July 17, 2015; Revised July 31, 2015; Re-revised August 11, 2015; Accepted August 12, 2015)

Case Report

DOI: 10.5582/irdr.2015.01013

Placental site trophoblastic tumor: A case report and literature review

Xianling Zeng[1], Xi Liu[2], Quan Tian[1], Yan Xue[1], Ruifang An[1,*]

[1] *Department of Obstetrics and Gynecology, the First Affiliated Hospital, Xi'an Jiao Tong University, Xi'an, China;*
[2] *Department of Pathology, the First Affiliated Hospital, Xi'an Jiao Tong University, Xi'an, China.*

Summary **Here, we report a case of a placental site trophoblastic tumor (PSTT) in a 36-year-old Chinese woman 10 months after a normal pregnancy. Two months postpartum, the woman presented with abnormal vaginal discharge and her condition was overlooked by her local hospital. The woman did not receive further attention until a mass with a heterogeneous echo was found in an ultrasound examination eight months postpartum. The final diagnosis was confirmed by histological examinations in conjunction with immunohistochemical studies. Since the patient had potential risk factors, she was successfully treated with a hysterectomy and peri- and post-operative chemotherapy. The latest follow-up (16 months after diagnosis) was uneventful, and the patient exhibited no signs of recurrence or metastasis.**

Keywords: Placental site trophoblastic tumor (PSTT), intermediate trophoblast (IT), diagnosis, treatment

1. Introduction

A placental site trophoblastic tumor (PSTT), originating from intermediate trophoblasts (ITs), refers to a special and rare type of gestational trophoblastic disease (GTD). In 1976, Kurman first described PSTT as syncytial endometritis and designated it a trophoblastic pseudotumor (*1*). In 1981, its malignant characteristics garnered attention when Twiggs reported a patient that died from the condition (*2*). At the same time, Scully reappraised the morphological aspects and malignant potential of the condition, designating it PSTT (*3*). In 1983, the World Health Organization (WHO) formally acknowledged the neoplastic nature of this lesion and adopted the terminology PSTT. PSTT has since become the third most common gestational trophoblastic neoplasm (GTN), second to invasive moles (IMs) and choriocarcinoma (CC). The incidence of PSTT is approximately 1/100,000 of all pregnancies and roughly 1-2% of all GTNs, while its mortality is 25% (*4*). To date, almost 300 cases of PSTT have been reported around the world (*5*). Its low mobility, uncharacteristic clinical presentation, and non-specific auxiliary examinations pose a substantial challenge to clinicians, leading to a low preoperative rate of diagnosis. In December 2013, the current authors encountered a case of PSTT, and this was the first such case seen at this hospital. This case is reported here and its clinical and pathological features have been analyzed based on the literature with the goal of enhancing the understanding of this disease.

2. Case presentation

A 36-year-old Chinese woman, gestation 2, miscarriage 1, underwent a cesarean section because of fetal distress and gave birth to a healthy full-term girl in February 2013. There was no postpartum hemorrhaging or puerperal fever during her postpartum course. The woman had no previous medical history and no personal or family history of GTD.

Two months postpartum, the woman visited her local hospital for abnormal vaginal discharge. The woman's condition was thought to be endometritis, and she was given antibiotic prophylaxis and medication to promote uterine contractions. Unfortunately, this treatment

Released online in J-STAGE as advance publication June 23, 2015.

**Address correspondence to:*
Dr. Ruifang An, Department of Obstetrics and Gynecology, the First Affiliated Hospital, Xi'an Jiao Tong University, Xi'an 710061, China.
E-mail: anruifangxj@163.com

was ineffective. The patient subsequently made repeated visits and was intermittently treated with anti-inflammatory therapy, but the therapy was ineffective. Eight months postpartum, a pelvic color Doppler ultrasound indicated a heterogeneous echo 17 mm × 10 mm in size in the uterine cavity, but no treatment was recommended except for monitoring by her doctor. Nine months postpartum, a second pelvic color Doppler ultrasound indicated that the heterogeneous echo was 56 mm × 20 mm in size, and the patient's serum beta human chorionic gonadotropin (β-hCG) level was 54.60 mIU/mL, compared to a normal level of lower than 5.4 mIU/mL. The patient subsequently underwent a hysteroscopy, and a pathological examination of endometrial curettage specimens suggested PSTT. Thus, the patient was referred to this hospital, a tertiary care center, for further management.

On admission, a physical examination revealed no abnormalities in the heart, lungs, or extremities. A gynecological examination revealed a normal vulva, little vaginal bleeding, no pain in the cervix upon lifting or manipulation, and an enlarged uterus about the size of that during the 8th week of pregnancy. In addition, the uterus was soft on palpation, and there was mild tenderness in the adnexa. The patient's β-hCG level was 66.14 mIU/mL. A pelvic color Doppler ultrasound indicated a region 12 mm × 10 mm in size with an abundant blood flow, and the patient's vascular resistance index (RI) was 0.41. Pelvic magnetic resonance imaging (MRI) indicated that the right posterior wall of the uterus was irregular, consistent with malignant changes.

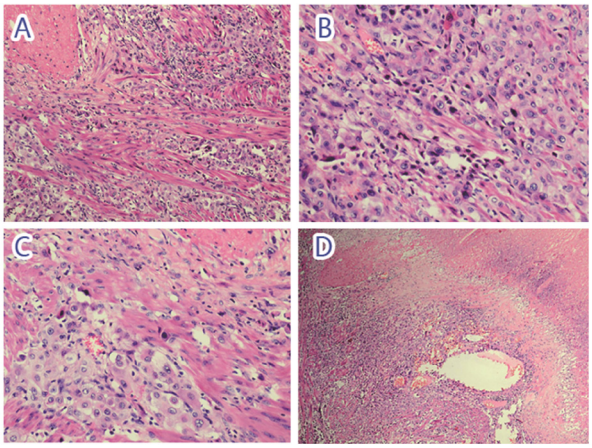


Figure 1. Microscopic findings. (A) The PSTT had monomorphic intermediate trophoblastic cells extensively infiltrating the myometrium, partly in nests and cords, separating myometrial muscle fibers, both individually and in groups. **(B)** These tumor cells were usually large and polygonal with irregular vesicular nuclei and displayed an abundance of dense eosinophilic to amphophilic cytoplasm. Abundant extracellular fibrinoid material was seen on occasion. **(C)** Multinucleated tumor giant cells are evident in places, but no syncytiotrophoblastic cells are evident. **(D)** Lesional cells displayed characteristic vascular invasion, replacing vessel walls, and tumor cells have vast areas of necrosis (hematoxylin and eosin staining, original magnification 100×, 200,× 200×, and 40×).

Pathological slides obtained from the referring hospital were viewed at this hospital. Ultimately, pathologists diagnosed the mass as a PSTT.

The mildly elevated level of serum β-hCG and pathological findings strongly suggested PSTT. The patient was treated with 5-fluorouracil, methotrexate, and etoposide (5-Fu, MTX, Vp16) for two days, followed by a total abdominal hysterectomy (TAH) with sparing of the ovaries. She finished the five-day chemotherapy regimen the day after surgery. Gross examination revealed an irregular mass, almost 1 cm × 1 cm in size, protruding from the uterine isthmus. Pathological examination of hysterectomy specimens (Figure 1) revealed a PSTT, along with extensive surface necrosis and vascular invasion, infiltrating the shallow muscle layer. Moreover, the tumor cells also displayed nuclear atypia, while chorionic villi and cytotrophoblasts were not evident. In order to confirm the diagnosis, pathologists were asked to perform immunohistochemical (IHC) studies (Table 1 and Figure 2), which indicated that the tumor cells

Table 1. Immunohistochemical results for the PSTT

Antigen	P(+)/N(-)
CK	+++
hPL	+++
Ki67	++
P53	++
P63	++
Calponin	++
HCG	+
PLAP	+
Vim	+
SMA	+
S-100	+
Inhibin	-

PLAP, placental alkaline phosphatase; hPL, human placental lactogen; SMA, smooth muscle actin; +++, strongly positive; ++, positive; +, focally positive; -, negative.

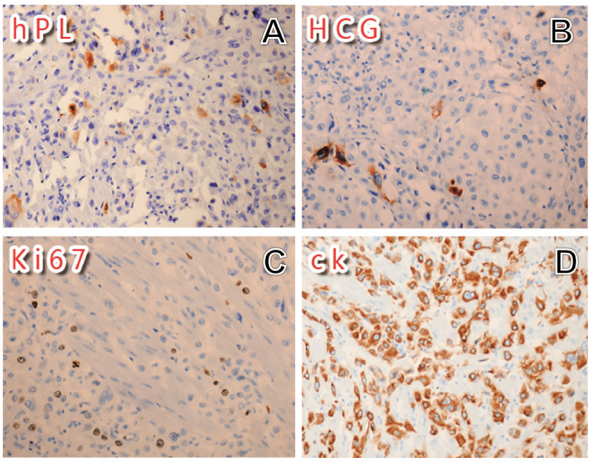


Figure 2. Immunohistochemical findings (200×) (A) Tumor cells were diffusely positive for hPL; **(B)** Tumor cells were focally positive for hCG; **(C)** About 10% of the tumor cells were slightly positive for ki67; **(D)** Tumor cells were strongly positive for CK.

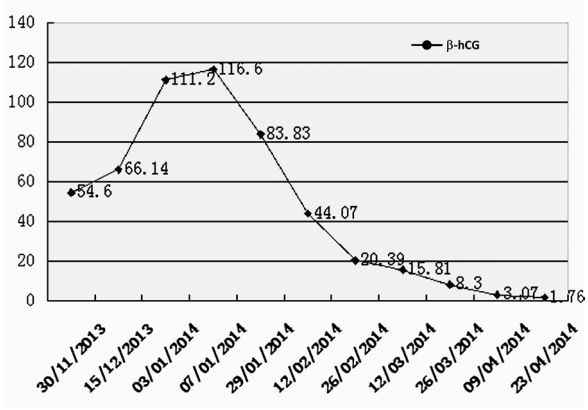


Figure 3. Changes in β-hCG levels (mIU/mL) during the treatment period.

were strongly positive for CK and human placental lactogen (hPL), positive for ki67, p53, p63, and calponin, and focally positive for HCG, placental alkaline phosphatase (PLAP), Vim, smooth muscle actin (SMA), and S-100. On the 10th day after surgery, the patient's serum β-hCG level was 111.20 mIU/mL, and that level rose to 116.60 mIU/mL on the 14th day after surgery. The presence of vast areas of necrosis and vascular invasion, together with a term pregnancy, all indicated that this PSTT possessed high-grade histological features that were suggestive of a poor prognosis. The patient subsequently underwent adjuvant chemotherapy. After the second cycle of the triple drug chemotherapy regimen (5-Fu, MTX, and Vp16), the patient's serum β-hCG level was 83.83 mIU/mL. Given the slow decline in the β-hCG level, treatment was changed to five cycles of the EMA-CO chemotherapy regimen (etoposide, methotrexate, actinomycin D, cyclophosphamide, and vincristine). During chemotherapy, the patient displayed obvious mouth ulcers and bone marrow suppression. When the level of β-hCG was under the measurable limit, two courses of consolidation chemotherapy were given. Changes in β-hCG levels during the treatment period were evident (Figure 3). At the latest follow-up (16 months after diagnosis), the patient's course was satisfactory, and she exhibited no signs of tumor recurrence or metastasis.

3. Discussion

PSTT frequently develops in women of reproductive age. The interval from prior pregnancy to tumor development is usually less than 2 years. PSTT is typically secondary to varieties of pregnancies and often follows a term labor for a female infant or after an abortion, molar pregnancy, or ectopic pregnancy (*6*). PSTT has also been reported in conjunction with a live twin pregnancy and was successfully resected during a caesarean section (*7*). Interestingly, PSTT can also develop in patients with no history of pregnancy, and PSTT has been noted in the ovary of a young child with isosexual precocious puberty (*8*) and in men (*9*).

The clinical presentation of PSTT is nonspecific and uncharacteristic. Patients usually present with amenorrhea or irregular vaginal bleeding. Gynecological examination reveals that the uterus is uniformly or irregularly enlarged. The overwhelming majority of PSTTs manifest as benign lesions and most frequently develop within the uterus. About 10%-15% of patients present with metastatic disease. However, several studies have suggested that metastases develop in over 30% of PSTTs upon presentation (32% according to Feltmate *et al*., 53% according to Newland *et al*., and 31% according to Chang *et al*.) (*10-12*). Recurrence occurs in over 30% of cases. Metastasis in the lungs, liver, kidneys, brain, abdomen, pelvic lymph nodes, or vagina is common. Based on 10 years of experience in dealing with PSTT, Schmid *et al*. noted that the probability of overall survival for patients with PSTT was 70% and the recurrence-free survival rate was 73% (*13*).

Unlike other GTNs (IM and CC), the level of serum β-hCG in PSTT is usually under the measurable limit or slightly elevated. The level of serum β-hCG in 79% of patients at Charing Cross Hospital was below 1000 mIU/mL (*14*), and the level was below 400 mIU/mL in 97% of the patients at the New England GTD Center (*15*). The level of serum β-hCG seldom reaches that noted in a CC is not proportional to the tumor load (*16*). In addition, the level of serum β-hCG is not associated with malignant behavior (*10*). Therefore, the level of serum β-hCG cannot accurately reflect the tumor burden and is of little value in assessing prognosis. However, a study found that hyperglycosylated hCG testing was more sensitive at detecting recurrent or persistent disease (*17*). A pelvic B ultrasound can display the signal of the tumor's blood flow. MRI has the advantage of displaying soft tissue in sharp contrast and allowing multi-aspect imaging, so it can be used to accurately determine the depth of myometrial invasion.

A macroscopic examination reveals that the tumors are localized nodules or polypoid tumors protruding into the uterine cavity. They may also present as masses infiltrating the myometrium with indistinct margins. Small bleeding foci may also be evident. Serosal infiltration may cause spontaneous perforation. A pathological examination reveals that the tumor only contains one form of trophoblastic cell that is either polygonal or circular. Spindle-shaped cells may be evident in some instances. Cells have a clear cell membrane and abundant cytoplasm that is eosinophilic or amphophilic. The nucleus is circular or ovoid and a few cells also have a macronucleus or multiple nuclei. A characteristic of tumor growth is diffuse infiltration. Bundles or clumps of tumor cells may invade muscle fibers. Histological findings from a PSTT are specific, but they are unable to distinguish the benign or malignant characteristics of a PSTT. A high mitotic rate of more than 5/10 HPF (and especially one more than 10/10

HPF) and substantial hemorrhaging and necrosis within the tumor are certainly suggestive of malignancy.

IHC is currently considered to be the "golden standard" for diagnosis of PSTT (*18*). On examination, tumor cells usually have a high level of Ki-67 expression (about 10%-15%). Inhibin is highly expressed by a PSTT. hPL, hCG, and PLAP are all secreted by syncytiotrophoblast cells, and these markers are rarely evident or even absent in a PSTT. In the current case, however, the tumor cells were positive for hPL and PLAP and slightly positive or negative for hCG. Accordingly, an IHC examination has distinct value in diagnosing a PSTT.

Tumor cells cannot be effectively removed through curettage because they invade uterine muscle fibers. In addition, they tend to spread through lymphatic pathways, resulting in relative resistance to chemotherapy (*19*). Thus, TAH is recommended for women if fertility need not be preserved, provided that the disease is localized to the uterus. Additionally, ovarian metastases are uncommon and an oophorectomy cannot prevent postoperative extrauterine metastasis or improve prognosis. Thus, ovaries and even fertility can be preserved in young women without ovarian metastasis (*20,21*). In contrast to other GTNs, the prevailing FIGO score as is routinely used to guide treatment for IM and CC is not valid for PSTT, so the choice of therapy should be made on grounds of possible related risk factors. Patients with low risk usually have a good prognosis after undergoing lesion resection. Patients with high risk may have a poor prognosis, so a hysterectomy along with chemotherapy is recommended. The risk factors associated with prognosis are: *i*) metastases from the uterus (*22*), *ii*) an interval from the preceding pregnancy of more than 4 years (*13*), *iii*) being over 40 years of age (*13*), *iv*) evidence of high-grade histological features such as deep myometrial invasion (> 1/2), a high mitotic figure (> 5 per 10 HPF), cells with a clear cytoplasm, coagulative necrosis, involvement of the vascular space, and a prior term pregnancy (*12,14,23*). The most critical of these risk factors is metastasis from the uterus. A patient with a PSTT has a good prognosis if lesions are limited to the uterus, but the mortality rate can reach as high as 25%. Chemotherapy is much more effective for cells that are positive for hCG than for cells that are positive for hPL. Therefore, several chemotherapy regimens have been found to successfully treat IM and CC, but which regimen is optimal for PSTT is still unknown. Single-agent chemotherapy and combination chemotherapy that are suitable for other GTNs with low-to-moderate risk only achieve partial remission of a PSTT or may fail to achieve any response at all. Chemotherapy has made a breakthrough with EMA/CO or EMA/EP regimens (etoposide, methotrexate, dactinomycin, and cisplatin). The two regimens are now mostly used as adjuvant therapy, and they play a significant role in the treatment of postoperative recurrence, residual tumors, and distant metastasis. Fortunately, the overall response rate to EMA/CO or EMA/EP is reported to be 71%, with a complete response in 38% of patients (*24*). Cytoreductive surgery combined with chemotherapy has become the standard treatment for metastatic PSTT. Recurrent PSTT after chemotherapy with EMA/CO or EMA/EP retreated with EMA/EP can still result in long-term complete remission. Therefore, the importance of using cisplatin to treat PSTT should be emphasized. Most researchers believe that EMA/EP has a definite effect on EMA/CO resistance, post-chemotherapy recurrence, and metastatic PSTT and should thus be used as the chemotherapy regimen of choice. For recurrent or progressive PSTT, the alternative second-line treatments are BEP (bleomycin, etoposide, and cisplatin) and VIP (etoposide, ifosfamide, and cisplatin) protocols (*24*). In addition, Feltmate *et al*. (*10*) noted that radiation may be effective in controlling local lesions and could be considered individually for recurrent disease. In general, the disease readily results in drug resistance and progresses rapidly once recurrence occurs or metastases develop, so metastatic multidrug-resistant PSTT was and still is the single leading cause of death due to PSTT (*25*).

In the current case, the patient was younger than 40 and she exhibited no evidence of extrauterine metastases, but the tumor developed after a term delivery of a female neonate. As Hassadia *et al*. (*15*) noted, the antecedent pregnancy resulted in a girl for 11 of 13 patients, and they also noted that 3 of 4 deaths occurred following a term delivery of a girl. This result is similar to the findings in the current case. Thus, full-term delivery of a girl is potentially linked to an adverse prognosis. Furthermore, the tumor in the current case displayed coagulation necrosis and vascular invasion. Therefore, the patient underwent TAH and was concurrently treated with peri- and post-chemotherapy, initially in a triple-drug regimen and then with EMA-CO, which further verified EMA-CO was the optimal regimen. The patient's ovaries were spared and displayed no signs of recurrence after more than 16 months of follow-up. The patient's β-hCG level was higher after surgery than before. This phenomenon is supposedly associated with the beta-subunit variant and excessive production of the beta-subunit as a result of the stimulation from surgery. However, literature focusing on this point has yet to be found.

In conclusion, PSTT is usually difficult to diagnose and routinely requires a combination of serum β-hCG testing, a radiological examination, a pathological examination, and IHC staining due to the heterogeneity of clinical manifestations. PSTT is potentially highly curable provided that the disease is confined to the uterus and that the disease is treated appropriately and regularly. Until recently, surgery has been the primary

treatment option. However, chemotherapy has also played an equally important role in cases of high risk. A close follow-up with serial serum β-hCG levels, a pelvic examination, and radiologic imaging such as MRI is suggested.

References

1. Kurman RJ, Scully RE, Norris HJ. Trophoblastic pseudotumor of the uterus: An exaggerated form of "syncytial endometritis" simulating a malignant tumor. Cancer. 1976; 38:1214-1226.
2. Twiggs LB, Okagaki T, Phillips GL, Stroemer JR, Adcock LL. Trophoblastic pseudotumor-evidence of malignant disease potential. Gynecol Oncol. 1981; 12(2 Pt 1):238-248.
3. Scully RE, Young RH. Trophoblastic pseudotumor: A reappraisal. Am J Surg Pathol. 1981; 5:75-76.
4. Piura B, Shaco-Levy R. Placental site trophoblastic tumor. Harefuah. 2007; 146:62-67.
5. Luiza JW, Taylor SE, Gao FF, Edwards RP. Placental site trophoblastic tumor: Immunohistochemistry algorithm key to diagnosis and review of literature. Gynecol Oncol Case Rep. 2013; 7:13-15.
6. Küçük Z, Ergün Y, Işik H, Kaya F, Akgün YA, Çaydere M. A rare case of uterine rupture due to a placental site trophoblastic tumour in the rudimentary horn. J Obstet Gynaecol. 2015; 35:97-98.
7. Liszka L, Wilk M, Wodolazski A, Palen P, Sikora J. Successful treatment of placental site trophoblastic tumor in twin pregnancy without hysterectomy. Tumori. 2009; 95:108-111.
8. Arroyo MR, Podda A, Cao D, Rodriguez MM. Placental site trophoblastic tumor in the ovary of a young child with isosexual precocious puberty. Pediatr Dev Pathol. 2009; 12:73-76.
9. Suurmeijer AJ, Gietema JA, Hoekstra HJ. Placental site trophoblastic tumor in a late recurrence of a nonseminomatous germ cell tumor of the testis. Am J Surg Pathol. 2004; 28:830-833.
10. Feltmate CM, Genest DR, Wise L, Bernstein MR, Goldstein DP, Berkowitz RS. Placental site trophoblastic tumor: A 17-year experience at the New England Trophoblastic Disease Center. Gynecol Oncol. 2001; 82:415-419.
11. Newlands ES, Bower M, Fisher RA, Paradinas FJ. Management of placental site trophoblastic tumours. J Reprod Med. 1998; 43:53-59.
12. Chang YL, Chang TC, Hsueh S, Huang KG, Wang PN, Liu HP, Soong YK. Prognostic factors and treatment for placental site trophoblastic tumor-report of 3 cases and analysis of 88 cases. Gynecol Oncol. 1999; 73:216-222.
13. Schmid P, Nagai Y, Agarwal R, Hancock B, Savage PM, Sebire NJ, Lindsay I, Wells M, Fisher RA, Short D, Newlands ES, Wischnewsky MB, Seckl MJ. Prognostic markers and long-term outcome of placental-site trophoblastic tumours: A retrospective observational study. Lancet. 2009; 374:48-55.
14. Papadopoulos AJ, Foskett M, Seckl MJ, McNeish I, Paradinas FJ, Rees H, Newlands ES. Twenty-five years' clinical experience with placental site trophoblastic tumors. J Reprod Med. 2002; 47:460-464.
15. Hassadia A, Gillespie A, Tidy J, Everard R G N J, Wells M, Coleman R, Hancock B. Placental site trophoblastic tumour: Clinical features and management. Gynecol Oncol. 2005; 99:603-607.
16. Yalinkaya A, Guzel AI, Kangal K, Buyukbayram H, Firat U. Two cases of placental site trophoblastic tumor. Taiwan J Obstet Gynecol. 2011; 50:372-374.
17. Khanlian SA, Cole LA. Management of gestational trophoblastic disease and other cases with low serum levels of human chorionic gonadotropin. J Reprod Med. 2006; 51:812-818.
18. Huang F, Zheng W, Liang Q, Yin T. Diagnosis and treatment of placental site trophoblastic tumor. Int J Clin Exp Pathol. 2013; 6:1448-1451.
19. Lan C, Li Y, He J, Liu J. Placental site trophoblastic tumor: Lymphatic spread and possible target markers. Gynecol Oncol. 2010; 116:430-437.
20. Saso S, Haddad J, Ellis P, Lindsay I, Sebire NJ, McIndoe A, Seckl MJ, Smith JR. Placental site trophoblastic tumours and the concept of fertility preservation. BJOG. 2012; 119:369-374.
21. Shen X, Xiang Y, Guo L, Feng F, Wan X, Xiao Y, Zhao J, Ren T. Fertility-preserving treatment in young patients with placental site trophoblastic tumors. Int J Gynecol Cancer. 2012; 22:869-874.
22. Hyman DM, Bakios L, Gualtiere G, Carr C, Grisham RN, Makker V, Sonoda Y, Aghajanian C, Jewell EL. Placental site trophoblastic tumor: Analysis of presentation, treatment, and outcome. Gynecol Oncol. 2013; 129:58-62.
23. Zheng Y, Bao L, Ning Y, Lu X, Hua K, Yi X. Retrospective analysis of the clinicopathologic and prognostic characteristics of stage I placental site trophoblastic tumor in China. Int J Gynaecol Obstet. 2015; 129:67-70.
24. Ajithkumar TV, Abraham EK, Rejnishkumar R, Minimole AL. Placental site trophoblastic tumor. Obstet Gynecol Survey. 2003; 58:484-488.
25. Kingdon SJ, Coleman RE, Ellis L, Hancock BW. Deaths from gestational trophoblastic neoplasia: Any lessons to be learned? J Reprod Med. 2012; 57:293-296.

(Received April 14, 2015; Revised May 27, 2015; Accepted May 28, 2015)

Case Report

DOI: 10.5582/irdr.2015.01016

Gonadal vein leiomyosarcoma: A case report with radiological findings

Tomoko Matsuzono[1,*], Cyrus Yin-Ho Chan[2], May Yuk-May Chan[1]

[1] *Department of Obstetrics and Gynaecology, Queen Elizabeth Hospital, Hong Kong, China;*
[2] *Department of Radiology, Queen Elizabeth Hospital, Hong Kong, China.*

Summary **A 56 year old postmenopausal lady presented with a rapidly enlarging pelvis mass. Clinical and ultrasonographic features were compatible with a rapidly enlarging fibroid with possible sarcomatous changes, and hence, computated tomography (CT) scan was performed to further delineate the nature and extent of the disease. However, CT scan revealed a huge tumour arising from the retroperitoneal space along the course of the left gonadal vein with typical radiological features of a gonadal vein leiomyosarcoma which were described in previous literatures. With joint collaboration with the surgeons, radical surgery with optimal debulking was subsequently performed for the patient and the diagnosis was confirmed intra-operatively and histologically.**

Keywords: Ovarian vein leiomyosarcoma, vascular leiomyosarcoma, computated tomography

1. Introduction

Leiomyosarcoma (LMS) is a rare malignant mesenchymal tumour of smooth muscle origin. Primary LMS of vascular origin are rare lesions, representing less than 2% of all LMS. The vena cava is the most commonly affected vessel, accounting for 60% of all tumours involving the vessel wall (*1*). LMS arising from the gonadal vein are particularly rare.

In this case report, we report a rare case of LMS arising from the ovarian vein. The patient first underwent preoperative conventional computated tomography (CT) for the initial diagnosis of rapidly enlarging fibroid with possible sarcomatous changes. However, the CT scan identified a retroperitoneal mass with intravascular growth patterns along the anatomical site of the left ovarian vein, hence confirmed the diagnosis of ovarian vein LMS. With joint collaboration with the surgeons, the patient subsequently underwent resection of the tumour en bloc with the uterus and ovaries, left kidney, descending and sigmoid colon to achieve a complete excision. However, despite optimal debulking, patient was found to have distant metastasis on subsequent follow up.

2. Case Report

A 56 year old post- menopausal woman had regular routine gynaecological checkup and they were all normal except a small 2 cm uterine fibroid. Her last ultrasound was performed one year ago, and the fibroid size was static. She complained of progressive abdominal distension and pressure symptoms for 3 months. On examination, her general condition was satisfactory, and there was no peripheral lymphadenopathy. However, abdominal and vaginal examinations revealed a 28 week gravid size irregular firm pelvic mass with decrease mobility, and uterus was not able to be felt separately. Ultrasonography performed showed a 20 cm irregular pelvic mass with echogenicity comparable to a uterine fibroid, uterus and ovaries were not separately identifiable.

In view of the rapid growth of the pelvic mass with heterogenicity comparable to fibroid on ultrasonography, the possibility of LMS was suspected. A CT scan with contrast of the thorax, abdomen and pelvis was performed, and it showed a 28 cm large heterogenous enhancing mass sited in the left retroperitoneal space with extensive cystic and necrotic components, small area of calcifications and dilated

Released online in J-STAGE as advance publication June 23, 2015.

**Address correspondence to:*
Dr. Tomoko Matsuzono, Department of Obstetrics and Gynaecology, Queen Elizabeth Hospital, 30 Gascoigne Road, Hong Kong, China.
E-mail: tomoko821@gmail.com

tortuous arteries seen within the tumor (Figure 1). Normal architecture of the left gonadal vein was absent. It extends along the expected course of left ovarian vein upwards to left renal vein, lower part of suprarenal vein and inferior vena cava. It was closely abutting and compressing onto the left ureter, leading to hydroureteronephrosis and reduced renal perfusion (Figure 2). It was also closely abutting the left psoas muscle, left ovary and sigmoid colon. No tissue plane between the tumor and left psoas was seen. Otherwise there were no ascites, lymphadenopathy or peritoneal metastasis. Uterus and right ovary were separately seen and were both normal. Based on the CT findings, a radiological diagnosis of LMS arising from the left gonadal vein was concluded.

Laparotomy, total abdominal hysterectomy and bilateral salpingo-oophorectomy, tumour excision en bloc with left kidney, descending and sigmoid colon, left pelvic and para-aortic lymph node dissection, and transverse colostomy were performed.

Final histology confirmed LMS with left renal vein invasion, all organs and margins were clear. After discussion by the multidisciplinary team which included the gynaecologist and clinical oncologist. They concluded that there was no role for adjuvant therapy. Unfortunately, despite optimal debulking, follow up scan of the patient 1 year post-operatively, revealed pulmonary and liver metastasis (Figure 3), and she was currently given palliative chemotherapy treatment.

3. Discussion

LMS should be suspected in postmenopausal women with rapidly growing leiomyoma. LMS of the vein arise from the smooth muscle cells of the tunica media of the vessel wall. They grow bilaterally along the wall of the

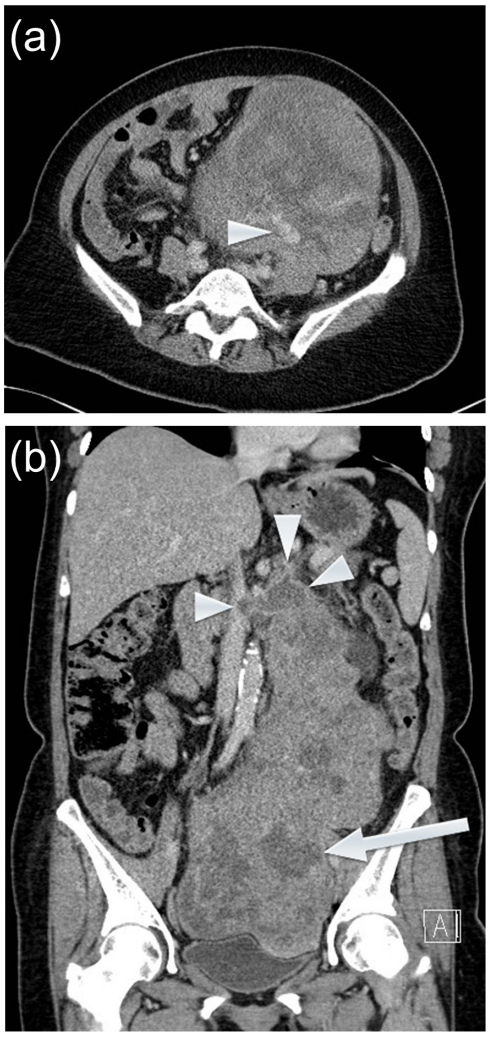


Figure 1. (a) axial and (b) coronal CT abdomen and pelvis with contrast. Longitudinally orientated huge soft tissue mass replacing normal left gonadal vein. Arrowheads in **(b)** indicate invasion of tumor into left renal vein, suprarenal vein and inferior vena cava. Necrotic areas (arrow in **(b)**) and hypertrophic vessels (arrowhead in **(a)**) are also seen.

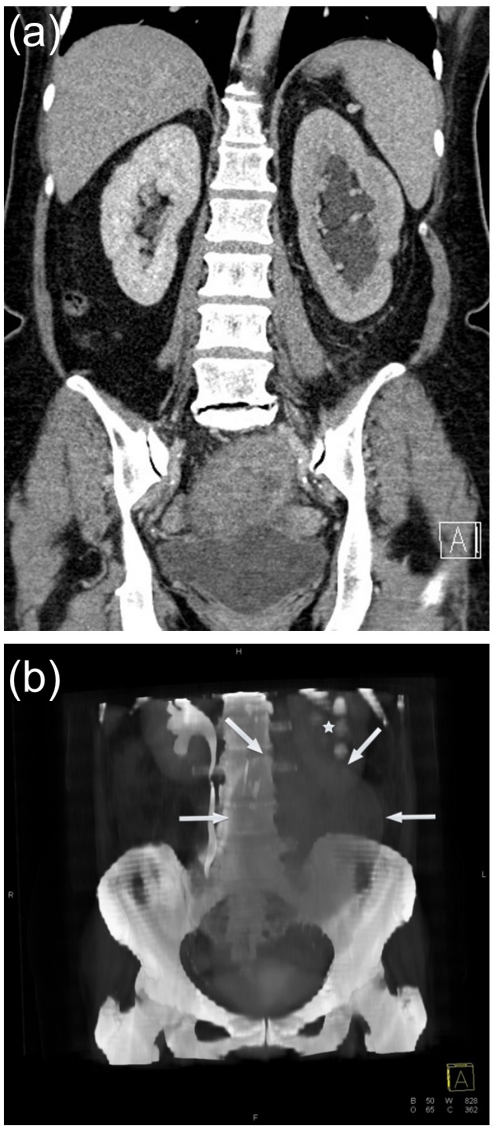


Figure 2. (a) Coronal CT abdomen and pelvis with contrast and (b) 3D reformatted image. Left gonadal vein leiomyosarcoma outlined by arrows in **(b)** compresses and obstructs left ureter resulting in left hydronephrosis and delayed contrast excretion (star in **(b)**).

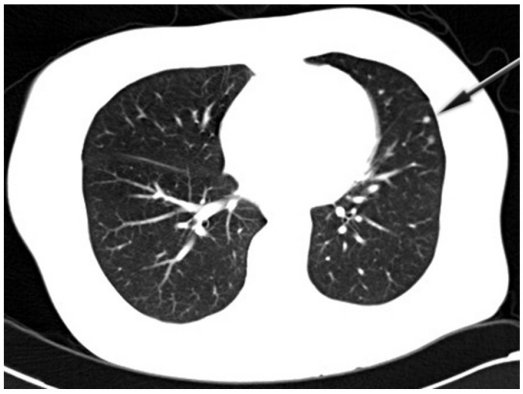


Figure 3. Axial CT thorax in lung window. Multiple small nodules in left lung base, which show interval growth in subsequent follow-up CT, in keeping with metastasis.

vessel. Gonadal vein LMS is extremely rare and only ten cases have been reported in the literatures (*1-10*).

Contrast enhanced CT is explicitly valuable to establish the pre-operative diagnosis, extent of the disease and guide further management. The general imaging findings in our case were compatible with those of other reported cases of venous LMS. According to literatures, gonadal vein LMS usually manifests as huge mass with heterogenous enhancement in longitudinal orientation, replacing normal gonadal vein. Cystic and necrotic component as well as hypertrophied vasculatures are often found in the mass. The claw sign, as demonstrated in our case, suggest that the tumor is intravascular in origin. With the advance of high spatial resolution of multi-detector CT over the last few decades, multiplanar reconstruction is possible, and these methods are useful in demonostrating the relationship between a mass and vessel, as well as detecting the tumour or venous thrombi (*1-5*). Combining the anatomical location and imaging features of the mass allows us to make the pre-operative diagnosis confidently.

It is universally accepted that surgery is the primary treatment for LMS. It has been recommended that aggressive surgical cytoreduction at the time of initial diagnosis offers the best possibility of prolonged survival (*11,12*). The role of adjuvant therapies have been investigated over the last decades, however, as many of the studies are underpowered with conflicting reports, there have been no randomized controlled trial (RCT) to date that have unambiguously demonstrated improved overall survival when using adjuvant radiotherapy or chemotherapy.

The prognosis for patients with LMS of vascular origin is very poor due to the high metastatic potential by haematogenous spread and non-specific presentations. For patients with tumours originating in the retroperitoneal space, the delay in subjective symptoms and diagnosis leads to a worse prognosis. Though surgical resection is the cornerstone of treatment, more than 50% of patients with complete macroscopic resection experience disease recurrence (*2*).

In conclusion, a suspicion of LMS should be raised in a rapidly enlarging fibroid or pelvic mass presenting in a postmenopausal lady. The use of contrast enhanced CT scan was the tool of choice to confirm the diagnosis of ovarian vein LMS radiologically. It has great benefit for the preoperative predictions of the location, extent, growth patterns of the tumour, as well as the relationship of the tumour and vessels to help surgeons in contemplating the extent of surgery.

References

1. Cho HJ, Kim HK, Suh JH, Lee GJ, Shim JC, Kim YH, Lee HS, Kang YK. Leiomyosarcoma of the ovarian vein: A case report with radiological findings. Korean J Radiol. 2008; 9:S14-S17.
2. Tsuyoshi H, Yoshida Y, Kurokawa T, Kotsuji F. Diagnosis and management of leiomyosarcoma arising from ovarian vein: Case report and literature review. J Obstet Gynaecol Res. 2012; 38:466-470.
3. Dzsinich C, Gloviczki P, van Heerden JA, Nagorney DM, Pairolero PC, Johnson CM, Hallett JW Jr, Bower TC, Cherry KJ Jr. Primary venous leiomyosarcoma: A rare but lethal disease. J Vasc Surg. 1992; 15:595-603.
4. Honoré LH, Robins RE, Taylor RH. Leiomyosarcoma of the right ovarian vein a case report. Angiology. 1977; 28:285-288.
5. Kim SH, Kwon HJ, Cho JH, Lee SW, Rha SH, Choi J, Nam KJ. Atypical radiological features of a leiomyosarcoma that arise from the ovarian vein and mimicked avascular tumour. Br J Radiol. 2010; 83:e95-e97.
6. Saigusa S, Ohi M, Inoue Y, Kusunoki M. Preoperative diagnosis by three-dimensional angiography of a leiomyosarcoma arising from the left ovarian vein. BMJ Case Rep. 2013; bcr2013008823. (doi:10.1136/bcr-2013-008823)
7. Kawai K, Horiguchi H, Sekido N, Akaza H, Koiso K. Leiomyosarcoma of the ovarian vein: An unusual cause of severe abdominal and flank pain. Int J Urol 1996; 3:234-236.
8. Iannelli A, Karimdjee BS, Fabiani P, Liolos J, Avallone S, Gugenheim J. Leiomyosarcoma of the ovarian vein: Report of a case. Int Surg. 2003; 88:6-8.
9. Chiarugi M, Pressi E, Mancini R, Fattori S, Galatioto C, Seccia M. Leiomyosarcoma of the right ovarian vein. Am J Surg. 2009; 197:e36-e37.
10. Yang SH, Chien JC, Chen CL, Chan WP. Leiomyosarcoma of ovarian vein compression as a cause of hydronephrosis. Eur J Gynaecol Oncol. 2011; 32:336-338.
11. Harry VN, Narayansingh GV, Parkin DE. Uterine leiomyosarcomas: A review of the diagnostic and therapeutic pitfalls. Obstet Gynaecol. 2007; 9:88-94.
12. Gage MJ, Patel AV, Koenig KL, Newman E. Non-vena cava leiomyosarcomas: A review of the literature. Ann Surq Oncol. 2012; 19:3368-3374.

(Received April 27, 2015; Revised May 27, 2015; Accepted May 28, 2015)

Case Report

DOI: 10.5582/irdr.2015.01021

Inflammatory pseudotumor of the liver: A case report and literature review

Yafei Zhang, Hongwei Lu, Hong Ji, Yiming Li*

Department of General Surgery, Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, China.

Summary **Reported here is the case of a 55-year-old man who had tarry stools for 3 days before he was seen at this Department. The man had weight loss and an intermittent fever for 3 months prior. Histopathology revealed an inflammatory pseudotumor of the liver. This case is reported here along with a review of the literature. Nine days after surgery, the patient passed bright red blood (150 mL) in the stool with no clear trigger. A colonoscopy a month later revealed no abnormalities. This is a rare report of an inflammatory pseudotumor featuring intractable bleeding. An inflammatory pseudotumor of the liver is a rare condition, and differentiating this pseudotumor from hepatic space-occupying lesions is crucial. An inflammatory pseudotumor of the liver may spontaneously regress and mimic other liver tumors. The treatment of choice for this pseudotumor is still surgical resection, and this is especially true for patients with severe symptoms or an indeterminate diagnosis.**

Keywords: Inflammatory pseudotumor (IPT), liver, bleeding

1. Introduction

An inflammatory pseudotumor (IPT) is a rare condition that was first described in the lung in 1939 (*1*). IPT most commonly occurs in the lung, but it can be found in other locations including the central nervous system, major salivary glands, the kidneys, the liver, the omentum, the ovaries, the larynx, the urinary bladder, the breasts, the pancreas, the spleen, lymph nodes, skin, soft tissues, and the orbit of the eye (*2*). An IPT of the liver (IPTL) is a rare benign lesion characterized by chronic infiltration of inflammatory cells and an area of fibrosis that sometimes mimics a malignant tumor (*3,4*). An IPTL was first reported by Pack and Baker in 1953 (*5*).

The etiology and pathogenesis of IPTL remain unknown. Biologically, there are no specific symptoms or laboratory or radiologic findings that are useful at diagnosing IPTL. Differentiating between IPTs and other focal hepatic lesions remains a major problem. The treatment of choice is still surgical resection, and this is especially true for patients with severe symptoms or an indeterminate diagnosis (*6,7*).

2. Case Report

This case involved a 55-year-old man who had an unremarkable medical history and tarry stools for 3 days before he was seen at this Department. The man also had weight loss (15 kg) and an intermittent fever for 3 months prior. He had no abdominal pain or night sweats and he presented without jaundice. On admission, a physical examination revealed no signs ("stigmataC") of chronic liver disease or hepatomegaly. A previous computed tomography (CT) scan showed a well-defined heterogeneous mass 4.0 cm × 4.0 cm in size situated in the left hepatic lobe (Figure 1). On CT, the lesion featured central necrosis, a hyper-dense rim, and mild enhancement starting in the arterial phase, thus corresponding to a hepatic abscess.

Laboratory results revealed an aspartate amino-transferase level of 122 IU/L (normal, 5-40 IU/L), an alanine aminotransferase level of 89 IU/L (normal, 8-40 IU/L), an alkaline phosphatase (ALP) level of 162 IU/L (normal, 40-140 IU/L), a gamma-glutamyl transpeptidase (GGT) level of 159 U/L (normal, 7-40 U/L), an erythrocyte sedimentation rate (ESR) of 45

Released online in J-STAGE as advance publication July 20, 2015.

**Address correspondence to:*
Dr. Yiming Li, Department of General Surgery, Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, 157 West 5th Road, Xi'an 710004, People's Republic of China.
E-mail: liyimingdoc@163.com

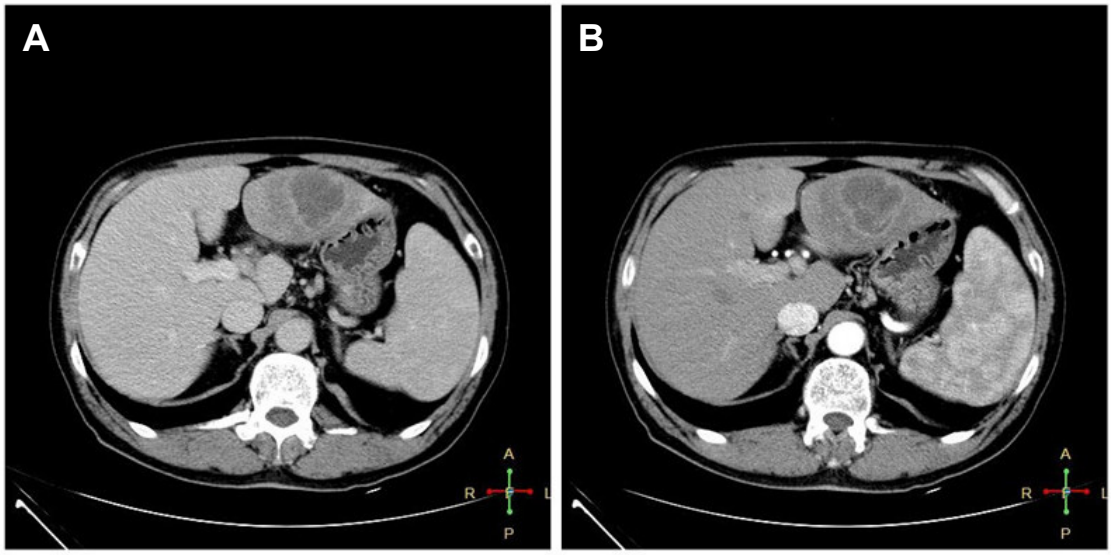


Figure 1. Computed tomography images of the abdomen. (A) A well-defined heterogeneous mass 4.0 cm × 4.0 cm in size was situated in the left hepatic lobe. **(B)** The lesion featured central necrosis, a hyper-dense rim, and mild enhancement starting in the arterial phase.

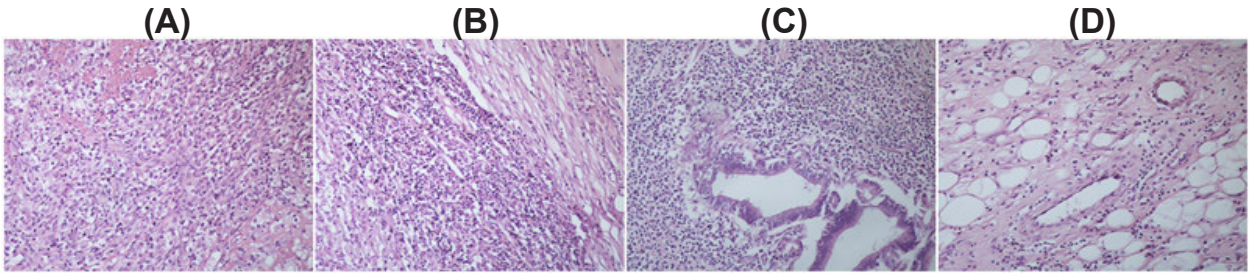


Figure 2. Histology of a surgical specimen. (A) Numerous lymphocytes and plasma cells are evident. **(B)** Cells mainly consist of inflammatory cells and eosinophils. **(C)** Shown are lymphocytes and macrophages. **(D)** Shown are inflammatory and xanthomatous changes. (hematoxylin and eosin, × 200).

mm/h (normal, < 7 mm/h), a CEA level of 1.34 ng/mL (normal, < 3.4 ng/mL), AFP level of 3.41 ng/mL (normal, < 7.02 ng/mL), and a CA 19-9 level of 6.64 U/mL (normal, < 39 U/mL). In addition, the patient had negative serologic results for hepatotropic viruses, cytomegalovirus, Epstein-Barr virus (EBV), and HIV 1 and 2. Standardized immunohistochemistry (Xijing Hospital, Fourth Military Medical University, Xi'an, China) showed monoclonal antibodies against broad-spectrum keratin AE/AE3(+), CD138(+), CK19(+), LCA(+), and Hep(-) (Dako, Glostrup, Denmark). Both antibodies reacted positively to cells from the lesion. An ultrasound-guided percutaneous liver biopsy with a tru-cut needle was performed and revealed hepatic tissue with proliferation of spindle-shaped cells mixed with an inflammatory infiltrate of histiocytes, suggesting a diagnosis of IPTL.

After surgery, a specimen of the liver parenchyma was examined histopathologically. The tumor had been completely removed. Microscopy revealed a host of benign cells, including numerous inflammatory cells, mature plasma cells, lymphocytes, eosinophils, and macrophages; most of these cells had xanthomatous changes (Figure 2). The patient received no further treatment. Nine days after surgery, the patient passed bright red blood (150 mL) in the stool with no clear trigger. A colonoscopy a month later revealed no abnormalities. Two months later, the patient's condition was still satisfactory.

3. Discussion

IPT is also known as an inflammatory myofibroblastic tumor or plasma cell granuloma, a xanthomatous pseudotumor, and inflammatory fibrosarcoma (*8*). Someren classified IPTs into 3 groups according to histology: xanthogranuloma-type pseudotumors, plasma cell granuloma-type pseudotumors, and sclerosing pseudotumors (*9*). Macroscopically, the lesion may mimic a malignancy and may be alone or several lesions may be present. The lesion may be as large as 25 cm. Microscopically, IPT is characterized by spindle-shaped cells, myofibroblasts, and mixed inflammatory cells (plasma cells, lymphocytes, and sporadic histiocytes). IPTL most often occurs in childhood and early adulthood (*10*). In adults, the ratio of males to females

affected ranges from 1:1 to 3.5:1 (*11*). IPTL appear to be more common in non-European populations (*12*). These IPTs are most likely inflammatory or infectious in origin. The lesions often appear to develop from a healing abscess or an inflammatory condition (*11*). The most common symptoms of IPTL are abdominal pain, a fever, and weight loss. IPTL frequently resolves spontaneously with a good prognosis (*13*).

IPTL is quite rare and accounts for 8% of extrapulmonary IPTs. The incidence of IPTL is reported to be around 0.7% according to recent studies (*14,15*). The etiology and pathogenesis of IPTL remain unknown since a variety of tumorous inflammatory lesions lack the features of other IPTs, but IPTL is thought to involve an inflammatory reaction (*16*). Infectious agents that have been hypothesized to cause IPTL include infections, trauma, vascular causes, and autoimmune disorders (*17*). Numerous studies have noted that the microorganisms responsible for IPTL include Bacteroides caccae, Actinomyces, Klebsiella, Escherichia coli, Gram-positive cocci, and b-hemolytic Streptococcus (*9*). Other studies reported that hepatopancreatobiliary autoimmune diseases, such as IgG4 sclerosing cholangitis, could also cause IPTL (*18*).

The diagnosis of liver pseudotumors is obviously difficult. Ultrasound and CT scans are not specific, revealing variable patterns of echogenicity or a liver mass mimicking hepatocellular cancer or an abscess (*19,20*). A CT scan usually reveals lesions with variable contrast enhancement. IPTs may display a hypovascular pattern because of fibrosis and also display delayed enhancement, similar to metastatic liver tumors and cholangio-carcinomas (*21*). MRI may produce low signal intensity (hypointensity) on T1-weighted images with moderate to high signal intensity (hyperintensity) on a T2 sequence (*16,21*). In general, differentiating IPTs from malignant tumors with radiographic studies is difficult. A definitive diagnosis of IPT can be made based on needle biopsy findings and, occasionally, in-needle aspiration, as long as the pathologist is aware of this possibility.

Biologically, there are no specific symptoms or laboratory or radiologic findings that are useful at diagnosing IPTL. Despite recent increases in the diagnostic capability of radiologic studies, differentiating IPTL from other focal hepatic lesions remains a major problem. Unfortunately, clinical and radiologic features of IPTL can mimic other liver tumors like lymphoma, malignant fibrous histiocytoma, hepatocellular carcinoma, metastatic tumor, tuberculosis, and sarcoidosis and thus lead to surgery (*16*). If an atypical solid mass is found in the liver, IPTL should be considered as a potential diagnosis, particularly if the mass is accompanied by clinical evidence of an inflammatory process: a recent history of asthenia, malaise, vague upper abdominal discomfort, and/or an intermittent fever; the presence of stigmata of chronic liver disease or splenomegaly; abnormal liver function test results; and a lack of specific imaging findings.

Although liver biopsy indisputably has a role in the investigation and management of liver metastases of unknown origin, its role is more contentious and possibly dangerous in cases of a solitary hepatic mass that is likely to be malignant (*22*). The main histopathological findings in all cases are the presence of myofibroblastic spindle cells, plasma cells, macrophages, and lymphocytes without cellular atypia or atypical mitotic figures (*23*). A biopsy of the tumor is not necessary when planning a surgical intervention for the liver.

Furthermore, an optimal treatment for IPTL and a method of determining its prognosis have yet to be established (*24,25*). Due to its diagnostic ambiguity, the lesion completely resolved in some patients that received antibiotics and/or corticosteroids, but some of these lesions recurred (*25*). In contrast, numerous studies have reported performing a hepatic resection, mainly due to evidence that the tumor is malignant according to preoperative radiography, after which IPTL never recurred (*26*).

Even though IPTL may spontaneously regress or regress following antibiotic treatment, the treatment of choice is still surgical resection, and this is especially true for patients with severe symptoms or an indeterminate diagnosis (*6,27*). Hepatectomy has become a safer option for non-cirrhotic patients over the past 20 years, with mortality converging to 0%. Therefore, the treatment of choice should be surgical resection in such cases (*28*). This approach is preferable because it minimizes the risk of a biopsy-related complication (dissemination in cases of malignancy) and because it eliminates the possibility of IPT recurring.

In conclusion, IPTL is a rare condition, and differentiating this pseudotumor from hepatic space-occupying lesions is crucial. IPTL may regress spontaneously and it may mimic other liver tumors. The treatment of choice is still surgical resection, and this is especially true for patients with severe symptoms or an indeterminate diagnosis.

References

1. Copin MC, Gosselin BH, Ribet ME. Plasma cell granuloma of the lung: Difficulties in diagnosis and prognosis. Ann Thorac Surg. 1996; 61:1477-1482.
2. Hsieh SC, Wu CH, Chan WP. Diagnosis of inflammatory pseudotumour of the urinary bladder aided by dynamic computed tomography. Acta Clin Belg. 2010; 65:360.
3. Faraj W, Ajouz H, Mukherji D, Kealy G, Shamseddine A, Khalife M. Inflammatory pseudo-tumor of the liver: A rare pathological entity. World J Surg Oncol. 2011; 9:5.
4. Torzilli G, Inoue K, Midorikawa Y, Hui AM, Takayama T, Makuuchi M. Inflammatory pseudotumors of the liver: Prevalence and clinical impact in surgical patients. Hepatogastroenterology. 2001; 48:1118-1123.

5. Pack GT, Baker HW. Total right hepatic lobectomy: Report of a case. Ann Surg. 1953; 138:253-258.
6. Nagarajan S, Jayabose S, McBride W, Prasadh I, Tanjavur V, Marvin MR, Rodriguez-Davalos MI. Inflammatory myofibroblastic tumor of the liver in children. J Pediatr Gastroenterol Nutr. 2013; 57:277-280.
7. Huang YH, Zhong DJ, Tang J, Han JJ, Yu JD, Wang J, Wan YL. Inflammatory myofibroblastic tumor of the liver following renal transplantation. Ren Fail. 2012; 34:789-791.
8. Fragoso AC, Eloy C, Estevão-Costa J, Campos M, Farinha N, Lopes JM. Abdominal inflammatory myofibroblastic tumor a clinicopathologic study with reappraisal of biologic behavior. J Pediatr Surg. 2011; 46:2076-2082.
9. Ntinas A, Kardassis D, Miliaras D, Tsinoglou K, Dimitriades A, Vrochides D. Inflammatory pseudotumor of the liver: A case report and review of the literature. J Med Case Rep. 2011; 5:196.
10. Levy S, Sauvanet A, Diebold MD, Marcus C, Da Costa N, Thiefin G. Spontaneous regression of an inflammatory pseudotumor of the liver presenting as an obstructing malignant biliary tumor. Gastrointest Endosc. 2001; 53:371-374.
11. Agaimy A, Markl B. Inflammatory angiomyolipoma of the liver: An unusual case suggesting relationship to IgG4-related pseudotumor. Int J Clin Exp Pathol. 2013; 6:771-779.
12. Schmid A, Janig D, Bohuszlavizki A, Henne-Bruns D. Inflammatory pseudotumor of the liver presenting as incidentaloma: Report of a case and review of the literature. Hepatogastroenterology. 1996; 43:1009-1014.
13. Yamaguchi J, Sakamoto Y, Sano T, Shimada K, Kosuge T. Spontaneous regression of inflammatory pseudotumor of the liver: Report of three cases. Surg Today. 2007; 37:525-529.
14. Tang L, Lai EC, Cong WM, Li AJ, Fu SY, Pan ZY, Zhou WP, Lau WY, Wu MC. Inflammatory myofibroblastic tumor of the liver: A cohort study. World J Surg. 2010; 34:309-313.
15. Park JY, Choi MS, Lim YS, Park JW, Kim SU, Min YW, Gwak GY, Paik YH, Lee JH, Koh KC, Paik SW, Yoo BC. Clinical features, image findings, and prognosis of inflammatory pseudotumor of the liver: A multicenter experience of 45 cases. Gut Liver. 2014; 8:58-63.
16. Goldsmith PJ, Loganathan A, Jacob M, Ahmad N, Toogood GJ, Lodge JP, Prasad KR. Inflammatory pseudotumours of the liver: A spectrum of presentation and management options. Eur J Surg Oncol. 2009; 35:1295-1298.
17. Tsou YK, Lin CJ, Liu NJ, Lin CC, Lin CH, Lin SM. Inflammatory pseudotumor of the liver: Report of eight cases, including three unusual cases, and a literature review. J Gastroenterol Hepatol. 2007; 22:2143-2147.
18. Kamisawa T, Okamoto A. IgG4-related sclerosing disease. World J Gastroenterol. 2008; 14:3948-3955.
19. Caramella T, Novellas S, Fournol M, Saint-Paul MC, Bruneton JN, Chevallier P. Imaging of inflammatory pseudotumors of the liver. J Radiol. 2007; 88:882-888.
20. Herek D, Karabulut N. Education and imaging. Hepatobiliary and pancreatic: inflammatory pseudotumors of the liver. J Gastroenterol Hepatol. 2011; 26:1217.
21. Brunello F, Caremani M, Marcarino C, Benci A, Menchetti D, Emanuelli G. Inflammatory pseudotumour of the liver: Diagnosis by fine needle biopsy in two cases and a review of the literature. Ital J Gastroenterol. 1994; 26:151-153.
22. Vicuña-González RM, Rivera-Salgado MI, Garcia-Velarde PM, de León-Bojorge B, Ortiz-Hidalgo C. Multicentric inflammatory pseudotumor with asynchronic presentation in meninges, liver, spleen and lymph nodes in a patient with seronegative spondiloarthropathy. Case report and review of the literature. Rev Neurol. 2008; 47:464-468. (in Spanish)
23. Iguchi H, Yamazaki H, Tsunoda H, Takahashi Y, Yokomori H. A case of inflammatory pseudotumor of the liver mimicking hepatocellular carcinoma on EOB-MRI and PET. Case Rep Med. 2013; 2013:594254.
24. Di Vita G, Soresi M, Patti R, Carroccio A, Leo P, Franco V, Montalto G. Concomitant inflammatory pseudotumor of the liver and spleen. Liver. 2001; 21:217-222.
25. Salakos C, Nikolakopoulou NM, De Verney Y, Tsamandas AC, Ziambaras T, Petsas T, Papanastasiou DA. Anaplastic lymphoma kinase (ALK) positive inflammatory pseudotumor of the liver: Conservative treatment and long-term follow-up. Eur J Pediatr Surg. 2010; 20:278-280.
26. Koea JB, Broadhurst GW, Rodgers MS, McCall JL. Inflammatory pseudotumor of the liver: Demographics, diagnosis, and the case for nonoperative management. J Am Coll Surg. 2003; 196:226-235.
27. Chablé-Montero F, Angeles-Ángeles A, Albores-Saavedra J. Inflammatory myofibroblastic tumor of the liver. Ann Hepatol. 2012; 11:708-709.
28. Imamura H, Seyama Y, Kokudo N, Maema A, Sugawara Y, Sano K, Takayama T, Makuuchi M. One thousand fifty-six hepatectomies without mortality in 8 years. Arch Surg. 2003; 138:1198-1206; discussion 1206.

(Received May 29, 2015; Revised July 2, 2015; Accepted July 6, 2015)

Case Report

DOI: 10.5582/irdr.2015.01030

Ventricular fibrillation development following atrial fibrillation after the ingestion of sildenaphil in a patient with Wolff-Parkinson-White syndrome

Sinan Inci[1,*], Ibrahim Izgu[1], Halil Aktas[1], Pinar Dogan[1], Ali Dogan[2]

[1] *Departmant of Cardiology, Aksaray State Hospital, Aksaray, Turkey;*
[2] *Departmant of Cardiology, Medical Faculty, Erciyes University, Kayseri, Turkey.*

Summary **Complications in the accessory pathway in Wolff-Parkinson-White (WPW) syndrome could cause different clinical conditions by inducing different arrhythmias. Atrial fibrillation (AF) is one of these arrhythmias and is important as it causes life-threatening arrhythmias. It is known that some drugs, underlying cardiac diseases, and the number of accessory pathways, cause a predisposition to this condition. In the current report, we presented a patient with WPW who was admitted to the emergency department with AF, wide QRS and a rapid ventricular response that progressed to ventricular fibrillation.**

Keywords: Atrial fibrillation, sildenaphil, Wolff-Parkinson-White syndrome

1. Introduction

Sildenaphil is an oral agent used in the treatment of erectile dysfunction (ED). It demonstrates its action by the potent inhibition of phosphodiesterase type 5 enzyme (PDE5) in cavernous tissue, thus increasing nitric oxide (NO) and cyclic 3', 5'-guanosine monophosphate (cGMP) levels and prolonging smooth muscle relaxation (*1*). In contrast to initial prejudgments, this drug continues to be used in individuals with cardiovascular disease. Moreover, in a few case reports this drug has been demonstrated to induce arrhythmias in the presence of underlying cardiac pathological conditions (*2-4*). We reported a patient with Wolff-Parkinson-White (WPW) syndrome in which atrial fibrillation developed a short period after the ingestion of sildenaphil citrate, and subsequently progressed to ventricular fibrillation.

2. Case Report

A thirty-seven year old male patient, without any atherosclerotic risk factors, was admitted to the emergency department complaining of palpitations and shortness of breath that had begun about an hour ago previously. In his anamnesis, he reported that his symptoms started 20 minutes after taking sildenaphil, prior to any sexual contact, and progressively increased. He had undergone an examination seven years previously, following complaints of palpitation, and was diagnosed with WPW syndrome. Although he had occasionally had palpitations, previously they had never been so severe. He said that he had used medication for one year after the initial diagnosis but he had then stopped using the drug. Following a physical examination his BP: 85/54 mm Hg, pulse: 180/min and other systemic examinations appeared normal. Electrocardiography (ECG) detected atrial fibrillation with wide QRS, and rapid ventricular response (Figure 1). There was no abnormality in laboratory results. Initially, his medical treatment was planned using Cordarone. As sinus rhythm could not be achieved, D&C with 200 joule was planned. Ventricular fibrillation developed while the patient was being sedated. Therefore defibrillation was performed and sinus rhythm was achieved. The patient's Basal ECG was consistent with Wolf-Parkinson-White syndrome (Figure 2). In transthoracic echocardiography, all wall motions and valve structures were found to be normal. The ejection fraction (EF) was 58%. There were not any findings to suspect myocardial ischemia. The patient was followed up in the coronary intensive care

**Address correspondence to:*
Dr. Sinan Inci, Aksaray State Hospital, Zafer Mahallesi Nevşehir caddesi No:117, Aksaray, Turkey.
E-mail: doktorsinaninci@gmail.com

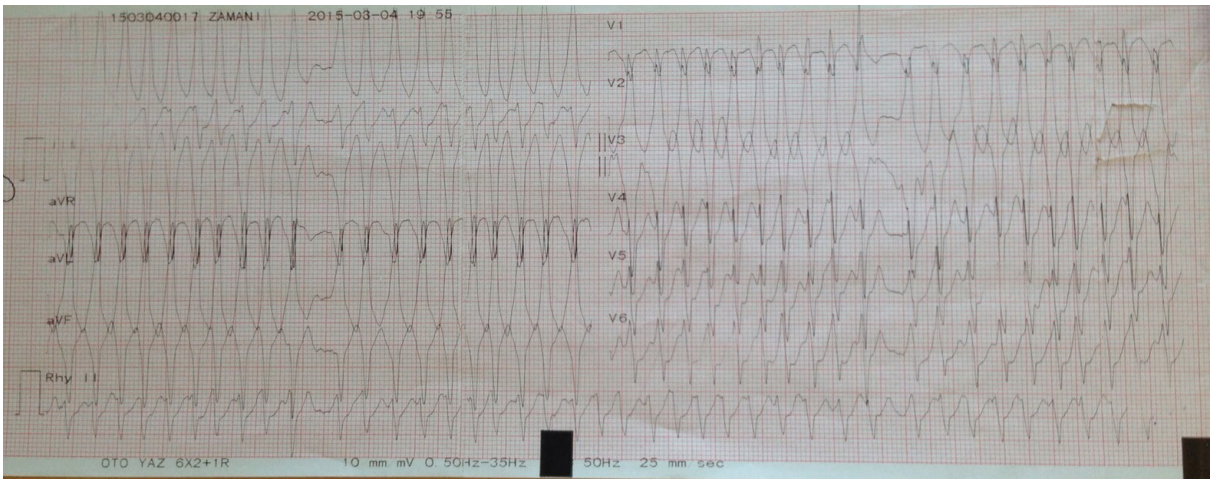


Figure 1. Atrial fibrillation with wide QRS and high ventricular response in the emergency department.

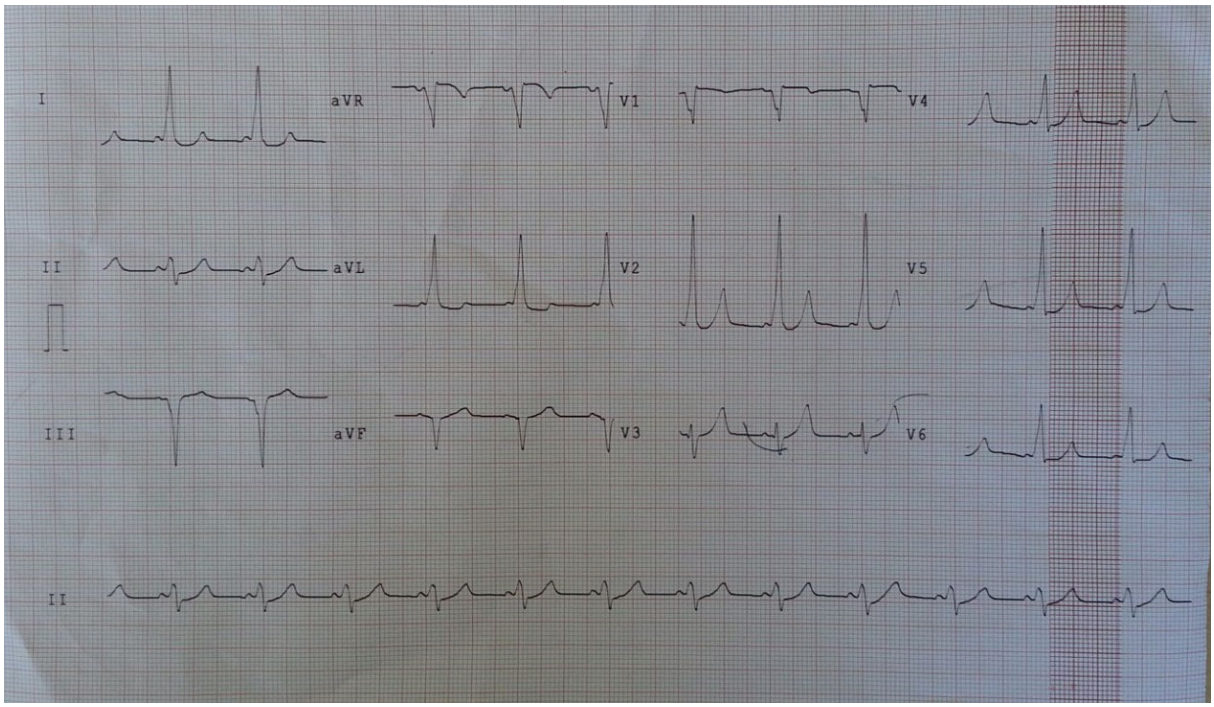


Figure 2. Twelve lead ECG showing characteristic delta waves and short PR interval of WPW Syndrome.

unit for three days and no complications developed. Electrophysiological study was performed and the accessory pathways were treated with catheter ablation. The patient was discharged without any complications. There were no cardiac complications on a third and six month follow-up.

3. Discussion

Incidences of AF in WPW syndrome is reported to be between 11% and 30%, higher than the normal population. In previous studies, it has been reported that sudden cardiac death occurs in patients with WPW each year, approximately at a rate of 0.15% and it has been demonstrated that the reason for these deaths are ventricular fibrillation caused by atrial fibrillation with rapid ventricular response (*5*).

Since its approval by the Food and Drug Administration (FDA) in 1998, sildenaphil has been used by millions of individuals worldwide (*6*) and has been well tolerated in the general population. Most of the side effects are minor, transient and dose dependent. In particular cases, when taken with organic nitrates, it can lead to severe hypotension. It is contraindicated in cases with acute coronary syndrome, life threatening arrhythmias and a recent history of stroke. The FDA has reported that it is necessary to use the drug carefully during the first six months following a recent myocardial infarction, in patients with hypotension, congestive heart disease (CHD) and severe hypertension (*7*).

Some predisposing factors for the development of AF in patients with WPW syndrome have been defined.

The presence of more than one accessory pathway, R-R interval shorter than 260 msec and drugs used in reciprocal tachycardias, such as verapamil, are the foremost among these (*1,8,9*). Hayashi *et al*. (*2*) have reported a similar case to this study, where the patient developed AF with rapid ventricular response and hypotension after using sildenaphil. As he had had no response to cardioversion, it is probable that the rhythm spontaneously returned to normal following the drug's action time. But unlike the current case, a fatal arrhythmia such as VF didn't develop. In the case presented by Awan *et al*. (*4*), AF with rapid ventricular response developed in a patient with HCMP on two occasions within a six-week period following the ingestion of sildenaphil but sinus rhythm returned with medical treatment. In the current case, AF with rapid ventricular response and subsequently fatal arhythmia, VF, developed a short period after sildenaphil use. It is not clear with these patients what mechanism from sildenaphil produces a predisposition to AF. The most probable explanation is that sildenaphil causes arterial vasodilatation, and as a result of developing hypotension causes increased activation in the sympathetic system. Increased sympathetic system activation might be one of the reasons that induces AF, as the effective refractory period of the accessory pathways is short, and the ventricular response in atrial fibrillation might have been quite rapid, causing ventricular fibrillation to develop.

In conclusion, the use of sildenaphil and similar drugs in patients with WPW, might cause atrial fibrillation and subsequently fatal cardiac arrhythmias. We believe that patients with an underlying cardiac pathology in particular, should be pre-warned and care should be taken before such drugs are recommended.

References

1. Ghofrani HA, Osterloh IH, Grimminger F. Sildenafil: From angina to erectile dysfunction to pulmonary hypertension and beyond. Nat Rev Drug Discov. 2006; 5:689-702.
2. Hayashi K, Minezaki KK, Narukawa M, Ookubo M, Mitsuhashi T, Shimada K. Atrial fibrillation and continuous hypotension induced by sildenafil in an intermittent WPW syndrome patient. Jpn Heart J. 1999; 40:827-830.
3. Shah PK. Sildenafil in the treatment of erectile dysfunction. N Engl J Med. 1998; 339:699.
4. Awan GM, Calderon E, Dawood G, Alpert MA. Acute, symptomatic atrial fibrillation after sildenafil citrate therapy in a patient with hypertrophic obstructive cardiomyopathy. Am J Med Sci. 2000; 320:69-71.
5. Centurión OA, Shimizu A, Isomoto S, Konoe A. Mechanisms for the genesis of paroxysmal atrial fibrillation in the Wolff Parkinson-White syndrome: Intrinsic atrial muscle vulnerability vs. electrophysiological properties of the accessory pathway. Europace. 2008; 10:294-302.
6. Sadovsky R, Miller T, Moskowitz M, Hackett G. Three-year update of sildenafil citrate (Viagra) efficacy and safety. Int J Clin Pract. 2001; 55:115-128.
7. Kloner RA, Jaron JP. Erectile dysfunction and sildenafil citrate and cardiologists. Am J Cardiol. 1999; 83:576-582.
8. Wellens HJJ, Bar FW, Fare J, Ross D, Vanagt EJ. Sudden death in the Wolff-Parkinson-White syndrome. In: Sudden Death, HE Kulbertus, HJJ Wellens (eds.). Sudden death, 1980. Martinus Nijhoff, Boston, 1980, pp.392-399.
9. Wyndham CR, Amat-y-Leon F, Wu D, Denes P, Dhingra R, Simpson R, Rosen KM. Effects of cycle length on atrial vulnerability. Circulation. 1977; 55:260-267.

(Received July 29, 2015; Revised August 6, 2015; Accepted August 12, 2015)

Commentary

DOI: 10.5582/irdr.2015.01017

The Birt-Hogg-Dubé cancer predisposition syndrome: Current challenges

Patrick R. Benusiglio[1,2,*]

[1] *Centre Expert National Cancers Rares PREDIR INCa, Hôpital Bicêtre AP-HP, Le Kremlin Bicêtre, France;*
[2] *Département de Médecine Oncologique, Consultation d'Oncogénétique, Gustave Roussy Cancer Campus, Villejuif, France.*

Summary **Birt-Hogg-Dubé is a rare syndrome in which carriers of germline mutations in the *FLCN* tumor suppressor gene are at risk of renal cell carcinoma of all histologies, most often of the chromophobe or hybrid chromophobe-oncocytoma type. Non-oncological manifestations such as lung cysts, pneumothoraces and skin fibrofolliculomas are also common. How germline mutations in a single gene can cause such different clinical features is intriguing and not fully explained, but involvement of the mTOR (renal cell carcinomas, lung cysts) and WNT (fibrofolliculomas) pathways has been described. Given the rarity of the condition, frequent exchanges of ideas between expert teams from around the world, multicentre international collaborations, and interactions between patients and researchers are essential. These needs are fulfilled through dedicated international symposia held every one to two years and through online resources aimed at patients and relatives.**

Keywords: Birt-Hogg-Dubé, fibrofolliculoma, hereditary neoplastic syndromes, pneumothorax, renal cell carcinoma

Birt-Hogg-Dubé (BHD) is a rare syndrome in which carriers of germline mutations in the *FLCN* gene are at risk of renal cell carcinoma (RCC) of all histologies, but most often of the chromophobe or hybrid chromophobe-oncocytoma type (*1,2*). Transmission is dominant. What is particularly interesting about this cancer predisposition syndrome is its association with non-oncological manifestations, more specifically lung cysts that often spare the apexes, spontaneous pneumothoraces and dome-shaped whitish skin lesions of the face and upper torso called fibrofolliculomas.

How germline mutations in a single gene can cause such different clinical features is intriguing and not fully explained. *FLCN* is a tumour suppressor gene, and gene inactivation follows the classic "two hit" model. BHD patients already have a germline mutation, and therefore a second somatic event is enough to initiate tumorigenesis (*e.g.* loss of heterozygozity, mutation, methylation). In the kidney, *FLCN* exerts its anti-tumour activity mainly by modulating the mTOR pathway (*3*). There are however conflicting data regarding the precise consequences of an inactivated FLCN protein, as both mTOR up- and down-regulation have been reported. Epithelial cells lining pulmonary cysts have no neoplastic or atypical characteristics but, like in kidney cancer cells, mTOR involvement is likely (*4*). Indeed, immunostaining studies suggest activation of the pathway and of its downstream effectors. As for cutaneous manifestations, fibrofolliculomas can be described as benign epithelial tumours of the hair follicles that could be caused by WNT pathway activation in neighbouring fibroblasts (*5*).

BHD illustrates the difficulties encountered with other rare diseases as awareness of the syndrome is limited, even among specialists involved in the management of associated clinical manifestations (*e.g.* urologists, oncologists, pulmonologists). As a result, the syndrome is often overlooked, patients are not referred to genetics clinics as often as they should and *FLCN* analysis is not performed. Underdiagnosing BHD in an index case and subsequently in his at-risk relatives can

Released online in J-STAGE as advance publication June 23, 2015.

**Address correspondence to:*
Dr. Patrick R. Benusiglio, Centre Expert National Cancers Rares PREDIR INCa, Hôpital Bicêtre AP-HP, 94275 Le Kremlin Bicêtre, France; Département de Médecine Oncologique, Consultation d'Oncogénétique, Gustave Roussy Cancer Campus, 94805 Villejuif, France.
E-mail: pbenusiglio.wk@gmail.com or patrick.benusiglio@gustaveroussy.fr

have damaging medical consequences, in particular with respect to RCC risk. Up to 34% of patients with BHD develop RCC (*6*), and regular screening with abdominal imaging is therefore essential (*1*).

Given the rarity of the condition, frequent exchanges of ideas between expert teams from around the world and multicentre international collaborations are essential. Every one to two years, scientists and clinicians working on BHD convene for an international symposium where they present and discuss the latest developements on the topic, start national and international collaborations, and review the available data in order to establish international guidelines or write state-of-the-art review articles. For example, a special issue on BHD adressing all the molecular and clinical aspects of the syndrome was published in Familial Cancer after the fourth BHD Symposium that took place in 2012 in Cincinatti, Ohio (*7*). The last symposium was held in Paris in June 2013, and from a French perspective it proved to be the springboard for a large national study on RCC characteristics associated with BHD (*8*). The next one will take place in Syracusae, New York State in September 2015, and will put an emphasis on drug development and therapeutics, and on the intra-operative management of patients with multi-focal RCC (*http://www.upstate.edu/urology/conference/index.php*). Sessions dedicated to patients are an essential component of these symposia, and a welcome opportunity to interact with physicians, surgeons, bench scientists and other affected individuals in an informal way. However, only a minority of patients have the chance to travel to these meetings, and alternative means of accessing reliable and up to date information are fundamental. Some online resources fulfil this need remarkably, and I would encourage all those interested in BHD, professionals and patients alike, to visit the BHD foundation website (*http://www.bhdsyndrome.org*).

Acknowledgements

The Centre Expert National Cancers Rares PREDIR is supported by the Ligue nationale contre le Cancer (Comités de l'Indre et du Cher), the Myrovlytis Trust (BHD Foundation) and the Fondation Gustave Roussy.

References

1. Stamatakis L, Metwalli AR, Middelton LA, Marston Linehan W. Diagnosis and management of BHD-associated kidney cancer. Fam Cancer. 2013; 12:397-402.
2. Menko FH, van Steensel MA, Giraud S, Friis-Hansen L, Richard S, Ungari S, Nordenskjöld M, Hansen TV, Solly J, Maher ER; European BHD Consortium. Birt-Hogg-Dubé syndrome: Diagnosis and management. Lancet Oncol. 2009; 10:1199-1206.
3. Schmidt LS. Birt-Hogg-Dubé syndrome: From gene discovery to molecularly targeted therapies. Fam Cancer. 2013; 12:357-364.
4. Furuya M, Nakatani Y. Birt-Hogg-Dube syndrome: Clinicopathological features of the lung. J Clin Pathol. 2013; 66:178-186.
5. Vernooij M, Claessens T, Luijten M, van Steensel MA, Coull BJ. Birt-Hogg-Dubé syndrome and the skin. Fam Cancer. 2013; 12:381-385.
6. Toro JR, Wei MH, Glenn GM, Weinreich M, Toure O, Vocke C, Turner M, Choyke P, Merino MJ, Pinto PA, Steinberg SM, Schmidt LS, Linehan WM. BHD mutations, clinical and molecular genetic investigations of Birt-Hogg-Dubé syndrome: A new series of 50 families and a review of published reports. J Med Genet. 2008; 45:321-331.
7. Czyzyk-Krzeska MF, McCormack FX. Birt-Hogg-Dubé syndrome. Fam Cancer. 2013; 12:355-356.
8. Benusiglio PR, Giraud S, Deveaux S, *et al*. Renal cell tumour characteristics in patients with the Birt-Hogg-Dubé cancer susceptibility syndrome: A retrospective, multicentre study. Orphanet J Rare Dis. 2014; 9:163.

(Received April 28, 2015; Revised May 19, 2015 ; Accepted May 21, 2015)

Letter

DOI: 10.5582/irdr.2015.01020

Electrocardiogram in anterior mid-ventricular Takotsubo syndrome variant

John E. Madias[1,2,*]

[1] *Icahn School of Medicine at Mount Sinai, New York, NY, USA;*
[2] *Division of Cardiology, Elmhurst Hospital Center, Elmhurst, NY, USA.*

To the Editor:

I enjoyed reading the report by Demirelli *et al*., published in the May, 2015 issue of the *Intractable & Rare Diseases Research*, pertaining to a 59 year old woman with emotional stress-induced anterior mid-ventricular Takotsubo syndrome (TTS) variant (*1*). The authors described the electrocardiogram (ECG) as showing T-wave inversions (TWI) in leads V1-V4. A recent paper reported an ECG correlate of TTS consisting of transient low voltage QRS (LVQRS) complexes (*2*). These ECG changes have been attributed to TTS-induced myocardial edema (ME), as detected by cardiac magnetic resonance imaging (cMRI) (*2*). Also TWI has been attributed to apicobasal ME gradient, as detected by cMRI (*3*). The present patient had a cMRI 4 weeks after her discharge, which confirmed the absence of chronic scar changes, compatible with TTS, but such late cMRI would not be suitable for the detection of ME, an early feature of TTS. Transient LVQRS often impacts the limb ECG leads (*2*), while affects leads I and aVL in patients with the midventricular TTS variant (*4,5*). Did this patient show transient LVQRS in the V1-V4 leads, which showed TWI? Also if serial ECGs were recorded, and if there was an ECG of this patient recorded prior to her admission with TTS, and at her 4 week follow-up, were there any transient LVQRS ECG changes, and in which leads?

References

1. Demirelli S, Ermis E, Hatem E, Uslu A, Askin L. Focal mid-ventricular anterior ballooning: An unusual pattern of Takotsubo cardiomyopathy. Intractable Rare Dis Res. 2015; 4:108-110.
2. Madias JE. Transient attenuation of the amplitude of the QRS complexes in the diagnosis of Takotsubo syndrome. Eur Heart J Acute Cardiovasc Care. 2014; 3:28-36.
3. Perazzolo Marra M, Zorzi A, Corbetti F, De Lazzari M, Migliore F, Tona F, Tarantini G, Iliceto S, Corrado D. Apicobasal gradient of left ventricular myocardial edema underlies transientT-wave inversion and QT interval prolongation (Wellens' ECG pattern) in Tako-Tsubo cardiomyopathy. Heart Rhythm. 2013; 10:70-77.
4. Madias JE. Electrocardiogram lead-specific QRS attenuation in an atypical midventricular case of takotsubo syndrome. J. Electrocardiol. 2013; 46:728-729.
5. Madias JE. Is the electrocardiogram different in recurrent episodes of Takotsubo syndrome involving 2 different left ventricular territories in the same patient? Int J Cardiol. 2015; 182:271.

(Received May 22, 2015; Accepted June 1, 2015)

Released online in J-STAGE as advance publication June 23, 2015.

*Address correspondence to:
John E. Madias, MD, FACC, FAHA, Division of Cardiology, Elmhurst Hospital Center, 79-01 Broadway, Elmhurst, NY 11373, USA.
E-mail: madiasj@nychhc.org

IRDR
Intractable & Rare Diseases Research

Guide for Authors

1. Scope of Articles

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

2. Submission Types

Original Articles should be well-documented, novel, and significant to the field as a whole. An Original Article should be arranged into the following sections: Title page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgments, and References. Original articles should not exceed 5,000 words in length (excluding references) and should be limited to a maximum of 50 references. Articles may contain a maximum of 10 figures and/or tables.

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

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

Policy Forum articles discuss research and policy issues in areas related to life science such as public health, the medical care system, and social science and may address governmental issues at district, national, and international levels of discourse. Policy Forum articles should not exceed 2,000 words in length (excluding references).

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

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

Letters should present considered opinions in response to articles published in Intractable & Rare Diseases Research in the last 6 months or issues of general interest. Letters should not exceed 800 words in length and may contain a maximum of 10 references.

3. Editorial Policies

Ethics: Intractable & Rare Diseases Research requires that authors of reports of investigations in humans or animals indicate that those studies were formally approved by a relevant ethics committee or review board.

Conflict of Interest: All authors are required to disclose any actual or potential conflict of interest including financial interests or relationships with other people or organizations that might raise questions of bias in the work reported. If no conflict of interest exists for each author, please state "There is no conflict of interest to disclose".

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

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

Copyright: A signed JOURNAL PUBLISHING AGREEMENT (JPA) form must be provided by post, fax, or as a scanned file before acceptance of the article. Only forms with a hand-written signature are accepted. This copyright will ensure the widest possible dissemination of information. A form facilitating transfer of copyright can be downloaded by clicking the appropriate link and can be returned to the e-mail address or fax number noted on the form (Please visit Download Centre). Please note that your manuscript will not proceed to the next step in publication until the JPA Form is received. In addition, if excerpts from other copyrighted works are included, the author(s) must obtain written permission from the copyright owners and credit the source(s) in the article.

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

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

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

4. Manuscript Preparation

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

Title page: The title page must include 1) the title of the paper (Please note the title should be short, informative, and contain the major key words); 2) full name(s) and affiliation(s) of the author(s), 3) abbreviated names of the author(s), 4) full name, mailing address, telephone/fax numbers, and e-mail address of the corresponding author; and 5) conflicts of interest (if you have an actual or potential conflict of interest to disclose, it must be included as a footnote on the title page of the

manuscript; if no conflict of interest exists for each author, please state "There is no conflict of interest to disclose"). Please visit Download Centre and refer to the title page of the manuscript sample.

Abstract: The abstract should briefly state the purpose of the study, methods, main findings, and conclusions. For article types including Original Article, Brief Report, Review, Policy Forum, and Case Report, a one-paragraph abstract consisting of no more than 250 words must be included in the manuscript. For News and Letters, a brief summary of main content in 150 words or fewer should be included in the manuscript. Abbreviations must be kept to a minimum and non-standard abbreviations explained in brackets at first mention. References should be avoided in the abstract. Key words or phrases that do not occur in the title should be included in the Abstract page.

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

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

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

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

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

References: References should be numbered in the order in which they appear in the text. Citing of unpublished results, personal communications, conference abstracts, and theses in the reference list is not recommended but these sources may be mentioned in the text. In the reference list, cite the names of all authors when there are fifteen or fewer authors; if there are sixteen or more authors, list the first three followed by *et al*. Names of journals should be abbreviated in the style used in PubMed. Authors are responsible for the accuracy of the references. Examples are given below:

Example 1 (Sample journal reference):
Inagaki Y, Tang W, Zhang L, Du GH, Xu WF, Kokudo N. Novel aminopeptidase N (APN/CD13) inhibitor 24F can suppress invasion of hepatocellular carcinoma cells as well as angiogenesis. Biosci Trends. 2010; 4:56-60.

Example 2 (Sample journal reference with more than 15 authors):
Darby S, Hill D, Auvinen A, *et al*. Radon in homes and risk of lung cancer: Collaborative analysis of individual data from 13 European case-control studies. BMJ. 2005; 330:223.

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

Example 4 (Sample web page reference):
World Health Organization. The World Health Report 2008 – primary health care: Now more than ever. *http://www.who.int/whr/2008/whr08_en.pdf* (accessed September 23, 2010).

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

Figure Legend: The figure legend should be typed on a separate page of the main manuscript and should include a short title and explanation. The legend should be concise but comprehensive and should be understood without referring to the text. Symbols used in figures must be explained.

Figure Preparation: All figures should be clear and cited in numerical order in the text. Figures must fit a one- or two-column format on the journal page: 8.3 cm (3.3 in.) wide for a single column, 17.3 cm (6.8 in.) wide for a double column; maximum height: 24.0 cm (9.5 in.). Please make sure that artwork files are in an acceptable format (TIFF or JPEG) at minimum resolution (600 dpi for illustrations, graphs, and annotated artwork, and 300 dpi for micrographs and photographs). Please provide all figures as separate files. Please note that low-resolution images are one of the leading causes of article resubmission and schedule delays. All color figures will be reproduced in full color in the online edition of the journal at no cost to authors.

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

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

5. Submission Checklist

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

6. Online submission

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

7. Accepted manuscripts

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

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

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

(As of February 2013)

Editorial and Head Office:

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

IRDR
Intractable & Rare Diseases Research

JOURNAL PUBLISHING AGREEMENT (JPA)

Manuscript No.:

Title:

Corresponding Author:

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

1. Authorship Criteria

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

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

2. Copyright Transfer Agreement

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

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

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

3. Conflict of Interest Disclosure

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

Corresponding Author's Name (Signature):

Date: