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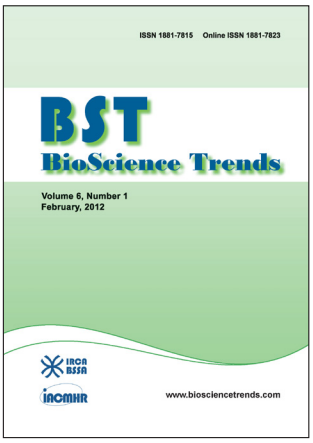

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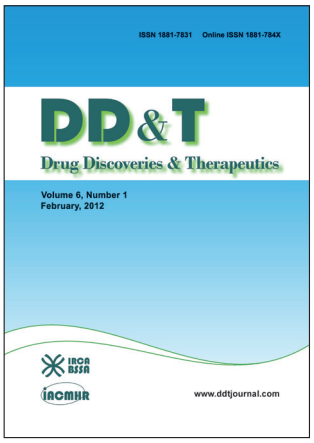

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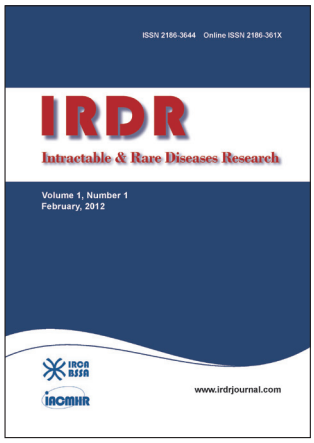

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Molecular genetics and general management of androgen insensitivity syndrome

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SUMMARY Androgen insensitivity syndrome (AIS) is a rare genetic disorder that affects the development of the male reproductive system in individuals with a 46,XY karyotype. In addition to physical impacts, patients with AIS may face psychological distress and social challenges related to gender identity and acceptance. The major molecular etiology of AIS results from hormone resistance caused by mutations in the X-linked androgen receptor (*AR*) gene. Depending on the severity of androgen resistance, the wide spectrum of AIS can be divided into complete AIS (CAIS), partial AIS (PAIS), or mild AIS (MAIS). Open issues in the treatment and management of AIS include decisions about reconstructive surgery, genetic counseling, gender assignment, timing of gonadectomy, fertility and physiological outcomes. Although new genomic approaches have improved understanding of the molecular causes of AIS, identification of individuals with AIS can be challenging, and molecular genetic diagnosis is often not achievable. The relationship between AIS genotype and phenotype is not well established. Therefore, the optimal management remains uncertain. The objective of this review is to outline the recent progress and promote understanding of AIS related to the clinical manifestation, molecular genetics and expert multidisciplinary approach, with an emphasis on genetic etiology.

Keywords AIS, androgen receptor, disorders of sex development (DSD), genetics

1. Introduction

The first case of androgen insensitivity syndrome (AIS; OMIM#300068) was reported in 1953 by Dr. John Morris, who called it testicular feminization, a phenomenon that causes feminization effects and involves the presence of testes observed in the body (*1*). AIS is a heterogeneous disease of hormone resistance (*2*) characterized by mutations of the androgen receptor (*AR*) gene. The incidence of AIS varies from 1:40,800 to 1:99,000 (*3*). AIS is a common disorder of sex development (DSD) with a 46,XY karyotype (*4*). It is distinguished by a variety of clinical features, including undermasculinization of the external genitalia at birth, abnormal secondary sexual development at puberty, and infertility. Depending on the degree of androgen resistance and the resulting physical characteristics, AIS is classified as complete (CAIS), partial (PAIS) or mild androgen insensitivity (MAIS). Although AIS mainly results from a loss-of-function in the *AR* gene (*4*), only approximately 85% of patients with a clinical diagnosis of CAIS and less than 30% with PAIS can be attributed to inactivating mutations in the *AR* gene (*5*). Not all individuals with clinical AIS exhibit mutations in the *AR* gene (*5*). The clinical diagnosis and optimal management remain challenging. The biology of masculinization depends on coordination among several signaling networks, such as androgen-dependent signals and downstream events (*6*).

This review provides an overview of the current research on AIS, covering its clinical manifestations, molecular genetics, and the importance of a multidisciplinary approach, with a particular focus on genetic etiology.

2. Clinical manifestation of AIS

AIS is classically characterized as an X-linked recessive genetic disorder due to absence or reduced functionality of the AR protein, which prevents the body from

responding to androgens. People with AIS are born with testes that produce androgens, but their bodies are unable to respond to these hormones. As a result, individuals with AIS may develop female-like physical traits such as breast development and lack of pubic hair.

Depending on the degree of androgen insensitivity (*5,7*), AIS manifests a broad spectrum of phenotypes from mild to partial or complete androgen insensitivity (*8*) (Table 1). CAIS occurs when an individual with the XY chromosome has a complete inability to respond to androgens, resulting in a typically female phenotype. The incidence of CAIS is estimated between 1 in 20,000 and 1 in 64,000 individuals with a 46,XY karyotype (*9*). PAIS occurs when there is some residual androgen receptor activity, leading to varying degrees of undervirilized male external genitalia or partially virilized. The incidence of PAIS is approximately 1 in 130,000 individuals with a 46,XY karyotype (*10*). PAIS patients usually present with clinical features such as micropenis, hypospadias and cryptorchidism. Individuals with PAIS may also have external genital anomalies such as bifid scrotum and penoscrotal transposition. MAIS is characterized by complete masculinization of the external genitalia, but individuals with MAIS typically show signs of incomplete masculinization, including gynecomastia at puberty and impaired spermatogenesis. Although the incidence of MAIS is much less than CAIS or PAIS, it has not been exactly measured.

The endocrine profile is responsible for producing and regulating hormones in the body. Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) play critical roles in the regulation of male reproductive function. LH is a gonadotropic hormone and stimulates the production of testosterone in testes (*11*), while FSH stimulates the production of sperm cells through its synergistic action with testosterone (*12*). In AIS, the characteristic feature of hormone spectrum is elevated levels of testosterone or normal basal testosterone levels that are associated with high serum LH, indicating impaired androgen negative feedback on the anterior pituitary (*13*). Despite the differences in the severity of androgen resistance among different types of AIS, there is no difference in hormonal levels (testosterone and LH) between them (*14*). Serum FSH levels were not different in individuals with MAIS, PAIS and CAIS (*14,15*). Therefore, hormone screening for AIS would be helpful, but lack specificity.

3. Molecular genetics of AIS

Androgens bind to the AR and activate its signaling pathway, which is essential for male sexual differentiation. The AR protein consists of 919 amino acid residues and is composed of four functional domains (Figure 1A): *i*) the N-terminal domain (NTD), which is encoded by exon 1 and initiates transcription of target genes; *ii*) the central DNA-binding domain (DBD) encoded by exons 2 and 3, which interacts with DNA and is critical for binding to hormone response elements (HREs); *iii*) the Hinge domain encoded by the proximal portion of exon 4, containing the phosphorylation site, which controls the AR activity; *iv*) the ligand-binding

Table 1. Clinical manifestation of different AIS phenotypes

Phenotypes	Prevalence	AIS With AR mutation	External Genitalia	Clinical characterization	LH (U/L)	FSH (U/L)	Testosterone (ng/dl)
 CAIS	1:20,000 to 1:64,000 (*9*)	85% (*5*)	Female	Absent or rudimentary Wolffian duct derivatives; Absence or presence of epididymides and/or vas deferens; Inguinal or labial testes; Short blind-ending vagina; Scant or absent pubic and/or axillary hair (*8*).	14–43 (*15*)	3.5–16 (*15*)	186–1,033 (*15*)
 PAIS	1:130,000 (*10*)	< 30% (*5*)	Predominantly female	Clitoromegaly and labial fusion, sinus urogenitalis with a wide opening, short, blind-ending vagina; Slight signs of androgen effects: slight clitoromegaly or partial labial fusion, distinct urethral and vaginal opening (*8*).	9–32 (*15*)	1.1–34 (*15*)	157–1,592 (*15*)
			Ambiguous	Microphallus with clitoris-like underdeveloped glans, labia majora like bifid scrotum, perineoscrotal hypospadias; Additional sinus urogenitalis with a short, blind ending vagina (*8*).			
			Predominantly male	Clitoromegaly and labial fusion, sinus urogenitalis with a wide opening, short, blind-ending vagina; Slight signs of androgen effects: slight clitoromegaly or partial labial fusion, distinct urethral and vaginal opening (*8*).			
 MAIS	NA	18% (*7*)	Male	Impaired spermatogenesis and/or impaired pubertal virilization (*8*).	2.7–25 (*14*)	0.6–50 (*14*)	141–2,047 (*14*)

AIS: androgen insensitivity syndrome. PAIS: partial androgen insensitivity syndrome. MAIS: mild androgen insensitivity syndrome. CAIS: complete androgen insensitivity syndrome. LH: luteinizing hormone. FSH: follicle-stimulating hormone. NA: not available.

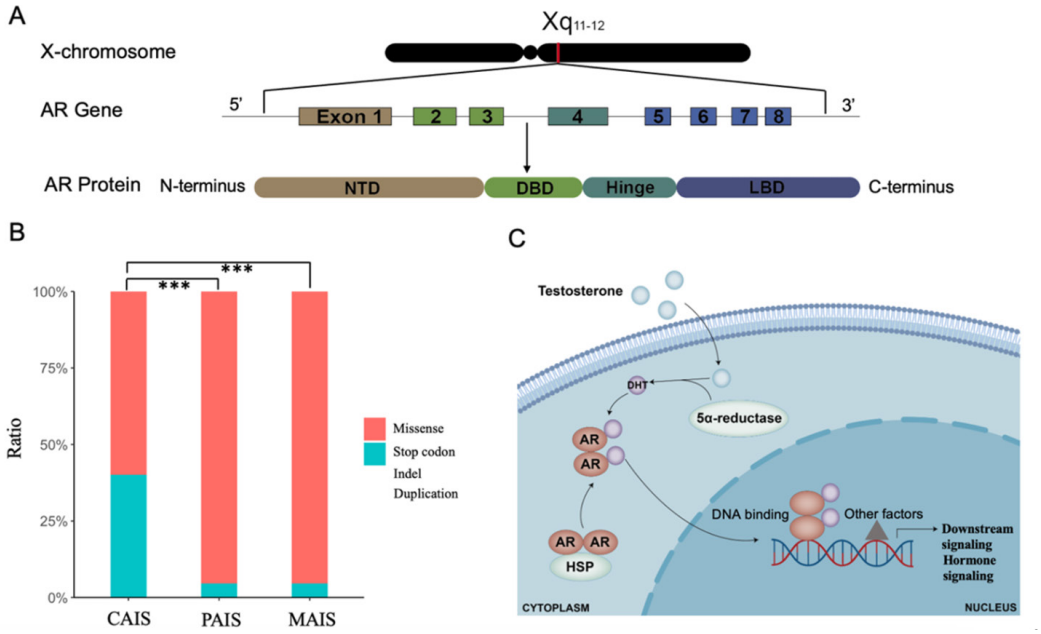

Figure 1. Structure and function of the androgen receptor (*AR*) gene, distribution of *AR* mutations in patients with AIS and androgen action. (A). A schematic representation of *AR* gene and AR protein. **(B)**. Distribution of different types of *AR* mutations in AIS showed that mutations with larger effect size (stop codon, insertion, deletion and duplication) are more frequently reported in individuals with CAIS in the androgen receptor mutations database. The asterisks indicate significant excess of mutations with larger effect size (***$p < 0.001$). **(C)**. Mechanism of AR activation through DHT binding.

domain (LBD) encoded by the remaining exons 4 and exons 5–8, which first facilitates interaction of AR with heat shock proteins (HSPs) in the cytoplasm, and then interacts with androgens, leading to translocation of the AR to the nucleus. In the androgen reporter databases (*www.mcgill.ca/androgendb*) (*7*), approximately 600 mutations in the *AR* gene were described in AIS. Mutations in the NTD domain are more common in patients with CAIS, whereas variants in LBD (exons 5 and 6) are more common in individuals with PAIS (*7*). Although almost all *AR* mutations associated with MAIS have been identified in the NTD domain, the number of *AR* mutations related to this phenotype remains relatively low (*15*). In the androgen receptor database, most *AR* variants identified in patients with AIS are missense mutations. Compared to missense variants, variants with larger effect size (stop codon, insertion, deletion and duplication) are more frequently reported in individuals with CAIS (Figure 1B).

Although the AIS diagnosis is characterized by the identification of mutations in the *AR* gene (*16*), there is not a robust correlation between AIS genotype and phenotype. The incidence of *AR* gene mutations tends to decrease progressively from CAIS to PAIS to MAIS. While mutations in *AR* gene were identified in more than 85% of individuals with CAIS, less than 30% of patients with PAIS (*5*) and about 18% of individuals with MAIS (*7*) are associated with a genetic abnormality in the *AR* gene. Especially in PAIS, partial loss of androgen action results in various phenotypes that depend on the overall fetal exposure to androgens. A new class of AIS group, AIS type II or *AR*-mutation negative AIS was proposed to better understand this type of AIS (*5*). Gene mutations outside the *AR* coding sequence have been discovered in patients with AIS, which may influence their response to androgens. For instance, a recurrent germline mutation in the 5'UTR of the *AR* resulted in aberrant translation in CAIS (*17*). In addition, epigenetic repression of *AR* transcription would contribute to *AR*-mutation negative class of AIS (AIS type II) (*18*). The aberrant methylation of CpG sites within the proximal *AR* promoter has been demonstrated to contribute to AIS (*19*). Furthermore, disruption of AR-dependent cofactors, such as *APOD*, would cause the *AR*-mutation negative class of AIS (AIS type II) (*5*). The duplication of an enhancer upstream of the *AR* gene leads to an increase in the expression of *AR* (*20*). This suggests that while AR is crucial for masculinization, multiple other components of the AR complex, including coactivators (*e.g.*, SRC and p300/CBP) (*21*), corepressors (SMRT, NCoR) (*22*), cofactors (*e.g.*, HSP56, HSP70, HSP90, β-catenin) (*23,24*) and androgen metabolism related genes (*25*) might be required for full masculinization.

The genetic etiology and underlying mechanisms leading to androgen resistance need to be further elucidated. Androgen-AR signaling plays a fundamental role in masculinization which involves many precisely regulated steps and biochemical interactions that are modulated by various types of cofactors (Figure 1C). The networks governed by core genes that interact with each other may offer valuable insights into comprehending the etiology of a disease (*26*). Dysregulation of any step regulated by androgen-AR signaling can potentially result in AIS. Hypospadias is a common feature in

individuals with AIS. In hypospadias, more than 70% of the proteins encoded by hypospadias risk genes that interact directly or indirectly with AR, thereby influencing androgen production and signaling (*27*). A recent study further demonstrated that triple compound rare damaging variants (one variant from *AR* and two variants from *SLC25A5*) rather than a single mutation yielded severe hypospadias (*25*). A following study demonstrated that hypospadias risk associated genes may influence *AR* expression through the AR-centered network (*27*) and genetic risk-associated transcription factors (TFs) (*28*). It has been indicated that *AR* may play a direct role in the genetic etiology of AIS, while genes that interact with *AR* and genes related to androgen-AR signaling have a minor impact on the etiology of AIS.

4. General management

Correct diagnosis of AIS patients is the basis for subsequent personalized treatment. In previous disease classifications, AIS was classified as intersex, while the current classification follows the DSD classification standards formulated by the Chicago consensus, which classifies AIS as 46, XY DSD (*29*). Therefore, the diagnosis of AIS patients may be confused due to the use of different disease classification standards. Since different types of AIS will be treated based on different strategies, it is recommended to re-diagnose some patients who were diagnosed using the old classification method according to the current standard before treatment (*2*). In addition, since other types of DSD may also exhibit phenotypes similar to AIS (*29*), it is necessary to perform a differential diagnosis through further endocrine evaluation and genetic sequencing.

The main principles of treatment for AIS focus on three main areas: performing surgery to reconstruct the external genitalia, removing abdominal gonads (such as undescended testes) to reduce the risk of cancer, and selecting the appropriate hormone therapy for the individual. To reduce the risk of testicular malignancy in individuals with CAIS, treatment options include prepubertal removal of the testes and provision for estrogen replacement therapy. Due to the ambiguous or indeterminate features of the external genitalia in PAIS individuals, surgical reconstruction requires more complex and extensive procedures compared to other types of AIS. Treatment for PAIS often involves surgery to reconstruct the external genitalia, removal of abdominal gonads to reduce the risk of cancer, as well as selection of appropriate hormone therapy for the individual. For PAIS, combined therapy is usually required. For example, endocrine and genetic evaluation should be considered for severe hypospadias (*30*). Additionally, reconstructive surgery can be performed to correct hypospadias, and testosterone therapy can be used to promote male secondary sexual differentiation. In MAIS, there is generally no need for surgical intervention, as affected individuals have fully developed male genitalia. However, counseling and hormonal therapy may be advised to those who experience gender dysphoria or other psychological effects.

Given the long-term consequences associated with the diagnosis of AIS, a collaborative approach involving physicians, patients and parents is crucial for making decisions. The formation of the external genitalia is one of the earliest and most visible signs of sex differentiation that occurs in the uterus during fetal development. Therefore, it may not be possible to initiate normal differentiation of external genitalia in individuals with AIS after birth without medical intervention. Due to androgen resistance, there are a variety of outcomes, including malignant disease (*31-35*), low bone mineral density and fractures (*36-38*), infertility (*14,39*), hypospadias and short vagina (*40,41*), impaired metabolism and cardiovascular disease (*42*), and mental disorders (*43-47*) (Table 2). The future aim of follow-up for AIS individuals is to identify the potential long-term health issues that may arise as a result of the condition, such as cancer risk, metabolic problems, cardiovascular disease, or mental health disorders.

5. Conclusions

Individuals with AIS may have impaired development of external genitalia and reproductive organs, leading to infertility and other consequences. Defining the genetic etiology for AIS is the foundation for understanding the pathogenesis of AIS and for long-term management of patients. The severity of AIS can vary widely and can be considered as an AR dosage-dependent condition. The largest-effect variants in *AR* play direct roles in AIS. Although molecular genetic diagnosis is achieved in almost all individuals with CAIS, identifying the genetic etiology of PAIS and MAIS is challenging and molecular genetic diagnosis is often not achieved. With the discovery of new genes contributing to androgen action and mechanisms involved in sexual differentiation, new concepts of AIS are emerging, especially for the *AR*-mutation negative class of AIS (AIS type II). We proposed that genetic contribution to PAIS is heavily concentrated in genes related to androgen-AR signaling or AR-centered network that are transcribed or expressed in relevant tissues. Rare damaging variants are likely to be causative than other classes of variants and hence explain some of the unknown genetic causes of birth defects, such as hypospadias (*48,49*). Furthermore, new genomic approaches for identifying non-coding, mosaic, structural or epigenetic variants will improve the understanding of the molecular causes (*50*). As additional genetic and epigenetic causes of AIS are identified, the diagnosis of these conditions will become more precise.

Patients with AIS, their parents, and healthcare providers face challenging decisions regarding gender assignment, genital surgery, and lifelong care. A

Table 2. Summary of the long-term consequences of AIS.

Long-term outcomes	CAIS	PAIS	MAIS
Malignant disease	Incidence rate (*31,34*): 1–2% in CAIS. Cause (*33*): the low rate of germ cell tumor in CAIS could be attributed to the rapid reduction of the germ cell population after the first year of life.	Incidence rate (*32*): > 15% in PAIS. Cause (*35*): untreated patients have a risk of up to 50% for undescended gonads, while the risk of scrotal testes remains unknown.	NA NA
Low bone mineral density and fractures	Outcome (*37*): the final height of CAIS and PAIS individuals was greater than the average height of women and lower than the average height of men.		NA
	Fracture rates among patients with CAIS who underwent gonadectomy ranged from 2% to 27% (*36,38*).		NA
Infertility, subfertility	No reported cases of biological fertility (*14,39*).	Usually infertility (*14*).	Fertility ay occur spontaneously or be induced through androgen treatment (*14*).
Gynaecomastia, hypospadias and short vagina	Patients with CAIS and phenotypically female individuals with PAIS have a short, blind-ending vagina (*40*).	All individuals with AR mutation develop gynecomastia; hypospadias cases with AR mutation are more likely to require additional surgical treatment than hypospadias cases without an *AR* mutation (*41*).	NA
Impaired metabolism and cardiovascular disease	Higher prevalence of obesity (16.7% *vs.* 3.6%), 56% higher total cholesterol, 33% higher low-density lipoprotein-cholesterol, 16% higher triglycerides, and 47% higher HOMA-Index (*42*).		
Mental disorder	Both PAIS and CAIS patients had lower quality of sexual life (*43,46*).		NA
	Patients with CAIS had a 5-fold higher risk of psychiatric disorders than the general population, a 3-fold higher risk of mood disorders, a 4-fold higher risk of anxiety disorders, and a 20-fold higher risk of obsessive-compulsive disorder (*44*).	Male PAIS patients often present with psychiatric and psychological problems due to clinical symptoms of poor virilization (*45,47*).	NA

NA: not available.

multidisciplinary team, such as geneticists, urologists, endocrinologists, and psychologists, can provide expertise in different areas of AIS diagnosis and treatment, and help facilitate shared decision-making. These advances are systematically improving the prediction of prognosis and improving the diagnosis and long-term management of patients with AIS.

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Review

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Urogenital sinus malformation: From development to management

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SUMMARY Urogenital sinus (UGS) malformation, also known as persistent urogenital sinus (PUGS), is a rare congenital malformation of the urogenital system. It arises when the urethra and vaginal opening fail to form properly in the vulva and fuse incorrectly. PUGS can occur as an isolated abnormality or as part of a complex syndrome, and is frequently associated with congenital adrenal hyperplasia (CAH). The management of PUGS is not well-established, and there are no standardized guidelines on when to perform surgery or how to follow up with patients over the long term. In this review, we discuss the embryonic development, clinical evaluation, diagnosis, and management of PUGS. We also review case reports and research findings to explore best practices for surgery and follow-up care, in hopes of increasing awareness of PUGS and improving patient outcomes.

Keywords UGS, PUGS, urogenital sinus malformation, embryology, management

1. Introduction

Urogenital sinus (UGS) malformation, also known as persistent urogenital sinus (PUGS), is a rare congenital pathological disease with an incidence of approximately 6/100,000 women (*1,2*). The urogenital sinus is a structure that typically develops during embryonic development. There is a confluence between the urethral and genital openings during the embryonic stage, and then the two structures separate and differentiate into the urinary and reproductive tracts. When the differentiation process is hindered or disrupted later, PUGS may occur. It can either be the sole malformation or a part of a complex syndrome. According to Campbell Walsh Wein Urology (*3*), PUGS occurs in four situations. It usually occurs in cases of unidentified external genitalia, and most frequently with congenital adrenal hyperplasia (CAH), which is an autosomal recessive disease with an incidence of 1/15,000-1/16,000 (*4,5*). Studies have reported that in atypical mild CAH, the incidence of PUGS can be as high as 1/500 (*6*). Additionally, it can occur with normal external genitalia, in cloacal malformations involving the rectum, as well as female exstrophy.

This review sought to summarize the relevant embryological development and pathogenesis, clinical assessment (manifestation and diagnosis), surgical and systematic management, case reports and research results, aiming to increase relevant medical personnel understanding of the PUGS.

2. Embryology and Etiology

Before the seventh week of pregnancy, the genitourinary system of male and female fetuses are in an identical undifferentiated precursor state. The differences in masculinization are mainly caused by the expression of the *Sry* gene and related downstream genes and their products, such as anti - Müllerian hormone and testosterone (*7*).

In normal female internal genitalia, without the presence of the *Sry* gene, the reproductive tract follows a feminization pathway (*8*). During embryonic development, the epithelium of the Müllerian ducts (pararenal ducts) fuse distally to form the ureterovaginal canal and migrate towards the caudal end of the urogenital sinus under the guidance of the Wolffian duct (mesonephric duct) (*9*), which contributes to the formation of the uterine cavity and most of the vagina. At approximately 10 weeks, the ureterovaginal tube attaches to the urogenital sinus, forming solid tissue coagulation commonly called sinus bulbs or sinus ridges. Previous studies have suggested that at weeks 10–20, the solid tissue portion of the sinus ridge extends caudally towards

the perineum to form the lower part of the vagina, while the upper part of the vagina is mostly derived from the ureterovaginal (*7*). However, as research progresses in humans, the intricacy of vaginal development may exceed prior assumptions. Robboy *et al*. (*10*) found that vaginal growth is not simply divided into upper and lower parts, but rather it dynamically proliferates by the pararenal epithelium and urogenital sinus epithelium during 10-21 weeks. Interestingly, vaginal development in mice is also dynamic. Kurita *et al.* reported that in embryonic and neonatal mice, the vagina consists of a fusion of the Müllerian duct epithelium and the urogenital sinus epithelium (*11*). However, as growth occurs into adulthood, the vaginal epithelium is derived only from the Müllerian duct (pararenal duct) epithelium (*11*). This was also confirmed by a recent study conducted by Harada M *et al.* (*12*). They found that postnatal descending growth of the Müllerian duct epithelium is attributed to rapid cell proliferation in the Müllerian duct epithelium and its surrounding mesenchymal tissue, as well as the apoptosis of urogenital sinus epithelial cells (*12*).

In the development of external genitalia, the cloaca subdivides to form the urogenital sinus, and the cloacal membrane ruptures to form the urogenital plate on the surface of the perineum. In front of the urogenital plate, interstitial condensation forms the genital tubercle. In females, the genital tubercle develops into the clitoris, the urogenital folds form the labial folds (labia major and minora), and the urogenital plate remains open, creating vaginal introversion (*13*). A study of female fetal external genitalia development in the second trimester found that the solid urethral plate opens through cell proliferation and extends laterally to form the vestibular groove in a zipper-like manner (*14*). However, unlike in males, where the double zippers close to form the tubular penis urethra, there is no evidence of zipper activity in females, and the vestibular sulcus remains open to form the vestibule and inner chamber (*14*). Between the 12th and 16th weeks of gestation, the junction between the developing lower vagina and urogenital sinus is displaced caudally until it stops at the urogenital sinus posterior wall, separating from the urethra in the vestibule to obtain a separate vaginal opening (*8*).

Although some studies have suggested that renal duct hypoplasia or insufficient growth of the tail urogenital wedge may cause some cases of PUGS (*15,16*), most are caused by the high androgen levels stimulated by CAH. CAH is an enzyme deficiency, commonly involving 21-hydroxylase deficiency (*17*). This deficiency leads to a blockade in hormone synthesis pathways, resulting in the accumulation of steroid precursors that ultimately convert to circulating testosterone *via* 4-androstenedione. Excessive circulating testosterone leads to variable degrees of virilization of the developing external genitalia in female fetuses (*18,19*). The formation of the urethral groove, which plays a role in both male and female, is independent of androgen stimulation, while the closure of the urethral groove to form the tubular male urethra by the closed zipper mechanism is an androgen-dependent process (*7*). In male fetuses, the formation of a tabular urethra occurs *via* a closed zipper mechanism, where the urethral plate fuses from the bottom to the tip which is an androgen dependent process (*20*). Disruptions in the process of tubulization are associated with disturbances in the androgen signaling pathway. Genetic variations in the androgen signaling pathway can affect the development of the urethra, such as the formation of hypospadias (*21*). Unlike males, in female fetuses, the urethral sulcus remains open to form the vestibule due to the low levels of testosterone. When this normal structure undergoes tubalization, which is stimulated by elevated androgen levels and involves a zipper-like closure mechanism, a urethral-vaginal fusion can occur, resulting in PUGS.

Although some individuals may appear male, those with a 46,XX karyotype have female internal sex organs, including the ovaries and vagina. However, the vaginal opening may be connected to the urethra instead of the vulva, with the junction of the vagina and urethra varying from the proximal confluence near the bladder neck to the distal confluence near the perineum, depending on the androgen dependent closure of the zipper mechanism. The urethral groove may be partially or completely closed, forming a tubular male urethra of variable length (*18*). Meanwhile, the location of the vaginal confluence depends on the descending position of the sinus ridge, which is related to androgen levels. Androgens inhibit the descending movement of the sinus ridge (*22,23*) and can prevent the formation of the vaginal opening in the vulva. Prenatal exposure to androgens in female mice has been found to inhibit the decline of the sinus ridge and prevent the formation of the vaginal opening in the vulva (*24*). The specific time and duration of androgen exposure determined the location of the confluence of the vagina and urethra (*24*). Mesenchymal cells adjacent to the urothelium are likely the primary target of androgen signaling for urogenital sinus ridge morphogenesis (*24*). Recent studies also showed that the position of the sinus ridge is influenced by the amount of androgen exposure, such that higher doses of androgen result in a proximal shift of the region where the vagina and urethra meet (*i.e.*, towards the bladder neck) (*25-27*).

3. Clinical manifestation and diagnosis

The clinical manifestations of PUGS are quite variable and mainly depend on the location of the confluence entrance and the size of the sinus ostium. Children with a lower confluence location and larger sinuses are more likely to be asymptomatic or prone to urinary tract infections, while those with smaller sinuses are more susceptible to urinary tract infections. If the confluence location is high, urinary incontinence or menstrual

hematuria may occur. In addition, when the sinus opening is small, it can lead to a congenital absence of the vagina, which can cause dyspareunia. In addition, PUGS can present as a pelvic mass (associated with bladder distention due to dilation of the vagina and uterus resulting from obstruction), hydrometrocolpos (atresia of the hymen or urogenital sinus stenosis), and hydronephrosis (dilation of the upper urinary tract due to obstruction). As most patients of PUGS are secondary to CAH, there is also genital ambiguity and possible hypertension (*3*).

Early diagnosis and timely decompression of PUGS is crucial due to the potential compression of the urinary and reproductive systems. Prenatal diagnosis is commonly performed using ultrasonography during the 20th to 24th weeks of gestation (*28*), the observation of a pelvic cystic mass containing fluid-filled debris situated posterior to the bladder may be representative of hydrometrocolpos (*1*), and the septum visible across the cystic structure is likely to be the urogenital septum (*29*), these may suggest the diagnosis of PUGS. Postpartum diagnosis should be considered for girls presenting with urinary incontinence, urinary tract infection at birth, vaginal swelling or fluid accumulation, and for adults with cyclical periods and vaginal atresia (*30*). Most patients are identified due to ambiguous genitalia, and diagnosis requires a combination of medical history, clinical examination, laboratory tests, and imaging tools. The medical history should include details about the mother's physical condition during pregnancy, the use of androgen-containing drugs during the first trimester, a family history of similar deformities, and the presence of monthly regular urine with blood during puberty or adulthood. The physical examination should include a thorough examination of the internal and external genitalia, such as groin examination, anus and abdominal examination to see if the uterus is palpable. The laboratory examination should include peripheral blood karyotyping to identify female or male pseudohermaphroditism, androgen levels and peripheral blood 17α-hydroxyprogesterone (17α-OHP). For patients with increased 17α-OHP levels, a further dexamethasone suppression test should be performed to determine the presence of CAH. The primary imaging modality used is ultrasound (*1,31*), while other imaging methods, including voiding cystourethrography (*3,32*), magnetic resonance imaging (MRI) (*3,33,34*), computed tomography (CT) (*35*), cystoscopy (*36-38*), and contrast-enhanced genital angiography (*39*) (Figure 1), are commonly utilized to provide assistance (*1*). The roles, advantages and disadvantages of these methods are summarized in Table 1.

4. Classification

The classification of PUGS is essential for the treatment team to reach a consensus in discussion and describing the condition, which in turn allows for a better design of subsequent surgical plans. The examinations used for classification include evaluating the shape and size of the penis or clitoris, the labia-scrotal fold, the position of the vagina and urethra, the length of the urethra and common channel. However, there is currently no internationally recognized standard. Typically, clinicians use two classification methods, Powell (*40*) and Prader (*41*), based on the confluence location of the vaginal urethra and the degree of virilization of the vulva (as shown in Table 2). However, some studies suggest that these two methods have limitations (*29*). In normal women, the urethra begins from the internal urethral orifice, passes through the urogenital diaphragm (UGD), and extends down some distance to the perineal opening. Therefore, the bladder neck and proximal urethra in normal women are located above the UGD. When the bladder neck is appropriately supported by the UGD , the urethral pressure remains higher than the bladder pressure. Any stressful event that increases intra-abdominal and intravesical pressure closes the urethra, preventing leakage and maintaining continence (*42*). Although the length of the common channel was previously believed to affect continence, some researchers have shown that the length of the urethra also plays an important role (*43*). Moreover, the relationship between the location of confluence and bladder neck is a crucial factor in surgical intervention, as opposed to the length of the common channel, for the management of urogenital sinus malformation (*3*). The goals of treatment include achieving voiding control and normal sexual function.

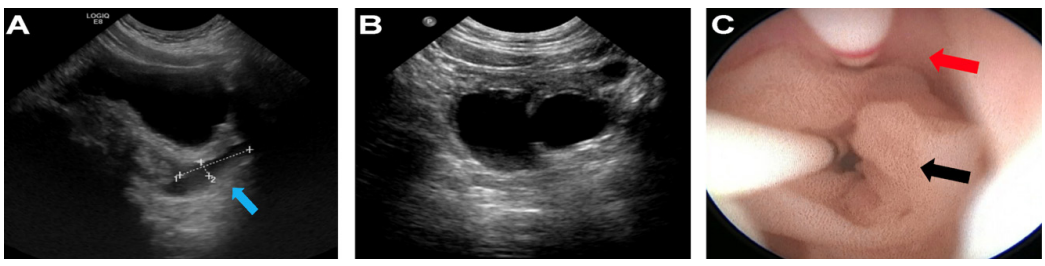

Figure 1. Imaging morphology of persistent urogenital sinus. Ultrasound scan shows two different imaging morphology: **(A)** vaginal effusion (indicated by blue arrows) and **(B)** double uterus and double vaginal malformation; cystoscopy shows **(C)** common channel of urethra (red arrow) and vagina (black arrow).

Table 1. Diagnostic imaging methods for persistent urogenital sinus

Image method (*Ref.*)	Application selection	Role	Advantages	Disadvantage
Ultrasound (*1,31*)	Prenatal diagnosis; Postpartum diagnosis.	Identify the location of the urinary and reproductive organs and whether the hydrops is expanding.	First-line diagnostic method; Simple and convenient; Prenatal diagnosis; No radiation.	Anatomy is unclear; Need to combine other imaging methods.
Voiding Cystourethrography (*3,32*)	Urethral malformation or dysfunction children at higher risk.	Identify the relationship between the urogenital sinus, vagina, urethra, bladder, and uterine contours.	No need for anesthesia; Urogenital permissible system anatomical assessment.	Can only be assessed during the excretion period; The effect depends on the operator's experience.
CT (*35*)	Children with complex malformations or rectal cloacal malformations.	With 3D reconstruction, a description of the cloacal malformation can be obtained.	Clearly, delineate regional anatomically relevant structures.	Radiation exposure; Not recommended in the absence of complex deformities.
MRI (*3,33,34*)	Children with pure PUGS or other sexual developmental abnormalities and cloacal malformations.	Accurate description of internal organs and evaluation of their relationship to the rectum.	No contrast agent required; No need to limit urination period.	Limited value in CAH diagnosis; Need anesthesia.
Cystoscopy (*36-38*)	Before or during surgical reconstruction.	Determining the anatomical relationship of the genitourinary tract.	Compared with other methods, the accuracy rate is very high; Can clearly determine the length of the urethra and vagina and opening position.	Need anesthesia; Invasive operation.
Enhance reproductive tract radiography (*39*)	Preoperative planning of surgical plan.	Determine the length of the urethra, genital tract, and the length of the confluence.	The uterus can be directly displayed at the same time.	Contrast agent required; Generally, not recommended.

Table 2. Preoperative classification of persistent urogenital sinus

Type name (*Ref.*)	Typing method	Specific type	Disadvantage
Powell (*40*)	Where the vaginal urethra joins	Type I is characterized by lip infusion; Type II is characterized by distal inflow; Type III is defined as proximal or high influx and long common tract; Type IV is characterized by vaginal absence.	Virilization assessment in individuals without external genitalia; No common channel and urethral length.
Prader (*41*)	The degree of virilization of the external genitalia and whether there is convergence of urethra and vagina	Type I: The clitoris is slightly larger, and the vagina and urethra are normal; Type II: The clitoris is larger and the vaginal opening is funnel-shaped, but the vagina and urethral opening are still separated; Type III: The clitoris is significantly enlarged, only one opening is visible in the vulva; Type IV: The clitoris is significantly enlarged like a penis, one opening at the base of the clitoris, similar to hypospadias; Type V: The clitoris resembles a male penis, the urethral opening is at the head of the clitoris, and complete fusion of labia majoris.	There is no exact correlation between the degree of virilization of the external genitalia and the location of the vaginal-urethral fusion; No common channel and urethral length.

5. General management

5.1. Surgical treatment

The current treatment for PUGS involves reconstructive surgery of the female genitalia, which comprises clitoroplasty, labioplasty and vaginoplasty. Vaginoplasty is the most critical step, and there are currently five surgical techniques in clinical practice: urogenital sinus incision, perineal flap vaginoplasty (Fortunoff flap, Ω-shaped flap), "pull-through" vaginoplasty (recumbent position, incision for lithotripsy position, and anterior sagittal transrectal approach, ASTRA), genitourinary mobilization (total urogenital mobilization or TUM, and partial urogenital mobilization or PUM) and total vaginal replacement. Urogenital sinus incision was the earliest technique used in vaginoplasty (*44*) and is only suitable for children with Prader grades I and II (*45*). Due to the lower Prader grade, the vulva shape is closer to that of a typical female, resulting in a better postoperative appearance. However, this technique does not address the narrow vaginal opening, leading to a relatively narrow vaginal opening in adulthood. The flap vaginoplasty technique widens the vaginal entrance using the posterior

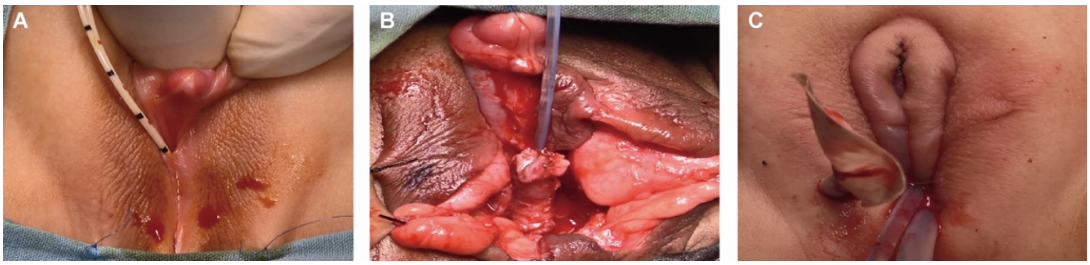

Figure 2. Total urogenital mobilization vaginoplasty method. (A) Initial perineal opening in a patient with PUGS; **(B)** Annular separation to release urogenital sinus; **(C)** Final appearance after surgery.

perineal flap. The insertion of the perineal U-shaped flap, also known as the Fortunoff flap, is a standard surgical approach for repairing low confluence genitourinary sinus abnormalities (*46*). In 2001, Jenak *et al.* modified this approach into a perineal omega-shaped flap (*36*). This procedure only opens the vaginal opening and urogenital sinus without changing the location of the vaginal confluence, making it generally unsuitable for high vaginal confluence due to the risk of complications such as vaginal urination, infection, and incontinence. This procedure is usually used alone for urogenital sinus plasty in patients below Prader III and more often in combination with other vaginoplasty procedures, such as urogenital mobilization (*36*). The "pull-through" vaginoplasty was first proposed by Hendren and Crawford in 1969, involves separating the vagina from the urogenital sinus and using the genital sinus to form the urethra. The free vagina is then pulled to the perineum, but most cases require surrounding flaps to form the vaginal opening (*47*). Among them, the separation of the anterior vaginal wall from the urethra and bladder neck is critical, as this area is difficult to fully expose and it is also the complex nerve supply area for the vagina and urethra (*48*). Complications such as vaginal urethral fistula, urinary continence disorder, vaginal stenosis, and poor appearance are prone to occur. Based on this, Salle *et al*. developed the ASTRA procedure to increase the exposure field of the surgical area and improve the free vagina, but it requires to change the patient's surgical position and involves the rectum (*49*). Therefore, this procedure is generally only used when the vaginal confluence location is high and urogenital mobilization cannot pull the vagina to the perineum without tension. Braga *et al*. (*50*) recommended it only when the common channel length exceeds 3 cm. TUM was first used as a vaginoplasty method when Pena completed cloacaplasty in 1997 (*51*). During the operation, the entire urogenital sinus was annularly separated and moved outward to the perineum (as shown in Figure 2). This method significantly shortens the operation time, improves the appearance of the vulva after surgery, and reduces the risk of complications such as urethrovaginal fistula and vaginal stenosis. Furthermore, in 2005, Rink *et al*. (*52*) proposed the use of PUM as an alternative to TUM due to the potential risk of nerve and sphincter between the urogenital sinus and the pubis during annular separation of the proximal urogenital sinus, which can result in complications such as urinary incontinence and sexual dysfunction. The difference is that in PUM, the circular anatomy of the urogenital sinus is performed but terminates at the level of the pubourethral ligament, which avoids damaging the nerve and sphincter between the urogenital sinus and the pubic bone (*52*). Acoording to Rink *et al*., the choice of these two surgical methods can be determined based on the location of the vaginal confluence. TUM is utilized when vaginal confluence is positioned high, and more urogenital sinuses need to be freed to make the vagina reach the perineal area without tension. PUM is recommended in cases where this is not necessary. Genitourinary mobilization is suitable for most patients, except in rare cases of poor vaginal development or extremely high vaginal confluence location. Total vaginal replacement, which is only used for hypoplastic or missing vaginas (*53*), is rarely performed.

However, the literature on PUGS surgical data is very limited. Most of the research data are retrospective and based on a small number of cases, with a focus only on short-term outcomes such as the appearance of the vulva and complications after surgery. These studies do not provide strong evidence on the long-term efficacy of these procedures, particularly with regards to sexual function. We summarized the available literature on PUGS surgical data in recent years (*36, 54-64*) (Table 3).

5.2. Surgical timing and selection

The optimal age for female genital reconstruction has been debated. At the IVth World Congress of the International Society of Hypospadias and Disorders of the Sex Development (ISHID), 78% of global delegates voted in favor of surgery before the age of 2, and most recommended one-stage plasty that includes clitoroplasty, labiaplasty, and vaginal surgery (*65,66*). This approach is designed to impart an early appearance consistent with the female parenting gender and cause less psychological harm than delayed surgery (*67*). However, there is a growing belief that surgical interventions in childhood, especially irreversible surgery, should be limited or postponed until the child

Table 3. Literature summary of persistent urogenital sinus surgical data

References (Year) (*Ref.*)	Type of study	Length of common channel	Surgical method	Complication	Key conclusion
Jenak R, *et al.* (2001) (*36*)	Case-report	> 3 cm	TUM	No lower urinary tract symptoms; No urinary incontinence.	TUM does not affect urination or urinary incontinence.
Kryger JV, *et al*. (2004) (*54*)	Review case study	2.1 cm (average)	TUM	2 cases lower urinary tract symptoms; 1 case urgent urination.	Patients with urinary control before surgery have immediate control ability after TUM; TUM does not appear to interfere with the normal development of urinary control in children undergoing surgery before the age of urinary control.
Kitta T, *et al.* (2004) (*55*)	Case-report	Not mentioned	Flap vaginoplasty	No lower urinary tract symptoms; No urinary incontinence.	Appropriate urinary care is crucial for preventing urinary complications in patients with simple PUGS.
Gosalbez R, *et al*. (2005) (*56*)	Review case study	Not mentioned	TUM + Fortunoff	No lower urinary tract symptoms; No urinary incontinence.	TUM does not affect urination or urinary incontinence.
Braga LH, *et al*. (2006) (*57*)	Prospective case study	3 cases < 2 cm 21 cases > 2 cm	PUM	No lower urinary tract symptoms; No urinary incontinence.	PUM enables urination control and good appearance, and fully exposes vaginal and urethral openings.
Palmer BW, *et al*. (2012) (*58*)	Review case study	13cases < 3cm 12cases > 3cm	18 cases TUM 7 cases PUM	5 cases after TUM with nocturnal enuresis for more than 1 year.	There was no significant difference between TUM and PUM in postoperative urinary incontinence, with a postoperative urinary control rate of 96%.
Ludwikowski BM, *et al*. (2013) (*59*)	Review	Not mentioned	TUM	No lower urinary tract symptoms; No urinary incontinence.	TUM does not affect urination or urinary incontinence.
Bailez MM, *et al*. (2014) (*60*)	Review case study	3.75 cm (19 cases, average) 6.34 cm (33 cases, average) 11.5 cm (3 cases, average)	PUM (low position) TUM (median position) TUM + ASTRA (high position)	No lower urinary tract symptoms; No urinary incontinence; Three cases were reported to have a normal sexual life.	TUM/PUM does not affect urination or urinary incontinence.
Jesus VM, *et al.* (2018) (*61*)	Review case study	Not mentioned	TUM/PUM	No lower urinary tract symptoms; No urinary incontinence.	TUM/PUM does not affect urination or urinary incontinence.
Fares AE, *et al*. (2019) (*62*)	Review case study	< 1.5 cm	Laparoscopic assistance "pull-through" vaginoplasty	No lower urinary tract symptoms; No urinary incontinence.	Laparoscopic assisted pull-through vaginoplasty provides good exposure, helps to separate the vagina from the urethra, and avoids damage to the urethral structure.
Ulusoy O, *et al.* (2021) (*63*)	Review case study	4.6 cm (average)	Posterior prone approach "pull-through" vaginoplasty	No lower urinary tract symptoms; No urinary incontinence.	Posterior prone approach "pull-through" vaginoplasty does not affect urination or urinary incontinence.
Yang J, *et al.* (2023) (*64*)	Case-report	Not mentioned	Robotic UGS mobilization	No lower urinary tract symptoms; No urinary incontinence.	Very high confluence PUGS can use robotic UGS mobilization.

becomes an adolescent, allowing them to be more involved in the surgical decision. Some researchers also suggest that the timing of surgery should depend on the location of the vaginal confluence point. Early one-stage plasty has become the standard for patients with a low vaginal confluence, but there is still much controversy for patients with a high vaginal confluence. Most believe that vaginoplasty should be performed at the same time as clitoroplasty and labiaplasty so that the excess clitoral foreskin can be used for reconstruction. However, some scholars suggest that these patients can participate in the choice of surgical methods during puberty, when their estrogen levels facilitate tissue healing, vaginal growth, and dilation. Therefore, delayed vaginoplasty is recommended for patients with short vaginas (< 3 cm) and high confluence points (*45*).

Accurate choosing of the surgical approach and implementing skilled surgical technique are key factors in ensuring a favorable postoperative appearance. Urogenital sinus incision may be used for patients with Prader class I, while perineal flap vaginoplasty may be employed for patients with Prader class I and II. For patients with Prader grade II or above, the surgical approach must be determined based on the location of the vaginal confluence point, the length of the common channel and the length of the urethra. First, the degree of external masculinization is not completely related to internal anatomy, and the depth of the vaginal confluence point is an indicator of the range of motion required for perineal approach surgery. PUM is suitable for low confluence and median confluence (the vaginal confluence point is at the level of the external urethral sphincter), and TUM can be used for high confluence. The "pull-through" method is suitable for very high confluence. Studies have shown that in 90% of cases (1–3 years old), this depth is < 20 mm, so PUM may be appropriate in most cases (*68*). Second, regarding the length of the common channel, Tugtepe *et al*. (*69*) recommended using PUM for patients with less than 2 cm and TUM for patients with a length of 2.5 to 3.5 cm. When it exceeds 4 cm, the "pull-through" method is recommended (*69*). Braga *et al*. (*57*) considered that PUM can still be used when the length exceeds 3 cm. Third, The bladder neck and proximal urethra of normal women are located above the urogenital diaphragm. When the bladder neck is well supported by the urogenital diaphragm, the urethral pressure is always higher than the bladder pressure. Stressful events that increase stress close the urethra, preventing leakage and maintaining continence (*42*). The previous view was that this depends on the length of the public channel, but some research teams believe that the length of the urethra also plays a key role (*43*). A sufficiently long urethra ensures that the bladder neck is placed over the urogenital diaphragm when reconstruction is complete, minimizing the risk of future incontinence. Researchers have reviewed voiding cystourethrography in 91 healthy women aged 6 to 36 months and measured urethral length of at least 1.5 cm in most normal control patients (*70*), whereas urethral length in children with PUGS was usually normal (*71*). According to the experience of Gonzalez R *et al*. (*72*) , the length of the urethra is always sufficient in PUGS with a high confluence position when performing PUM/TUM. Therefore, most of the patients above Prader II are suitable for PUM/TUM.

5.3. Hormone replacement

Since most PUGS are secondary to CAH, the main follow-up treatment after surgical female genital reconstruction is long-term glucocorticoid therapy, which aims to suppress excess hormones, replace deficient hormones, and avoid potential Cushing-like side effects (*17*). Generally, high doses of glucocorticoids are required to fully suppress the massive secretion of adrenocorticotropic hormone (ACTH) and reduce androgen production, as insufficient cortisol supplementation is not enough. In pediatric patients with non-classical forms congenital adrenal hyperplasia, low-dose glucocorticoid therapy is generally employed as a hormone treatment strategy when necessary (*73*). All children with CAH who receive glucocorticoid therapy are at risk of growth retardation and short stature, as the effects of glucocorticoids on growth are dose-dependent (*74*). Therefore, it is necessary to cooperate with endocrinologists to use low effective doses of glucocorticoids (medium- and short-acting preparations) as much as possible and adjust dosage based on 17α-OHP, testosterone levels, and clinical manifestations of cortisol deficiency or excess. Estrogen replacement therapy with progesterone (women) should be initiated around physiological puberty to induce periodic bleeding (menstruation) and gradually transition to an adult regimen (*75*).

5.4. Following

A survey of 62 pediatric urologists conducted by the American Association of Urology (AUA) indicates that establishing appropriate long-term follow-up is crucial for a successful transition to adulthood in pediatric patients with complex genitourinary conditions (*76*). According to the 2006 "Chicago Consensus Statement", in addition to evaluating appearance and lower urinary tract symptoms follwing surgical reconstruction of the female genitalia, attention should be given to future sexual function (*77*). This is because complications such as sexual dysfunction may take decades to develop, and pubertal development may significantly affect the final outcome (*78,79*). However, current literature data are limited, and the results of a few studies differ. For example, Ellerkamp V *et al*. reported (*80*) that perineal flaps with partial urogenital mobilization provided normal anatomical results with normal sexual function

in patients following female genital repair. In contrast, several other studies reported unsatisfactory follow-up outcomes after female genital reconstruction. A meta-analysis reported impaired clitoral sensitivity, vaginal stenosis, and pain and discomfort during intercourse (*81*). Two other studies with long-term follow-up showed that postoperative outcomes in children with CAH in terms of sexual function and clitoral sensitivity were unsatisfactory (*82,83*). Therefore, more research is needed to evaluate long-term genital sensitivity and sexual function in children after surgery. Close collaboration between the pediatric urologist and the adult urologist and provision of long-term monitoring is considered a better long-term follow-up modality, ensuring continuity and eliminating the anxiety about being transferred to another team (*84*). Another option is to transition from a pediatric urologist to an adolescent or adult specialist with an interest in the field, usually at the age of 16–20 (*85*). The transition specialist must have an understanding of pediatric diagnosis and treatment and training in urology (*86*).

6. Conclusion

PUGS is a very rare congenital malformation of the genitourinary system, and in this manuscript, we found that the management of PUGS remains a very thorny challenge throughout childhood, adolescence and adulthood, lacking of consensus, especially the timing of surgery and long-term follow-up of sexual function. Multidisciplinary engagement is required to overcome the rarity of this PUGS and a combined team of specialists in psychology, endocrinology, pediatric urology, and adult urology should be established to conduct long-term collaborative management. In addition to CAH, which causes PUGS as a result of abnormally high androgen levels, the pathogenesis of other causes of PUGS remains unclear. Further research on etiology and genetic factors will enhance our understanding of this rare disease.

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Health-related quality of life (HRQoL) and psychological impact of the COVID-19 pandemic on patients with myasthenia gravis

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SUMMARY The aim of this study was to compare the effects of the pandemic on health-related quality of life (HRQoL), anxious-depressive symptoms, feelings of loneliness, and fear of COVID-19 between people with myasthenia gravis (MG) and healthy controls. We also wanted to know in which group the variable fear of COVID-19 interfered the most with the results. This cross-sectional study involved 60 people with MG and 60 healthy controls. Participants using an online platform completed a sociodemographic questionnaire, the Short Form-36 Health Survey (SF-36), the Hospital Anxiety and Depression Scale (HADS), the revised UCLA Loneliness Scale and the Fear of COVID19 Scale (FCV-19S). The MG group reported worse levels in HRQoL indicators (p = 0.043- <.001), more severe anxiety-depressive symptoms (p = 0.002), and greater fear of COVID-19 (p < 0.001), but there were no differences in feelings of loneliness (p = 0.002). Furthermore, after controlling for the effect of the fear of COVID-19 variable, the differences remained for physical health indicators, but not for the most of psychosocial indicators (Social Functioning p = 0.102, η^2_p = 0.023; Role Emotional p = 0.250, η^2_p = 0.011; and HADS Total p = 0.161, η^2_p = 0.017). The harmful effect of the COVID-19 pandemic was greater in the MG group, and the perceived fear of COVID-19 had also a greater impact among this group, which has increased its negative effect on their psychosocial health.

Keywords MG, HRQoL, anxious-depressive symptoms, feelings of loneliness, fear of COVID-19

1. Introduction

Rare diseases (RDs) are clinical conditions that individually affect fewer than five people per 10,000 population (*1-3*). Together they affect more than 300 million people around the world (*2*). So far, about 7,000 different RDs have been recognised, which show great heterogeneity and are vastly dispersed geographically (*2-3*). Most of these diseases are chronic and lead to reduced life expectancy for those affected (*4*).

Myasthenia gravis (MG), also called myasthenia gravis acquisita, is a RD. This is a neuromuscular and autoimmune condition that affects the production of antibodies against acetylcholine receptor (AChR), muscle-specific kinase (MuSK) or other AChR-related proteins in the postsynaptic membrane of the neuromuscular junction, which impairs the muscle contraction (*5,6*). The overall prevalence of MG is around 150–250 cases per million people (*7*), affecting both sexes equally. In women it tends to develop before the age of 40, while in men it usually begins after the age of 50 (*8*). MG is characterised by weakness and fatigue affecting different muscle groups, with the most significant signs and symptoms in this population being ptosis, dysarthria, dysphagia, diplopia, fatigue, dyspnoea and weakness in arms and legs. In addition, weakness can become generalised and lead to a full paralytic crisis, also known as a myasthenic crisis, which can require hospitalisation (*5,8-9*).

In addition to physical health status, people with MG report high levels of depression, anxiety, loneliness, and isolation, as well as poorer health-related quality of life (HRQoL) compared to the general population (*10,11*). All of this interferes with the daily functioning of these individuals (*9,11*). Furthermore, the social stigma attached to visible symptoms, such as ptosis, can have a negative impact on the individual's self-perception (*9*). It is therefore essential to address the psychological aspects in people with MG (*9,12*).

Pandemic period due to the SARS-CoV-2 virus (COVID-19) brought a new and difficult challenge in this respect. Since it started in winter 2019, both its

impact on daily life and the resulting restrictions and lockdowns affected the physical and mental health of the population around the world (*13*). In Spain, due to the rapid spread of the virus, a state of alarm was declared in March 2020 and mandatory confinement was imposed for three months. Thereafter, until early 2022, Spain remained in a state of health emergency. During this time, there were some specific restrictions according to mobility (*e.g.* leaving your district and imposed curfew) and health/social measures, such as mandatory mask wearing, social distancing and limited capacity of people in both indoor and outdoor public settings. The demand for health care reached the point of saturation of health services (*14*). All these factors related to the pandemic situation, both during the state of alarm and the state of health emergency, were a major source of stress for the Spanish population, as in other countries around the world (*15*).

According to the worldwide COVID-19 psychological impact, some meta-analyses conducted during pandemic confirmed an increase in the prevalence of anxious and depressive symptoms (*16-18*), as well as an increasing number of people with post-traumatic stress disorder (*16,18*). Other clinical variables that have also become important due to their impact include feelings of loneliness and HRQoL (*19,20*).

However, the impact of the COVID-19 pandemic on people living with RD, especially in terms of their mental health, has been and continues to be under-represented (*21,22*). In this regard, it is important to understand that people with MG often manifest respiratory muscle weakness, which leaves them in a position of increased vulnerability as a population group who are more at risk if they contract COVID-19. In addition, immunosuppressive treatment for MG may limit the immune response to viral infection, and even drugs to combat COVID-19 infection may have adverse effects on the neuromuscular system. Therefore, the pandemic has become a potential stressor for these individuals, which adds to the long list of concerns and needs related to their health (*21,23-30*). Specifically, some studies conducted in Spain highlighted the importance of considering the perspective of patients with chronic diseases during the pandemic era, as the quality and continuity of care for the management of their clinical condition was interrupted or disrupted by the lack of health resources (*31,32*). The preliminary findings of one of the few studies conducted in a small MG sample have shown that the COVID-19 pandemic is particularly associated with anxious-depressive symptoms and poorer HRQoL (*33*).

This study aims to analyse the effects and psychological impact of the COVID-19 pandemic after lockdown and during health emergency state on people living with MG as a pre-existing rare and chronic condition, and to address the urgent need for better understanding of their situation (*34,35*). Specifically, in terms of HRQoL, anxiety-depressive symptoms, feelings of loneliness and fear of COVID-19. Furthermore, given the psychological effect that the pandemic has had across the world's population, the influence that the COVID-19 fear variable may have had on the rest of the clinical variables reported was controlled for. This led to a more detailed analysis of the differences found between a sample with MG and a sample from the general population. Based on the objectives, we expected to find a greater psychological impact of the pandemic among people with MG. Secondly, it was hypothesised that the perception of fear of COVID-19 would also increase the other variables studied to a greater extent in the MG group.

2. Materials and Methods

2.1. Participants

This cross-sectional study was performed on a quasi-control group of 120 Spanish participants, who were recruited in the first half of 2021 (during the state of emergency for COVID-19 in Spain). The total sample consisted of 60 patients diagnosed with MG and 60 control-matched participants. Table 1 shows the sociodemographic data.

The inclusion criteria for the clinical group were: *i*) a diagnosis of MG given by a neurologist, *ii*) being aged 18 or over, *iii*) informed consent provided prior to participation, *iv*) being resident in Spain, and *v*) speaking Spanish as one of their main languages. The exclusion criteria were a diagnosis of any clinical condition

Table 1. Socio-demographic characteristics of the total sample

Variable	Clinical group (n = 60) M (SD) / n (%)	Control group (n = 60) M (SD) / n (%)
Sex		
Female	44 (77.3%)	44 (77.3%)
Male	16 (26.7%)	16 (26.7%)
Age (years)	51.90 (14.73)	51.93 (14.60)
Educational level		
Primary education or equivalent	5 (8.3%)	16 (26.7%)
Secondary education or equivalent	11 (18.3%)	10 (16.7%)
Baccalaureate or equivalent	13 (21.7%)	13 (21.7%)
Higher Level of Vocational Training	7 (11.7%)	3 (5%)
University degree or equivalent	17 (28.3%)	13 (21.7%)
Master's degree	5 (8.3%)	5 (8.3%)
Doctorate	2 (3.3%)	0 (0%)
Employment status		
Employed	24 (40%)	33 (55%)
Self-employed	3 (5%)	2 (3.3%)
Unpaid work	1 (1.7%)	2 (3.3%)
Unemployed for health reasons	3 (5%)	1 (1.7%)
Unemployed (for other reasons)	2 (3.3%)	4 (6.7%)
Retired	26 (43.3%)	14 (23.3%)
Student	1 (1.7%)	4 (6.7%)

Note: n = number of participants; M = mean; SD = standard deviation.

other than MG. The control group consisted of healthy participants from the general Spanish population. Both groups were homogeneous with respect to gender, $\chi 2$ (1) = 0, p = 1, and age (U = 1818.000, p = 0.925).

2.2. Instruments

Socio-demographic and participants' exposure experience to the pandemic data were collected through an ad hoc questionnaire. The items related to exposure experience to COVID-19 refer to the following information: *If the person…i*) Has been infected by COVID-19 at any time; *ii*) Has gone into voluntary lockdown after the end of the alarm state / during state of health emergency; *iii*) Has lived with someone infected; and *iv*) Have relatives, friends or colleagues who have been infected.

The following variables were included in the assessment protocol: HRQoL, anxiety-depressive symptoms, feelings of loneliness and fear of COVID-19. All tests were adapted to Spanish and had appropriate psychometric properties.

2.2.1. Short Form-36 Health Survey (SF-36)

The Short Form-36 Health Survey (SF-36) (*36*; Spanish version: *37*) is an instrument designed to assess HRQoL. It consists of 36 items that are made up of eight different scales ("Physical Functioning", "Role Physical", "Bodily Pain", "Vitality", "Social Functioning", "Role Emotional", "Mental Health" and "General Health"). Administration time is around 10 minutes and the range of scores is between 0 and 100 points, with 0 being the worst possible health status for that dimension and 100 being the best. In addition, the instrument offers two standardized components summaries: Physical and Mental. The overall test-retest reliability is above 0.79, reaching a Cronbach's alpha of 0.94 for some scales (*38*).

2.2.2. Hospital Anxiety and Depression Scale (HADS)

The Hospital Anxiety and Depression Scale (HADS) (*39*; Spanish version: *40*) consists of 14 items divided into two subscales (anxiety and depression). Each of these is made up of seven items that presented by alternating the order with a Likert-type choice of four responses to each item (ranging from 0 to 3 points). The total scores, which are obtained by adding the scores of each item, range from 0 to 21 points for each subscale and from 0 to 42 points for the overall test, with a higher score implying a higher level of anxious-depressive symptoms. The full scale has high values for internal consistency (α = 0.90) (*40*).

2.2.3. Revised UCLA (University of California, Los Angeles) Loneliness Scale

The revised UCLA Loneliness Scale (*41*; Spanish version: *42*) is a self-reporting measure that assesses feelings of loneliness. It is made up of 20 items, with Likert-type response options (1 = often, 2 = sometimes, 3 = rarely and 4 = never) and scores range from 20 to 80 points. A higher score suggests a greater feeling of loneliness. The instrument adapted for use with the Spanish population has high internal consistency (α = 0.94) (*42*).

2.2.4. Fear of COVID-19 Scale (FCV-19S)

The Fear of COVID-19 Scale (FCV-19S) (*43*; Spanish version: *44*) is a seven-item Likert-type test that assesses fear of COVID-19. The participant is asked to report their degree of agreement with some statements, 1 meaning "strongly disagree" and 5 meaning "strongly agree". The minimum possible score per question is 1 and the maximum 5. The total score is obtained by adding up the scores for each item (ranging from 7 to 35). Thus, the greater the score, the greater the fear of COVID-19. The psychometric validation of the Spanish version (*45*) achieved acceptable internal consistency (α = 0.82).

2.3. Procedure

Given the characteristics of the target population, convenience sampling was carried out in the first part. Participants were recruited by contacting the Spanish Myasthenia Gravis Association (*Asociación Española de Miastenia Gravis* (AMES)), which was responsible for disseminating the study information letter to its members. All questionnaires used in this research were adapted to an online format using the "Qualtrics platform (XM version)". In this way, each participant self-administered the survey *via* a link to the evaluation protocol. It took approximately 25 minutes to complete the questionnaire. The platform used allowed the protocol to be completed at different times by recording the information previously covered, provided that it was accessed from the same electronic device (smartphone, tablet, *etc*.). This protocol also contained an *ad hoc* questionnaire and an informed consent form for participation in the study. It was specified that participation would be voluntary and without financial remuneration. In the second part, the participants in the homogeneous control group were recruited and provided with the information included in the information letter. They also received the link to access the evaluation protocol in the same way as the clinical group. In addition, the study was carried out between 2021–2022. Finally, participants were informed that the study complied with the criteria of the Code of Ethics, ensured compliance with the international standards proposed in the Declaration of Helsinki and was approved by the Research Ethics Committee of the institution (Ref: ETK-39/20-21).

2.4. Data analysis

Statistical Package for Social Sciences (SPSS) version 28.0 was used for the analyses. The Kolmogorov-Smirnov test was applied to determine the normal distribution of the variables. The direct scores were converted into *z* scores to carry out the analyses.

The Mann-Whitney *U*-test for quantitative variables and the Chi-square statistic for categorical variables were used to compare sociodemographic data, COVID-19 exposure, and clinical variables between groups. Cramer's V and Pearson's r measures of effect size were taken as appropriate.

A multivariate analysis of covariance (MANCOVA) was also carried out to analyse the influence of the COVID-19 fear variable on the differences found in the rest of the clinical variables analysed between the clinical and control groups. As an indicator of effect size, the partial *eta* squared η^2_p was established. The significance level was set at $p < 0.05$.

3. Results

The data collected on exposure experience to COVID-19 for each group can be found in detail in Table 2. In this case, statistically significant differences were found only in the question about voluntary lockdown $\chi 2$ (1) = 5.507, $p = 0.025$, with participants in the clinical group going into voluntary lockdown more often in comparison to control ones.

Table 3 shows the scores obtained from the different psychometric instruments. The analyses carried out showed statistically significant differences between the clinical group and the control group. Specifically, people with MG reported a worse HRQoL in almost all indicators of the SF-36: "Physical Functioning" ($p < 0.001$, $r = 0.613$), "Role Physical" ($p < 0.001$, $r = 0.487$), "Bodily Pain" ($p = 0.003$, $r = 0.273$), "Vitality" ($p < 0.001$, $r = 0.579$), "Social Functioning" ($p = 0.002$, $r = 0.283$), "Role Emotional" ($p = 0.002$, $r = 0.287$), "General Health" ($p = 0.043$, $r = 0.184$), and "PCS" ($p < 0.001$, $r = 0.567$). In addition, higher levels of anxious ($p = 0.003$, $r = 0.269$) and depressive ($p = 0.006$, $r = 0.250$) symptoms in the different subscales and total score ($p = 0.002$, $r = 0.288$) of the HADS and greater fear of COVID-19 ($p < 0.001$, $r = 0.335$) assessed through the FCV-19S instrument. According to effect sizes, the magnitudes of the differences ranged from small to large. However, no statistically significant differences were found between the clinical group and the control group in the HRQoL indicator "Mental Health" and "MCS" of the SF-36 test nor in the variable of feelings of loneliness analysed using the UCLA test.

Given the differences found in the level of perceived fear of COVID-19 between the two study groups, a MANCOVA analysis was performed in order to control for the effect of this variable on HRQoL and anxious-depressive symptoms, and to analyse whether fear of COVID-19 might exacerbate its impact on participants' physical and mental health. Table 4 shows how differences were eliminated for the SF-36 "Social Functioning" ($F = 2.718$, $p = 0.102$) and "Role Emotional" ($F = 1.337$, $p = 0.250$) variables and for the anxiety ($F = 1.497$, $p = 0.224$) and depression ($F = 1.197$, $p = 0.276$) subscales and the HADS total score ($F = 1.989$, $p = 0.161$), while for "Physical Functioning", "Role Physical", "Bodily Pain", "Vitality", "General Health" and "PCS" of SF-36 differences remained between the groups. In other words, the statistically significant differences in the physical health and in the "Vitality" psychosocial indicators between groups remained even after controlling for fear of COVID-19, but not in the other psychosocial variables. This means that the differences between the clinical and control group in psychosocial health were influenced by the perceived fear of COVID-19.

Table 2. Exposure experience to COVID-19 data of the total sample

Variable	Clinical group ($n = 60$) *n* (%)	Control group ($n = 60$) *n* (%)	$\chi 2(1)$	*p*	*V*
Have you been infected by COVID-19?			1.365	0.243	0.107
Yes	5 (8.3%)	2 (3.3%)			
No	55 (91.7%)	58 (96.7%)			
Have you gone into voluntary lockdown after the end of the state of alarm?			5.507	0.025*	0.205
Yes	22 (36.7%)	11 (18.3%)			
No	38 (63.3%)	49 (81.7%)			
Have you lived with someone who has been infected with COVID-19?			2.157	0.142	0.134
Yes	9 (15%)	4 (6.7%)			
No	51 (85%)	56 (93.3%)			
Has any member of your family been infected with COVID-19?			2.727	0.099	0.151
Yes	20 (33.3%)	12 (20%)			
No	40 (66.7%)	48 (80%)			
Have any of your friends or colleagues been infected with COVID-19?			0.037	0.847	0.018
Yes	39 (65%)	40 (66.7%)			
No	21 (35%)	20 (33.3%)			

Note: *n* = number of participants; $\chi 2$ = Chi-squared test; *$p < 0.05$; **$p < 0.001$; *V* = Kramer's *V* (effect size).

Table 3. Differences in HRQoL, anxiety-depressive symptoms, feelings of loneliness and fear of COVID-19 between the clinical group and the control group

Variable	Clinical group (n = 60) Mdn (Range)	Control group (n = 60) Mdn (Range)	U	Z	p	r
SF-36						
Physical Functioning	65.00 (95)	97.50 (75)	541.000	-6.704	< 0.001**	0.613
Role Physical	25.00 (100)	100.00 (100)	860.000	-5.341	< 0.001**	0.487
Bodily Pain	52.00 (100)	72.00 (90)	1233.500	-2.999	0.003*	0.273
Vitality	47.50 (85)	55.00 (90)	594.000	-6.345	< 0.001**	0.579
Social Functioning	68.75 (100)	87.50 (100)	1211.000	-3.101	0.002*	0.283
Role Emotional	67.70 (100)	100.00 (100)	1214.000	-3.149	0.002*	0.287
General Health	41.00 (82)	67.00 (75)	1454.000	-2.021	0.043*	0.184
Mental Health	60.00 (64)	64.00 (56)	1535.000	-1.397	0.162	-
PCS	38.13 (44.34)	54.64 (48.97)	620.000	-6.193	< 0.001**	0.567
MCS	42.19 (45.18)	44.89 (50.14)	1684.000	-0.609	0.543	-
HADS						
Total	18.50 (23)	15.00 (16)	1199.000	-3.161	0.002*	0.288
Depression subscale	9.00 (10)	8.00 (13)	1281.500	-2.748	0.006*	0.250
Anxiety subscale	9.00 (15)	7.00 (12)	1240.500	-2.949	0.003*	0.269
UCLA	39.00 (39)	37.00 (45)	1572.000	-1.198	0.231	-
FCV-19S	19.00 (27)	16.50 (19)	1101.500	-3.673	< 0.001**	0.335

Note: n = number of participants; Mdn = median; U = Mann-Whitney U test; Z = z scores; PCS = Physical Component Summary; MCS = Mental Component Summary; *$p < 0.05$; **$p < 0.001$; $r = r$ coefficient (effect size). Raw scores have been used in the Table.

Table 4. MANCOVA for HRQoL and anxiety-depression symptoms after controlling for the effect of fear of COVID-19 symptoms

Variable	Clinical group (n = 60) M (SD)	Control group (n = 60) M (SD)	F	p	η^2_p
SF-36					
Physical Functioning	-0.55 (0.99)	0.55 (0.63)	39.196	< 0.001 **	0.251
Role Physical	-0.48 (.095)	0.48 (0.79)	22.491	< 0.001**	0.161
Bodily Pain	-0.26 (0.98)	0.26 (0.95)	4.668	0.033*	0.038
Vitality	-0.28 (0.92)	0.28 (0.99)	5.629	0.019*	0.046
Social Functioning	-0.25 (0.99)	0.25 (0.94)	2.718	0.102	0.023
Role Emotional	-0.19 (1.06)	0.19 (0.90)	1.337	0.250	0.011
General Health	-0.58 (0.80)	0.58 (0.82)	37.988	< 0.001**	0.245
PCS	39.28 (9.90)	51.84 (9.60)	35.468	< 0.001**	0.233
HADS					
Total	0.29 (1.02)	-0.29 (0.89)	1.989	0.161	0.017
Depression subscale	0.22 (0.97)	-0.22 (0.98)	1.197	0.276	0.010
Anxiety subscale	0.27 (1.05)	-0.27 (0.86)	1.497	0.224	0.013

number of participants; M = mean; SD = standard deviation; F = MANCOVA; $\eta 2p$ = partial eta squared (effect size); PCS = Physical Component Summary; *$p < 0.05$; **$p < 0.001$. Raw scores have been used in the Table.

4. Discussion

The COVID-19 pandemic has had an impact on the mental health of the world's population. Therefore, it is particularly important to specifically address the psychological state of people who were already living with a pre-existing chronic disease (*46*). This becomes even more vital for RD patients, given the under-representation of scientific evidence on their situation in this health emergency (*21,22*). Therefore, the purpose of the present study was to analyse the impact on the mental health status of people diagnosed with MG compared to a healthy control group. This aimed to better understand the experience and care needs of people with MG during the COVID-19 pandemic.

According to the first hypothesis, the results obtained in this study confirmed that people with MG had poorer levels of HRQoL for most of the indicators analysed, as well as higher levels of anxiety-depressive symptoms and greater fear of COVID-19 compared to their healthy peers. On the one hand, results are consistent with the clinical psychopathology associated with the neuromuscular condition, which involves particularly impaired physical and psychosocial HRQoL and increased comorbidity with anxiety-depressive disorders (*16-18*). On the other hand, while there are no prior studies that have analysed fear of COVID-19 using the FCV-19S in the population with MG, some authors have used this instrument in patients with chronic diseases related to MG. For example, one study found that people with fibromyalgia reported significantly higher scores on the FCV-19S test compared to healthy controls, with

similar results to this study (*47*). Moreover, in the case of the population with MG, this greater fear could be justified by the consequences of being a vulnerable group, since they are at a greater risk. Treatment for MG may weaken their ability to fight the virus and their disease may worsen either as a result of it or because of the drugs prescribed to treat it (*21,23-30*). Furthermore, this study also found that people with MG had a greater tendency to go into voluntary lockdown, which also supports the idea that the impact of fear of COVID-19 is greater in the clinical population.

Nevertheless, no statistically significant differences were found between the clinical group and the control group for the HRQoL "Mental Health" indicator of the SF-36 test or for the variable of feelings of loneliness analysed by the UCLA test. These results may be due to the fact that the SF-36 test is a generic assessment instrument (*48*) and instruments such as the HADS are more sensitive to detecting psychological symptoms in people with MG compared to control subjects. This is evidenced by the fact that the HADS has been more widely used and recommended for studies in people with MG (*8,49-51*). Regarding feelings of loneliness, one possible explanation for the lack of statistically significant differences for this variable between the two groups could be that that the pandemic and lockdowns have posed a strong risk for the entire population; therefore, there has been overall isolation, causing feelings of loneliness to grow across the board. In fact, this problem elicited studies aimed at targeting urgent and effective interventions to prevent psychological and physical comorbidities, especially in vulnerable groups such as older adults (*20,52-54*).

Regarding second hypothesis, this study also aimed to find out whether fear or perceptions about the pandemic might have a greater impact on the mental health of people with MG compared to their healthy peers. The results showed that, controlling for the effect generated by the fear of COVID-19 variable, significant differences remained only for the indicators of general health and the different indicators referring to physical health. This is consistent with the fact that it is mainly the physical sphere in MG that is compromised, given the muscle weakness and fatigue MG patients suffer, which limits their daily functioning and interferes with their HRQoL (*5,8-11*). However, differences in anxiety-depressive symptoms and socio-emotional health indicators were not maintained, which suggests that the fear of COVID-19 seems to have a stronger negative influence on the psychosocial aspects of people with MG. This finding may be reinforced by more recent scientific literature, as it was stipulated early in the pandemic that those with pre-existing chronic illnesses would be one of the groups most likely to suffer adverse psychosocial effects (*55*).

In addition, there are contextual variables that may have increased this fear and the way in which people with MG perceive the threat that COVID-19 poses to their health. In particular, there are currently no quality COVID-19 pandemic guidelines for patients with MG, and the continuous and sometimes variable media coverage of recommendations to prevent transmission of the virus may have hindered their awareness or perception, while contributing to their anxiety (*30*). However, it has been argued that the MG population is considered even more vulnerable, making it essential that they receive both specific health education, as a preventive measure against COVID-19, and psychological care and counselling (*33*). Undoubtedly, promoting the provision of accurate COVID-19-related and MG-specific information by healthcare authorities, as well as patient associations, would lead to more informed and therefore less anxious MG patients (*30*). In this sense, strengthening telehealth strategies and services is an ideal, safe, and efficient resource for managing MG patients during the pandemic (*30,56*); especially, telecare psychological interventions have proven to be effective in the neuromuscular patient group on previous occasions (*e.g.*, *10*).

Despite the importance of the evidence and conclusions drawn, this study had many limitations. Firstly, it did not have a large sample size, compared to other studies conducted during the pandemic with participants from the general population. However, the difficulty in recruiting patients to participate in research studies needs to be taken into consideration, which is even more complex in the case of RDs (*57*). Secondly, as this was a cross-sectional study, there were no "pre-COVID-19" measurements of the same subjects; if these had been available, the conclusions drawn about the impact of COVID-19 on people with MG could have more accurately reflected. In addition, although guidelines were considered to ensure the validity and ethical implications of the self-report instruments used, many of them did not have a proper adaptation to the remote model (*58*). Finally, the specific psychosocial intervention required was not offered during this study.

Therefore, it is hoped that future research will follow up the variables discussed here using longitudinal studies and larger numbers of participants. It would also be useful to conduct psychosocial interventional studies, especially through telecare, for people with MG with the aim of mitigating the harmful effects of the pandemic. It is also hoped that the issues highlighted in this study can be extended across the RD community to reach specific patient groups, so that evidence on their experience during the pandemic can be obtained and appropriate guidelines for action can be developed accordingly.

To conclude, this study has shown that, during the pandemic, people with MG have had poorer levels of HRQoL, stronger anxiety-depressive symptoms and greater fear of COVID-19 compared to their healthy peers. Perceived fear of COVID-19 has also had a greater impact among people with MG, with an increased negative impact on their psychosocial health. Therefore,

this study provides additional evidence, and joins the rest of the literature in calling for regular attention to the mental and psychosocial health of people with MG, especially in this time of unprecedented large-scale pandemic.

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Trust in physicians and definitive diagnosis time among Japanese patients with specific intractable diseases: A cross-sectional study

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SUMMARY Trust in physicians is an important metric in shared decision-making. Many patients with rare diseases experience misdiagnosis or delayed diagnosis because of difficulties in diagnosis or access to specialists. What impact do these have on trust in physicians? This study focused on patients with rare diseases, evaluated the effects of a delayed diagnosis and misdiagnosis on trust in physicians, and clarified the backgrounds of patients who have experienced delayed diagnoses. Patients with any of the 334 intractable diseases in Japan were registered, and a questionnaire survey was conducted on 1,000 valid registrations. Scores were calculated on a five-point Likert scale, and Cronbach's alpha coefficient was calculated to determine internal consistency, which was 0.973. Independent sample t-tests and analysis of variance were used to compare average trust scores based on patient demographics. The mean trust in physician score of patients who waited ≤ 1 year until definitive diagnosis was 47.66 ± 11.69, while those of patients who waited > 1 year was 45.07 ± 11.63 (p = 0.004). The average trust scores of patients with or without a misdiagnosis were 46.69 ± 11.96 and 47.22 ± 11.65 (p = 0.550), respectively. Among patients with time to a definitive diagnosis of > 1 year, 62.8% had a period from symptom onset to initial hospital visit of > 1 year. A longer time to definitive diagnosis lowered the degree of trust in physicians. Many patients who experienced delayed diagnoses also had a long time from symptom onset to the initial medical visit. This aspect is important for understanding the background of patients who experienced delayed definitive diagnoses.

Keywords delayed diagnosis, misdiagnosis, rare diseases, shared decision-making, symptoms

1. Introduction

The conventional relationship between patients and physicians is a passive-active relationship between a helpless patient suffering from an illness and a physician trying to save the patient. Physicians use their knowledge and skills to select treatment methods that effectively restore health and relieve pain. Subsequently, information is provided to the patient, but the mainstream perspective assumes that the patient would agree with the physician's choice or so-called paternalism (*1*). However, in recent years, shared decision-making (SDM) has been established, in which physicians and patients share information and deepen mutual understanding to make appropriate treatment decisions (*2*). This patient-centered approach to medicine is described as one in which "physicians seek to enter the patient's world and see the disease through the patient's eyes" (*3*). The patients make their final medical decision based on all of the information provided by the physician. Various mechanisms are believed to influence this important decision-making process. One of these is the patient's trust in their physician. Trust in physicians is an important metric when implementing SDM (*2*).

When a patient visits a hospital, an appropriate diagnosis is promptly made, and the patient selects a treatment approach from several options provided by a physician. In other words, SDM is conducted sincerely, and the patient is involved in the decision-making. This process is acceptable for patients in today's advanced medical care. However, there are diseases for which this process is unclear due to the difficulty of diagnosis and treatment methods. One of these is rare diseases. It can take a long time to reach a definitive diagnosis for many rare diseases owing to the difficulty of diagnosis and the lack of access to specialists (*4,5*). Furthermore, treatment options are limited, and patients face many challenges (*4,6*). Even under such circumstances, sincere SDM should still be implemented, but can trust in physicians, which mainly influences this mechanism, be maintained at a high level? Alternatively, what types of changes will occur in that trust? Although the individual prevalences

of these diseases are small, rare diseases represent a significant public health challenge in terms of the cumulative number of patients since there are thousands of rare disease cases worldwide (*1,5*). Few studies have examined trust in physicians among patients with rare diseases. The first purpose of this study was to evaluate how delayed diagnosis or misdiagnosis of rare diseases affects patients' trust in physicians. The International Rare Diseases Research Consortium (IRDiRC) has been working to improve international collaboration and take action to reduce the time required for patients with rare diseases to receive a definitive diagnosis after visiting a medical institution (*4*). Reducing the time to a definitive diagnosis of a rare disease as much as possible is important in improving a patient's quality of life (QOL) and implementing SDM. In addition to evaluating changes in trust in physicians, we also aimed to understand the backgrounds of patients who experienced a delayed diagnosis. In this study, we evaluated patients with rare diseases with a definitive diagnosis of any of the 334 diseases specified as intractable in Japan (*7*).

2. Materials and Methods

This study was conducted in August 2022 using the Rakuten Insight patient panel. A questionnaire survey was conducted on 1,000 patients with a definitive diagnosis of any of the 334 diseases specified as intractable in Japan. The data used in this study were outsourced to Rakuten Insight, Inc., and were obtained using their panel. All data obtained from Rakuten Insight, Inc. were anonymized before analysis. In addition, the data were unconnected and completely anonymized. We did not have access to the anonymization correspondence table or any personal identifiable information. Therefore, this study was exempted from ethical approval by the Research Ethics Review Committee of the Graduate School of Health Innovation, Kanagawa University of Human Services, as the study used a fully anonymized questionnaire survey (notification number SHI No. 52). Participants were informed about the purpose of the research and their participation implied consent.

A 13-item questionnaire was used to obtain a trust score that indicated the patient's degree of trust in their physicians. A questionnaire survey was conducted among physicians currently treating the patients. Questionnaire responses were given scores of 5, 4, 3, 2, and 1 for the options of "extremely strongly agree", "strongly agree", "somewhat agree", "somewhat disagree", and "disagree completely". These were calculated on a five-point Likert scale. Trust analysis was conducted by calculating Cronbach's alpha coefficient for internal consistency of trust in physicians.

Independent sample *t*-tests and analysis of variance were used to compare the average trust scores by sex, age group, marital status, educational background, and occupation. Trust in doctors' scores was used as the dependent variable, and sex, age group, marital status, educational background, and occupation as independent variables. The *t*-test was conducted for average physician trust scores according to the presence or absence of a misdiagnosis experience. $P < 0.05$ was considered statistically significant. Patients with a misdiagnosis were asked to provide free responses to specify which diagnosis they were given in cases where a definitive diagnosis was not made.

The IRDiRC has set the goal of ensuring that everyone with a rare disease receives an accurate diagnosis, care, and available treatment within 1 year by 2027 (*4*). Since no standard definition of a delayed diagnosis exists, this consortium's guidelines were used as a standard. We divided patients into two groups: those with a time to a definitive diagnosis of ≤ 1 year and those with > 1 year and calculated the respective average physician trust scores. We further divided the two groups into four categories according to the presence or absence of misdiagnosis, calculated the respective physician trust scores, and conducted analyses of variance.

We asked, "When did you start to suspect this disease?" from the time of definitive diagnosis. Patients were divided between those with a period of ≤ 1 year from that date to the date of definitive diagnosis and those with > 1 year. These two categories are presented using a pie graph.

Answers to the question, "How long did it take from the time you felt something was wrong with your body until you went to the hospital for the first time?" were used to divide patients between those with a time to a definitive diagnosis of ≤ 1 year and those with > 1 year. These two categories are presented in a pie graph. This study used IBM's SPSS statistical software ver. 28 for statistical analysis.

3. Results

A questionnaire survey was conducted on 1,000 patients diagnosed with any of Japan's 334 diseases specified as intractable. Table 1 shows the characteristics of the participants. Men comprised 60.4% of participants. The most common age group was 30–49 years, accounting for 35.0%. Married participants comprised 63.1% of the total population. The most common educational level was university graduate, accounting for 39.2% of the total. By occupation, those employed by companies were the most common (34.7 %), followed by those unemployed (31.9 %).

Table 2 shows the number and percentage of patients with major specified intractable diseases who participated in this survey and the number and percentage of patients registered with specified intractable diseases reported by the Ministry of Health, Labor, and Welfare in 2020 (*8*). According to a Ministry of Health, Labor, and Welfare report, Parkinson's disease was the most registered intractable disease, accounting for 13.8% of all

registrations. However, in the present survey, the disease prevalence was low, accounting for only 2.9%. The overall registration profile was similar to the Ministry of Health, Labor, and Welfare data.

Table 1. Characteristics of the study sample

Characteristic	Categories	Number	Percentage (%)
Sex	Male	604	604
	Female	396	396
Age	< 29 years	18	18
	30–49 years	350	350
	50–59 years	295	295
	60–69 years	242	242
	> 70 years	95	95
Marriage	Married	631	631
	Others	369	369
Education background	Junior school	21	21
	High school	299	299
	Professional school	134	134
	Junior college	94	94
	Bachelor degree	392	392
	Master degree and above	54	54
	Other	6	6
Occupation	Company employee	347	347
	Self-employed	79	79
	Government employee	35	35
	Teacher	20	20
	Contract worker / Temporary worker	52	52
	Part-time job	88	88
	Student	3	3
	Unemployed	319	319
	Other	57	57

We asked 13 questions about patients' trust in their physicians (Table S1, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=144*). The average patients' physician trust scores were calculated using a Likert scale. Trust in these question items was evaluated using Cronbach's alpha for internal consistency. The result was 0.973, indicating a sufficiently explainable internal consistency level for these question items. The overall average score was 47.10 (total score of 65.00). This trust score was used in subsequent research.

We compared average trust scores according to sex, age, marital status, educational level, and type of occupation using an independent *t*-test or analysis of variance to investigate the influence of each characteristic on trust in physicians by category (Table 3). Significant differences were observed regarding age ($p = 0.005$) and marital status ($p = 0.012$). For age, subsequent multiple regression analysis (Bonferroni method) showed that the trust in physicians score of those aged 30–49 years was significantly lower than that of those aged 60–69 ($p = 0.008$). Previous studies have focused on sociodemographic characteristics when investigating physician trust scores (*9-13*). Among these, a statistically significant difference has been reported in the respective trust scores of age and marital status (*13,14*). This trend was repeated in the present study in patients with specified intractable diseases.

The time to definitive diagnosis in patients with specified intractable diseases in Japan is shown

Table 2. Major patients with specified intractable diseases registered in this survey

Specified intractable disease	This survey		Data from the Japanese MHLW*	
	Number	Percentage	Number	Percentage (%)
Ulcerative colitis	223	22.3	140,574	13.6
Systemic lupus erythematosus	57	5.7	64,468	6.2
Sjögren's syndrome	49	4.9	17,628	1.7
Crohn's disease	49	4.9	47,633	4.6
Posterior longitudinal ligament ossification	37	3.7	36,401	3.5
Idiopathic dilated cardiomyopathy	30	3.0	20,387	2.0
IgA nephropathy	30	3.0	12,699	1.2
Parkinson's disease	29	2.9	142,375	13.8
Multiple sclerosis/neuromyelitis optica	29	2.9	21,437	2.1
Moyamoya disease	27	2.7	13,894	1.3
Myasthenia gravis	25	2.5	25,416	2.5
Eosinophilic sinusitis	25	2.5	13,404	1.3
Polycystic kidney disease	24	2.4	11,935	1.2
Behcet's disease	23	2.3	15,537	1.5
Sarcoidosis	21	2.1	16,138	1.6
Dermatomyositis/polymyositis	20	2.0	24,894	2.4
Idiopathic thrombocytopenic purpura	17	1.7	18,793	1.8
Idiopathic femoral head osteonecrosis	17	1.7	20,003	1.9
Spinocerebellar degeneration (excluding multiple system atrophy)	16	1.6	27,365	2.6
Systemic scleroderma	15	1.5	27,647	2.7
Retinitis pigmentosa	14	1.4	23,979	2.3
Anterior hypopituitarism	13	1.3	18,653	1.8
Primary biliary cholangitis	13	1.3	17,993	1.7
Pustular psoriasis (disseminated)	11	1.1	2,058	0.2
Idiopathic interstitial pneumonia	11	1.1	17,589	1.7

*Excerpt from an example of a health administration report by the Ministry of Health, Labor, and Welfare in 2020 (*http://www.mhlw.go.jp/toukei/list/36-19.html*).

in Table S2 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=144*). The time to definitive diagnosis was ≤ 1 year in 78.2% of patients. Furthermore, 21.8% of all patients had a time of > 1 year. Patients aged > 21 years comprised 3% of all patients. The data showed that patients with intractable diseases required a long time to obtain a definitive diagnosis. Subsequently, we investigated patients who were misdiagnosed before the definitive diagnosis, and the results showed that those who were misdiagnosed comprised 22.8% of the total. A *t*-test was used to compare the average trust scores of patients with or without a misdiagnosis, and the results showed no significant differences. Table 4 shows the major intractable diseases in patients with time to a definitive diagnosis of > 1 year. The ratio of each disease to this study's total number of registrations is also shown. Sjögren's syndrome and ulcerative colitis each accounted for 17 cases. Regarding Sjögren's syndrome, the percentage of registered cases was relatively high (34.69 %). Among the diseases with at least 10 cases, eosinophilic sinusitis and polycystic kidney disease were high (48.00% and 41.67%, respectively). Additionally, ankylosing spondylitis had a high probability. However, there is a need to further expand the scale of the study in the future to make a clear judgment because the number of cases was small.

Table S3 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=144*) shows the results of the free responses from patients who experienced misdiagnosis and were asked to specify the misdiagnosis. Depending on the disease, patients experienced multiple misdiagnoses before a definitive diagnosis. Figures 1A and 1B show a pie graph depicting the time to definitive diagnosis for patients who experienced misdiagnosis and

Table 3. Comparison of trust in doctors score based on participants' characteristics

Characteristic	Categories	Trust in doctor scores (mean ± SD)	*p* value
Sex	Male	47.55 ± 11.51	0.131
	Female	46.40 ± 12.02	
Age	< 29 years	47.56 ± 9.55	0.005*
	30–49 years	45.24 ± 11.73	
	50–59 years	47.54 ± 12.10	
	60–69 years	48.53 ± 11.70	
	> 70 years	48.80 ± 10.07	
Marriage	Married	47.81 ± 11.53	0.012*
	Others	45.88 ± 11.96	
Education background	Junior school	49.38 ± 10.97	0.548
	High school	46.33 ± 11.93	
	Professional school	46.72 ± 12.47	
	Junior college	46.69 ± 11.03	
	Bachelor's Degree	47.48 ± 11.62	
	Master's degree and above	48.65 ± 10.63	
	Other	52.50 ± 13.52	
Occupation	Company employee	46.18 ± 11.95	0.574
	Self-employed	46.20 ± 10.95	
	Government employee	47.06 ± 11.06	
	Teacher	47.55 ± 11.01	
	Contract worker / Temporary worker	46.04 ± 12.63	
	Part-time job	48.73 ± 10.80	
	Student	49.67 ± 13.28	
	Unemployed	47.97 ± 11.76	
	Other	47.16 ± 12.27	

*Statistical significance: $p < 0.05$; independent sample *t*-test and ANOVA performance for comparison of means.

Table 4. Highest number of patients with rare diseases with a definitive diagnosis exceeding 1 year

Diseases	Number of patients	Number of registrations	Percentage (%)
Sjögren's syndrome	17	49	34.69
Ulcerative colitis	17	223	7.62
Crohn's disease	14	49	28.57
Systemic lupus erythematosus	13	57	22.81
Eosinophilic sinusitis	12	25	48.00
Polycystic kidney disease	10	24	41.67
IgA nephropathy	9	30	30.00
Posterior longitudinal ligament ossification	8	37	21.62
Parkinson's disease	7	29	24.14
Idiopathic dilated cardiomyopathy	6	30	20.00
Anterior hypopituitarism	5	13	38.46
Moyamoya disease	5	27	18.52
Multiple sclerosis/neuromyelitis optica	5	29	17.24
Spinal muscular atrophy	4	9	44.44
Muscular dystrophy	4	9	44.44
Idiopathic interstitial pneumonia	4	11	36.36
Sarcoidosis	4	21	19.05
Behçet's disease	4	23	17.39
Ankylosing spondylitis	3	3	100.00
Amyotrophic lateral sclerosis	3	4	75.00
Malignant rheumatoid arthritis	3	8	37.50
Ligamentum flavum ossification	3	8	37.50
Pustular psoriasis (disseminated)	3	11	27.27
Spinocerebellar degeneration (excluding multiple system atrophy)	3	16	18.75
Idiopathic femoral head osteonecrosis	3	17	17.65
Myasthenia gravis	3	25	12.00

those who did not. Patients who did not experience a misdiagnosis and had a time to a definitive diagnosis of > 1 year were 18.1% of the total. In comparison, patients who were misdiagnosed and had time to a definitive diagnosis of > 1 year were 34.2% of the total.

Subsequently, we used the IRDiRC guidelines as a standard to divide patients with time to a definitive diagnosis of ≤ 1 year and > 1 year and calculated the average physician trust score for each group (Table 5). The results showed that the physician trust score for patients with time to a definitive diagnosis of > 1 year was significantly lower than that for patients with a time of ≤ 1 year ($p = 0.004$). Additionally, we compared the physician trust score among patients with time to a definitive diagnosis of ≤ 1 year and > 1 year according to whether the patient experienced a misdiagnosis. There were no significant differences in physician trust scores among patients with time to a definitive diagnosis of ≤ 1 year, according to the experience of misdiagnosis. However, patients with time to a definitive diagnosis of > 1 year who experienced a misdiagnosis had the lowest physician trust scores. We performed an analysis of variance of the average physician trust scores between these four groups, and the results showed statistically significant differences.

Subsequently, we asked, "When did you start to suspect this disease, starting from the time of definitive diagnosis?" We divided the answers to this question between patients with time to a definitive diagnosis of ≤ 1 year and those with a time > 1 year and presented the results in a pie graph (Figure 1B and 1C).

The results showed that the number of patients with no suspicion of their underlying disease from the beginning was the highest for both categories. The number of patients who suspected the disease for > 1 year was 28.9%, higher than that of those who suspected the disease for ≤ 1 year. Therefore, patients who experienced a delayed diagnosis had suspected their disease for > 1 year but took a long time to reach a definitive diagnosis.

The answer to the question, "How long did it take from the time you felt something was wrong with your body until you went to the hospital (for the first time)?" was divided into two categories: time to a definitive diagnosis of ≤ 1 year and > 1 year, and investigated. The

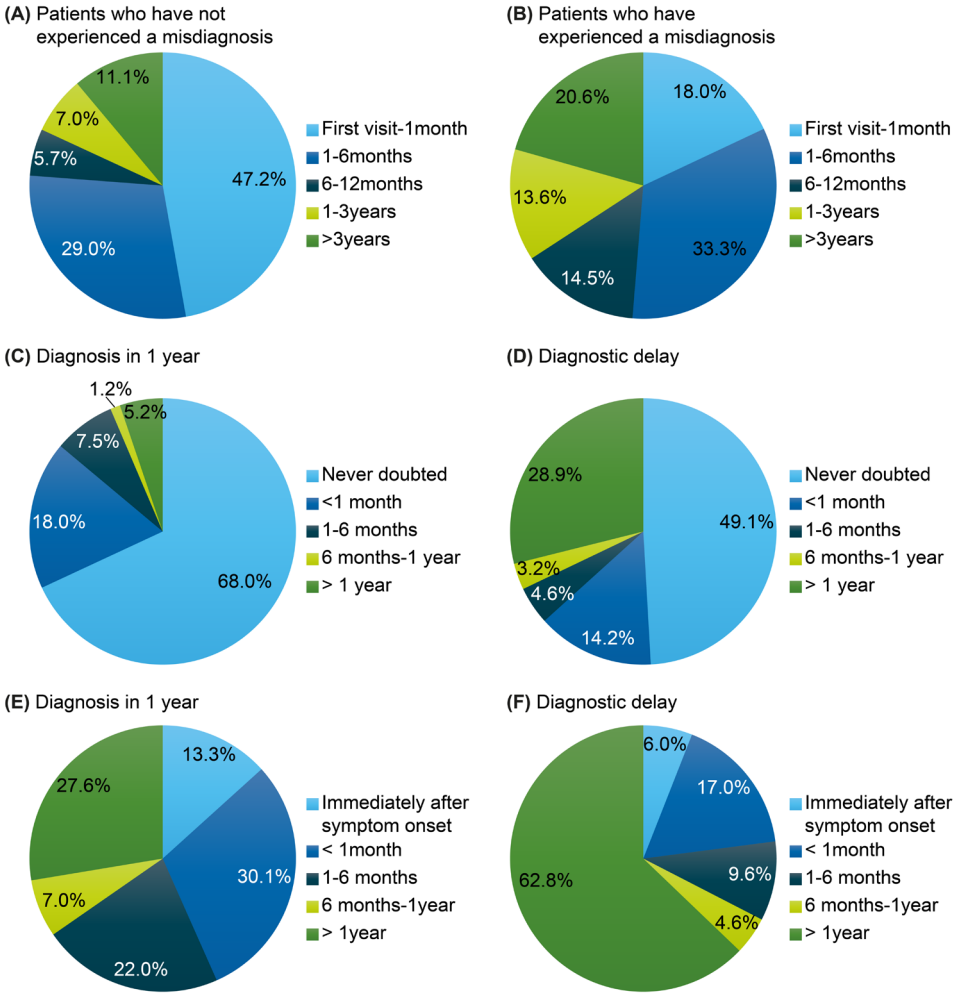

Figure 1. (A) and (B) reflect the period to a definitive diagnosis of patients who have or have not experienced misdiagnosis; (C) and (D) note the period patients suspected the disease before a definitive diagnosis; (E) and (F) reflect the period between symptom onset and first hospital visit.

Table 5. Comparison of trust in doctors between the period of definitive diagnosis and misdiagnosis

Period until definitive diagnosis	Categories	Trust in Doctor Score (mean ± SD)	*p* value
First visit ≤ 1 year	All	47.66 ± 11.69	0.004*
> 1 year	All	45.07 ± 11.63	
First visit – 1 year	Without misdiagnosis experience	47.63 ± 11.63	0.035*
First visit – 1 year	With misdiagnosis experience	47.79 ± 11.72	
> 1 year	Without misdiagnosis experience	45.35 ± 11.72	
> 1 year	With misdiagnosis experience	44.56 ± 11.72	

*Statistical significance: $p < 0.05$; independent sample *t*-test and ANOVA performance for comparison of means.

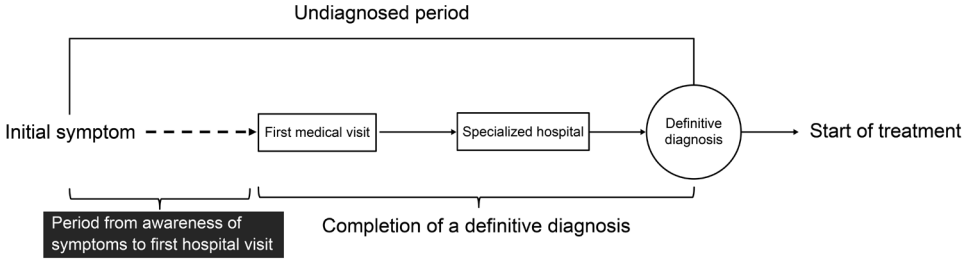

Figure 2. Diagram of the process from initial symptoms to a definitive diagnosis.

results are presented in Figures 1E and 1F.

For the category with time to a definitive diagnosis of > 1 year, patients who responded that the time from when they felt something was wrong with their body to the first hospital visit was > 1 year comprised 62.8% of the total. This was higher than the 27.6% in the category with a time to a definitive diagnosis of ≤ 1 year. Patients who experienced a long time from the initial hospital visit to a definitive diagnosis reported a long period from when they felt something was wrong with their body to the first hospital visit.

4. Discussion

Access to an appropriate diagnosis is difficult for rare diseases, and delays frequently occur (*4*). Patients face many difficulties because of delayed diagnosis. To solve this problem, the IRDiRC aims for everyone with a rare disease to have an accurate diagnosis and receive prompt care and available treatments by 2027 (*4*). From the perspective of SDM, it is important to maintain a relationship of trust between the physician and patients, from diagnosis to available treatment.

The first important achievement of this study is that we clarified the changes in patient's trust in physicians during the period leading to definitive diagnoses in patients with rare diseases. We used the IRDiRC statement as a standard and compared the physician trust scores of patients with time to a definitive diagnosis of ≤ 1 year and those with > 1 year. Physician trust scores declined significantly when the time to definitive diagnosis was > 1 year. The time to definitive diagnosis had a greater influence on the degree of trust in physicians than the presence or absence of a misdiagnosis experience. These results will be of great interest. On the other hand, many patients who experienced a misdiagnosis had a time to a definitive diagnosis of > 1 year. In other words, misdiagnosis prolongs the time to a definitive diagnosis. This result demonstrates the difficulty of definitively diagnosing rare diseases. If a rare disease is suspected, one solution is to use the domestic medical network and prepare a medical environment where an appropriate diagnosis can be made. Furthermore, efforts to create networks of information on a global scale by organizations such as IRDiRC are anticipated in the future. In this study, 28.9% of patients with time to a definitive diagnosis of > 1 year were suspected of having an intractable disease for > 1 year. Patients experienced a long time to definitive diagnosis; however, it was suspected to be an intractable disease from their symptoms. We hope that our future in-depth follow-up interviews with patients with intractable diseases will provide a more detailed interpretation of this observation.

The second important achievement of this study is that we clarified that patients with time to a definitive diagnosis of > 1 year included those with a period from symptom onset to the initial hospital visit of > 1 year. Many studies have focused on shortening the time from the initial hospital visit to a definitive diagnosis in patients with rare diseases. It is important to use the above-mentioned information network to establish a system that can appropriately test for rare diseases; however, the undiagnosed period includes the period from symptom onset to the time of hospital visits. In other words, giving patients a strong motivation to see a physician as soon as possible after the first symptoms appear is important to improve the QOL of patients with rare diseases (Figure 2). In the future, we would like to clarify why patients took a long time from the symptom

onset to the initial hospital visit in additional in-depth interviews; however, one possibility is that the diagnosis was delayed because of the patient's residence locations or physical disabilities. Spreading awareness of remote diagnoses and eliminating disparities in medical care between rural and urban areas may improve this.

Finally, we showed that a delay in definitive diagnosis reduces patients' trust in physicians. In other words, avoiding diagnosis delays may help maintain trust in physicians and promote SDM. Furthermore, we showed that motivating patients to visit the hospital as soon as possible after symptom onset is necessary to improve the QOL of patients with rare diseases. In addition, the time to diagnosis may be further shortened if the government and related medical services encourage people to visit medical institutions and support such actions.

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Original Article

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Genetic diagnostic approach to intellectual disability and multiple congenital anomalies in Indonesia

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SUMMARY Intellectual disability (ID) and multiple congenital anomalies (MCA) are major contributors to infant mortality, childhood morbidity, and long-term disability, with multifactorial aetiology including genetics. We aim to set a diagnostic approach for genetic evaluation of patients with ID and MCA, which can be applied efficiently with a good diagnostic rate in Indonesia or other low resources settings. Out of 131 ID cases, twenty-three individuals with ID/global developmental delay (GDD) and MCA were selected from two-steps of dysmorphology screening and evaluation. Genetic analysis included chromosomal microarray (CMA) analysis, targeted panel gene sequencing, and exome sequencing (ES). CMA revealed conclusive results for seven individuals. Meanwhile, two out of four cases were diagnosed by targeted gene sequencing. Five out of seven individuals were diagnosed using ES testing. Based on the experience, a novel and comprehensive flowchart combining thorough physical and dysmorphology evaluation, followed by suitable genetic tests is proposed as a diagnostic approach to elucidate the genetic factor(s) of ID/GDD and MCA in low resources settings such as Indonesia.

Keywords intellectual disability, multiple congenital anomalies, genetic testing, diagnostic procedures, low- and middle-income countries

1. Introduction

Intellectual disability (ID) is defined in the latest DSM-5 as a disorder with onset during a developmental period, that includes both intellectual and adaptive functioning deficits in conceptual, social, and practical domains (*1*). ID is considered a global lifelong health problem, which affects around 1–3% of the world's population. In Indonesia, the estimated proportion of children aged 5 to 17 years with disability is 3.3 percent (*2*). Children with special needs, which includes those with ID are estimated at around 1.6 million, or 2% of all children in the population (*3,4*), but this number is likely to be higher because of the disparity on available data. Genetic ID comprises approximately 50–65% of moderate and severe ID, while only 20% of mild ID cases are of genetic predominant aetiology (*5*). Understanding the aetiology of ID, as well as multiple congenital anomalies (MCA) will help the parents, family, and health care providers to give more appropriate medical and supportive care.

Genetic testing methods have improved significantly from conventional cytogenetic analysis to next-generation sequencing (NGS), which can identify pathogenic variants from the whole human exome or genome (*6*). However, the availability of genetic testing varies across countries, and in Indonesia, it is only available in some research-based institutions. Other challenges include the lack of health professional and public awareness, medical genetic infrastructure including the recognition of its profession, law and regulation, limitation of national health insurance coverage, minimum government support, and lack of expertise and interests from researchers in genetic diseases (*7,8*). Availability of diagnosis will give better comprehension of genetic counselling and family understanding to facilitate autonomous decision making, avoid unnecessary testing or medical treatment, and eventually increase patient's quality of life (*9,10*).

To evaluate and diagnose patients with MCA, global developmental delay (GDD), and ID, several strategies have been established. First-line genetic testing, such as routine karyotyping and/or chromosomal microarray, is generally applied for patients with noticeable dysmorphic features (*11*). When there is evidence for autistic features or a family history with ID, *FMR1* gene

analysis is warranted to elucidate Fragile X syndrome (FXS). Then, second-tier testing is performed to find common monogenic disorders (*12*). Further analysis by next-generation sequencing (NGS), a high-throughput technology, is warranted for unexplained ID and MCA after conventional testing. Although still deemed costly, especially in developing countries, the use of exome sequencing (ES) and whole genome sequencing (WGS) increase the diagnostic yield of individuals with ID and MCA and may end a diagnostic odyssey (*13-15*). There are two approaches applied to discover pathogenic variants, which are the phenotype-first approach which investigates patients based on the clinical features, while the genotype-first approach offers unbiased examination to find causative genetic variants by simultaneously analyzing sequences of the patient's and the respective parents' DNA (trio approach) (*16*). These strategies are believed cost-effective in most countries, and therefore even some developed countries are implementing a genotype-first approach in genetic diagnosis (*13,17*). Still, it is not feasible to have this approach put into practice in Indonesia. In most developing countries where advanced molecular facilities are limited, a phenotype-first approach is more favorable. For example, in Morocco, genetic testing methods such as cytogenetic, molecular cytogenetic, and several molecular diagnostics are available for several conditions such as constitutional chromosomal abnormalities, inborn error of metabolisms, chronic myeloid leukemia, and thalassemia (*18*). Similarly in Pakistan, the number of genetic diagnostic laboratories are limited, and no newborn screening program is conducted at the national level (*19*). Meanwhile in Sri Lanka, aside from cytogenetics service, NGS is available for university-based cancer genetic diagnosis (*20*).

We aim to set a systematic flowchart for the genetic evaluation of patients with ID and MCA, which can be applied efficiently in Indonesia or other low resources settings, while attempting to achieve a good diagnostic yield.

2. Materials and Methods

2.1. Patients

Our study population consisted of 155 individuals, of which 133 individuals were included from an institution for ID individuals, and 22 MCA patients with global developmental delay (GDD) were referred from clinicians to our laboratory of Center for Biomedical Research through Diponegoro National University Hospital, from 2016 to 2018. We excluded 24 individuals with clinical features suggestive of Down syndrome (DS) for further analysis (*21*). Blood sampling from all individuals were performed for chromosomal and molecular analyses. We obtained blood from the index cases, and follow-up when necessary for trio blood collection. Patients with GDD and MCA with unexplained aetiology were referred by clinicians/pediatricians for genetic testing. All individuals were investigated through physical and dysmorphology examination, and the clinical data was recorded using a standardized form. Clinical photographs were taken from all examined individuals.

All parents/legal guardians signed a consent form, including for publication of photographs prior to study. The study complied with the Declaration of Helsinki. Ethical clearance was obtained from Health Research Ethic Committee, Faculty of Medicine, Universitas Diponegoro, Semarang (No. 1.032/EC/FK-RSDK/XII/2016). We applied the study procedure as followed.

2.2. Study procedure

Patient inclusion and procedure of this study are shown on Figure 1. Routine cytogenetic analysis using G-banding was performed on all individuals with ID/DD and dysmorphic features (except one patient due to the patient's poor condition) to exclude the possibility of a chromosomal abnormality. Individuals with mild to moderate ID from institutions were screened for FXS, using FastFrax™ Identification, Sizing, and Methylation Status kits (The Biofactory Pte Ltd, Singapore) as previously described (*22*).

We performed dysmorphology evaluation in two steps. The first step of screening aimed to identify individuals with syndromic ID/GDD, by evaluating facial dysmorphic features and existing comorbidities (*e.g.*, congenital heart abnormalities). Clinical and dysmorphic features were analyzed using the London Dysmorphology Database which is available online from the Face2Gene system software (FDNA Inc, Boston, MA, USA). From this screening, we selected 50 individuals for further evaluation. The second step of phenotypic evaluation was performed by researchers and experienced clinical geneticists (IvB and BvB), in order to determine accurate genetic testing for each individual based on its disease mechanism and mode of inheritance. From the comprehensive clinical screening, we selected 23 patients for further analysis, *i.e.*, chromosomal microarray (CMA) for 13 individuals, targeted gene sequencing (panels) for 4 individuals and exome sequencing (ES) for 6 individuals. One individual was added to ES analysis as a follow-up of CMA examination.

2.3. Chromosomal microarray (CMA)

CMA was performed at the Division of Genome Diagnostics of the Radboud university medical center (Nijmegen, Netherlands) for 13 individuals using the CytoScan HD array platform (Thermo Fisher Scientific Inc., Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions. Interpretation was made according to the following categories, based on

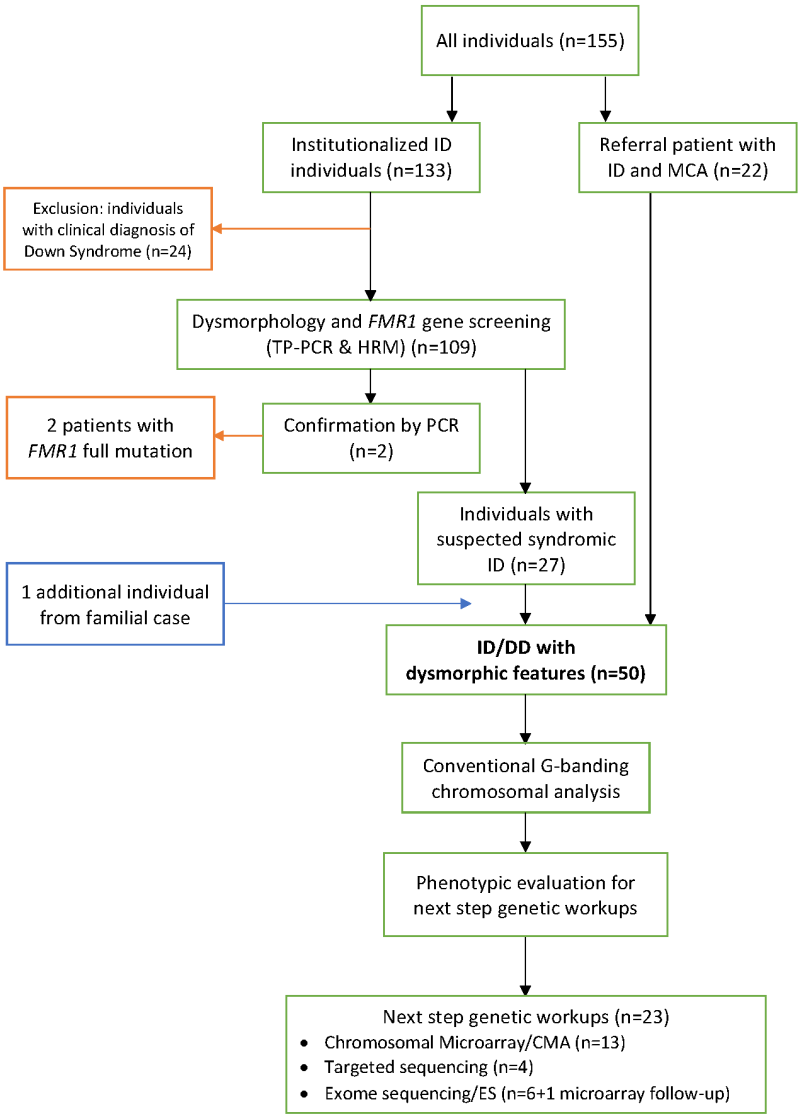

Figure 1. Flowchart and patient selection for the research. Red boxes and arrows indicate excluded individuals, blue box and arrows indicate additional individual from a familial case (hereafter referred to as P6).

the ACMG standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants (*23*).

2.4. Next generation sequencing (NGS)

Exome sequencing (ES) was performed on DNA from seven individuals at the Division of Genome Diagnostics (Radboudumc, Netherlands). Exome capturing was carried out using Agilent SureSelect Target Enrichment V5 (Agilent Technologies, Santa Clara, CA, USA) as described previously (*24*). Next, sequencing was performed using the Illumina HiSeq 2000 platform (Illumina, Inc. San Diego, CA). Illumina base calling software v1.7 was performed using the Roche Newbler software (v2.3) using human genome build hg19/ GRCH37.

Seven major steps were taken to select all high-quality potentially pathogenic variants, as previously described. The exome sequencing results were confirmed by Sanger sequencing. Primers for the amplification of the exons carrying variants were designed using Primer3. PCR reactions were performed on 50 ng of genomic DNA with Taq DNA polymerase (Invitrogen, Carlsbad, CA). PCR amplicons were purified with NucleoFast 96 PCR plates (Clontech Lab, Mountain View, CA), according to the manufacturers protocol. ABI PRISM Big Dye Terminator Cycle Sequencing V3.1 Ready Reaction Kit and the ABI PRISM 3730 DNA Analyzer were used to perform sequencing (Applied Biosystems, Foster City, CA, USA) (*25*).

Targeted gene sequencing was performed on DNA from four individuals with suggestive clinical features of syndromic ID. Targeted gene sequencing was done on *BCOR* and *NAA11* genes for one individual and on the *KMT2A* gene on another. Panel sequencing was done on DNA from two patients for Stickler syndrome and Noonan syndrome, respectively. The Stickler syndrome panel consisted of *COL11A1, COL11A2, COL2A1, COL9A1, COL9A2, COL9A3, SLC26A2, VCAN* genes,

and the Noonan syndrome panel included 17 genes (*BRAF, CBL, HRAS, KRAS, LZTR1, MAP2K1, MAP2K2, NRAS, PPP1CB, PTPN11, RAF1, RIT1, RREB1, SHOC2, SOS1, SOS2, SPRED1*).

3. Results

We examined individuals with ID/GDD and MCA by combining conventional analysis, stringent patient selection from two-steps evaluation, and advanced genetic testing. Out of 109 individuals from the institution who underwent *FMR1* gene analysis, two individuals were found to have a full mutation of the *FMR1* gene with a methylated repeat allele (*22*). Subsequently, based upon first dysmorphology evaluation using the dysmorphology database and facial analysis software of Face2Gene (Facial Dysmorphology Novel Analysis, FDNA), 27 (out of the 107) individuals with the most prominent dysmorphisms and/or congenital anomalies and comorbidities were selected for further genetic testing.

The second group of samples came from referred patients (n = 22) with ID/GDD and MCA. All but one patient underwent routine cytogenetic analysis with no visible aberration. Finally, one individual was added from a cascade testing of a family with ID and MCA.

Following two steps of dysmorphology evaluation, 23 individuals were included to next step genetic analysis. The main clinical findings and the genetic test results are summarized in Table 1 (Online Data, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=145*). The frontal facial photographs of each diagnosed individuals are shown in Figure 2.

3.1. Chromosomal microarray (CMA)

CMA was performed on DNA from 13 individuals (Table 1). Of these 13 cases, seven individuals received conclusive results, yielding a diagnostic rate of 54% in this selected group of patients.

From thirteen cases who underwent microarray, P4, P5, and P6 were siblings with ID and similar dysmorphic features. Individuals P5 and P6 were twins, with P6 having more severe clinical features compared to the twin sister. Subject P6 was included from the family cascade testing prior to further dysmorphology evaluation. Since no genomic aberrations were found, P6 was subjected to further analysis using exome sequencing to search for possible pathogenic variants (*26*).

Subject P11 was included for array analysis due to the suggestive features of split hand foot malformation (SHFM). Routine cytogenetic analysis was not performed due to the patient's poor condition (*i.e.*, insufficient blood collection) and high suspicion of this syndrome. Array analysis revealed a complete trisomy 18, as reported earlier (*27*).

3.2. Next generation sequencing (NGS)

Seven individuals were subjected to ES, while some other patients underwent targeted sequencing due to high suspicion of a specific syndrome diagnosis, as shown in

Figure 2. Frontal documentation of diagnosed individuals with ID/GDD and MCA. Supplementary data on P16 is provided.

Table 1.

In this cohort, P13 and P14 were two sisters with developmental delay and similar clinical features, who were first suspected of having Angelman syndrome. The older sister (P13) was sent in for array analysis and P14 was subjected to exome sequencing. Both results showed an interstitial gain in band q31.3q41 of chromosome 1, as reported previously (*28*). Further analysis using exome sequencing on P6 detected a nonsense pathogenic variant in the *NFIX* gene, which was also found in the two other affected sisters. A clinical report of this family has been reported in detail elsewhere (*26*). Taken together, the diagnostic rate of ES was 71% (5 out of 7 individuals diagnosed) in this selected group of individuals. Meanwhile in targeted gene sequencing, 2 out of 4 cases were concluded, yielding a 50% diagnostic rate.

4. Discussion

Our study applied a stepwise phenotype-first approach to elucidate the aetiology of ID/GDD and MCA patients. The first step was performing thorough physical examination to identify dysmorphic features in each individual. The existing physical characteristic found was defined using standardized terms from The Elements of Morphology (*29*). Additionally, facial recognition software was utilized to aid phenotyping towards a presumptive syndrome diagnosis (*30*). The purpose of this step is to recognize syndromic ID. Meanwhile, the second step applied thorough evaluation and reassessment, which was conducted by experienced clinical geneticists, aimed to determine appropriate advanced genetic tests. Although genetic testing in recent years has rapidly advanced and genotype-first approach has progressed, dysmorphology examination still remains an important component to make a presumptive diagnosis (*31*). Evidently, the phenotype-first approach is still widely used and obligatory, especially in developing countries with limited access or resources to be able to perform next generation sequencing in individuals with ID/GDD and MCA (*32*). Many different diagnostic approaches on individuals with ID/GDD and MCA individuals have been established within different settings (*32-34*). We described our experience in Indonesia, a low-middle income country setting without the possibility to perform MCA and ES in all individuals with ID/GDD without performing patient selection. Hence, we propose a genetic diagnostic approach for individuals with ID/GDD and MCA, which is applicable to developing countries setting as shown in Figure 3.

In countries with limited resources setting such as Indonesia, cytogenetic analysis is still deemed useful in evaluating individuals with ID/GDD and MCA. Routine cytogenetic analysis with G-banded karyotyping has been applied for more than three decades in Indonesia. A study by Mundhofir *et al.* suggested that cytogenetic analysis could detect chromosomal aberrations in 16.5% of ID population including 14% of trisomy 21. A similar study was done in a Rwandan ID/GDD and MCA population, resulting in a 39% diagnostic yield, including 30% of individuals with Down syndrome (*35,36*). However, due to the low sensitivity, most individuals with smaller chromosomal abnormalities or microdeletion/ monogenic disorders remain undiagnosed. Here, we were able to use CMA as the next approach for a selected group of individuals with a normal karyotype result.

CMA analysis provided a diagnosis in seven out of 13 individuals. From seven individuals, there were two individuals with deletions, two cases with a copy number gain, two cases with large regions of homozygosity, and one trisomy 18 case in whom no karyotyping was done prior to array analysis because of his poor condition and unsuccessful karyotyping. Meanwhile, from the six undiagnosed cases, we found one case where the genome contained large homozygous regions, confirming parental consanguinity. Since 2010, CMA has been recommended as the first-tier diagnostic test for individuals with ID/GDD and MCA, instead of G-banded karyotyping (*11*). CMA can detect copy number variations (CNVs), as well as regions of homozygosity if a SNP-based array platform is used to reveal conditions such as uniparental isodisomy (UPD) and parental consanguinity, as shown in our results. One of the limitations of CMA is the inability to detect balanced chromosomal rearrangements, which was apparent in case P9 with a 46,XY, (t1;2)(q43;q10) karyotype. No genomic imbalances were observed with CMA, even at the translocation breakpoints in the long arms of chromosomes 1 and 2, respectively.

In the CMA study, we obtained a diagnosis in 4 out of 7 cases after finding relatively large chromosomal imbalances (ranging from 16.71 Mb–20.48 Mb), including two cases in whom the imbalance was not previously identified upon routine cytogenetic analysis. Several factors may have led to these missed diagnoses in the first-tier karyotype test. First, in these cases the resolution was low, the band size was approximately 400–450 bands, and as a result, structural chromosomal abnormalities such as deletions or duplications of up to 20 Mb were not resolvable. For postnatal indications karyotyping using a banding technique such as G-banding requires a minimum banding quality of 550 bands per haploid set (*37*), but preferably the chromosome resolution is at or above the 650-band stage (resolution at the 850-band level may be necessary) for structural abnormalities (*38*). Second, initial clinical suspicion may hinder the unbiased decision to evaluate the karyotype or to enroll the patient in a specific diagnostic test. These potential pitfalls have been discussed in our previous reports (*27,28*).

We performed ES and targeted sequencing as

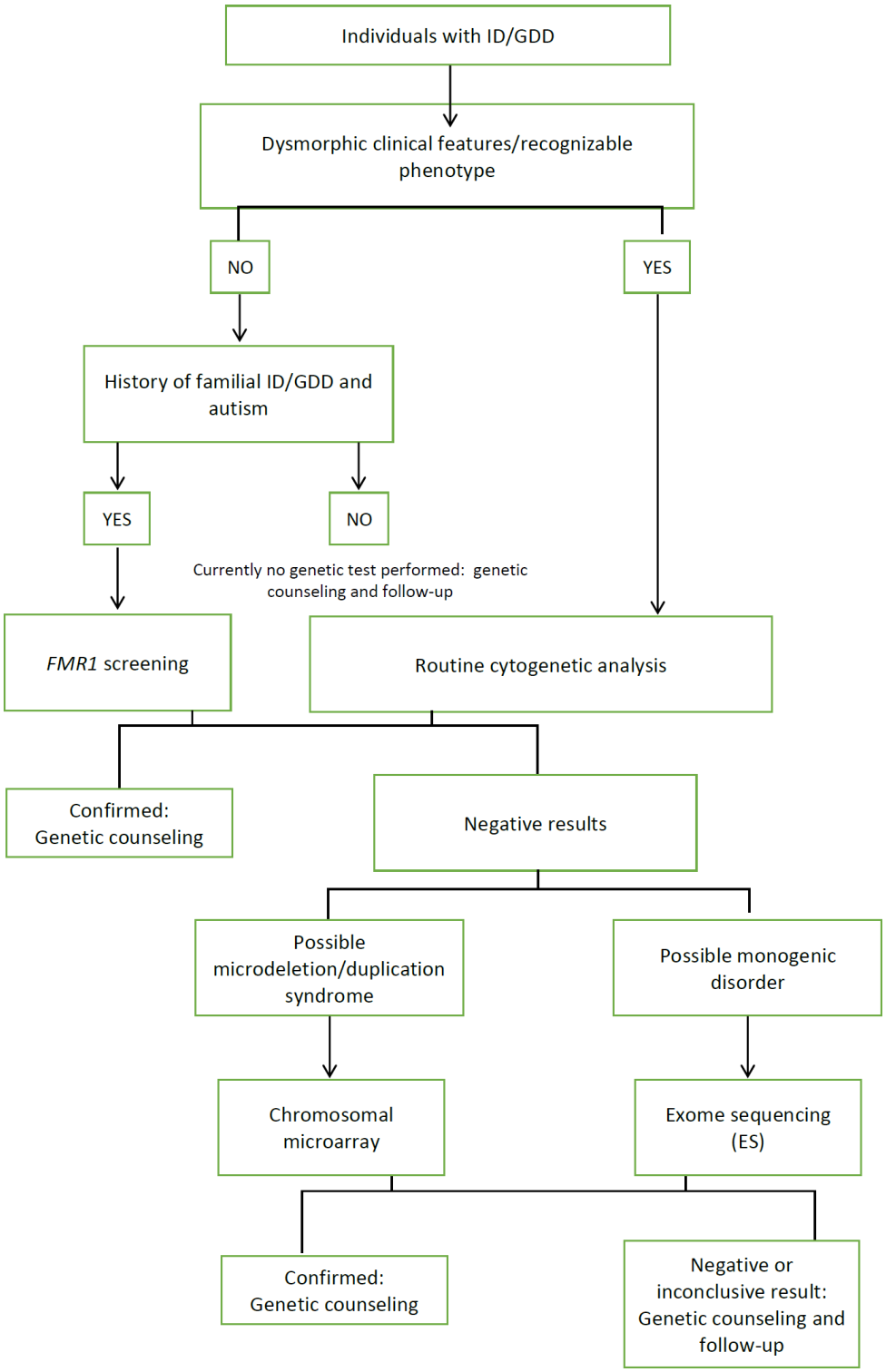

Figure 3. Proposed diagnostic approach for individuals with ID/GDD and MCA in Indonesia. Stepwise phenotype-first approach starts from identifying dysmorphic features, conducting routine cytogenetic analysis, and follow-up with CMA/ES according to possible clinical diagnosis.

the next approach in undiagnosed cases. From seven individuals who underwent ES, pathogenic variants were obtained in four cases, and one individual (P14) yielded a large CNV similar to the sister's (P13) CMA results. The availability of exome data in this familial case could further delineate possible breakpoints, whether it occurred in a functional gene region, and whether any pathogenic nucleotide variants were found in the patients. The use of ES is highly efficient, since it can detect both single nucleotide variants as well as CNVs at the sequence-level in the protein-coding exome and at the intron-exon boundaries (*28,39,40*).

By utilizing ES, a diagnosis was finally established in a male baby (P6) after more than one year in which he underwent several evaluations and follow-ups with various differential diagnoses, from Robinow

syndrome on the first examination to Antley-Bixler syndrome on the second evaluation, due to clinical characteristics and dysmorphic features alteration. When all genetic testing results came back with no pathogenic variant found, a trio ES was performed to elucidate the causative pathogenic variant in the patient, which revealed a de novo, pathogenic variant in the Filamin-A (*FLNA*) gene (NM_001456.3:c.3425A>T; p(Asp1142Val)). This variant has been described as a gain-of-function pathogenic variant, which causes frontometaphyseal dysplasia type 1 (FMD1, OMIM #305620), a spectrum of otopalatodigital syndrome (see Supplementary File, *http://www.irdrjournal.com/action/getSupplementalData.php?ID=146*) (*41*). The accessibility and availability of a diagnosis in this patient has finally put an end to his diagnostic odyssey, which is described as a condition where a strong diagnostic hypothesis is absent even after clinical evaluation, or a negative diagnostic work-up including array analysis, FXS screening, and targeted testing for monogenic disorders (*42*). In a study from Brazil, ES identified underlying pathogenic variants in almost half (9 out of 19) of individuals with undiagnosed ID/GDD with MCA (*43*). ES is beneficial compared to conventional approaches in terms of diagnosing atypical forms of known syndromes, recently described genes and/or syndromes, and ultra-rare conditions (*42*). Moreover, although the utility of ES did not directly change the treatment, therapy or prognosis, the results are important to improve the family members' understanding of the psychological condition, especially because the diagnostic odyssey could be ended. In addition, genetic counselling can be done when the disease is inherited to improve the understanding of the disease, to identify the family member(s) who may be at risk to be a carrier or affected (adult onset disease), calculating the recurrence risk, and to provide continuing support to the family member who needs more information in the future (*44,45*). When the recurrence risk can be calculated, it helps the family, especially young couples, to plan future reproductive options or to consider invasive prenatal diagnosis or pre-implantation genetic testing (PGT) when available.

By using targeted sequencing (single-gene sequencing and panel sequencing), we obtained a genetic diagnosis in 2 out of 4 individuals. Both solved cases were diagnosed with panel sequencing on specific syndromes, *i.e*., Stickler syndrome and Noonan syndrome, respectively. Meanwhile, in the other two individuals, we only attempted sequencing of one or two genes. It is important to notice that targeted Sanger sequencing will be most beneficial for individuals with recognizable syndromes (*46*).

The detection rate of each genetic test in our study varied between 50% and 71%, with the highest rate using ES. Current evidence suggests that for ES, the diagnostic yield is around 34% for patients with ID/GDD (*14*). The high detection rate in our relatively small cohort was due to selection bias, because stringent selection was made by experienced clinicians beforehand (*32,47,48*).

There are some limitations to our study. Although the aetiology on half of the cases evaluated using exome sequencing have been found, some cases remained elusive, thus further follow-up is needed for possible retesting or reanalysis. This could be due to the unavailability of a trio *de novo* analysis. In addition, other genetic factors, such as methylation abnormalities, repeat expansion disorders or intronic variants are not possible to detect by ES. Finally, non-genetic factors will not be detected using this diagnostic strategy. This study involved individuals from an institutionalized intellectually disabled population as well as referred patients from clinicians. Some clinical data was incomplete, for example on severe cases of MCA, information on clinical characteristics including dysmorphic features were limited at times, and patients had passed away after only a few hours or days of life, thus making diagnostic workups difficult. To deal with this issue, it is important for the referring clinicians to document comprehensive notes on patients, including good photographs, which include a frontal facial photo and detailed pictures of dysmorphic features.

Although the advancement of ES technology is promising, there are some challenges in applying ES in a routine clinical setting. For instance, while ES can facilitate the diagnosis in atypical and heterogeneous cases, it should not replace the need for thorough clinical evaluation by the clinician to narrow down the clinical diagnosis and select the appropriate panel testing, if available (*49*). A good diagnostic approach includes awareness of positive signs during history taking, pedigree construction, physical and dysmorphology evaluations, which prompt further genetic testing, and performing comprehensive analysis based on suspected conditions or syndromes, starting from routine cytogenetic analysis to NGS. The presence of recognizable dysmorphic features, growth abnormalities or peculiar comorbidities should prompt clinicians on the possibility of a genetic origin. Additionally, parental reproductive issues and family history of ID/GDD and MCA are considered as a "warning sign" to initiate genetic evaluation.

When no conclusive results are obtained in routine cytogenetic analysis, further genetic testing should be done together with parental samples, in order to do trio analysis. Conducting the flowchart does not warrant conclusive results in all patients. In patients with moderate to severe ID/GDD, accumulated diagnostic yield from cytogenetic analysis to whole genome sequencing can be achieved in mostly around 55–70% of all cases (*50*). Thus, pre-test and post-test genetic counselling is important and take a longer time in order to plan for follow-up or re-testing when more causative

pathogenic variants are elucidated. Genetic counselling in most families has been done in the clinic or by conducting home visits. However, several others were done virtually by video call or phone call, since patients would have to come from various parts of Java Island, including remote areas. Virtual counselling may hinder full comprehension about the patient's condition and diagnosis, consequently, patients or family may decide for a less suitable option for (follow-up) testing.

Recently, the Indonesia Health Ministry took a step forward by launching the Biomedical and Genome Science Initiative (BGSi), which is aimed to integrate genomics capacity into health services for rare diseases, metabolic syndrome, infectious diseases, cancer, and wellness (*51*). In the future, NGS will become widely available for genetic diagnosis services in Indonesia. However, considering the current policy national healthcare insurance, which does not cover genetic testing, a phenotype-first approach will remain more cost-effective compared to a genotype-first approach.

In conclusion, our flowchart is applicable for the genetic diagnosis of ID/GDD and MCA in Indonesia and similar countries with limited resources available for genetic services. Here we established a diagnosis in 17 of 23 patients. By recognizing the phenotype and categorizing syndromic ID, followed by conducting appropriate genetic testing, most syndromes are explained, and diagnostic odysseys have been solved using this comprehensive approach. While some laboratory facilities such as array analysis and exome sequencing are not widely available in Indonesia, close collaboration between clinical and laboratory centers with a research institution, both nationwide and international, government, and stakeholders will improve the possibility of providing a genetic diagnosis for individuals with ID/GDD and MCA.

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Evaluation of the efficacy and safety of pegloticase for the treatment of chronic refractory gout through meta-analysis

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SUMMARY Gout is the most common arthritis that affects more than 2% of adults in developed countries. 3% to 4% of gout is chronic refractory gout. Conventional treatments are considered invalid. A new drug, pegloticase is used to treat chronic refractory gout, and there are still many questions about efficacy and safety. We searched PubMed, web of science, and the Cochrane Library. Preprints and references of related literature were also considered. Related efficacy and safety indicators were statistically analyzed by Review Manager 5.4 to conduct meta-analysis. A total of one article and one clinical trial were included. Pegloticase is able to reduce serum uric acid and reduce tender joints, thereby improving joint function. But pegloticase has more adverse events. Pegloticase can be used to treat chronic refractory gout. However, Pegloticase has a higher risk of adverse events. Considering the efficacy and safety, the scope of clinical applications of pegloticase can be further widened in patients in good medical condition.

Keywords chronic refractory gout, pegloticase, meta-analysis

1. Introduction

Gout is the most common inflammatory arthritis, with prevalence now exceeding 2% in adults in developed countries (*1*). The worldwide incidence of gout has increased gradually due to poor dietary habits such as fast foods, lack of exercise, increased incidence of obesity, and metabolic syndrome. Gout is caused by the deposition of monosodium urate crystals in the joints following chronic hyperuricemia (*2*). In gout, the therapeutic goal is to lower serum uric acid below 6 mg/dL, but the British Society for Rheumatology gives a lower target that serum uric acid should be reduced to less than 5 mg/dL (*3*). However, gout is a chronic disease. Even after serum uric acid is reduced to the target, gout can still flare up in the next 12 to 18 months (*4*).

During gout exacerbations, patients experience severe pain. After about 5 to 10 days, gout will go to a chronic-phase. Uric acid crystals can accumulate in joints and other positions causing disability, especially in metatarsophalangeal 1 joints. Due to joint pain, inflammation, and flares, gout poses a major burden on the patient's daily life, and even causes disability (*5*). Patients with gout have a higher risk of death from all causes, especially cardiovascular disease (*6*). There is a case report decreasing the accumulation of urate crystals in the retina causing macular degeneration (*7*). The prevention and treatment of gout flares become especially important.

Available treatment strategies for gout include two major types of treatment: xanthine oxidase inhibitors, and uricosuric agents. But all these drugs have side effects (*8*). Even worse, these drugs are less effective for chronic refractory gout (CRG).

2. Pegloticase dilemma

Pegloticase (Krystexxa®), a pegylated recombinant mammalian uricase, is a US Food and Drug Administration–approved medication for treatment of uncontrolled gout in 2010. Pegloticase provides a new approach to the treatment of CRG. Pegloticase, a recombinant uricase, catalyzes the conversion of uric acid to allantoin (*9*). Allantoin is more soluble and easily excreted. Uric acid-lowering therapy improved when 8 mg was injected every two or four weeks, enabling serum uric acid below 6 mg/dL in some patients with CRG (*9*).

There are still some unclear issues regarding the safety of pegloticase to date. On 30 June 2016, the European Commission withdrew marketing authorization for pegloticase in the European Union. Consequently,

comprehensive efficacy and safety assessments are still required for pegloticase before more approval for clinical use by the regulatory agents.

3. Meta-analysis

Our study was registered in PROSPERO (CRD42022322978). The PRISMA guideline was followed throughout. Literature search, data extraction and data analysis were performed independently by two researchers. Divergence was examined by a third researcher. If something appeared different, a third investigator intervened in data extraction. Specific details are given in PROSPERO (*https://www.crd.york.ac.uk/PROSPERO/display_record.php?RecordID=322978*).

4. Main finding

Figure 1 shows the detailed literature search and screening process. A total of 7 articles fulfill the inclusion criteria. Among six included studies completed according to NCT00325195 and/or NCT01356498. NCT01356498, an open label extension study was excluded because the control group did not meet the criteria. Albert 2020(10) and NCT00325195 were included in the meta-analysis finally.

Albert *et al*. believe that pegloticase can be used to treat CRG, and that methotrexate/pegloticase co-therapy have a higher response rate than pegloticase alone (*10*). NCT00325195 found that pegloticase produces good clinical curative effects in CRG. Although advance events were experienced, most patients tolerated pegloticase.

According to the meta-analysis, pegloticase has a noticeable effect in the reduction of uric acid. Pegloticase has poor safety profiles. Pegloticase potently decreases the number of tender joints without significant reduction in swollen joints. Eight mg pegloticase every 2 weeks significantly reduces pain in patients, while 8 mg pegloticase every 4 weeks is ineffective, according to the Health Assessment Questionnaire. Regardless of the dose used, pegloticase improves the quality of life of patients, according to the SF-36 Physical Component Summary Score. The specifics are detailed in Table 1.

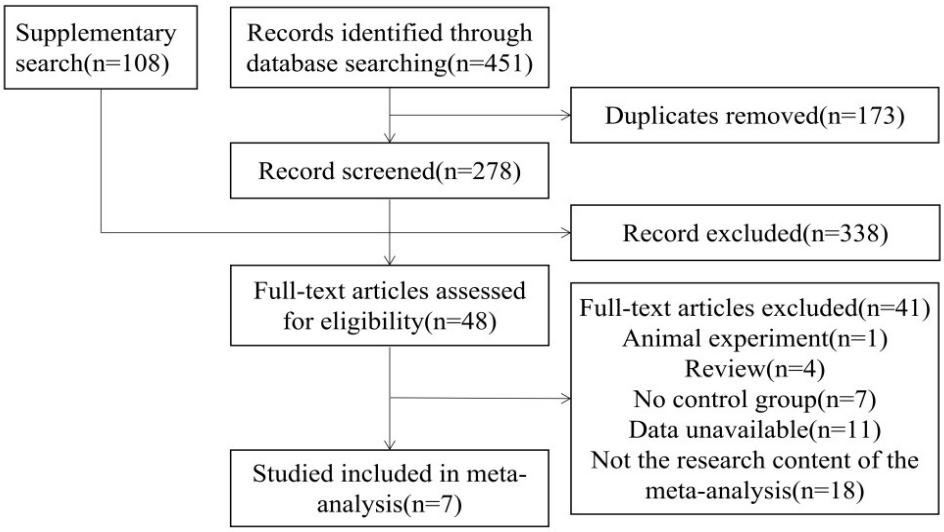

Figure 1. Flowchart of the systematic literature search.

Table 1. Pooled results for meta-analysis about the efficacy and safety of pegloticase

Outcome Measures	Subgroup	Number of Trials	Statistical Method	Effect Estimate	*p* for Heterogeneity
Plasma Uric Acid Responder	q2 Wks	2	OR (M-H, Random, 95% Cl)	67.26 [8.18, 552.87]	< 0.0001
	q4 Wks	1	OR (M-H, Random, 95% Cl)	46.24 [2.75, 778.33]	0.008
Advance Events	q2 Wks	2	RR (M-H, Random, 95% Cl)	8.04 [1.59, 40.70]	0.01
	q4 Wks	1	RR (M-H, Random, 95% Cl)	8.70 [1.20, 63.22]	0.03
Change in Number of Swollen Joints	q2 Wks	1	MD (IV, Random, 95% Cl)	-2.90 [-7.08, 1.28]	0.17
	q4 Wks	1	MD (IV, Random, 95% Cl)	-2.50 [-6.39, 1.39]	0.21
Change in Number of Tender Joints	q2 Wks	1	MD (IV, Random, 95% Cl)	-6.20 [-10.73, -1.67]	0.007
	q4 Wks	1	MD (IV, Random, 95% Cl)	-4.90 [-9.28, -0.52]	0.03
Health Assessment Questionnaire	q2 Wks	1	MD (IV, Random, 95% Cl)	-12.80 [-24.52, -1.08]	0.03
	q4 Wks	1	MD (IV, Random, 95% Cl)	-8.30 [-19.11, 2.51]	0.13
SF-36 Physical Component Summary Score	q2 Wks	1	MD (IV, Random, 95% Cl)	4.70 [1.29, 8.11]	0.007
	q4 Wks	1	MD (IV, Random, 95% Cl)	5.20 [1.91, 8.49]	0.002

q2 Wks: 8 mg pegloticase every 2 weeks; q4 Wks: 8 mg pegloticase every 2 weeks; Cl: confidence intervals.

5. Discussion and evaluation

In the meta-analysis, only two studies met the criteria and were included. At present, the clinical research of pegloticase has the problem of a small sample size and insufficient research centers. These studies have predominantly focused on North America cohorts. Considering the differences in enzymes due to genetic differences in races, the relationship between safety and race remains to be studied. The most important finding of this study is that we have not been able to apply pegloticase to a wide range of clinical applications.

Pegloticase has a significant effect compared with placebo in the treatment of CRG. The Health Assessment Questionnaire and SF-36 Physical Component Summary Score reflect the quality of life and physical function, respectively. Except for 8 mg pegloticase every 4 weeks in the Health Assessment Questionnaire, the remaining subgroups showed that pegloticase was beneficial to patients. Although the reduction in swollen joints was not statistically significant, tender joints were significantly reduced. Considering that the swelling does not go away in the short term, swollen joints may decrease as tophi dissolves. However, the effective rate of pegloticase is very low. In NCT00325195, the treatment efficacy of CRG is less than 50%.

Given that more than 40% of patients develop antibodies against pegloticase, strategies for delivering pegloticase are being reassessed (*11*). Methotrexate has been the most investigated agent as a drug that reduces the tolerability of pegloticase. Immunosuppression with methotrexate may prevent loss of pegloticase tolerability and also be used to recover sensitivity after the development of intolerance (*12,13*). There is a potential impact on liver/kidney toxicity with methotrexate (*14*). Mycophenolate mofetil has enticing clinical potential to prolong the efficacy of pegloticase. No drug, however, is truly perfect. The use of mycophenolate mofetil was associated with an increase in gastrointestinal adverse events, hematological adverse events, and minute virus of canine infection (*15*). But it is not clear whether this association is specific. Large-scale clinical experiments are currently lacking.

According to the meta-analysis, the adverse events of pegloticase were significantly higher than placebo. The most common adverse events reported with pegloticase in the published trials include gout flares and infusion reactions associated with antibody response. 91% of infusion reactions occurred in patients with serum uric acid concentrations > 6 mg/dL (*16*). Infusion reactions decrease with decreasing serum uric acid concentration. Khanna *et al*. carried out a phase II, randomized, double-blind, placebo-controlled trial that concluded mycophenolate mofetil prolongs the efficacy of pegloticase (*14*). And mycophenolate mofetil can be used in patients with chronic kidney disease (*17,18*). However, pegloticase does not improve renal function, either with or without tophi (*19*). The infusion reaction of pegloticase can be effectively prevented with immunotherapy. The infusion reaction of pegloticase can be effectively controlled. It cannot be ignored that patients using pegloticase in NCT00325195 died. Specific causes of death are unclear, but it is cautionary. With the further use of pegloticase, new adverse events were discovered. A study reported hemolytic anemia using pegloticase in patients with G6PD deficiency (20). The use of pegloticase in CRG patients with G6PD deficiency has only received extensive attention in recent years.

In conclusion, the efficacy and safety of pegloticase are unsatisfactory. However, for CRG, pegloticase is currently the best treatment. The adverse events of pegloticase can be prevented. Based on current research, there are no serious safety concerns with pegloticase. Pegloticase can be used clinically for the treatment of CRG if the patients are in good medical condition.

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A very rare cause of leukoencephalopathy: Lymphomatosis cerebri

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SUMMARY Leukoencephalopathy is a common finding on Magnetic Resonance Imaging (MRI), particularly in the elderly. A differential diagnosis may represent a very bet for clinicians when clear elements for diagnosis are lacking. Diffuse infiltrative "non mass like" leukoencephalopathy on MRI may represent the presentation of a very rare aggressive condition known as lymphomatosis cerebri (LC). The lack of orienting data, such as contrast enhancement on MRI or specific findings on examination of Cerebrospinal Fluid (CSF) or blood tests, may even far more complicate such a difficult diagnosis and orientate toward a less aggressive but time-losing mimic. A 69-old man initially presented to the Emergency Department (ED) complaining the recent appearance of unsteady walking, limitation of down and upgaze palsy, and hypophonia. Brain MRI revealed the presence of multiple, confluent hyperintense lesions on T2/Flair Attenuated Imaging Recovery (FLAIR) sequences involving either the withe matter of the semi-oval centres, juxtacortical structures, basal ganglia, or bilateral dentate nuclei. DWI sequences showed a wide restriction signal in the same brain regions but without any sign of contrast enhancement. Initial 18F-labeled fluoro-2-deoxyglucose positron emission tomography (FDG PET) and CSF studies were not relevant. Brain MRI revealed a high choline-signal, abnormal Choline/N-Acetyl-Aspartate (NAA), and Choline/Creatine (Cr) ratios, as well as reduced NAA levels. Finally, a brain biopsy revealed the presence of diffuse large B-cell lymphomatosis cerebri. The diagnosis of lymphomatosis cerebri remains elusive. The valorisation of brain imaging may induce clinicians to suspect such a difficult diagnosis and go through the diagnostic algorithm.

Keywords lymphomatosis cerebri, MRI, Primary Central Nervous System lymphoma (PNCSL), leukoencephalopathy, ^{1}H-MRS

Leukoencephalopathy may present a diagnostic challenge, even in the elderly. A long list of different diseases such as vascular diseases, immune-mediated disorders, infections, neurodegenerative, dysmetabolic patterns, and myelin-dystrophic diseases may underlie such a condition. Although rare, lymphomatosis cerebri (LC) may be indistinguishable from other less aggressive and more frequent conditions responsible for diffuse leukoencephalopathy at an initial brain MRI assessment (*1*). Clinical presentation might not help in the orientation of the diagnostic work-up, as initial symptoms may include subacute cognitive impairment, focal motor deficits, movement disorders, and incoordination. The MRI features of LC are described as diffuse, infiltrative T2W, or FLAIR hyperintense lesions, but they lack contrast enhancement on T1 sequences (*2,3*). Lesions may involve any structure belonging to either the white or grey matter of the Central Nervous System, including the eye. These findings are very different from those of Primary Nervous System Lymphoma (PNCSL) (*4*), which is typically characterized by nodular lesions emerging either on T2W imaging or enriched by contrast enhancement on T1W sequences, especially of brain structures lying on the median line.

On June 1, 2022, a patient in the 60s presented to the ED complaining of unsteady walking, frequent falls, downgaze, and up gaze palsy, and hypophonia. Symptoms had started and slowly progressed in the previous two months. The patient was referred to

the neurological ward. Extensive blood examination included routine and rheumatologic tests, neoplastic markers, and an autoimmune panel. All results were un-relevant. Computed tomography (CT) scans of the chest and abdomen were obtained, and pathological findings were disclosed. Brain MRI showed diffuse infiltrative encephalopathy involving either the cerebral deep and juxtacortical white matter, basal ganglia, dentate nuclei, or cerebellar medulla (Figure 1, a-c). Notably, DWI and ADC sequences demonstrated wide restriction signals in the same areas (Figure 1, d and e). CSF studies demonstrated only a slight increase in protein concentration (2 cells/μL), normal glucose, lactate, and absent bilirubin. HIV, VDRL, and TPHA tests were negative. IgM and IgG titers in Borrelia Burgdoferi were normal. Polymerase Chain Reactions failed to detect the presence of DNA from all herpes viruses, coxachies, enterovirus, and JC virus. Antibodies to both surface and intracellular neuronal antigens were detected in the patient's serum and CSF and were found to be undetectable. Tests included anti-Hu, anti-Ri, anti-Yo, anti-CV2/CRMP5, anti-MA2, anti-amphiphysin, anti-GAD 65, anti-MA 1, anti-SOX 1, anti-TR (Dner), anti-Zic4, anti-LGI1, anti-CASPR2, anti-AMPA1, anti-AMPA2, anti-NMDA, anti-DPPX, and anti-IgLON5. Initial FGD-PET was not relevant for pathologic accumulation of radiotracer within the brain (data not shown). ^{1}H-MRS revealed increased Cho/Naa and Cho/cr ratios with reduced NAA absolute peak levels (Figure 1, f). We suspected a brain tumor and performed a brain biopsy. Meanwhile, awaiting results from microscopic studies on brain tissue, distressed by diagnostic uncertainty, we offered a trial of steroids at a therapeutic dosage to the patient in order to fight against his rapid clinical deterioration. Despite initial clinical improvement, the patient's condition worsened after a few days, prompting us to stop the steroid course definitively. Immunohistochemical analysis of the brain specimens demonstrated diffuse infiltration of lymphocytes, which were positively marked for CD3, CD20, and KI67 (Figure 2, a-e). A new brain MRI performed 30 days after the former imaging showed a T1

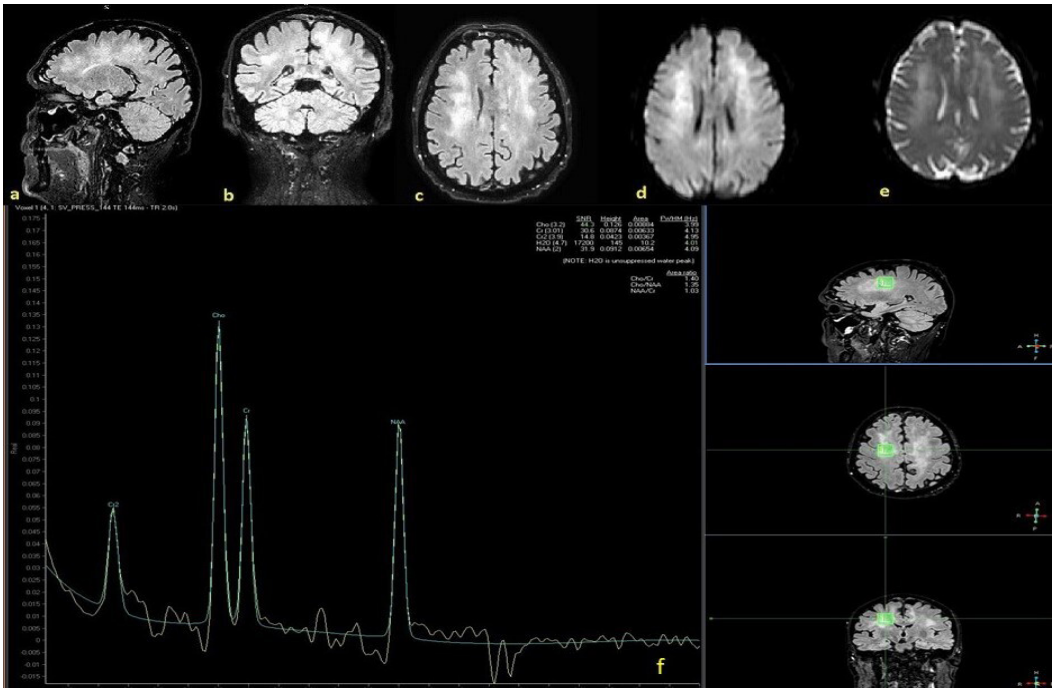

Figure 1. Imaging of Lymphomatosis cerebri. Sagittal **(a)**, coronal **(b)**, and axial **(c)** FLAIR images of the brain showing diffuse high signal intensity involving deep and juxtacortical white matter, basal ganglia, dentate nuclei and cerebellar medulla. DWI **(d)** and ADC **(e)** images showing reduced water diffusivity in the same affected regions, thus suggesting high cellularity. ^{1}H-MRS disclosing high Cho/NAA and Cho/Cr ratio indicating enhanced cell membrane turnover and reduced neuronal viability **(f)**.

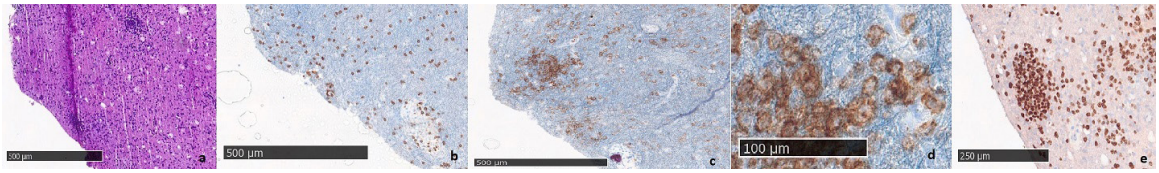

Figure 2. Microscopical Features of Lympomathosis cerebri. Hematoxylin-eosin staining of brain specimens showed diffuse infiltration of lymphocytes in the perivascular and in tissue brain **(a)**. Immunohistochemistry showed infiltration of CD3+ reactive lymphocytes **(b)**, CD20+ large lymphomatous cells **(c,d)**, all characterized by increased expression of KI67 receptor, a marker of cellular proliferation **(e)**.

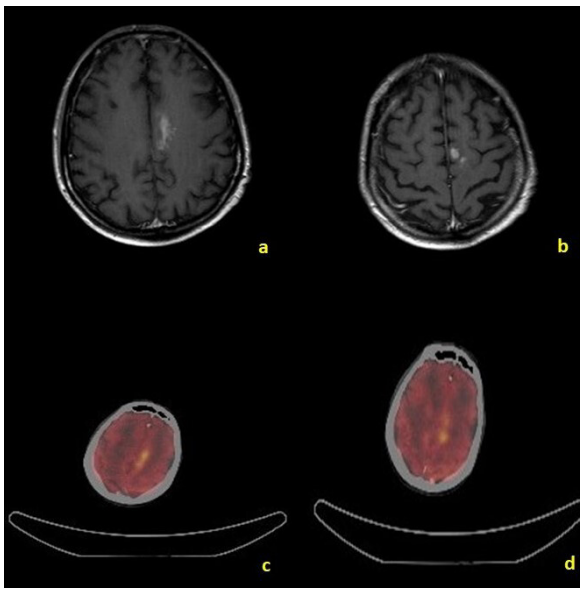

Figure 3. Imaging of Primary Central Nervous System Lymphoma as evolution of Lymphomatosis cerebri. T1-wheighed contrast enhanced sequences showed nodular lesions characterized by intense contrast enhancement in the cortex and subcortex of frontal left hemisphere **(a,b)**. FDG-PET showed focal glucose hypermetabolism of the frontal lesions **(c,d).**

contrast-enhanced subcortical nodular lesion within the left hemisphere (Figure 3, a and b), which also showed pathologic accumulation on brain FDG PET (Figure 3, c and d). Finally, a diagnosis of primary large B-cell brain lymphoma was made, and the patient underwent treatment according to international protocols including Metotrexate (MTX) and Cytosine Arabinoside (ARA-c). The patient died in March 2023, six months after having received diagnosis and having started specific therapeutics.

Lymphomatosis cerebri (LC) is a rare and aggressive variant of Primary Central Nervous System lymphoma (PNCSL) and may be undistinguishable from other less aggressive and more frequent conditions responsible for diffuse leukoencephalopathy at an initial brain MRI assessment (*1-3*). In contrast to the classic form of cerebral lymphoma, LC is characterized by diffuse infiltration of brain structures, predominantly of the withe matter, without forming a cohesive mass of malignant lymphoid cells (*1,2*). Sugie *et al*. (*4*) demonstrated that PNCSL may initially present as diffuse leukoencephalopathy without any T1W contrast-enhancing nodular lesions on brain MRI, thus resembling the imaging features of LC. As a result, the brain imaging of LC may give rise to dangerous misdiagnosis, as it is not specific of any disease (*1*).

Diagnosis in our patient was extremely challenging due to the lack of pathological findings, including normal initial brain FDG PET and CSF studies, the last showing only a mild increase in protein concentration with a slight increase in the albumin ratio.

Given that Apparent Diffusion Coefficient (ADC) maps are particularly low in cerebral lymphoma (*2*) and positively correlate with the expression KI 67 (*5*), we emphasized Diffusion Weighted Imaging (DWI) and ADC imaging from the initial brain MRI of our patient and, suspecting a brain tumour, decided him undergo MRI spectroscopy.

^{1}H-MRS was critical to our diagnostic workup due to the finding of abnormal Cho/NAA and Cho/Cr ratios as well as reduced NAA levels, strongly suggesting a proliferative disease involving the brain (*6*). These results prompted us to perform a diagnostic brain biopsy and immunohistochemical study. Initial results from brain specimens were interlocutory, and we administer high-dose steroids to stop the rapid worsening of the patient's clinical condition. Such behaviour, although frequent, should be avoided when suspecting a brain LC unless necessary as a lifesaving therapy. Steroids may delay diagnosis and selection of resistant clones (*1*). Accurate microscopic analysis of specimens and immunohistochemical studies later revealed the presence of LC and allowed the application of internationally accepted therapeutic protocols.

Notably, in contrast to the initial imaging, a brain MRI performed one month after hospital admission showed the appearance of a T1-contrast-enhanced nodular lesion, which was also characterized by increased glucose metabolism on brain FDG PET. Similar behaviour of LC has already been reported (*4*). It is tempting to suggest that LC might be an early step of CNS invasion by malignant lymphomatous cells, which later aggregate to form nodular lesions of classic PNCSL. This might depend on the differential and time-dependent expression of adhesion molecules such as CD31 (PECAM), whose expression was not detected by immunohistochemical study of brain biopsy in our patient. Similarly, the Lack of CD29 and CD54 adhesion molecules has been described in intravascular lymphomatosis (*7*).

The relevance of data from diffusion MRI and MR spectroscopy has already been suggested for diagnosing lymphomatosis cerebri, especially when basic MRI sequences are un-informative (*4,6*). Our case report adds significance to both imaging studies in the diagnostic workup of LC, before lymphomatous cells aggregate in the solid masses typical of PNCSL.

Suspecting lymphomatosis cerebri based on a picture of aspecific leukoencephalopathy is a challenge for clinicians. Increased Cho/cr and Cho/NAA ratios, reduced NAA peak as revealed by brain ^{1}H-MRS, and abnormal signals in ADC sequences from brain MRI should prompt clinicians to perform biopsy in undetermined leukoencephalopathy, even in the elderly.

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Autoantibodies, clinical phenotypes and quality of life in Lebanese patients with myasthenia gravis

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SUMMARY Myasthenia gravis (MG) is a rare autoimmune disease that affects the neuromuscular junction. It is characterized by the production of heterogeneous autoantibodies that bind to the neuromuscular junction and alter neural transmission. Recently, more attention was given to MG-related antibodies and their clinical influence. In Lebanon, studies about MG are very rare. To date, there is still no research on the different autoantibodies developed by Lebanese MG patients. We conducted a study aimed at detecting the prevalence of different antibodies in a group of seventeen Lebanese patients with MG, and exploring their associations with clinical phenotypes and quality of life (QOL). MG antibody test in Lebanon is restricted only to two antibodies: acetylcholine receptor (anti-AChR) and muscle-specific kinase (anti-MUSK) antibodies. Results showed that 70.6% of patients were anti-AChR positive and all of them were anti-MUSK negative. Association between MG serological profiles, clinical outcomes and QOL was not significant. Together, current findings suggest that anti-MUSK antibody is not common and difference in antibody profile may not change the clinical phenotypes and QOL of MG Lebanese patients. In the future, it is recommended to check also for autoantibodies other than anti-AChR and anti-MUSK, which may reveal new antibody profiles and possible associations with clinical outcomes.

Keywords myasthenia gravis, anti-AChR, anti-MUSK, Clinical phenotype, QOL

1. Introduction

Myasthenia gravis (MG) is a rare autoimmune disease but is the most common neuromuscular junction disorder. It can be divided into juvenile, early-onset, and late-onset MG. The annual incidence of MG was 10–29 cases per million and the prevalence was 100–350 cases per million (*1*). Autoantibodies targeted against components of the neuromuscular junction disrupt neurotransmission and lead to skeletal muscle weakness at ocular, bulbar, or general levels. The Myasthenia Gravis Foundation of America (MGFA) classified MG into five main classes according to disease severity (*2*). Anti-AChR antibody directed against the postsynaptic nicotinic acetylcholine receptor is the most common type of MG autoantibodies (*3*). It is detected in 85% of patients with generalized/bulbar MG and in 60% of ocular MG patients (*4*). The autoantibodies against muscle-specific kinase (anti-MUSK) and low-density lipoprotein receptor-related protein 4 (LRP4) are found in 6% and 2% of MG cases, respectively. Intriguingly, around 60% of anti-AChR negative cases were anti-MUSK positive. Some patients present autoantibodies against other less common neuromuscular targets such as anti-agrin, anti-collagen Q, anti-cortactin, anti-titin, or anti-ryanodine antibodies (*5*).

Some of the previous studies showed a correlation between the presence of specific autoantibodies and the onset of disease, prognosis, as well as response to treatment but no solid data supports these results (*6,7*). A Chinese study showed that the severity of double positive (anti-AChR and anti-MUSK) cases was between that of AChR-MG and MUSK-MG (*8*). Nagappa *et al.*, showed that anti-AChR positivity and clinical severity were positively correlated, and that good clinical results were associated with anti-MUSK positivity (*9*). Furthermore, the disease severity increased with the presence of anti-Agrin and anti-LRP4 antibodies in double seronegative patients (*7*). AChR antibody titer was also associated with severity (*10*). In the Middle East, few studies only had looked at MG antibodies (*11*).

Lebanese studies about MG were very rare and limited to few case reports. These works had only presented some clinical observations and operational methods to manage MG without investigating

autoantibodies (*12-15*). Therefore, the present study aimed at determining the prevalence of different autoantibodies and their association with clinical phenotypes and quality of life (QOL) in a Lebanese group with MG. It was conducted at the American University of Beirut Medical Center (AUBMC). Seventeen participants were eligible to enter the study. Pediatric patients and those who had received plasmapheresis and/or intravenous immunoglobulin (IVIG) in the preceding four weeks were excluded.

Ethical boards at American University of Beirut (AUB) and Beirut Arab University (BAU) approved the present study that is conformed to the provisions of the Declaration of Helsinki (IRB BAU approval code: 2020-H-0104-H5-R-0375 and AUB approval code: BIO-2020-0011). All informed consent was obtained from the subject(s) and/or guardian(s).

2. Clinical data

Around 70% (n = 12) were males and 30% (n = 5) females. Most of the patients (around 78%, n = 13) had late-onset MG at an age > 50 years. Participants were from all Lebanese districts (South Lebanon, North Lebanon, Bekaa, Baalbeck, Akkar, Mount Lebanon, and Beirut). Two patients were diagnosed in 2021 and the rest between 2018 and 2020.

Only two antibodies (anti-AChR and anti-MUSK) were investigated in our group of MG patients. Tests for other antibodies are not routinely performed in Lebanese hospitals. All participants were anti-MUSK negative and the majority were anti-AChR positive (n = 12). Subsequently, MG patients were classified into two groups: group I (anti-AChR negative; anti-MUSK negative) and group II (anti-AChR positive; anti-MUSK negative). Of these, 35.3 % of participants (n = 6) had ocular MG, 5.9 % (n = 1) had bulbar MG, and 17.7 % (n = 3) belonged to ocular-bulbar MG class. The others were diagnosed with generalized MG 41.2% (n = 7). According to MGFA classification, MG classes of patients were distributed as follows: 35.3% (n = 6) were class I, 17.7 % (n = 3) were class II (33% class II a and 67% class II b). In addition, 23.5% (n = 4) were class III with 50% class III a and 50% class III b, 17.7 % (n = 3) belonged to class IV, with 67% to class IV a and 33% to class IV b and 5.9% (N=1) belonged to class V.

Interestingly, 17.7 % (n = 3) of patients underwent thymectomy, while 82.3 % (n = 14) showed no evidence of thymoma. One patient only had a respiratory crisis after thymectomy. The majority were on Mestinon and azathioprine (around 29.4%, n = 5), 23.5% (n = 4) received IVIG at certain point of time, 29.4% (n = 5) were on Mestinon alone, and 11.8% (n = 2) were on Mestinon and steroids.

For the quality-of-life characteristics, we used the questionnaire (MG-QOL15r) that checks for difficulties in eating, speaking, eye-opening, mood, independence, and ambulation (*16*). A higher score indicates a more severe case of MG. The mean score (MG-QOL15r) of group I patients was 12.8/30 at baseline, 8.2/30, and 7/30 at three and six months; respectively. On the other hand, group II had a mean score (MG-QOL15r) of 13.3/30 at baseline, 8.3/30, and 5.08/30 at 3 and 6 months respectively (Table 1).

Both groups I and II showed similar clinical phenotypes and QOL patterns ($p > 0.05$) (Table 2). This indicates that there was no significant association between difference in these two antibody profiles and clinical phenotype, and QOL.

3. Discussion

Today, autoantibody testing became essential for the diagnosis and management of MG. However, very little research on antibody profiles and their possible association with clinical outcomes was conducted in the Middle East. In Lebanon, this was the first study looking at MG-related antibodies. Based on PubMed search with the combination of the keywords "Myasthenia Gravis" and "Lebanon", less than twenty articles only had been identified between 1991 and 2023. Most of studies were case reports. None had aimed to investigate the MG autoantibodies. They had mainly described clinical observations like a paraneoplastic MG with central nervous system lymphoma (*12*), an association of MG with polyarteritis nodosa (*13*), and a thymoma accompanied by MG, PRCA, and Good's syndrome (*14*). Other discussed management of MG like the radiotherapy for thymoma resection (*15*), the safety of cardiopulmonary bypass

Table 1. Clinical phenotypes of MG Lebanese cohort

Characteristics	Frequency	Percentage (%)
MG category		
Ocular	6	35.3
Bulbar	1	5.9
Ocular-bulbar	3	17.7
Generalized	7	41.2
MGFA Class		
I	6	35.3
II	3	17.7
III	4	23.5
IV	3	17.7
V	1	5.9
Thymus condition		
Thymectomy	3	17.7
Absence of thymoma	14	82.3
Severe respiratory crisis		
Yes	1	5.9
No	16	94.1
Treatment		
Mestinon	5	29.4
Mestinon + Steroid	2	11.8
Azathioprine	1	5.9
Mestinon + Azathioprine	5	29.4
IVIG	4	23.5
Rituximab	0	0

Table 2. Association between antibodies profiles, clinical phenotypes, and QOL.

Characteristics	Group I (AChR negative; MUSK negative)	Group II (AChR positive; MUSK negative)	*p* value
Gender			0.117
Male	2	10	
Female	3	2	
MG Category			0.879
Generalized	1	6	
Ocular	2	4	
Bulbar	1	0	
Ocular-bulbar	1	2	
MGFA Class			1.00
Class I	2	4	
Class II	1	2	
Class III	1	3	
Class IV	1	2	
Class V	0	1	
Thymus status			0.515
Absence of thymoma	5	9	
Thymectomy	0	3	
History of severe respiratory crisis			1.00
No	5	11	
Yes	0	1	
Treatment			1.00
Mestinon	2	3	
Mestinon+Steroids	1	1	
Mestinon+Azathioprine	1	4	
IVIG	1	3	
Azathioprine	0	1	
Response to treatment			0.413
No/Poor	2	2	
Complete Remission	1	1	
Partial remission	2	9	
MG-QOL15 r score (Baseline)			1.00
0 to 10/30	2	5	
11/30 to 20/30	2	5	
21/30 to 30/30	1	2	
MG-QOL15 r score (3 months)			1.00
0 to 10/30	2	6	
11/30 to 20/30	3	6	
21/30 to 30/30	0	0	
MG-QOL15 r score (6 months)			1.00
0 to 10/30	4	8	
11/30 to 20/30	1	4	
21/30 to 30/30	0	0	

Fisher's exact test ($p < 0.05$ is considered statistically significant).

(*17*), new anesthetics conditions and treatments (*18,19*).

In our group, antibody testing had included only anti-AChR and anti-MUSK antibodies. Antibody test of other MG-related autoantibodies such as LRP4, agrin, collagen Q, titin *etc.* is not prescribed in Lebanon. Therefore, Lebanese MG patients can be divided into two groups based on serological findings: anti-AChR negative/anti-MUSK negative and anti-AChR positive/anti-MUSK negative groups. Results showed that most Lebanese patients with MG were anti-AChR positive like the other MG populations worldwide (*20*). However, all patients were anti-MUSK negative. Thus, it becomes of interest to check also for the other MG antibodies that may reveal new antibodies profiles and possible effects. Results showed no significant association between the different serological profiles and gender, MGFA Class, MG category, thymus status, history of respiratory crisis, choice of treatment, and response to treatment. However, three patients who underwent thymectomy belonged to the seropositive group with titers higher than the rest of the patients. Interestingly, patients with MGFA class I and II had a lower titer of anti-AChR antibodies compared to other classes and patients that went into remission showed lower titers of anti-AChR antibodies. Two patients went into total remission after around a year of diagnosis. One of them was seronegative and had remission following resection of a lung adenocarcinoma and the other had a low anti-AChR titer. Importantly, some patients had other diseases prior to MG such as lung cancer and Systemic Lupus Erythematosus. This supports the idea that other diseases may trigger

autoimmune disorder. Results showed no significant association between serological profiles and QOL. However, all participants presented an overall improvement in the QOL at 3 months and 6 months after starting treatment.

Based on this study, we conclude that MG patients in Lebanon should not only be tested for anti-MUSK and anti-AChR antibodies but also for the other types of MG autoantibodies. This is essential in order to set up all antibody profiles and their possible associations with clinical phenotypes and QOL.

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Letter

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Pseudoxanthoma elasticum is associated with cardiocirculatory inefficiency

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SUMMARY Pseudoxanthoma elasticum (PXE) is a rare, genetic, metabolic disease characterized by dystrophic calcification of elastic fibres in the skin, retina and vascular wall. Data on cardiac involvement are inconsistent. Hence, we aimed to evaluate cardiorespiratory response to incremental cardiopulmonary exercise testing (CPET) in PXE. A total of 30 PXE patients (54.0 ± 11.2 years, 40.0% male) and 15 matched controls underwent symptom-limited incremental CPET. PXE patients presented an impaired peak work rate as compared to controls (84.2 ± 16.0% *vs*. 94.7 ± 10.4%, $p = 0.03$) that was accompanied by a lower peak oxygen uptake (in % predicted and mL/min/kg), reduced increments in oxygen uptake per increments of work rate ($\Delta V'O_2/\Delta WR$, 8.4 ± 3.0 mL/min/W *vs*. 11.3 ± 4.9 mL/min/W, $p = 0.02$), lower peak oxygen pulse (78.0 ± 12.3% *vs*. 90.6 ± 19.6%, $p = 0.01$) and reduced minute ventilation at peak exercise ($V'E$, 66.2 ± 16.8% *vs*. 82.9 ± 25.2%, $p = 0.02$). To summarize, we presently observed impairment in mainly cardiocirculatory parameters, whilst no substantial ventilatory limitation was detected. The potential implications of this finding for PXE management warrant further study.

Keywords pseudoxanthoma elasticum, cardiopulmonary exercise testing, cardiocirculatory limitation

Pseudoxanthoma elasticum (PXE) is a rare, multisystem disorder with autosomal recessive inheritance in which ectopic calcification affects elastin-rich connective tissues such as the skin, eyes and cardiovascular system (*1,2*). First clinical manifestations of PXE occur in childhood to adolescence and comprise diverse cutaneous lesions. Ophthalmological changes become apparent in the third to fourth decade of life, affect the ocular fundus, predispose to choroidal neovascularization and entail the risk of blindness as early as in the fifth to sixth decade (*3*). Cardiovascular manifestations occur years after the onset of dermal and retinal lesions. They result from an extensive calcification of both the medial and intimal layer of small- and medium-sized arteries. Vascular symptoms primarily encompass intermittent claudication in consequence of peripheral vascular compromise. However, arterial narrowing has casuistically also been described to affect the coronary vascular bed (*4*). Moreover, autoptic studies have identified degenerated elastic fibres with calcification in the subendocardium, suggesting cardiac involvement in PXE (*1,5*). In keeping with this, the aim of the present study was to evaluate cardiorespiratory response to incremental cardiopulmonary exercise testing (CPET) in PXE patients and to compare the results with those obtained in matched healthy controls.

Between January and May 2018, 30 consecutive patients with PXE were included in this case-control study. PXE diagnosis relied on the revised diagnostic criteria by Plomp *et al*. (*6*). Exclusion criteria for study participation comprised preexisting cardiac disorders or symptomatology. 15 age-, gender- and body mass index-matched healthy controls were recruited from the general population by screening invitation. All study participants gave written informed consent for partaking in the study. The study was approved by the medical ethics committee of the University Hospital Bonn, Germany (No. 349/17) and complied with the Declaration of Helsinki. All subjects underwent symptom-limited incremental CPET (Cardiovit AT-104, Schiller, Feldkirchen, Germany) using a cycle ergometer in semi-supine position. The same step protocol was applied for patients and controls. It provided baseline measurements before exercise for 2 min, followed by an initial workload of 10–30 W which was increased by 10–20 W every 1–2 min. Participants were encouraged to exercise until exhaustion. Prior to CPET, detailed pulmonary function testing was performed. Statistical analyses were conducted using

Table 1. Demographics, PFT- and CPET-derived measurements in PXE patients and controls

Variables	PXE patients (n = 30)	Controls (n = 15)	p value
Demographics			
Age [years]	54.0 ± 11.2	56.6 ± 10.4	0.46
Male sex	12 (40.0%)	7 (46.7%)	0.67
BMI [kg/m²]	27.1 ± 4.2	24.7 ± 4.4	0.08
Pulmonary function testing			
TLC [L]	6.3 ± 1.3	7.1 ± 0.9	**0.04**
TLC [% predicted]	106.2 ± 15.7	112.5 ± 17.2	0.23
FVC [L]	3.8 ± 0.9	3.8 ± 0.9	0.90
FVC [% predicted]	101.7 ± 14.1	93.2 ± 14.5	0.07
FEV_1 [L]	3.2 ± 0.7	3.2 ± 0.7	0.76
FEV_1 [% predicted]	102.7 ± 14.2	95.4 ± 10.8	0.09
FEV_1/VC [%]	87.8 ± 8.7	83.8 ± 4.9	0.11
DL_{CO} [% predicted]	73.2 ± 13.5	80.3 ± 7.7	0.07
DL_{CO}/VA [% predicted]	81.0 ± 14.6	85.6 ± 12.6	0.31
CPET			
Work rate peak [W]	125.5 ± 40.1	141.1 ± 37.8	0.22
Work rate peak [% predicted]	84.2 ± 16.0	94.7 ± 10.4	**0.03**
RER peak	1.11 ± 0.11	1.13 ± 0.10	0.45
Heart rate peak [per min]	133.9 ± 16.1	143.3 ± 17.0	0.08
Heart rate peak [% predicted]	89.7 ± 10.3	100.2 ± 9.7	**0.003**
O_2 pulse peak [mL/beat]	10.7 ± 3.6	12.0 ± 2.1	0.22
O_2 pulse peak [% predicted]	78.0 ± 12.3	90.6 ± 19.6	**0.01**
$\dot{V}O_2$ peak [L/min]	1.41 ± 0.44	1.66 ± 0.39	0.08
$\dot{V}O_2$ peak [% predicted]	75.3 ± 11.6	89.5 ± 16.4	**0.002**
$\dot{V}O_2$ peak/kg [mL/min/kg]	18.1 ± 4.7	21.9 ± 3.7	**0.009**
$\dot{V}E$ peak [L/min]	41.7 ± 12.2	49.7 ± 13.1	0.05
$\dot{V}E$ peak [% predicted]	66.2 ± 16.8	82.9 ± 25.2	**0.01**
$\dot{V}E/\dot{V}CO_2$ (AT)	22.9 ± 4.4	22.0 ± 4.8	0.54
$\Delta\dot{V}O_2/\Delta$WR [mL/min/W]	8.4 ± 3.0	11.3 ± 4.9	**0.02**
Breathing reserve [%]	45.4 ± 15.9	43.7 ± 15.1	0.74
$PETO_2$ peak [mmHg]	105.4 ± 6.8	105.5 ± 5.2	0.96
$PETCO_2$ peak [mmHg]	42.2 ± 4.9	43.1 ± 4.3	0.55

Data are presented as n (%) or mean ± standard deviation. P values are significant at < 0.05. Statistically significant differences are given in bold. *Abbreviations*: BMI: body mass index; CPET: cardiopulmonary exercise testing; DL_{CO}: diffusion capacity of the lung for carbon monoxide; $\Delta\dot{V}O_2/\Delta$WR: increments in oxygen uptake per increments in work rate; FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; $PETO_2$: end-tidal oxygen tension; $PETCO_2$: end-tidal carbon dioxide tension; PFT: pulmonary function testing; PXE: pseudoxanthoma elasticum; RER: respiratory exchange ratio; RV: residual volume; TLC: total lung capacity; VA: alveolar volume; $\dot{V}E$: minute ventilation; $\dot{V}E/\dot{V}CO_2$ (AT): minute ventilation/carbon dioxide production (ventilatory equivalent for carbon dioxide) at anaerobic threshold; $\dot{V}O_2$: oxygen uptake.

SPSS statistics version 26.0 (IBM, Armonk, NY, USA). Continuous variables were evaluated by use of unpaired *t*-test, categorical parameters by Pearson's Chi-squared test. Statistical significance was assumed when the null hypothesis could be rejected at $p < 0.05$.

Demographic and clinical data are displayed in Table 1. Overall, PXE patients were middle-aged (54.0 ± 11.2 years) with slight female predominance (n = 18/30, 60.0%). Pre-CPET, detailed pulmonary function testing revealed no substantial intergroup differences in measurements, except for absolute lung capacity that was significantly lower in the patient group than amongst controls (6.3 ± 1.3L *vs*. 7.1 ± 0.9L, $p = 0.04$). In terms of CPET results, PXE patients presented an impaired peak work rate as compared to controls (84.2 ± 16.0% *vs*. 94.7 ± 10.4%, $p = 0.03$) that was accompanied by a lower peak oxygen uptake (in % predicted and mL/min/kg), reduced increments in oxygen uptake per increments of work rate ($\Delta\dot{V}O_2/\Delta$WR, 8.4 ± 3.0 mL/min/W *vs*. 11.3 ± 4.9 mL/min/W, $p = 0.02$), lower peak oxygen pulse (78.0 ± 12.3% *vs*. 90.6 ± 19.6%, $p = 0.01$) and reduced minute ventilation at peak exercise ($\dot{V}E$, 66.2 ± 16.8% *vs*. 82.9 ± 25.2%, $p = 0.02$). By contrast, end-tidal oxygen and carbon dioxide tensions did not differ between groups.

This is the first study to investigate functional capacity by CPET in PXE patients. Of note, we presently observed impairment in mainly cardiocirculatory parameters, whilst no substantial ventilatory limitation was detected. Maximum respiratory exchange ratio (RER) slightly exceeded 1.10, implying excellent exercise effort in both study groups. The vast majority of PXE patients (n = 23/30, 76.7%) exhibited exhaustion as exercise limiting factor, only 1 patient aborted CPET due to claudication in lower limbs. By now, available evidence on cardiac involvement in PXE is inconsistent. Whilst Prunier *et al*. reported no elevated number of cardiac complications in PXE (*7*), other trials have reiteratedly observed diastolic dysfunction in PXE patients (*8,9*). Cardiovascular disease, foremost accelerated atherosclerosis, at an early age despite the absence of established risk factors has been described by Lebwohl and colleagues (*10*). Our findings support

the assumption that cardiac dysfunction seems to be truly related to PXE and not just coincidental. Potential underlying pathophysiological mechanisms that have previously been proposed comprise subendocardial deposition of altered elastic fibres affecting ventricular relaxation (*5*), silent myocardial ischaemia derived from premature atherosclerosis and compromised myocardial oxygen supply by disturbed myocardial microcirculation.

Study limitations arise from the relatively small number of patients studied inherent to the rarity of the disease. Additional cardiac imaging would have been a valuable adjunct to further define CPET observations.

To summarize, our data suggest that PXE is accompanied by cardiocirculatory inefficiency. The potential implications of this finding for PXE management warrant further study.

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Letter

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End-stage renal disease due to retroperitoneal fibrosis in neurofibromatosis type I

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SUMMARY Retroperitoneal fibrosis (RF) commonly leads to renal impairment due to compression of ureters, and around 8% of patients eventually progress to end-stage renal disease (ESRD). We present a case of RF in a 61-year-old female patient with neurofibromatosis type 1 (NF1) who developed ESRD. She presented with a postrenal acute kidney injury, being initially treated with an ureteral catheter. A magnetic resonance imaging of the abdomen showed parietal thickening of the right ureter, and she underwent right ureter reimplantation through bladder flap and psoas hitch. There was an extensive area of fibrosis and inflammation over the right ureter. Biopsy disclosed nonspecific fibrosis, which was consistent with RF. Although the procedure was successful, she developed ESRD. We review atypical presentations of RF and causes of renal injury in NF1. RF should be considered a possible cause of chronic kidney disease in patients with NF1, perhaps due to an unknown underlying mechanism.

Keywords end-stage renal disease, neurofibromatosis type 1, retroperitoneal fibrosis.

Neurofibromatosis type 1 (NF1), also known as von Recklinghausen's disease, is an autosomal dominant disease that affects approximately 1 in 3,000 individuals worldwide. It is characterized by multiple benign peripheral nerve sheath tumors, as well as other nervous system neoplasms. Other common manifestations include multiple flat, light-brown patches of skin pigment (café-au-lait spots), skinfold freckling, visible subcutaneous neurofibromas, and small nodules of the iris (Lisch nodules) (*1*).

Retroperitoneal fibrosis (RF) is a rare disease which causes fibrosis and inflammation in the retroperitoneum, most commonly affecting the abdominal aorta. The ureters are commonly affected by RF as well, leading to postrenal acute kidney injury (AKI) and chronic kidney disease (CKD) (*2*). We present a case of a female NF1 patient with a single kidney who developed end-stage renal disease (ESRD) due to RF. As of April 2023, a literature search on PubMed using the terms "retroperitoneal fibrosis" and "neurofibromatosis" disclosed three results, with none of them describing a case of RF in a person with NF1. This is the first time this association is being reported.

On october 2020, a 61-year old female patient presented to the emergency department with nausea, vomiting, asthenia, decreased appetite, and a 3-month history of recurrent urinary tract infections. She denied fever, dysuria, oliguria, or hematuria. She had a previous history of smoking, weight loss of 6 kg in one week, hypertension, NF1, and a left radical nephrectomy in 2007 because of a renal hematoma from a biopsy-proven chronic pyelonephritis. She was on olmesartan and hydrochlorothiazide.

Laboratory studies revealed leukocytosis, hyperkalemia, uremia, and elevated creatinine levels (6.85 mg/dL). Physical examination was unremarkable, except for multiple neurofibromas and café-au-lait spots in her trunk. Urinalysis revealed microscopic hematuria and proteinuria. A computed tomography scan of the abdomen disclosed right hydronephrosis with proximal dilation of the ureter. There were, however, no signs of obstruction. A diagnosis of AKI was made. She was transferred to the Urology service, with ureteroscopy revealing mild stenosis of the median ureter. An ureteral catheter was placed and treatment with ceftriaxone was started.

On follow-up six-months later, she still had dysuria and suprapubic pain. A magnetic resonance imaging (MRI) of the abdomen disclosed a diffuse parietal thickening of the middle ureter up to the ureterovesical junction (Figure 1). Neurofibroma of the ureter, retroperitoneal fibrosis, and desmoid type fibromatosis

were suspected. An urinary tract X-ray showed correct positioning of the ureteral stent. She then underwent right ureter reimplantation through bladder flap and psoas hitch. There was an intense area of fibrosis and inflammation over the ureteral crossing of the iliac vessels. Biopsy of the ureter revealed nonspecific fibrosis and no signs of malignancy, excluding neurofibroma of the ureter due to a negative S100 staining and desmoid fibromatosis due to negative beta-catenin staining (Figure 2). A diagnosis of RF was made by exclusion. IgG4 titers could not be measured.

A follow-up renal ultrasound (US) in December 2021 showed a normal renal parenchyma. Due to her continuously rising creatinine levels, she was referred to the Nephrology service, ultimately being diagnosed with ESRD, with a glomerular filtration rate (GFR) of 11.9 mL/min/1.73 m² at the first consult. ESRD was attributed to a probable long-standing RF, which was diagnosed after an AKI. In August 2022, with a GFR of 11.15 mL/min/1.73 m², another follow-up renal US revealed hydronephrosis. As of her last follow-up visit, on January 2023, she has not started kidney replacement therapy and has no urgent indications for it, since her GFR has remained stable. Her creatinine levels and timeline are summarized in Figure S1 (*http://www.irdrjournal.com/action/getSupplementalData.php?ID=147*). Written informed consent was obtained from the patient prior to publication.

This is the first described case of NF1 with concurrent RF. Renal function impairment in NF1 is usually related

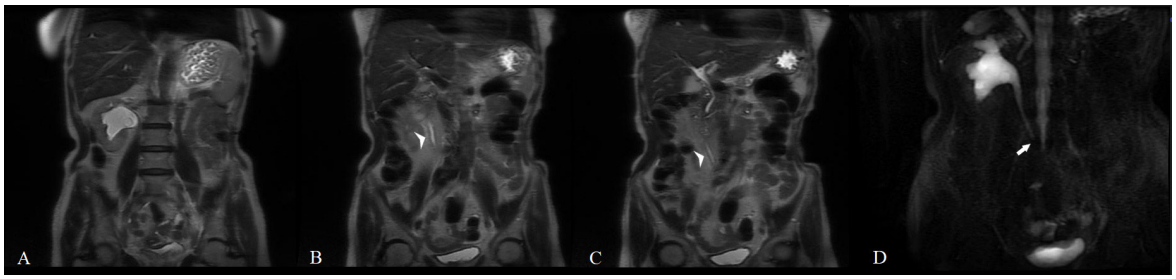

Figure 1. T2-weighted abdominal MRI scan showing right hydronephrosis (A) and progressive thickening of the right ureter (B,C) (arrowheads). Magnetic resonance urography showing the classic triad of retroperitoneal fibrosis: medial deviation of the ureter, tapering of the ureteral lumen (arrow), and hydronephrosis (D).

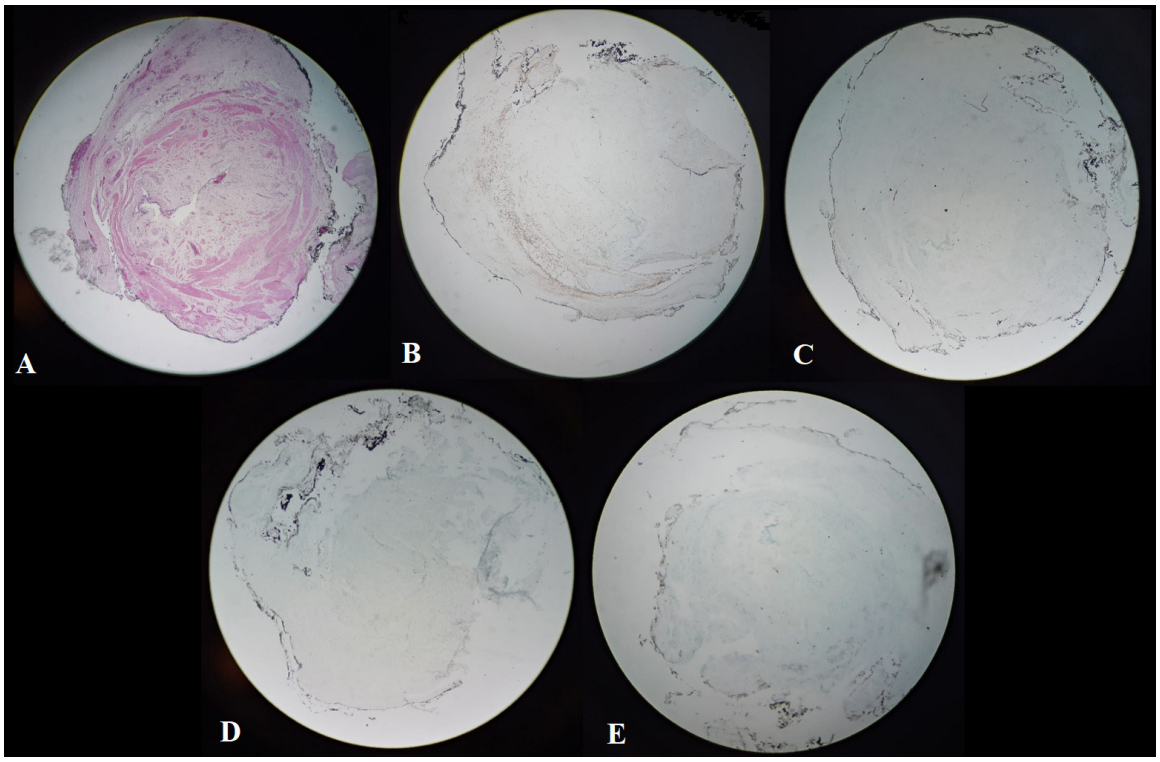

Figure 2. Ureter biopsy showing fibrous thickening of the lamina propria and serosal fibrosis (A). Immunohistochemistry panel revealed a slightly positive CD34 staining (B) and negativity for S100 (C), beta-catenin (D), and collagen type IV (E).

to vessel disease, such as renal artery stenosis, which is the most common cause of death after malignancies in NF1 (*3*). RF most commonly presents as a fibrous retroperitoneal plaque surrounding the abdominal aorta and some of its branches, the inferior vena cava, and the ureters. However, atypical locations have been described, such as purely periureteral (as in our case), around the renal hilum, periduodenal, and peripancreatic. In these cases, a biopsy is warranted for diagnostic confirmation. There were no signs of chronic inflammation in our patient biopsy, a hallmark of RF (*4*). Other cases of RF without evidence of chronic inflammation have been published (*5-7*).

Hydroureteronephrosis is the most common complication of RF, affecting 60-70% of patients, which occurs due to the extrinsic compression of the ureters (*8*). Bilateral disease may be found in 40% of cases (*4*), and a similar proportion may suffer from unilateral disease. The latter may be asymptomatic for years but may lead to kidney atrophy, a finding which is present in up to 30% of patients at time of diagnosis (*8*). Accordingly, around 40% of cases develop CKD and 8% progress to end-stage renal disease (*9,10*).

We presented for the first time a case of RF with atypical location and histopathological findings in a patient with NF1 who developed ESRD. Although rare, RF should be considered a differential diagnosis when evaluating an NF1 patient with CKD. Finally, further studies are needed to elucidate whether NF1 predisposes to RF.

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