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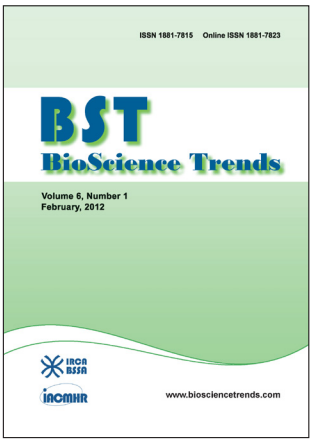

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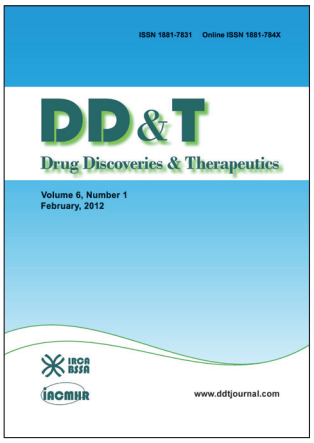

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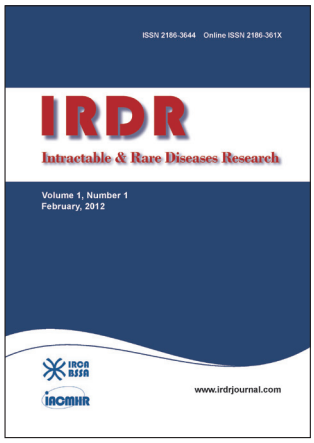

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Review

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Acute intermittent porphyria: focus on possible mechanisms of acute and chronic manifestations

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SUMMARY Porphyrias are a group of inherited metabolic diseases that include eight types, each of which is caused by a mutation that affects an enzyme of the heme biosynthetic pathway. When an enzyme defect has physiological significance, it leads to overproduction of pathway precursors prior to the defective step. The partial absence of the third enzyme in the heme biosynthetic pathway, porphobilinogen deaminase (PBGD) also known as hydroxymethylbilane synthase (HMBS), results in acute intermittent porphyria (AIP), which affects mainly women. Subjects who had AIP symptoms were deemed to have manifest AIP (MAIP). Clinical manifestations are usually diverse and non-specific. Acute AIP episodes may present with abdominal pain, nausea, and vomiting, and repeated episodes may result in a series of chronic injuries. Therefore, studying the mechanisms of acute and chronic manifestations of AIP is of great significance. This review aims to summarize the possible mechanisms of acute and chronic manifestations in patients with AIP.

Keywords acute intermittent porphyria, mechanisms, aminolevulinic acid (ALA), attack

1. Introduction

Porphyria is a rare metabolic disease caused by abnormal enzyme activity in the heme synthesis pathway (*1,2*). Acute intermittent porphyria (AIP) is due to a partial deficiency in porphobilinogen deaminase (PBGD), also known as hydroxymethylbilane synthase (HMBS) (*3,4*), which is autosomal dominant and has low clinical penetrance (*5-7*). A PBGD enzyme deficiency leads to the significant accumulation of certain porphyrin precursors, such as aminolevulinic acid (ALA) and porphobilinogen (PBG). Women are more susceptible (*8*). Most patients develop symptoms after puberty (*6*).

AIP has varied clinical manifestations, and its attacks can be induced by a number of factors, such as drugs, hormones, starving, infection, psychological stress, and other unknown factors (*9*). Porphyrin precursors may affect the automatic, central, and peripheral nervous systems. In addition, porphyrin precursor deposition in the liver, kidneys, and other organs can cause damage to these organs and cause metabolic disturbances.

2. Neurologic symptoms

Some patients with AIP may have neurological manifestations. The automatic, central, and peripheral nervous systems can all be involved (*6,10-12*).

2.1. Automatic nervous system symptoms

Most automatic nervous system symptoms are non-specific, including abdominal pain, vomiting, *etc*. (*13*).

More than 80% of patients have abdominal pain, which is intermittent and involves cramping (*6,8*). Abdominal pain usually lasts hours to days and does not respond well to narcotic pain medications (*14*). In addition, the degree of abdominal pain is often disproportionate to the physical examination findings (*6,15*). Evacuation disorders may also be seen, including constipation, abdominal distention, and intestinal obstruction. Cardiovascular symptoms such as arrhythmia and hypertension may also be observed in some patients. The condition can also manifest as bladder dysfunction, such as urinary retention, urinary incontinence, and dysuria (*6*).

There is a hypothesis that increased serotonergic activity is associated with autonomic neuropathy. Tryptophan dioxygenase (TDO) is a rate-limiting enzyme that catalyzes the degradation of tryptophan. Heme deficiency in the liver will lead to decreased TDO activity, which will cause an increase in tryptophan. Tryptophan is activated by tryptophan hydroxylase to produce serotonin (5-HT), so if tryptophan levels increase, the levels of 5-HT will also increase to some

extent. The following related studies further confirm this hypothesis. Puy *et al*. reported increased blood tryptophan and 5-HT levels, and the concentration of 5-hydroxy indoleacetic acid (5-HIAA) in urine also increased in patients with AIP. Injection of heme can correct these changes. The results of these studies add to the credibility of the hypothesis. 5-HT receptors are distributed in many tissues, such as the digestive tract, urinary bladder, adrenal glands, heart, and blood vessels. Several clinical manifestations of acute porphyrias, and autonomic neuropathy in particular, are similar to the effects of increased 5-HT activity (*16*).

2.2. Central nervous system damage

There are many clinical features associated with CNS damage, such as seizures, coma, syndrome of inappropriate antidiuretic hormone (SIADH), and porphyria-induced posterior reversible encephalopathy syndrome (PRES). The following will discuss possible mechanisms of three specific clinical manifestations.

2.2.1. Epileptic seizures

Seizures have been reported in patients with AIP suffering an acute attack (*17-22*). Types of seizures include myoclonic jerks, tonic-clonic seizures, partial seizures followed by secondarily generalized seizures, and generalized seizures (*23-26*). The actual mechanisms are poorly understood, though the following are the major hypotheses for those mechanisms. First, hyponatremia may be a pathogenic factor (*6,14,27*). Corroborating the hypothesis is the fact that seizures have been reported to be associated with hyponatremia in some cases (*23,28-30*). Second, the pathogenesis of seizures may be related to ALA and PBG (*12*). One study has indicated that injections of ALA and PBG into the brain of mice, respectively, cause seizures (*31*). In addition, ALA is known to be toxic to neuronal and glial cells in culture (*32*). Third, sympathetic excitation results in increased production of catecholamine, which can cause vasoconstriction (*6*). Constriction of blood vessels in certain parts of the CNS may lead to seizures. Fourth, the loss of normal gamma-aminobutyric acid (GABA) function is thought to cause epilepsy. GABA is thought to suppress the CNS. ALA is structurally similar to GABA. One study has shown that ALA can inhibit the release of K^+-stimulated GABA from preloaded synaptosomes (*33*). Thus, ALA accumulation is assumed to impair normal GABA function, causing damage to the CNS (*34*). Finally, PRES may be a possible cause of seizures in patients with AIP because some patients with PRES present with seizures (*12,35-38*).

2.2.2. SIADH

An important characteristic of SIADH is hyponatremia, and SIADH can manifest as varying degrees of loss of appetite, nausea, vomiting, convulsions, and coma. There is a theory that excess PBG and ALA might cause SIADH through neurotoxigenic mechanisms (*6*). Suarez *et al*. reported such a case, and a histopathological examination of that patient revealed a marked decrease in the number of hypothalamic cells. Those results indicate that hypothalamic-hypophyseal tracts may be damaged, leading to SIADH (*39*).

2.2.3. PRES

PRES can present as headaches, nausea, seizures, visual disturbances, *etc*. (*12,21,40,41*). Recently Jaramillo-Calle *et al*. reviewed previous cases to describe several features of PRES and discuss possible pathogenesis. Neuroimaging often reveals bilateral vasogenic edema characterized by asymmetry and major involvement of the parieto-occipital regions, and these imaging changes usually disappear during reexamination (*10*).

Endothelial dysfunction is considered to be a major pathogenic process. Many factors contribute to endothelial dysfunction. First, if the blood pressure rises significantly beyond the upper limit of autoregulation (mean arterial pressure ~150 mmHg), endothelial damage may occur. Second, a theory contends that toxic damage can cause endothelial dysfunction. This theory may explain the pathogenesis of PRES in patients with normal blood pressure and patients with hypertension within the range of autoregulation. Finally, a primary inflammatory injury might lead to endothelial dysfunction. One study showed that the concentrations of several proinflammatory cytokines and vascular endothelial growth factor (VEGF) were higher in symptomatic patients with AIP than control groups (*10*). This result further validates the hypothesis.

2.3. Peripheral neuropathy

Peripheral neuropathy is a common neurological manifestation of AIP (*42*). Patients may present with pain, muscle weakness, paresis, sensory neuropathy, *etc*. (*23,43-46*). Pain may involve the limbs, chest, back, *etc*. (*6*). Paresis usually begins at the proximal end of the upper limb (*46,47*). Sensory neuropathy may include paresthesia, hypoesthesia, numbness, and neuropathic pain (*5,47*). If bulbar paralysis is present with cranial nerve involvement, the symptoms are usually severe, including dysphagia, dysarthria, and dysphonia. Severe cases may require intubation and mechanical ventilation (*6*). Paralysis usually can be completely reversed with proper treatment and months of recovery (*5*).

Electrophysiological findings in patients who have acute porphyric neuropathy reveal primary axonal motor neuropathy (*26,48-50*). An autopsy study of patients with AIP conducted by Cavanagh and Mellick

found that axonal motor fibers were affected more significantly than sensory fibers (*34,51*).

Fast axonal transport depends on energy. Therefore, a reduction in heme production may disrupt this process and result in axonal degeneration (*52,53*). Heme is required for aerobic metabolism and the production of adenosine triphosphate (ATP) (*52,54,55*). Therefore, a heme deficiency may lead to axonal damage, causing peripheral neuropathy.

3. Psychiatric symptoms

Some patients with AIP may also experience a variety of psychiatric symptoms, such as depression, anxiety, and insomnia (*56-61*). Severe cases can even manifest as schizophrenia and hallucinations (*25,62-64*). Psychiatric symptoms may occur during an acute episode or as a chronic complication.

Several studies have been conducted to observe the incidence of psychiatric symptoms in patients with AIP (*64-67*). Other studies were done to screen psychiatric patients for acute intermittent porphyria (*68,69*). First, genetics may play a role. One study showed that patients with AIP and their first-degree relatives were at varying degrees of increased risk of being diagnosed with schizophrenia and bipolar disorder. The results suggest that there may be a genetic link between AIP and psychiatric symptoms (*64*). Another study examined the association between the PBGD genetic variation and schizophrenia, and it found that the PBGD *Msp*I 2.2-kb allele is significantly associated with schizophrenia. However, subsequent studies failed to replicate this finding (*70,71*). Second, the activity of the disease may be associated with psychiatric symptoms. Two studies have shown that psychiatric symptoms are more common in patients with manifest AIP (MAIP) (*65,66*). Finally, rare homozygous mutations may cause depression-like mental behavior. Most HMBS mutations are known to be heterozygous mutations. Berger *et al*. conducted an animal study which found that severe HMBS deficiency led to depression-like behavior in a mouse model of homozygous dominant AIP (*72*).

4. Hepatic impairment

An association between AIP and hepatocellular carcinoma (HCC) was reported for the first time in 1984 (*73*). Since then, several studies have estimated the relative risk (RR) or standardized incidence ratio (SIR) for HCC in patients with AIP. In a Finnish study, the RR was 61 (*74*). A French study reported an SIR of 36 (*75*). A Swedish study reported an SIR of 64 in 2011 and 86 in 2013 (*76,77*). The risk of HCC in patients with AIP may be underestimated because better diagnostic methods have been developed in recent years, some patients could not be traced, or for other reasons (*73,74,78*). In conclusion, the results of different studies have varied greatly, but these results still indicate that the risk of developing HCC may be greater in patients with AIP.

The exact mechanism by which HCC develops in patients with AIP is not completely known. The following are several possible risk factors.

4.1. Cirrhosis may be a possible risk factor

Some patients with AIP have both HCC and liver cirrhosis (*73,75,77-79*). In some samples, the number of patients with non-neoplastic parenchyma was too small to adequately assess possible liver cirrhosis. Thus, the number of patients with known liver cirrhosis may be underestimated (*74*). These findings suggest that cirrhosis may play a significant role in the development of HCC in patients with AIP.

4.2. Elderly patients with AIP may have an increased risk of developing HCC

The mean age at diagnosis of HCC was over 60 in numerous studies (*1,73,76,77,79,80*). However, a French study reported that 50 years was the mean age at diagnosis. Two patients with hepatitis were included in the French study, which may account for the slightly younger age at diagnosis of HCC, because hepatitis may contribute to the development of HCC to some extent (*75,76*). These results suggest that HCC develops more often in patients over 60 years of age, which may be related to the physical condition of the elderly patients and the course of AIP.

4.3. The frequency of AIP attacks may affect the development of HCC

In several previous studies, the ratio of the number of patients with HCC who had a history of AIP symptoms to the total number of patients with AIP and HCC was 6/9, 16/22, 3/5, or 3/23 (*75-78*). Although the ratios vary, HCC seems to be more common in symptomatic patients with AIP.

4.4. An HMBS enzyme deficiency may diminish the inhibition of tumor growth

One study excluded several key genes associated with HCC, including KRAS, TP53, BRAF, and CTNNB1 (*80*). However, the study found a somatic mutation in the HMBS gene, L220X, which was present only in cancer tissues, and the study did not find that somatic mutation in the liver tissues of ten patients with non-porphyria-related HCC. These results strongly suggest that the HMBS gene defect may play a role in tumor growth in the AIP patient studied. Although it is only a single study, the hypothesis it generated cannot be ignored.

4.5. ALA may be carcinogenic

Since ALA is the main cause of AIP episodes, numerous studies have hypothesized that ALA itself may be carcinogenic (*78,81,82*). Further research is needed to explore the carcinogenicity of ALA and the mechanism by which ALA induces HCC.

4.6. Decreased heme production may influence the development of HCC

Recent studies have shown that hemin has obvious antigenotoxic and anti-inflammatory effects (*83-86*). Unfortunately, these studies used non-HCC cells, so there is no conclusive evidence that heme inhibits the development of HCC.

In summary, patients with AIP are at risk of developing HCC, so patients with AIP over 50 years of age can be screened for HCC annually to detect HCC early (*87-91*).

5. Renal impairment

Renal impairment is more common in AIP, and particularly in patients with frequent porphyric attacks (*92*). Kidney damage in patients with AIP is mainly reflected in laboratory results, such as elevated creatinine and urea nitrogen (*93,94*).

Pallet *et al*. conducted a follow-up study from 2003 to 2013, and their experimental data showed that more than 50% of patients with AIP are diagnosed with chronic kidney disease (CKD), compared with about 10% of asymptomatic patients (*95*). In addition, the kidney function of asymptomatic patients is analogous to that of the general population, indicating that repeated attacks can exacerbate kidney damage. Multiple regression analysis revealed that AIP may independently induce estimated glomerular filtration (eGFR) damage. Histological findings from renal biopsies of patients with AIP revealed varying degrees of glomerulosclerotic and interstitial changes (*95,96*).

Current research has yet to reveal the exact mechanism underlying renal impairment.

5.1. Porphyrin precursors may have a negative effect on the kidney

5.1.1. Porphyrin precursors promote tubular injury

In a previous study, human renal epithelial cells (HRECs) were cultured using ALA and PBG, and results showed that ALA and PBG can cause cell death and endoplasmic reticulum stress. DNA damage is thought to induce apoptotic cell death. Consistent with this finding, a marker of tubular damage, urinary neutrophil gelatinase-associated lipocalin (NGAL), was found to be at higher levels in patients with AIP during attacks than levels in asymptomatic carriers (*95*). In one study, nine patients with AIP in clinical remission underwent an isotopic renography, which revealed tubular damage (*97*). These findings may explain the interstitial changes in renal biopsy results from patients with AIP (*95,96*).

5.1.2. Porphyrin precursors promote severe arteriolar lesions

ALA is an effective vasoconstrictor that can promote injury in target organs. Studies have shown that the presence of excessive ALA may cause vasospastic renal vascular lesions, leading to chronic vasculopathy with a narrowed lumen and tissue ischemia that contribute to CKD (*92,98,99*). Other cardiac and ophthalmic symptoms were reported during the onset of AIP, suggesting arterial spasms (*100,101*). These findings may correspond to glomerular sclerosis as revealed by renal biopsies (*95,96*).

5.1.3. Porphyria-related nephropathy is due to genetic variations in renal ALA transporters

The reabsorption of ALA occurs in the S2 and S3 segments of the proximal tubule, and the transporter of its reabsorption is the human peptide transporter 2 (PEPT2). Tchernitchko *et al*. conducted an observational study to explore the relationship between PEPT2 genotypes and porphyria-associated kidney disease (PAKD). Notably, immunohistochemistry revealed PEPT2 expression in the renal proximal tubules of both patients with porphyria-associated kidney disease (PAKD) and individuals without PAKD. According to haplotype analysis, PEPT2*1 and PEPT2*2 are the two main variants of PEPT2. The Km values for different PEPT2 genotypes indicated that the PEPT2*1 genotype features a stronger ability to bind to the substrate than the PEPT2*2 genotype. Experimental data revealed that patients with PEPT2*2 had less severe PAKD and slower progression. In addition, PEPT2*1/*1 independently predicts PAKD severity, and the PEPT2 genotype is an independent risk factor for eGFR degradation over time (*102*).

Although the aforementioned study described the effect of different genotypes of PEPT2 on the kidneys, there is no histological evidence of increased ALA in the kidney. This finding leads to the question of whether the ALA level in the kidney can be measured using immunohistochemistry or other methods.

In contrast to previous studies, a study by Unzu *et al*. found no significant renal dysfunction or histologic changes other than a few small inflammatory infiltrates. The model used in that study was an HMBS-deficient mouse, and the accumulation of porphyrin precursors was induced via a phenobarbital challenge over 14 weeks to produce acute AIP episodes (*92*). A number of factors can account for the experiment's results. First,

only if 30% of the HMBS activity was retained could the mice survive. Second, the duration of the experiment (14 weeks) may not have been long enough for kidney damage to occur. Finally, germ-line differences in mice may have affected the results (*95*).

5.2. Hypertension

High blood pressure (HHP) occurs frequently in patients with AIP, and especially in patients with recurrent attacks (*96,99*). A population-based study on the clinical aspects of AIP in northern Sweden involving 356 gene carriers suggested that hypertension was significantly associated with MAIP, adjusted for age and sex (*66*). First, excessive porphyrin metabolites lead to cytotoxic or vasospastic renal vascular lesions, which may lead to HHP (*96*). Second, sympathetic excitation may occur during an acute episode, leading to increased release of catecholamines (*96,99*). Other factors, like vasopressin and angiotensin, may also play a significant role (*96*).

Chronic hypertension can lead to thickening of the vessel wall and narrowing of the lumen, which can trigger damage to the kidneys and aggravate renal arteriolosclerosis. These findings may explain the glomerular sclerosis observed in kidney biopsies (*95,96*).

5.3. Repeated hemin therapy

Renal damage may be caused by repeated hemin therapy. A case of renal injury following heme infusion has been reported (*103*). Whether the renal injury was caused by heme infusion is not known, but this factor

Table 1. Summary of possible mechanisms of acute and chronic manifestations

Acute and chronic manifestations	Symptoms	Possible mechanisms
Neurologic symptoms	*Automatic nervous system symptoms*: abdominal pain, vomiting, constipation, intestinal obstruction, arrhythmias, hypertension, *etc*.	The effects of increased 5-HT activity are similar to some symptoms of autonomic neuropathy
	Central nervous system damage: *Epileptic seizures*: myoclonic jerks, tonic-clonic seizures, partial seizures followed by secondarily generalized seizures, generalized seizures, *etc*.	1. Hyponatremia 2. ALA is toxic to neuronal and glial cells 3. Increased production of catecholamine can cause vasoconstriction 4. CNS suppression induced by GABA is diminished 5. PRES itself can trigger seizures
	SIADH: loss of appetite, nausea, vomiting, convulsions, coma, *etc*.	Excess PBG and ALA cause damage to hypothalamic-hypophyseal tracts
	PRES: headaches, nausea, seizures, visual disturbances, *etc*.	Endothelial dysfunction caused by HHP, toxic damage, and a primary inflammatory injury is a major pathogenic process
	Peripheral neuropathy: pain, muscle weakness, paresis, sensory neuropathy, *etc*.	A reduction in heme production disrupts fast axonal transport
Psychiatric symptoms	Depression, anxiety, insomnia, schizophrenia, hallucinations, *etc*.	1. Genetic factors 2. AIP attacks 3. Homozygous HMBS mutations (animal study)
Hepatocellular carcinoma	Elevated transaminases and hepatalgia	1. Liver cirrhosis 2. Elderly patients may have an increased risk 3. AIP attacks 4. HMBS enzyme deficiency may diminish the inhibition of tumor growth 5. The carcinogenicity of ALA 6. Decreased heme production reduces hemin's antigenotoxic and anti-inflammatory effects
Renal impairment	Elevated creatinine and urea nitrogen	1. Porphyrin precursors can cause tubular injury and severe arteriolar lesions, and PEPT2, a transporter of ALA, plays an important role. 2. Hypertension may aggravate renal arteriolosclerosis 3. Repeated hemin therapy
Metabolically related changes	Elevated serum uric acid and hypercholesterolemia	The exact mechanism is unclear

cannot be ruled out.

Based on the above mechanism, corresponding treatment can be given to improve the state of the kidneys. Damage to the kidneys can be limited by reducing attacks, such as by avoiding predisposing factors and providing preventive treatment. In addition, PEPT2 may be an alternative therapeutic target. It works by inhibiting the tubular reabsorption of ALA, but it still needs to be tested.

6. Metabolically related changes

Few studies have examined elevated serum uric acid and hypercholesterolemia in patients with AIP. According to existing research, the exact mechanism is unclear (*104*). The factors associated with hypercholesterolemia are as follows. First, hypercholesterolemia may be related to porphyria itself and acute AIP attacks (*105-109*). Second, hypercholesterolemia is associated with an increase in low-density lipoprotein (LDL) (*105,106*). Studies have found that increases in cholesterol are sometimes accompanied by increases in LDL. LDL is known to be a transporter of human plasma cholesterol, which transports endogenous cholesterol to extrahepatic tissues. If LDL is elevated, more cholesterol is transported outside of the liver, causing hypercholesterolemia. Relevant studies need to be designed to explore the possible mechanism of these changes.

7. Conclusion

The possible mechanisms of acute and chronic manifestations of AIP are briefly summarized in Table 1. Although several studies have examined acute and chronic manifestations in patients with AIP, the specific mechanisms behind those manifestations are not fully understood. Therefore, future research on this disease should continue to explore the mechanisms of those manifestations. Understanding the relevant mechanisms can help us to better understand the disease and to take corresponding actions to delay its incidence and progression.

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Review

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Recent advances in the epidemiology and genetics of acute intermittent porphyria

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SUMMARY Acute intermittent porphyria (AIP) is a dominant inherited disorder with a low penetrance that is caused by mutations in the gene coding for hydroxymethylbilane synthase (HMBS). Information about the epidemiology and molecular genetic features of this rare disorder is crucial to clinical research, and particularly to the evaluation of new treatments. Variations in the prevalence and penetrance of AIP in various studies may due to the different inclusion criteria and methods of assessment. Here, the prevalence and penetrance of AIP are analyzed systematically, and the genetic traits of different populations and findings regarding the genotype-phenotype correlation are summarized. In addition, quite a few studies have indicated that AIP susceptibility was affected by other factors, such as modifying genes. Findings regarding possible modifying genes are documented here, helping to reveal the pathogenesis of and treatments for AIP. The status of research on AIP in China reveals the lack of epidemiological and genetic studies of the Chinese population, a situation that needs to be promptly remedied.

Keywords acute intermittent porphyria, prevalence, penetrance, genetic traits, genotype-phenotype correlation, modifying genes

1. Introduction

Porphyrias are a group of metabolic diseases that result from a specific abnormality in one of the eight enzymes of the heme biosynthetic pathway (*1,2*). In general, porphyrias are classified either as acute porphyrias or cutaneous porphyrias based on their clinical presentation or as hepatic and erythropoietic porphyrias based on the tissue where heme precursors are overproduced (*3*).

Acute intermittent porphyria (AIP, OMIM#176000), the most common and severe form of acute hepatic porphyrias (*4*), is an inherited metabolic disease that exhibits an autosomal dominant pattern of inheritance caused by partial deficiencies in hydroxymethylbilane synthase (HMBS; EC 2.5.1.61), the third enzyme in heme biosynthesis (*5*). AIP has significant molecular genetic heterogeneity and low penetrance (*6*). It leads to accumulation of upstream metabolites δ-aminolevulinic acid (ALA) and porphobilinogen (PBG), which induce toxicity to the neurologic system, and then trigger episodic, acute neurovisceral symptoms that can even be life-threatening (*7-11*). Studies of the prevalence, penetrance, and molecular genetic traits of AIP are crucial to its early diagnosis and rational management. Thanks to the rapid development of next-generation sequencing (NGS) technology, genetic sequencing has been widely used to detect *HMBS* gene mutations (*12,13*). Some studies based on genetic testing have revealed that the prevalence of *HMBS* variants was substantially underestimated, with extremely low penetrance in the general population but higher penetrance in families with AIP (*14,15*).

Findings regarding modifying genes and the correlation between genotype-phenotype warrant more attention in recent studies on AIP. Although a genotype-phenotype correlation has not been identified, certain mutations may be relevant to penetrance or the severity of clinical manifestations. Some modifying genes that have been identified in recent years are reviewed here, including the *PEPT2* gene, *PPARA* gene, cytochrome P450 gene, and *ABCB6* gene.

In China, concern about AIP has increased, but most studies on AIP are various case reports and small series. The prevalence, penetrance, and genetic traits of Chinese patients with AIP are still unclear and need to be fully evaluated.

2. Prevalence and penetrance of AIP

AIP is an autosomal dominant metabolic disorder with

a variable prevalence among different countries (*16*). Because it is a rare disease with multiple phenotypes, its prevalence is difficult to evaluate (*17*). Therefore, information about the prevalence of AIP is most often based on estimates. Variations in the prevalence and penetrance of AIP in various studies are probably due to different inclusion criteria and methods of assessment (*18*). Information is classified in Table 1 as the prevalence of symptomatic AIP, the prevalence of pathogenetic *HMBS* mutations (including data on the general population and families with AIP), and penetrance (including data on the general population and families with AIP).

Much of the information on AIP comes from a 3-year prospective study of newly diagnosed symptomatic patients with AIP in 11 European countries, and the annual incidence of symptomatic AIP was reported to be 0.13 per million. Its prevalence, which was calculated based on the incidence and mean disease duration, was 5.9 per million in Europe (19). Moreover, the numbers were similar in all countries except Sweden (*19*). The rate of recurrence, which was 3-5%, was also been estimated in that study. In addition, the sliding prevalence of symptomatic AIP indicates the importance of improved management and educational strategies (*19-22*). Similarly, a 60-year retrospective study in Finland revealed a decrease in patients with active AIP (*23,24*). In light of the number of patients with AIP referred to a French facility, the prevalence of overt AIP was estimated to be 7.6 per million, which was in line with the aforementioned figure of 5.9 per million (*25*).

Due to founder effects, the prevalence of AIP was markedly higher in some regions, such as 17.7 per million in southeastern Spain (*26*) and 23 per million in Sweden (*19*). In northern Sweden, it was even 192 per million (*27*). Information from Argentina and Western Australia may less credible because no gene sequencing was performed (*28,29*).

Although symptomatic patients with AIP are quite rare, some findings based on population have indicated that the prevalence of pathogenic *HMBS* mutations was higher than previously estimated. A study based on Caucasians indicated that the prevalence of pathogenic *HMBS* mutations was 1/1,782 (*14*). This result was consistent with the findings of two studies in France, which estimated the prevalence of mutations in the *HMBS* gene to be 1/1,675 to 1/1,299 (*15,25*).

Thus, this information indicates a marked discrepancy in the estimated prevalence of *HMBS* mutations and the occurrence of acute attacks in patients with AIP, which means that its penetrance is very low. As of the current point in time, penetrance is estimated to be approximately 20-50% in families with AIP but only ~1% in the general population, with the exception of 23% in the Swedish and 42% in the Northern Swedish (*14,19,25,27,31,32*). This finding strongly suggests that other factors act as a catalyst for AIP attacks, such as modifying genes and environmental factors.

Table 1. The estimated prevalence and penetrance of AIP

Country (region) or Population	Prevalence of symptomatic AIP (case per million inhabitants)	Prevalence of pathogenetic *HMBS* mutations		Penetrance (%)		*Ref.*
		in the general population	in families with AIP (%)	in the general population	in families with AIP	
Caucasian		1/1,782		< 1		14
Europe	5.9					19
France	7.6	1/1,299		0.5-1	22.9	25
		1/1,675				15
	5.5					19
Sweden	23			23		19
		1/10,000				30
Northern Sweden		1/1,000				30
	192	1/2,000		42		27
Switzerland	10-15		50		52	31
	9.9					19
Southeastern Spain	17.7					26
Spain	6.3				52	19
Finland			47		35	32
	5.9					19
Norway	6.3					19
Northern Italy	5.0					19
Poland	7.2					19
UK	7.2					19
Netherlands	8.1					19
Russia			59		57	58
Argentina*	8				40	28
Western Australia*	24				29	29

*No gene sequencing was performed in the study

3. Genetics of AIP

3.1. Molecular bases

Genetically, AIP is an autosomal dominant disorder resulting from mutations in the *HMBS* gene (*33*), which is located at the chromosomal region 11q24.1-24.2 (GRCh38.p11:119,084,003-119,094,417). There are two isoforms of HMBS, the housekeeping and the erythroid isoforms (*34*). The transcript including exons 1 and 3-15 is directed by housekeeping promoter located in the 5' flanking region upstream of exon 1, and the transcript containing exons 2-15 is produced by an erythroid-specific promoter located in a region 3 kb downstream of intron 1 (*35-37*). Most patients with AIP carry mutations in exons 3-15, which affect not only the housekeeping but also the erythroid isoforms of HMBS, which are the classic form of AIP. When mutations occur within or close to the coding region of exon 1, the activity of HMBS in erythroid cells is normal (*38-40*). Regulatory gene defects in the 5' -promoter regions of the *HMBS* gene have been found in patients with AIP (*41*). To date, no variants have been found in the erythroid-specific promoter or in exon 2 (*42*).

3.2. Molecular genetic features

Thus far, over 500 different mutations in the *HMBS* gene have been identified (Human Gene Mutation Database HGMD, *http://www.hgmd.cf.ac.uk/ac/index.php*). There are 31 CpG dinucleotides, at which most of the *de novo* events in human genes appear, in the 1,086 base-pair coding sequence of *HMBS* (*14,43*). Because of oxidative deamination of methylated cytosines, the CpG dinucleotides are believed to be hypermutable (*44*). Most of the mutations resulting in AIP are private or present only in a few unrelated families, a fact that highlights the molecular genetic heterogeneity of AIP (*45-53,73*). Nevertheless, a few mutations are relatively common either due to CpG dinucleotide mutational hotspots in the gene (*43*), such as the mutations encoding p.R173W and p.R167Q, or due to a founder effect, such as *HMBS* P.G111R in Argentina and p.W198X in Sweden (*54,55*). (Table 2 shows the founder effect mutations causing AIP among different populations).

Since AIP is an autosomal dominant disease, no sex differences in gene carriers are expected (*60,61*), but all studies have found that acute attacks affect females much more frequently than males (*19,54,59,60,62-64*). A recent study in Finland found a high penetrance of 41% for AIP acute attacks and 50% for all acute manifestations associated with AIP in female patients (*32*). Similar findings have been gleaned from families with the founder mutation p.W198X in northern Sweden (*27,65*). These studies emphasize that female hormones trigger AIP attacks (*23,66,67*).

3.3. Genotype-phenotype correlation

To date, no clear genotype-phenotype correlation for AIP has been determined (*24,63,68*). However, some studies have indicated that some mutations may be related to a higher penetrance and/or more severe clinical manifestations, such as p.W198X, c.1073delA, and p.R26C (*32,69*), while some variants may be associated with a lower penetrance and/or milder manifestations, including p.R167W, p.R225G, and c.G33T(*24,32,69*). Interestingly, conflicting findings were obtained from studies on P.R173W; a study in Finland reported a lower penetrance but studies in Northern Sweden and Spain reported a higher penetrance (*16,32,69*). Although a study in Finland found similar genotypes, penetrance differed greatly among families (32). This shows that the phenotype of AIP is not determined by the *HMBS* genotype alone but that other factors such as modifying genes and the environment also play a vital role in the pathogenesis of AIP attacks (*6,24,25,32,70,71*). Moreover, protein function analysis and bioinformatic tools have been used to identify a genotype-phenotype correlation. Some mutations in the *HMBS* gene including p.R116W, p.R173W, p.R149X, p.Q217H, p.G218R, p.A219P, and p.A330P have been predicted to lead to severe clinical manifestations (*72*). The correlation between genotype and phenotype is still a topic of interest in research on AIP.

3.4. Modifying genes

The low penetrance of AIP and the significant difference between the penetrance found in families with AIP and that found in the general population indicate that AIP susceptibility is affected by the inheritance of *HMBS* gene mutation as well as other genetic or environmental factors. A hypothesis has been put forth that AIP

Table 2. Founder effect mutations causing AIP among different populations

Population	Mutation (cDNA)	Mutation (Amino Acid)	Exon	*Ref.*
Dutch	c.346C > T	P.R116W	8	51
Canadian (Nova Scotia)	c.517C > T	P.R173W	10	56
Swedish	c.593G > A	P.W198X	10	55
Swiss (German-speaking)	c.848G > A	P.W283X	14	31
Argentinean	c.331G > A	P.G111R	7	54
Spanish (Murcia, southeastern region of Spain)	c.669_698del		12	57
Russian	c.53delT	p.Met18ArgfsX3	3	58

inheritance does not follow the classical autosomal dominant pattern but an oligogenic or polygenic inheritance pattern with environmental modifiers (*25,58,74,75*).

Many attempts using various approaches have been made to search for those modifying genes in order to both further understand the pathogenesis of AIP and to identify reliable therapeutic targets.

3.4.1. *PEPT2* gene

Genetic variation in human peptide transporter 2 (*PEPT2*, also known as *SLC15A2*) has been identified as an aspect in modulating the severity of renal and neurologic impairment (*6,76-78*). ALA is reabsorbed in the proximal tubules by PEPT2 variants. Variants of PEPT2 have different affinities for ALA: PEPT2*1 has a higher affinity for ALA while PEPT2*2 has a lower affinity (*79,80*). Thus, more significant neurotoxicity may occur in *PEPT2*2* carriers because they have lower ALA brain efflux (*81-83*). That said, PEPT2 *1 with a higher affinity for ALA is associated with an increased risk of renal disease (*6,76*).

3.4.2. Cytochrome P450 gene

The enzyme 5-aminolevulinic acid synthase (ALAS) 1 catalyzes the rate-limiting step in the production of heme and is regulated by heme through a negative feedback loop (*84*). Many of the substances that induce cytochromes (CYPs) in the endoplasmic reticulum of the liver can also result in increased hepatic ALAS1 activity (*85,86*).

Studies have indicated that the hepatic cytochrome P450 gene may play a role in AIP attacks (*87*). A study found that *Cyp2c40*, *Cyp2c68*, and *Cyp2c69* were upregulated in mice with induced AIP but downregulated in wild-type mice. These genes are respectively equivalent to *CYP2C8*, *CYP2C9*, and *CYP2C19*, which are the primary human CYP450 enzymes involved in drug metabolism. Cyp21a1, the homolog of human CYP21A2 or CYP17A1 (-37), is a crucial enzyme in corticosteroid and sex hormone synthesis; *Cyp21a1* is downregulated in mice with induced AIP but upregulated in wild-type mice (*88,89*). A study based on the population in the Spanish region of Murcia revealed parallel findings: the alleles *CYP2D6*4* and *5 may prevent acute attacks in patients with AIP while *CYP2D6* may constitute a penetrance-modifying gene (*26*).

In short, the cytochrome P450 gene may be related to the pathogenesis of AIP, but further studies are needed to confirm this hypothesis.

3.4.3. *PPARA* gene

Peroxisome proliferator-activated receptor alpha (PPARα) is a transcription factor belonging to the nuclear receptor superfamily, and ALAS1 has been identified as a target (*90*). In general, nuclear receptors regulate transcription through interactions with coactivator or corepressor molecules (*91-95*). Binding of agonists to PPARα leads to an enhanced binding of co-activator proteins, such as the proliferator-activated receptor γ coactivator 1α (PGC- 1α) (*96,97*). PGC-1α, which acts as a bridge from the activated PPARs to the basal transcriptional machinery (*90*), activates the *ALAS1* promoter by coactivating the nuclear respiratory factor-1 (NRF-1) and the forkhead box O1(FOXO1), both of which directly bind to the *ALAS1* promoter (*98*).

Recently, a study based on protein functional analysis indicated that the *PPARA* gene has obvious functional overlap with *ALAS1* (*72*). That study also indicated that transcription of *CYP2C8* and *CYP3A4* is directly regulated by the *PPARA* gene (*99*), which is similar to previous findings that genes in CYP classes 1-3 are specifically regulated by *PPARA* in humans (*100-103*). Thus, mutations in *PPARA* may affect the heme biosynthesis pathway by regulating the cytochrome P450 system and eventually lead to AIP attacks (*104*).

3.4.4. Some genes regulating the nervous system

Currently, a few defects in genes regulating the nervous system (*UNC13A*, *ALG8*, *FBXO38*, *AGRN*, *DOK7*, and *SCN4A*) have been detected in a study in Russia (*58*). The latter three genes are related to congenital myasthenic syndrome (CMS), whose symptoms bear a remarkable resemblance to neurologic features of AIP attacks. Thus, the combination of a mutation in the *HMBS* gene with defects in genes regulating the nervous system may play a key role in triggering acute AIP attacks and prompting the development of specific symptomatic traits (*58*). However, this assumption needs to be verified in future studies.

3.4.5. *ABCB6* gene

Elevated porphyrins are a hallmark of various types of porphyria. ABCB6, a type of porphyrin transporter, has been investigated as a modulator of porphyria severity through deep sequencing and biochemical analysis. A study found that patients with severe porphyria have variant alleles in the *ABCB6* gene (*105*). Plasma membrane ABCB6 exports multiple disease-related porphyrins. Functional studies have revealed that most of these ABCB6 variants are poorly expressed and also dysfunctional. Therefore, ABCB6 is presumably a genetic modifier of porphyria that alleviates its severity by expelling porphyrins.

Nevertheless, most reports of modifying genes are only preliminary findings, and further clinical studies are needed to verify their reliability.

4. Status of research on AIP in China

For a long time, China' s healthcare system has paid less attention to rare diseases, including AIP (*106,107*). An early study in the Mainland found a high rate of AIP misdiagnosis (*33*). Over a decade ago, a study involving 24 unrelated Chinese patients with AIP in Taiwan found no genotype/phenotype correlations, but the spectrum of *HMBS* gene mutations detected in these patients with AIP coincided with those observed in patients of other ethnic origins (*61*).

In recent years, this rare disorder has garnered attention from Chinese physicians, and small series of and case reports on AIP, and especially reports of novel mutations, have increased in China, indicating that AIP may not be as "rare" as was previously assumed (*108-114*). In addition, some studies have analyzed clinical features of Chinese patients with AIP, such as posterior reversible encephalopathy syndrome (*110,115-118*). Peking Union Medical College Hospital first described how acute attacks affected the quality of life and psychological state of patients with AIP in Northern China (*119*).

Unfortunately, there are as of yet no studies on the prevalence, penetrance, and genetic traits of Chinese patients with AIP. Findings from Western counties are most likely inapplicable to the Chinese population due to the obvious differences in ethnic characteristics. Therefore, data on Chinese patients need to be evaluated in order to promptly identifying patients with *HMBS* mutations that have not yet suffered attacks, to properly manage patients, and to improve prognosis.

5. Conclusion

AIP is an inherited metabolic disease that exhibits an autosomal dominant pattern of inheritance caused by partial deficiencies in *HMBS*. In Europe, the estimated prevalence of AIP was 5.9 cases per million population, while the prevalence of *HMBS* variants was 1/1,299~1/1,782, with penetrance estimated at 20-50% in families with AIP but only ~1% in the general population. AIP has marked molecular genetic heterogeneity. No clear correlation between genotype and phenotype has been confirmed for AIP, although studies have reported that some mutations may be relevant to its penetrance or the severity of clinical manifestations. Consequently, the prevailing view is that other factors, such as modifying genes, may play an essential role in AIP attacks. Studies have identified some likely modifying genes, but most of those studies are just initial studies based on trials or predictions. Further clinical studies are needed to verify their reliability. Recently, studies on AIP have increased in China, but the prevalence, penetrance, and genetic traits of AIP in Chinese patients are unclear. Studies are urgently needed to reveal those aspects.

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Review

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Therapeutic strategies for acute intermittent porphyria

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SUMMARY Acute intermittent porphyria (AIP) is an autosomal dominant disease caused by mutations in porphobilinogen deaminase (PBGD), the third enzyme of the heme synthesis pathway. Symptoms of AIP usually manifest as intermittent acute attacks with occasional neuropsychiatric crises. The management of AIP includes treatment of acute attacks, prevention of attacks, long-term monitoring and treatment of chronic complications. Intravenous injection of heme is the most effective method of treating acute attacks. Carbohydrate loading is used when heme is unavailable or in the event of mild attacks. Symptomatic treatment is also needed during attacks. Prevention of attacks includes eliminating precipitating factors, heme prophylaxis and liver transplantation. New treatment options include givosiran (siRNA) to down-regulate ALA synthase-1 (ALAS1) and the messenger RNA of PBGD (PBGD mRNA) delivered to the liver cells of patients with AIP. Long-term monitoring of chronic complications includes regular liver-kidney function and hepatocellular carcinoma (HCC) screening.

Keywords acute intermittent porphyria, heme, carbohydrate loading, givosiran (siRNA), mRNA therapy

1. Introduction

Acute intermittent porphyria (AIP, MiM 176000) is an inherited autosomal dominant disorder characterized by hepatic deficiency of hydroxymethylbilane synthase (HMBS,HGNC 4982)/porphobilinogen deaminase (PBGD, EC 4.3.1.8), the third enzyme of the heme synthesis pathway (*1-3*). Regulated by heme stores, ALA synthase-1 (ALAS1) is the first enzyme of the hepatic heme synthesis pathway, and is also a rate-limiting enzyme. In patients with AIP, increased heme demand will lead to the up-regulation of ALAS1 and increase the production and accumulation of aminolevulinic acid (ALA) and porphobilinogen (PBG) (*1,2*).

Clinical symptoms are related to the accumulation of high levels of porphyrin precursors (*4-6*). Symptoms of AIP usually manifest as intermittent acute attacks. The most common symptoms during attacks are abdominal pain accompanied by nausea, vomiting, constipation, hypertension and tachycardia (*1-3,7*). Some patients develop severe neurological complications, including peripheral neuropathy and central nervous system (CNS) involvement, such as acute encephalopathy, convulsions, anxiety, insomnia, depression, seizures and paralysis. After many years of repeated attacks, symptoms may become chronic (*8,9*).

The management of AIP includes treatment of acute attacks, prevention of attacks, long-term monitoring and treatment for complications (*10*). The current work summarizes existing and recently developed AIP treatments for those three purposes.

2. Treatment of acute attacks

2.1. Specific therapy

Pathophysiologically, the aim of treating acute attacks is to suppress hepatic ALAS1 activity. The current treatments for acute attacks involve heme replacement and carbohydrate loading.

2.1.1. Heme

Intravenous heme is the most effective therapy; it provides exogenous heme and down-regulates ALAS1 transcription. This, in turn, results in a rapid reduction in the overproduction of ALA and PBG. Heme is isolated and purified from human red cell concentrates, and it has been used for more than 30 years (*4,12*). Currently, two forms of heme for human use are available: Normosang® heme arginate in Europe and South Africa and Panhematin® heme (Recordati Rare Diseases) in the

US. Both must be freshly prepared and delivered over 30 min. Heme should be reconstituted in human albumin because of its instability in aqueous solution. Due to the possibility of painful phlebitis as a result of intravenous injection of heme into a peripheral vein, heme should be given *via* central intravenous. A standard heme regimen is 3-4 mg/kg/day for 4 days; additional courses may be required if symptoms continue to worsen (*13*). Due to rapid clinical improvement (often within 1-2 days) once an acute attack occurs, heme should be used as soon as possible, and especially when there are convulsions, hyponatremia, neuropathy or psychosis. Intravenous heme is efficacious even in the late stage of progressing motor neuropathy (*14*).

Although heme therapy is well tolerated in most cases, repeated treatment increases the risk of hepatic fibrosis and liver iron overload (a heme arginate dose of 250 mg contains 22.7 mg of iron) (*15*). A point worth mentioning is that experience shows that heme can be used safely during pregnancy (*16*).

2.1.2. Carbohydrate loading

Adequate caloric support (carbohydrates and proteins) is essential to the treatment of AIP (*17*). Carbohydrate loading was considered to be a standard treatment for acute attacks before the appearance of heme, but it does not alleviate symptoms as quickly as heme. Glucose inhibits ALAS1 by affecting peroxisome proliferator-activated receptor gamma coactivator1-alpha (PGC1-α) (the "glucose effect") (*18-20*).

According to guidelines for the treatment of an acute attack in the US and South Africa, glucose is limited to the treatment of mild attacks (mild pain, no vomiting, no paresis, no seizures, or no hyponatremia) or when heme is not available locally (*21*). The latter is a challenge in lower-to-middle-income countries (*22*).

Mild attacks should initially be treated with oral glucose, but patients who are not tolerating oral glucose can be given glucose intravenously (300-500 g/day, preferably 10% dextrose in 0.45% saline) as a preferred source of energy (*16,23*), in order to down-regulate the activity of ALAS1 and prevent fasting (*24*). Moreover, the combination of glucose with insulin can be more effective because insulin can also hamper ALA synthesis induced by PGC1-α (*25*). However, hyponatremia worsens with hemodilution caused by large amounts of 10% glucose. At the same time, the blood sugar level should be monitored regularly to avoid the osmotic effects of glucose (hyperglycemia or hypoglycemia causes additional neurological complications). After emergency intravenous glucose, oral nutrition with carbohydrates should be initiated as soon as possible. Of course, intravenous glucose does not prevent recurrent attacks or long-term consequences (*26*).

2.2. Symptomatic treatment

2.2.1. Digestive system

2.2.1.1. Abdominal pain management

Pain stress contributes to neuroendocrine reactions that activate ALAS1 and exacerbate symptoms, so pain management in patients with AIP is crucial and remains a challenge.

Generally, abdominal pain is severe (visual analog scale (VAS) >7 cm, scale from 0 to 10 cm) (*27*). Acetaminophen and non-steroidal anti-inflammatory medicines are first-line agents in mild cases (*21*), but parenteral narcotic analgesics (fast-acting opioid analgesics) are universally used to treat incapacitating pain associated with acute attacks (*28*). Morphine and buprenorphine are the safest of those medications. An experimental study indicated that fentanyl, tramadol, nalbuphine, oxycodone, and hydrocodone resulted in different degrees of porphyrin accumulation (*29*). Addiction to medication warrants attention, although few cases of opioid dependence in patients with AIP have been reported (*30*). In addition, neurolysis of the celiac ganglion with absolute alcohol has been performed as an analgesic strategy in patients with refractory neurovisceral pain, subsequent pain control, and opioid medication withdrawal (*31,32*). Thus, appropriate therapies (intravenous glucose, heme, and safe analgesics) are administered without delay, and alleviation of pain should result within 3 to 5 days.

2.2.1.2. Management of other symptoms

Phenothiazines, such as chlorpromazine and promethazine, play an important role in managing nausea and vomiting (*33*). Moreover, studies have indicated their usefulness in managing anxiety, restlessness, pain, and reducing the requirement for opioid analgesics. Of course, ondansetron is also a good choice for treating nausea and vomiting while metoclopramide can trigger an acute attack.

Intestinal obstruction in patients with AIP is considered to be a dynamic obstruction, and constipation is a possible cause of patients' abdominal pain. The treatment strategies for constipation and an intestinal obstruction include eliminating risk factors, giving adequate caloric support, and symptomatic treatment. Most patients improve after discharge. Symptomatic treatment can be given with lactulose (*22*) or repeated enemas (*34*).

2.2.2. Cardiovascular system

Sympathetic hyperactivity results in tachycardia and systemic arterial hypertension during acute attacks (*29*). Beta blockers, angiotensin-converting enzyme inhibitors, and calcium channel blockers (diltiazem is preferred over nifedipine) are preferred agents, but

patients need to be monitored for hypotension and bradycardia (*35*).

2.2.3. Nervous system

Involvement of the CNS manifests as a combination of seizures, syndrome of inappropriate antidiuretic hormone (SIADH), posterior reversible encephalopathy syndrome (PRES), or psychiatric symptoms (agitation, hallucinations, anxiety, and depressive behaviors) (*36,37*). Overproduction of ALA *via* neurotoxicity, oxidative damage, modification of glutamatergic release, and increased metabolites of the kynurenine pathway initiates dysfunction of the CNS as mentioned above (*38-41*). Moreover, the accumulation of ALA is assumed to impair normal γ-aminobutyric acid (GABA) function, leading to seizures and psychiatric symptoms (*42-45*). Hypothalamic impairment due to the neurotoxicity of ALA might cause SIADH, which is most often characterized by hyponatremia (*37,44*). Seizures can occur in the event of ALA neurotoxicity, decreased GABA activity, hyponatremia, hypertension, or PRES (*36,44*). ALA neurotoxicity and hyponatremia may cause psychiatric symptoms in patients with AIP.

2.2.3.1. CNS

2.2.3.1.1. Treatment of seizures

Treatment of seizures is difficult because several commonly used medications are highly risky. Gabapentin, vigabatrin, levetiracetam, and probably bromides or magnesium sulfate can be given safely (*46,47*). Benzodiazepines including diazepam are relatively safe (*48*). The safety of valproate and clonazepam is a subject of debate (*49*). Status epilepticus seizures require sedation with propofol. Intravenous infusion of magnesium sulphate can effectively treat adrenergic symptoms (*45,50,51*).

Bromides have been successfully used in an attempt to avoid other enzyme-inducing antiepileptic drugs, but they frequently have adverse effects on the skin, gastroenteric, renal systems, and CNS (*52*). Taylor reported that intravenous magnesium can be used to safely treat seizures in AIP (*53*). Tatum and Zachariah described successful seizure control with gabapentin in two patients with AIP without exacerbating acute attacks (*54*). Zadra *et al.* reported a female patient who had both partial and generalized seizures with AIP and who remained seizure-free and attack-free on gabapentin alone (*47*). Another report described a patient with hereditary coproporphyria treated with levetiracetam, and that medication did not exacerbate porphyria (*55*). Zaatreh described a patient with AIP and status epilepticus who was successfully treated with a combination of intravenous magnesium and oral levetiracetam and who was thereafter maintained on oral gabapentin and levetiracetam without any adverse reactions (*56*). A case involving a 12-year-old boy with AIP and status epilepticus was resolved with intravenous propofol (in addition to the continuation of gabapentin and levetiracetam in the event of episodes of status epilepticus) (*57*). The management of seizures can be a potential source of iatrogenicity when using carbamazepine, phenytoin, or barbiturates; all can induce acute attacks of porphyria (*54,58,59*).

Careful correction of hyponatremia and hypertension is necessary, particularly when those conditions are associated with seizures (*48*). Patients with neurological complications (convulsions, progressive neuropathy, respiratory insufficiency, and encephalopathy) must be cared for in a High Dependency or an Intensive Care Unit (*33*).

2.2.3.1.2. Treatment of SIADH

Hyponatremia is a common symptom of SIADH in patients with AIP. The correction of hyponatremia should be performed according to hyponatremia guidelines developed by professional organizations (*60-63*). The strategy should be guided by the symptoms and not just by a reduction in serum [Na^+] (*64*).

First, SIADH is associated with euvolemic hyponatremia, and fluid restriction (1 L/day) is the best option (*65*). However, carbohydrate loading is the only specific therapy in areas where heme is not available. During carbohydrate loading, fluid restriction is not practical. Moreover, fluid restriction may be poorly tolerated because of increased thirst, resulting in poor compliance (*66,67*).

Second, patients with severe hyponatremia (serum [Na^+] < 125 mmol/L) should be intravenously infused with a 3% saline solution as a bolus of 100-150 mL over 10-20 min; this should be repeated 2-3 times or until the goal of a 5-mEq/L increase in serum [Na^+] is met (*65*). For patients with moderate hyponatremia (serum [Na^+] between 125-129 mmol/L), a 3% hypertonic saline may be administered as a continuous infusion. A formula can be used to calculate the rate of infusion (*68*). An initial 4-6 mEq/L increase is considered sufficient to reduce the risk of brain herniation and neurological damage from cerebral ischemia. The risk of osmotic demyelination syndrome (ODS) increases with chronic hyponatremia. Therefore, a limit of 10-12 mEq/L in 24 h or 18 mEq/L in 48 h seems to be appropriate for patients without risk factors for ODS. A correction > 8 mEq/L in 24 h is sufficient for high-risk patients (*62*).

Third, loop diuretics that increase free water excretion rates have proven effective in the treatment of SIADH. A combination of low-dose loop diuretics and oral sodium chloride is only recommended in European guidelines (*60,63*).

Finally, vaptans, and tolvaptan in particular, are selective competitive vasopressin receptor-2 antagonists

that have been used in recent years (*69*). The usual starting dose is 15 mg/day. However, lower doses (*e.g.* 7.5 mg/day) may be effective in clinical practice to guarantee a desired serum [Na^+] increase (*64*). If the [Na^+] rise is less than 5 mEq/L in 24 h, the daily dose can be increased up to a maximum of 60 mg (*64*).

However, overcorrections and adverse reactions, such as hypotension or liver toxicity, are relatively frequent (*70,71*). Tolvaptan must be discontinued when liver injury is suspected. The use of vaptans differs according to the US recommendations and European guidelines (*59-62*). The US recommendation suggest using vaptans only in the event of chronic hyponatremia as a second option after fluid restriction.

2.2.3.1.3. Psychiatric symptoms

A variety of psychiatric symptoms, such as depression, anxiety, insomnia, and even schizophrenia and hallucinations, may occur during an acute attack (*40*). Severe agitation, insomnia, and anxiety can be treated with low-dose short-acting benzodiazepines and chlorpromazine (*33*). Tricyclic antidepressants (TCAs) have the worst security profile among antidepressants. Of all selective serotonin reuptake inhibitors (SSRIs), only fluoxetine is categorized as non-porphyrogenic. A 25-year-old woman with AIP received fluoxetine (20 mg/day) for 9 weeks for depressive symptoms. Fluoxetine seems to be efficacious without causing any adverse reactions (*72*). Duloxetine is the serotonin norepinephrine reuptake inhibitor (SNRI) with the most favorable profile (*40*). Hallucinations should be treated with phenothiazine or olanzapine (*22*). Databases should be consulted to determine the porphyrogenicity of different medications. The Norwegian Porphyria Centre (NAPOS) and American Porphyria Foundation (APF) databases are now available in Porphyria Drug Safety finder (*73-75*).

2.2.3.2. Peripheral nervous system

Peripheral neuropathy caused by ALA neurotoxicity and heme deficiency is a common neurological manifestation of AIP (*60*). Patients may present with muscle weakness, paresis, bulbar paralysis, sensory neuropathy (include paresthesia, hypoesthesia, and neuropathic pain), or the like (*61-63*). The pain score and the severity of muscle weakness should be evaluated daily (*22*). Bulbar paresis and increasing muscle weakness are signs of an acute attack, and the patient should be transferred to the intensive care unit. Vital capacity, reduced by paralysis of the intercostal muscles, increases the risk of pneumonia and may require early mechanical ventilation (*22*). It may be necessary for several months if respiratory failure has occurred. Patients with paresis require rehabilitation therapy as soon as possible. Paralysis can usually be reversed with proper management and months of recovery (*46*).

3. Prevention of attacks

3.1. Eliminating precipitating factors

The most important preventive measure for AIP is to eliminate all predisposing factors that may cause an acute attack. This includes avoiding unsafe drugs (such as barbiturates, anticonvulsants, some sedatives, antibiotics, antifungals, and hormones), fasting, alcohol use, smoking, infections, and stress and encouraging adequate calorie and carbohydrate intake (*25,33,44*). These triggers significantly increase the hepatic heme requirement, and the reduction in hepatic free heme leads to the synthesis of ALAS1. Given that drugs are an important cause of acute attacks of AIP, patients with AIP should be referred to the American Porphyria Foundation (APF), the Norwegian Porphyria Centre (NAPOS), or the Porphyria Drug Safety finder to ensure safe use of medications (*28*). Extreme dieting, severe caloric restriction, and starvation should be avoided (*76*). Patients should be advised to maintain a balanced diet with a slightly higher carbohydrate content (60-70% of total calories). There is little evidence that extra carbohydrates in the diet help further prevent attacks, and trying to increase carbohydrate intake may only lead to unwanted weight gain. Obese patients should lose weight gradually while they are clinically stable (*10*). In addition, patients should give up smoking and limit alcohol. Generally, no more than two drinks a day for men and no more than one for women are recommended (*16*).

Studies have reported that 10-30% of cyclical AIP episodes occur in the luteal phase of the menstrual cycle (*77,78*). Probably the main factor provoking a crisis is the ovarian hormone progesterone (*79-80*). Progesterone is a porphyrin inductor and an effective inducer of ALAS1 (*78,81*). Therefore, women with recurrent premenstrual attacks of AIP can be given GnRH analogues (leuprolide or histrelin) to prevent ovulation and alleviate symptoms (*16*). GnRH treatment is initiated during the first 1-3 days of a menstrual cycle to reduce the risk of an AIP attack precipitated by transient ovarian stimulation (*15*). Possible adverse reactions include depression, hot flushes, reduced libido, osteoporosis, and other menopausal symptoms (*33*). Women with frequent attacks that appear to be associated with their cycle can tentatively receive a GnRH analogue for 3 months, and it can be discontinued if ineffective (*15*). Receiving the GnRH analogue continuously for more than 6 months carries an irreversible risk of bone loss. If, therefore, such treatment continues, a low dose of estradiol (preferably *via* the percutaneous route) or bisphosphonate may be added to prevent bone loss and other adverse reactions, or the treatment may be switched to a low-dose oral

contraceptive (*10*). Bone density should be assessed annually to ensure there is no ongoing bone loss. In addition, there is a continuing risk of endometrial dysplasia in the absence of progesterone, so endometrial monitoring by a gynecologist should be performed at least annually (*15*). The efficacy of GnRH analogues should be reviewed after one year (*33*).

3.2. Heme prophylaxis

Heme prophylaxis may be effective in preventing attacks, and it is particularly appropriate in preventing frequent (four or more episodes per year), noncyclic attacks (*15*). The prophylactic dose of heme is 3-4 mg/kg of body weight, once or twice a week, administered intravenously (*10,81*). Heme is a short-acting drug. Therefore, prophylactic administration of heme is less likely to be effective if administered less frequently than weekly (*81*). The main adverse reaction to frequent use is damage to the superficial venous system, which may necessitate the use of a central venous catheter. Iron overload is a major complication, and serum ferritin should be monitored in the event of repeated use (*44*). If necessary, a phlebotomy should be performed to treat iron overload (*15*). Serum ferritin may not be a reliable indicator of iron overload in some circumstances, as ferritin levels may rise sharply after hemin infusion (*81*). Other potential adverse reactions to intravenous heme include transient thrombocytopenia and prolongation of prothrombin time (*82,83*).

After 6-12 months of repeated prophylactic treatment, the need for continued prophylactic use of heme should be reassessed. A study has reported that stopping treatment and starting again if necessary may be more effective than gradually reducing the dose or the number of doses given (*15*).

Compared to the treatment of acute attacks, weekly planned prophylactic use of heme significantly reduced the number of hospitalizations and emergency department visits by patients with AIP, thus improving their overall quality of life (*84*). Another important benefit was an improved doctor-patient relationship, which is crucial to better management of patients with AIP.

3.3. Liver transplantation

Correcting genetic defects in PBG deaminase through orthotopic liver Transplantation (OLT) is an alternative to suppressing ALAS activation. This approach has been found to be an effective treatment option that alleviates symptoms and normalizes biomarkers within 3 days of transplantation (*44*). Indications for OLT include intractable acute attacks not responsive to medical treatment, recurrent acute attacks severely affecting quality of life, and repeated severe life-threatening acute attacks leading to prolonged ventilation (*26,33*). In AIP, common complications of OLT include hemorrhage, bile leak, and renal dysfunction (*87*). Studies have reported an increased risk of hepatic artery thrombosis (HAT) in patients with AIP who undergo OLT. Up to 40% of patients with AIP after liver transplantation have HAT (*85,86*). Ten patients with AIP have undergone OLT in the UK and Ireland and have been cured with biochemical and clinical remission. However, HAT occurred in 4 of the 10 patients who received liver transplants for AIP (*85*). For this reason, routine anticoagulation is administered post-operatively (*44*). In addition, hemin injection may lead to an iron overload and vascular damage, which may limit the the conduct of OLT (*86*). Lastly, patients who have hepatic iron overload pre-transplant have worse long-term outcomes following transplantation (*87*). Moreover, transplantation cannot completely repair the long-standing injury to motor nerves and the CNS (*88*).

3.4. Emerging therapy-Givosiran (siRNA-*ALAS1*)

3.4.1. Mechanism

With advances in small interfering RNA (siRNA) and its targeting technology, siRNA targeting liver ALAS1 expression has become a new treatment for AIP (*89,90*). Givosiran (Alnylam Pharmaceuticals, Cambridge, MA, USA), a double-stranded *ALAS1* specific siRNA, is linked to a ligand containing three N-acetylgalactosamine (GalNAc) residues that targets and interacts with liver asialoglycoprotein receptor (ASGPR) (*91,92*). Within the hepatocyte, RNA is cleaved by cellular enzymes into fragments of approximately 20 bp and then separated into single strands, which bind to and silence *ALAS1* mRNA, therefore inhibiting the translation and expression of the ALAS1 protein (*1,91,92*).

3.4.2. Trials

In preclinical studies of rodent and non-human primate models of AIP, use of an siRNA targeting *ALAS1* (siRNA-*ALAS1*) was associated with a rapid reduction in urine and plasma ALA and PBG levels and effective prevention of acute attacks (*93,94*). In a phase I clinical trial (NCT02452372) in adults with AIP, a single 2.5- mg/kg dose of givosiran similarly resulted in a maximum average reduction in the urinary ALA, PBG, and *ALAS1* mRNA levels by 86%, 91%, and 96%, respectively (*91,95*). In patients with recurrent acute attacks, a once-monthly dose of givosiran (2.5 or 5 mg/kg) resulted in a maximum reduction of *ALAS1* mRNA from baseline levels of 67% or 74% (*95,96*). Urinary *ALAS1* mRNA levels were significantly associated with ALA and PBG levels ($P < 0.001$) (*93*).

In a multinational phase III clinical trial (NCT03338816), 89 patients with AIP were randomly

assigned to receive subcutaneous givosiran (2.5 mg/kg) monthly or a placebo for 6 months (*97*). Compared to the placebo group, monthly subcutaneous injection of givosiran 2.5 mg/kg significantly reduced the composite annualized attack rate (ARR) for AIP attacks (mean composite ARR 3.2 *vs.* 12.5) (*91*). The median composite ARR was 10.7 for givosiran and 1.0 for the placebo. During the period of the intervention, the percentage of patients without acute attacks in the treatment group increased threefold compared to the placebo group (50 *vs.* 17%) (*91,95*). In the treatment group, the mean annualized number of days of heme use was markedly lower than that in the placebo group (6.8 *vs.* 29.7days) (*91*). The percentage of patients that used any opioids in the treatment group decreased by 20% compared to the placebo group (67 *vs.* 88%) (*97*). The average ALA and PBG levels in the givosiran group were 77% and 76%, respectively (*86,96,97*).

3.4.3. Pharmacokinetics

Givosiran is absorbed from the subcutaneous (SC) injection site, reaches a peak plasma concentration within 0.5-5 hours, and is then eliminated with a half-life of 4-10 hours (*96*). Givosiran and its active metabolite AS(N-1) 3'givosiran have equal potency (*91,96,98*). Givosiran and AS(N-1) 3'givosiran reduce the levels of ALA and PBG in a dose-dependent manner over the 0.35 to 2.5 mg/kg dose range (*95,96*). However, increasing the dose to 5.0 mg/kg did not result in an additional reduction in PBG at a dose of 2.5 mg/kg (*96*). Compared to quarterly administration, monthly administration results in greater and more sustained reductions in ALA and PBG (*95*).

Preclinical research indicated that siRNA-*ALAS1* did not cause hepatic heme deficiency and did not decrease cytochrome P450 2E1 activity (*94,95*). In a drug-drug interaction study, however, givosiran had a moderate effect on CYP2D6 and CYP1A2, a weaker effect on CYP3A4 and CYP2C19, and no effect on CYP2C9. The simultaneous use of givosiran with CYP1A2 (*e.g.* caffeine) and CYP2D6 (*e.g.* opioid) substrates should be avoided since this medicine may increase or prolong their therapeutic effect (*98,91*).

3.4.4. Adverse reactions

In a phase III clinical trial of givosiran, common adverse reactions occurred more frequently in the givosiran group than in the placebo group (*91,97*). Those reactions were elevated transaminase (15 *vs.* 2%), increased serum creatinine (CRE) (15 *vs.* 4%), injection site reactions (25 *vs.* 0%), nausea (27 *vs.* 11%), a rash (17 *vs.* 4%), and fatigue (10 *vs.* 4%). Sardh *et al.* reported that one patient had a serious adverse event (spontaneous abortion) after receiving givosiran at a dose of 1.0 mg/kg (*95*). In placebo-controlled and open-label studies, one patient (0.9%) had an allergic reaction, and another patient (0.9%) developed treatment-induced anti-drug antibodies (ADA) during treatment (*98*). The levels of alanine transaminase (ALT) increased mainly within 3-5 months after administration, and elevated levels in most patients return to normal after givosiran was continued (*91,98*). In a phase III clinical trial, one patient (0.9%) in the givosiran group discontinued treatment due to elevated ALT 9.9 times the upper limit of normal (ULN). One patient with an elevated ALT of 5.4 times the ULN temporarily discontinued treatment and resumed at a lower dose (1.25 mg/kg), without elevated ALT recurring (*97*). Decreased liver detoxification capacity maybe a potential complication of long-term downregulation of ALAS1 (*11,96*). However, givosiran has not been linked to acute liver impairment with jaundice (*99*). The increased CRE level and associated decreases in the glomerular filtration rate (eGFR), which occurred early during the 6-month period, are considered mostly transient and reversible (*97*). For chronic diseases, the 6-month follow-up period is relatively short, and a longer follow-up period is needed to evaluate the safety of treatment (*11*). There are limited data on the use of givosiran by pregnant women. Studies in animals have found that administration of givosiran during organogenesis resulted in adverse developmental outcomes (*97,98*). Human heme oxygenase 1(HMOX1) is an enzyme that plays a key role in placental biology, heme is a substrate of HMOX1, and the placenta expresses givosiran-targeted ASGPR (*100*). To minimize the risk of pregnancy, all programs require women to use birth control when receiving givosiran (*97*). Injection-site reactions to givosiran is similar to those of other RNA interference drugs (*e.g.* patisiran), indicating that this may be related to this type of drug (*11*).

3.4.5. Medication instructions

Givosiran is approved by the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) as a drug for acute hepatic porphyria (AHP) in adults and adolescents 12 years of age and older (*98,101*). The dose for patients ≥ 12 years to < 18 years is the same as for adults (*91,98*). The approved dose for an SC injection of givosiran is 2.5 mg/kg once a month; if a dose is missed, it should be given as soon as possible. The dose does not need to be adjusted for patients with mild liver impairment, bilirubin ≤ 1 × ULN and aspartate aminotransferase (AST) > 1 × ULN, or bilirubin > 1 × ULN to 1.5 × ULN. Discontinuing the drug and reusing at dose of 1.25 mg/kg once monthly should be considered for patients with clinically relevant elevated ALT (*98*). No studies have been conducted in patients with end-stage renal disease and moderate or severe liver impairment (*91,98*). (features and applications of givosiran are shown in Table 1)

Table 1. Features and applications of givosiran

Alternative names	Givlaari™
Mechanism of action	small interfering RNA (siRNA); targeting ALAS1 mRNA
Indications	AHP in adults and adolescents 12 years and older
Posology and method of administration	2.5 mg/kg , once monthly, subcutaneous injection
Interaction with other medicines	CYP1A2 Theophylline; caffeine CYP2D6 Pain management (opioid , NSAIDs, and triptan) Antidepressants (TCAs, SSRIs, and SNRIs) Antipsychotics
Adverse reactions	Hypersensitivity, nausea, rash, elevated transaminase, fatigue, decreased eGFR, injection site reactions
Dose modification for adverse reactions	1.25 mg/kg dose per month
Fertility, pregnancy, and lactation	Uncertain

3.5. Potential treatment

New therapeutic advances indicate that AIP can be addressed etiologically by correcting the deficiency in PBGD. The first approach involves increasing the expression of the deficient protein *via* recombinant adeno-associated virus (rAAV)-mediated transfer of human *PBGD* (*hPBGD*) cDNA. In the second method, *hPBGD* mRNA is packaged into lipid nanoparticles (LNP) and taken up by hepatocytes (*102*).

3.5.1. *PBGD* cDNA

Due to their poor transfection efficiency, AIP gene therapies with a non-viral vector and first-generation adenovirus vector were unsuccessful (*103-104*). Now, rAAV vectors have good biological safety and can maintain a high level of liver transgene expression for a long time (*105*). The introduction of the *PBGD* gene into mice with AIP *via* non-viral vectors does not produce sufficient levels of PBGD (*103*). Annika *et al.* successfully transfected adenovirus-mediated *PBGD* cDNA into PBGD-deficient mice (*104,106*). This led to increased PBGD expression in the liver and eliminated the accumulation of ALA and PBG in mice with AIP induced by phenobarbital. However, the first-generation adenovirus-mediated transgene is not suitable for clinical use because of transient expression and host cell immune response (*104*).

Makiko *et al.* injected the rAAV8 serotype vector (rAAV2/8-*hPBGD*) encoding the mouse *hPBGD* gene into the peritoneum of mice with AIP, resulting in an increase in liver PBGD activity. During acute attacks, it prevented an increase ALA and PBG over 36 weeks (*105*). rAAV8-mediated PBGD activity may increase the biosynthesis of heme and then down-regulate the expression of liver ALAS1. Importantly, rAAV2/8-*hPBGD* treatment improved neuromotor function in mice with AIP (*106,107*). Adding specific enhancer factors to the promoter of the AIP gene vector can enhance gene expression in mice with AIP (*108,109*).

In 2009, this gene therapy vector was utilized as an orphan drug for the treatment of AIP (*110*). In an initial human phase I clinical trial (NCT02082860), therapy with the rAAV5 vector encoding *hPBGD* (rAAV2/5-*hPBGD*) proved safe in patients with AIP (*105,111*). No serious adverse events related to treatment were observed after 8 patients were intravenously injected. Although ALA and PBG levels remain unchanged, the number of hospitalizations and frequency of heme treatment tend to decrease. All patients developed neutralizing antibodies against rAAV5. An important safety issue is the potential genotoxicity of the vector (*111*). Nault *et al.* reported the presence of integrated wild-type (wt) AAV genomes in HCC samples from 11 of 193 patients (*112*). However, the AAV vectors detected in liver biopsies from 6 patients with AIP after 1 year of treatment did not involve the carcinogenic regions reported in patients with HCC (*105,111*).

Liver-directed gene therapy may be effective in correcting AIP. However, this technology has many unresolved problems. First, there are no biochemical indicators of the effectiveness of gene therapy (*102,105*). Second, repeated administration is required. However, initial exposure to the viral vector will lead to the development of neutralizing antibodies (*17,102*). Therefore, rAAV-*hPBGD* gene therapy requires a balance between sufficient transgene expression and destructive immune responses, and this may be affected by the serotype of the rAAV vector. Finally, individual differences in transfection efficiency lead to inherent variability in levels of PBGD protein expression (*17,105*).

3.5.2. *PBGD* mRNA

Compared to rAAV-*hPBGD* gene therapy, *hPBGD* mRNA packed into LNP may be a cheaper and less immunogenic strategy. In addition, mRNA does not require nuclear localization, so it has minimal risk

of causing an insertion mutation (*17*). LNP protects mRNA from nuclease-mediated degradation (*113*). It also shields mRNA from the immune system (*114*). LNP interacts with apolipoprotein E (ApoE) and low-density lipoprotein (LDL) receptors, which mediate the internalization of LNP into hepatocytes (*113*). *hPBGD* mRNA can produce PBGD protein in the liver of patients with AIP through the cell's endogenous translation mechanism (*113-115*).

In animal models of AIP, Jiang *et al.* gave mice a single intravenous injection of LNP encoding *hPBGD* mRNA at different doses (0.2 or 0.5 mg/kg). More than 90% of hepatocytes expressed high levels of hPBGD protein 2 h after administration. On day 10 post-injection, this protein is still detectable, indicating that it can remain in the liver for a long time (*116*). During an acute attack of AIP induced with phenobarbital, a single injection of *hPBGD* mRNA (0.2 or 0.5 mg/kg) can rapidly normalize ALA and PBG levels. Intravenous injection of mRNA can prevent acute attacks of AIP. There is a link between the dose and efficiency of a single administration (*17*). Mice with AIP that received *hPBGD* mRNA (0.05 or 0.1 mg/kg) had partial protection against PBG accumulation and pain. In contrast, doses of *hPBGD* mRNA (0.2 or 0.5 mg/kg) provided full protection against PBG accumulation and pain (*102,117*).

Surprisingly, intravenous injection of *hPBGD* mRNA can protect against the significant up-regulation of hepatic ALAS1 during recurrent acute attacks of AIP in mice (*117*). *hPBGD* mRNA can normalize the storage of heme in hepatocytes. *hPBGD* mRNA can not only deal with abnormal biochemical indicators but also protect against pain and movement disorders. It also normalizes high blood pressure during acute attacks (*17,117*). Repeated intravenous injection of *hPBGD* mRNA was well tolerated by mice and non-human primates. No adverse events occurred during administration or follow-up (*117*). After single and repeated intravenous injections of *hPBGD* mRNA (0.5 mg/kg), PBGD protein activity increased by 80% while liver function was still within the normal range. In addition, there were no significant changes in antibodies against the hPBGD protein (ADA) (*113,117*).

In general, intravenous injection of *hPBGD* mRNA can induce the expression of PBGD protein in non-human hepatocytes and rapidly normalize the excretion of porphyrin precursors during acute attacks (*117*). Repeated administration has sustained efficacy without causing adverse events. Moreover, *hPBGD* mRNA protects against hypertension, pain, and movement disorders. Clinical trials need to be conducted to verify the safety and feasibility of this promising treatment (*102*).

4. Long-term monitoring and treatment of chronic complications

Chronic complications of AIP include systemic arterial hypertension, renal impairment, liver impairment, HCC, chronic pain, a few psychiatric symptoms, and peripheral neuropathy. AIP causes a high incidence of systemic arterial hypertension, which is a key cause of renal impairment, so timely antihypertensive treatment should be provided. Annual examination of creatinine and eGFR are also recommended for all patients. In addition, patients with AIP should refrain from using nephrotoxic drugs and drink more water (*16*). The angiotensin-converting enzyme inhibitor losartan may delay the development of renal impairment (*10,102*). If the patient has already developed renal impairment, blood purification or kidney transplantation is often helpful. For patients with AIP and kidney failure, renal transplantation or combined liver-kidney transplantation can have a good curative effect (*16, 102*). Liver impairment is common in patients with AIP. The incidence of HCC in symptomatic patients with AIP over 50 years of age is about 3%, which is about 80 times higher than that in individuals without AIP (*1,10,118*). Liver enzymes and liver function should be regularly checked in patients to determine if liver impairment has occurred (*16,46*). Alpha-fetoprotein (AFP) should be checked and ultrasound should be performed annually on patients over the age of 50 years to facilitate the early discovery of HCC (*10,16,46*). Screening for HCC is even more important if PBG and ALA levels continue to rise (*10*). Treatment with ursodeoxycholic acid and cholestyramine is a potential option for patients with liver impairment. Elimination of redundant protoporphyrin *via* plasma exchange can also delay the progression of liver impairment (*119*). OLT is the most thorough treatment for liver failure and HCC. Pain due to porphyria can be chronic and should be prevented from becoming acute pain. Medications such as gabapentin and amitriptyline can alleviate chronic pain (*28*). Patients with AIP may also experience a variety of psychiatric symptoms, such as depression and anxiety. In addition, individuals with AIP are four times more likely to suffer from bipolar disorder or schizophrenia than those without AIP (*120*). Peripheral neuropathy can also become chronic and may present as multiple mononeuritis or myasthenic syndrome (*28*). Therefore, patients with AIP should be monitored for psychiatric symptoms and peripheral neuropathy, and rehabilitation therapy should be provided.

5. Conclusion

As a rare disease, AIP can pose a challenge in terms of treatment and management. Heme and glucose are specific treatments for acute attacks. In addition, symptomatic treatment is necessary. Educating patients to eliminate precipitating factors is the key to preventing attacks. Heme prophylaxis can effectively control recurrence. The emergence of givosiran represents

Table 2. Symptomatic therapy and safe medicines for acute attacks

Symptoms	Medication/therapy
Autonomic neuropathy	
Abdominal pain	Acetaminophen Non-steroidal anti-inflammatory drugs Morphine and buprenorphine
Nausea	Ondansetron
Vomiting	Chlorpromazine Promethazine
Intestinal obstruction	Glycogen and symptomatic treatment
Constipation	Lactulose
Urinary retention	Urethral catheter
Tachycardia	Beta blockers
Hypertension	Angiotensin-converting enzyme inhibitors Beta blockers Calcium channel blockers
Central neuropathy	
Seizures	Correction of hyponatremia and hypertension Gabapentin Levetiracetam Diazepam Propofol
SIADH	Fluid restriction Infusion of saline solution Vaptans Loop diuretics
Peripheral neuropathy	
Muscle weakness	Rehabilitation
Respiratory muscle paresis	Mechanical ventilation
Psychosis	
Insomnia and/or anxiety	Benzodiazepines including zopiclone and lorazepam Chlorpromazine
Depression	Fluoxetine Duloxetine
Hallucinations	Phenothiazines Olanzapine

great progress in treating AIP based on its etiology, and *hPBGD* mRNA is a promising treatment. Liver transplantation is the last resort for patients with AIP. Generally, patients with AIP need long-term monitoring. Symptomatic therapies and safe medicines to treat acute AIP attacks are summarized in Table 2.

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Original Article

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Reactive astrocytes express Aggregatin (*FAM222A*) in the brains of Alzheimer's disease and Nasu-Hakola disease

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SUMMARY By combining genomic data and brain imaging data, a recent study has identified a novel gene named *FAM222A* that participates in the formation of amyloid-β (Aβ) plaques and brain atrophy in Alzheimer's disease (AD). *FAM222A* encodes a 47-kDa protein designated Aggregatin that accumulates in the center of amyloid plaques and physically interacts with Aβ to facilitate Aβ aggregation. Aggregatin is expressed predominantly in the central nervous system (CNS) and its levels are increased in brains of the patients with AD and in mouse models of AD. However, at present, the precise cell types that express Aggregatin in the human CNS remain unknown. By immunohistochemistry, we studied Aggregatin expression in the frontal lobe of the patients with AD, Nasu-Hakola disease (NHD), and the subjects who died of non-neurological causes (NNC). We identified the clusters of Aggregatin-positive reactive astrocytes distributed widely in the cerebral cortex of most cases examined. In contrast, small numbers of cortical neurons showed variable immunoreactivities for Aggregatin, whereas microglia and oligodendrocytes did not express Aggregatin. Importantly, amyloid plaques were not clearly labelled with anti-Aggregatin antibody. These results suggest that Aggregatin plays a primarily role in generation of reactive astrocytes in the human CNS.

Keywords Aggregatin, Alzheimer's disease, amyloid plaque, FAM222A, Nasu-Hakola disease, reactive astrocytes

1. Introduction

Alzheimer's disease (AD) is a progressive intractable dementia that chiefly affects elderly persons. Pathologically, it is characterized by the extracellular plaque deposits of the amyloid-β (Aβ) peptide and the intracellular accumulation of the neurofibrillary tangles composed of the microtubule binding protein tau, resulting in profound activation of microglia, reactive gliosis, and extensive neurodegeneration (*1*). Recently, by combining genomic data and brain imaging data, a research group has identified a novel gene named *FAM222A* that contributes to the formation of Aβ plaques and brain atrophy in AD (*2*). *FAM222A* encodes a single 47-kDa protein designated Aggregatin that highly accumulates in the center of amyloid plaques. Aggregatin physically interacts with Aβ *via* its N-terminal Aβ binding domain (NABD), and facilitates Aβ aggregation. Aggregatin cross-seeds Aβ *via* direct binding. Actually, in a direct binding assay, recombinant Aggregatin (rAggregatin) co-precipitates with $Aβ_{1-40}$ and $Aβ_{1-42}$, while immobilized $Aβ_{1-40}$ or $Aβ_{1-42}$ binds to rAggregatin. Recombinant NABD of Aggregatin by itself is capable of binding to amyloid deposits of $Aβ_{1-42}$. Importantly, Aggregatin protein levels were upregulated in the brains of AD and mouse models of AD (*2*). Overexpression of Aggregatin in neurons enhances amyloid deposition and associated neuroinflammation, whereas deletion of Aggregatin inhibits amyloid deposition and subsequent neuroinflammation. Thus, Aggregatin acts as a potent seeding factor for Aβ oligomerization and aggregation. Reducing aberrant accumulations of Aggregatin might serve as a therapeutic approach for preventing progression of AD. However, at present, the precise cell types that express Aggregatin in AD and non-AD diseases remain unknown.

Nasu-Hakola disease (NHD), also designated polycystic lipomembranous osteodysplasia with sclerosing leukoencephalopathy (PLOSL; OMIM 221770), is a rare autosomal recessive disorder, characterized by progressive presenile dementia and formation of multifocal bone cysts, caused by loss-of-function mutations of either

TYROBP(DAP12) or *TREM2* (*3*). Clinically, the patients with NHD show recurrent bone fractures during the third decade of life, a frontal lobe syndrome during the fourth decade of life, and progressive dementia and death until the fifth decade of life (*4*). Pathologically, NHD brains exhibit extensive demyelination designated leukoencephalopathy, astrogliosis, accumulation of axonal spheroids, and remarkable activation of microglia predominantly in the white matter of frontal and temporal lobes and the basal ganglia (*5*). Although NHD patients are clustered in Japan and Finland, approximately 200 NHD cases are presently reported worldwide. Because TREM2 and DAP12 constitute a receptor/adaptor signaling complex expressed exclusively on osteoclasts, dendritic cells, macrophages, and microglia, it is generally supposed that a complete loss of function of the TREM2-DAP12 signaling pathway in microglia induces NHD (*6*). Notably, a rare variant of the *TREM2* gene encoding p.Arg47His causes a 3-fold increase in the risk for late-onset AD (*7*). Thus, neurodegenerative processes in NHD and AD share a common pathological mechanism of aberrant TREM2 signaling in microglia.

In the present study, we studied Aggregatin expression in the frontal lobe of AD, NHD, and non-neurological controls (NNC) by immunohistochemistry.

2. Materials and Methods

2.1. Human brain tissues

The brain autopsies were performed at the National Center Hospital, National Center of Neurology and Psychiatry (NCNP), Japan, Kohnodai Hospital, National Center for Global Health and Medicine (NCGM), Japan, and affiliated hospitals of Research Resource Network (RRN), Japan. The comprehensive examination by established neuropathologists (YS and TI) validated the pathological diagnosis. In all cases, written informed consent was obtained. The Ethics Committee of the NCNP for the Human Brain Research, the Ethics Committee of the NCGM on the Research Use of Human Samples, and the Human Research Ethics Committee of the Meiji Pharmaceutical University (MPU) approved the present study.

For immunohistochemical studies, serial sections of the frontal cortex were prepared from four subjects who died of non-neurological causes (NNC), composed of three men and one woman with a mean age of 79.0 ± 9.2 years, ten AD patients, composed of five men and five women with a mean age of 69.9 ± 8.2 years, and five NHD patients, composed of three men and two women with a mean age of 40.8 ± 6.1 years. The homozygous mutation of a single base deletion of 141G (c.141delG) in exon 3 of DAP12 was identified in three NHD patients. All AD cases were satisfied with the Consortium to Establish a Registry for Alzheimer's Disease (CERAD) criteria for diagnosis of definite AD (*8*). They were categorized into the stage C of amyloid deposition and the stage VI of neurofibrillary degeneration, following the Braak's staging (*9*).

2.2. Immunohistochemistry

After deparaffination, tissue sections were heated in 10 mM citrate sodium buffer, pH 6.0 by autoclave at 110℃ for 15 min in a temperature-controlled pressure chamber (Biocare Medical, Pacheco, CA, USA). They were treated at room temperature (RT) for 15 min with 3% hydrogen peroxide-containing methanol to block the endogenous peroxidase activity. They were then incubated with phosphate-buffered saline (PBS) containing 10% normal goat serum at RT for 15 min to block non-specific staining, followed by incubation in a moist chamber at 4℃ overnight with rabbit polyclonal anti-Aggregatin antibody (C12orf34; ab122626, Abcam, Cambridge, UK). The specificity of anti-Aggregatin antibody was validated by western blot analysis of recombinant human Aggregatin protein expressed in HEK293 cells, which were transfected with the pcDNA4/HisMax TOPO vector (Thermo Fisher Scientific, Carlsbad, CA, USA) containing the full-length Aggregatin sequence. The antigen sequence that anti-Aggregatin antibody recognizes is composed of PSIHSLLYQL NQQCQAPGAA PPACQGMAIP HPSPAKHGPV PSFPSMAYSA AAGLPDCRKG TELGQGATQA LTLAGAAKPA GYADSGLDYL LWPQKP, corresponding to amino acids 197-292 of human Aggregatin.

After washing with PBS, tissue sections were incubated at RT for 30 min with horseradish peroxidase (HRP)-conjugated secondary antibody (Nichirei, Tokyo, Japan), followed by incubation with diaminobenzidine tetrahydrochloride (DAB) substrate (Vector, Burlingame, CA, USA). Enhancement was not applied to DAB staining. They were processed for a counterstain with hematoxylin. Negative controls underwent all the steps except for exposure to primary antibody. In limited experiments, double immunolabeling was performed using rabbit anti-Aggregatin antibody in combination with mouse monoclonal antibodies against glial fibrillary acidic protein (GFAP) (GA5, Nichirei, for an marker of astrocytes), gp91phox (ab139371, Abcam, for a marker of microglia), apolipoprotein E (ApoE; ab1906, Abcam), phospho-tau (AT8, Thermo Fisher Scientific) or Aβ peptide ($A\beta_{11-28}$; 12B2, Immunobiological Laboratories, Gunma, Japan), followed by incubation with HRP-conjugated or alkaline phosphatase-conjugated anti-rabbit or anti-mouse secondary antibody and exposure to DAB substrate and Warp Red chromogen (Biocare Medical).

2.3. RT-PCR analysis

Total cellular RNA was extracted from human neural

cell lines and tissues with TRIZOL (Thermo Fisher Scientific). Pooled human frontal lobe total RNA (#636563, Clontech, Mountain View, CA, USA) was utilized to prepare human brain cDNA. RNA treated with DNase I was processed for cDNA synthesis by using oligo$(dT)_{20}$ primers and SuperScript II reverse transcriptase (Thermo Fisher Scientific). PCR cocktails were prepared by mixing with sense and antisense primer sets following: 5'agcacggtggccagcaagtcccctgag3' and 5'ttatctgtagacgggtaggcggatgtg3' for detection of a 444 bp product of the *FAM222A* gene (NM_032829.3). Then, cDNA was amplified by PCR with HotStarTaq DNA Polymerase (Qiagen, Valencia, CA, USA) on a PC815 thermal cycler (Astec, Fukuoka, Japan) at 95°C for 1 min for denaturation, at 62°C for 40 sec for annealing, and 72°C for 50 sec for extension for 35 cycles.

3. Results

First of all, we studied the expression of Aggregatin in human neural cell lines by RT-PCR analysis. We found that Aggregatin is ubiquitously expressed in various human neural cell lines, along with astrocytes in culture (Figure 1, lanes 1, 3-10), while we did not detect Aggregatin in cDNA samples of the human frontal lobe prepared without inclusion of reverse transcription step (Figure 1, lane 2). Next, we validated the specificity of anti-Aggregatin antibody ab122626 by western blot analysis of Xpress-tagged recombinant human Aggregatin protein expressed in HEK293 cells (Figure 2, panels a-c, lane 2). Then, by immunohistochemistry using ab122626, we identified an intense expression of Aggregatin immunoreactivity chiefly in reactive astrocytes forming clusters and surrounding glial scar distributed in the grey matter of the frontal cortex derived from AD and NHD patients (Figures 3, panels a-c and Figure 4, panel a).

In addition, Aggregatin immunoreactivity was detectable in reactive astrocytes occasionally found in the brains of NNC (Figure 4, panel b). Aggregatin immunoreactivity was located in the cell soma and proximal processes of reactive astrocytes. By double immunolabelling, Aggregatin-positive reactive astrocytes expressed GFAP (Figure 5, panel b). Some of the Aggregatin-expressing astrocytes showed a binuclear morphology, suggesting that they underwent active proliferation (Figure 6, panel a, arrows).

In contrast, surviving oligodendrocytes distributed in the white matter and gp91phox-positive microglia in AD brains did not display Aggregatin (Figure 4, panel c and Figure 5, panel a), while we found inconsistent staining of blood vessel walls and variable immunoreactivities for a subset of cortical degenerating neurons (Figure 3, panel d and Figure 4, panel d). No immunoreactivity was detected, when the primary antibody was omitted from the reaction (Figure 5, panel c). Although reactive astrocytes forming clusters were often closely associated with amyloid plaques, the plaques themselves were not clearly labelled with anti-Aggregatin antibody (Figure 5, panel d and Figure 6, panel a, b). Furthermore, the centers of amyloid plaques were devoid of Aggregatin immunoreactivity (Figure 6, panel b). By double immunolabelling, phospho-tau-positive intraneuronal inclusions in AD brains were not stained with anti-Aggregatin antibody (Figure 6, panel c). In addition, the majority of Aggregatin-immunoreactive astrocytes in AD brains did not express ApoE (Figure 6, panel d).

4. Discussion

By combining genomic data and brain imaging data, a recent study has identified a novel gene named

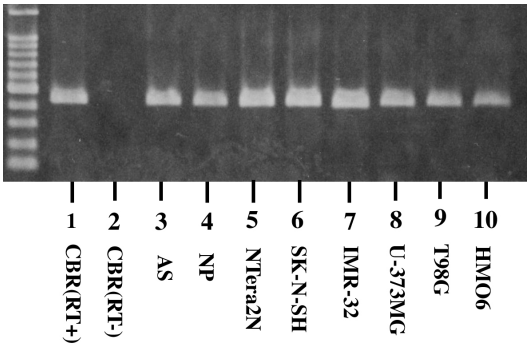

Figure 1. Aggregatin expression in human neural cell lines. The mRNA expression was studied by RT-PCR in human tissues and cultured cells. The lanes **(1-10)** indicate **(1)** the frontal cortex of the human cerebrum (CBR) with inclusion of the reverse transcription (RT) step, **(2)** CBR without inclusion of the RT step, **(3)** astrocytes (AS), **(4)** neuronal progenitor (NP) cells, **(5)** NTera2 teratocarcinoma-derived neurons (NTera2N), **(6)** SK-N-SH neuroblastoma, **(7)** IMR-32 neuroblastoma, **(8)** U-373MG glioblastoma, **(9)** T98G glioblastoma, and **(10)** HMO6 immortalized microglia. cDNA was amplified by PCR for 35 cycles.

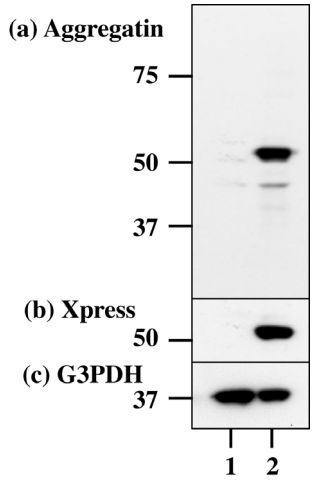

Figure 2. The specificity of Aggregatin antibody. Western blot of non-transfected HEK293 cells **(lane 1)** and the cells transfected with the vector containing the full-length Aggregatin sequence **(lane 2)**. **(a)** Aggregatin (ab122626), **(b)** Xpress tag, and **(c)** G3PDH as a loading control. The position of molecular weight markers is shown on the left.

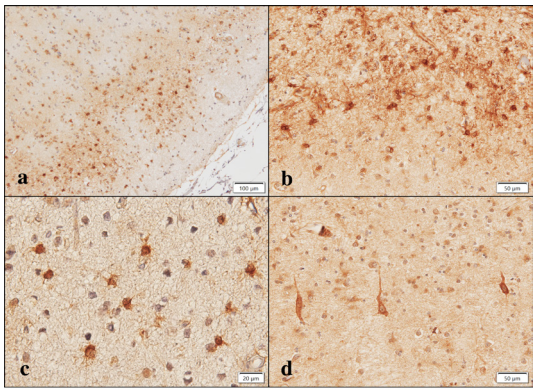

Figure 3. Aggregatin expression in AD brains. Aggregatin is expressed in **(a)** reactive astrocytes, **(b)** reactive astrocytes and glial scar, **(c)** reactive astrocytes at higher magnification, and **(d)** degenerating neurons.

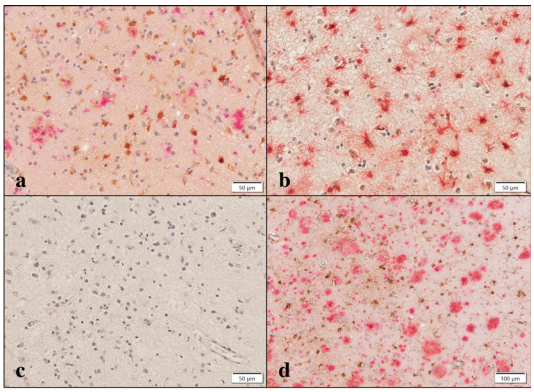

Figure 5. Aggregatin expression in AD brains. (a) double immunolabeling of Aggregatin (brown) and gp91phox (red), **(b)** double immunolabeling of Aggregatin (brown) and GFAP (red), **(c)** the negative control without inclusion of the primary antibody, and **(d)** double immunolabeling of Aggregatin (brown) and Aβ (red).

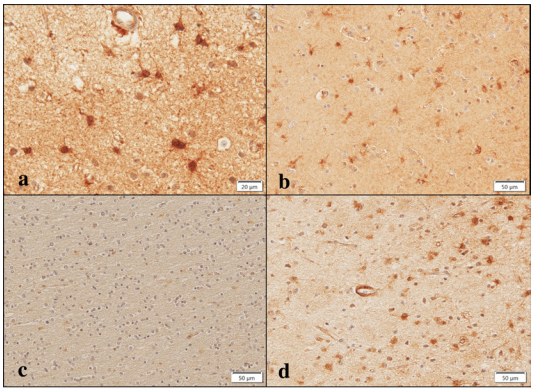

Figure 4. Aggregatin expression in NHD, NNC, and AD brains. Aggregatin is expressed in **(a)** reactive astrocytes at higher magnification, NHD, **(b)** reactive astrocytes, NNC, **(c)** no immunolabeling on oligodendrocytes, AD, and **(d)** a small blood vessel and reactive astrocytes, AD.

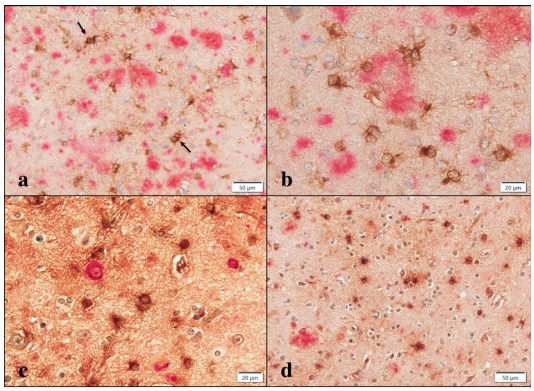

Figure 6. Aggregatin expression in AD brains. (a) double immunolabeling of Aggregatin (brown) and Aβ (red). Arrows indicate reactive astrocytes with a binuclear morphology, **(b)** double immunolabeling of Aggregatin (brown) and Aβ (red) at higher magnification. The center of amyloid plaque is devoid of Aggregatin immunoreactivity, **(c)** double immunolabeling of Aggregatin (brown) and AT8 (red) at higher magnification. AT8-expressing intraneuronal inclusions are negative for Aggregatin immunoreactivity, and **(d)** double immunolabeling of Aggregatin (brown) and ApoE (red). Aggregatin-expressing reactive astrocytes are negative for ApoE immunoreactivity.

FAM222A encoding Aggregatin involved in the formation of Aβ plaques and brain atrophy in AD (*2*). The authors found that Aggregatin is concentrated on the center of amyloid plaques and physically interacts with Aβ to facilitate Aβ aggregation. However, the previous study did not characterize the precise cell types expressing Aggregatin in the central nervous system (CNS). By immunohistochemistry, we found an intense immunoreactivity for Aggregatin expressed predominantly in reactive astrocytes in AD, NHD, and NNC brains. Therefore, we supposed that Aggregatin plays a pivotal role in generation of reactive astrocytes in the human CNS. A subpopulation of degenerating neurons also expressed Aggregatin in AD and NHD brains, suggesting that Aggregatin expression is not purely specific for astrocytes. In contrast, amyloid plaques and cores were negative for Aggregatin immunoreactivity in most AD cases. An obvious difference in the results of the previous study and the present study is in part attributable to different tissue sections and immunohistochemical protocols utilized, although the primary antibody tested (ab122626) is the same in both studies (*2*). Importantly, human astrocytes in culture exhibited Aggregatin by RT-PCR, being consistent with our observations.

Astrocytes represent the most numerous cell type uniformly distributed throughout the CNS. Astrocytes play a pivotal role in various physiological processes, including glutamate homeostasis, blood-brain barrier permeability, metabolic support of neurons, and synaptic development and plasticity (*10*). Reactive astrocytes are induced by exposure to various stressful insults in the brain, such as brain injury, ischemia, inflammation, and neurodegeneration, associated with marked upregulation of GFAP, vimentin, and nestin, those of which represent the markers of astrocyte reactivity (*10*). Recent studies indicate that reactive astrocytes are composed of two distinct subtypes with different pathological properties designated neurotoxic A1 and neuroprotective A2

astrocytes (*11*). Numerous A1 astrocytes induced by activated microglia accumulates in AD brains (*11*), suggesting that Aggregatin-expressing reactive astrocytes surrounding amyloid plaques belong mostly to A1.

In NHD brains, along with AD brains, Aggregatin expression was actively induced in reactive astrocytes. NHD is pathologically characterized by extensive demyelination and profound astrogliosis (*5*). The molecular mechanisms underlying upregulation of Aggregatin in reactive astrocytes are overlapping between NHD and AD, and even in aged NNC.

Under neurodegenerative and neuroinflammatory circumstances, reactive astrocytes capable of producing a detectable amount of Aβ, constitute an integral component of amyloid plaques in AD (*12*). We found that Aggregatin-expressing reactive astrocytes are frequently associated with amyloid plaques in AD brains, as previously described (*13*). These results suggest that Aggregatin-expressing reactive astrocytes might contribute to the continuous production of amyloid plaques in AD.

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Original Article

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Two new missense mutations in the protein interaction ASH domain of OCRL1 identified in patients with Lowe syndrome

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SUMMARY The oculocerebrorenal syndrome of Lowe is a rare X-linked disease characterized by congenital cataracts, proximal renal tubulopathy, muscular hypotonia and mental impairment. This disease is caused by mutations in the *OCRL* gene encoding membrane bound inositol polyphosphate 5-phosphatase OCRL1. Here, we examined the *OCRL* gene of two Lowe syndrome patients and report two new missense mutations that affect the ASH domain involved in protein-protein interactions. Genomic DNA was extracted from peripheral blood of two non-related patients and their relatives. Exons and flanking intronic regions of *OCRL* were analyzed by direct sequencing. Several bioinformatics tools were used to assess the pathogenicity of the variants. The three-dimensional structure of wild-type and mutant ASH domains was modeled using the online server SWISS-MODEL. Clinical features suggesting the diagnosis of Lowe syndrome were observed in both patients. Genetic analysis revealed two novel missense variants, c.1907T>A (p.V636E) and c.1979A>C (p.H660P) in exon 18 of the *OCRL* gene confirming the clinical diagnosis in both cases. Variant c.1907T>A (p.V636E) was inherited from the patient's mother, while variant c.1979A>C (p.H660P) seems to have originated *de novo*. Analysis with bioinformatics tools indicated that both variants are pathogenic. Both amino acid changes affect the structure of the OCRL1 ASH domain. In conclusion, the identification of two novel missense mutations located in the OCRL1 ASH domain may shed more light on the functional importance of this domain. We suggest that p.V636E and p.H660P cause Lowe syndrome by disrupting the interaction of OCRL1 with other proteins or by impairing protein stability.

Keywords *OCRL*, Lowe syndrome, missense mutation, ASH domain

1. Introduction

The oculocerebrorenal syndrome of Lowe (OMIM #309000) is a rare X-linked disorder characterized by congenital cataracts, intellectual impairment, and progressive renal tubular dysfunction (*1,2*). Other features include muscular hypotonia, severe growth retardation and behavioral problems. Cataracts and muscular hypotonia are evident at birth whereas the proximal tubulopathy is frequently diagnosed later in life. The tubular dysfunction involves low-molecular-weight proteinuria, aminoaciduria and hypercalciuria, and usually results in progressive renal failure leading to end-stage renal disease in adulthood (*1,3*). The disease is caused by mutations in the *OCRL* gene encoding a membrane bound inositol polyphosphate 5-phosphatase (OCRL1), which dephosphorylates the 5' position of the inositol ring in membrane phospholipids (*4*). The *OCRL* gene is located on chromosome Xq26.1 and is expressed in nearly all tissues (*5*). It contains 24 exons including exon 18a, which is alternatively spliced and mostly expressed in brain (*4,6*). Lowe syndrome affects almost exclusively males, although a few female carriers with the clinical symptoms of the disease have been reported (*7,8*). Interestingly, some mutations of the *OCRL* gene cause Dent-2 disease (OMIM #300555), which presents a similar proximal tubule dysfunction but only mild or no additional clinical defects (*9-11*). There seems to be a continuous range of symptoms, varying from very severe features of Lowe syndrome to mild forms of Dent-2 disease that are mostly restricted to the kidney (*1*). In most cases, genotype–phenotype

correlations have not been established.

OCRL1 is a multi-domain protein that is comprised of an N-terminal pleckstrin homology (PH) domain followed by a 5-phosphatase catalytic domain, an ASPM-SPD-2-Hydin (ASH) domain, and a C-terminal RhoGAP-like domain that is catalytically inactive (*12-14*). The PH domain, ASH domain and RhoGAP-like domain specify the subcellular localization and function of the enzyme. The OCRL1 protein localizes predominantly at the trans-Golgi network and early endosomes, and is also present at the plasma membrane, endocytic clathrin-coated pits and vesicles, intercellular junctions of polarized cells, and primary cilium (*2*). This wide subcellular distribution is mediated by its interactions with different proteins, such as clathrin heavy chain, the clathrin adaptor AP-2, several Rab and Rho GTPases, and the endocytic proteins APPL1, Ses1 and Ses2. The ASH-RhoGAP module regulates the interactions of OCRL1 with many proteins. In connection with its diverse cellular locations and interactions, OCRL1 is involved in different functions including endocytic trafficking, cell migration and polarity, actin polymerization and ciliogenesis (*2*). However, its precise role in disease is not fully comprehended.

Lowe syndrome has been described in all ethnicities and its prevalence in the general population has been estimated to be between 1:500,000 and 1:1,000,000 (*1,2,15*). Approximately 63% of *OCRL* mutations identified in patients with Lowe syndrome are nonsense, frameshift, or splice site mutations that lead to premature termination of the OCRL1 protein or mRNA decay, while missense mutations comprise about 33% (*16-18*). Most mutations causing Lowe syndrome are located in exons 9-23, and affect the 5-phosphatase, the ASH and the RhoGAP-like domains (*2*). Missense mutations in the ASH and RhoGAP domains usually abolish interactions with other proteins and affect OCRL1 targeting (*19-23*).

Here, we report the identification of novel missense variants affecting the ASH domain of OCRL1 in two patients with Lowe syndrome. The potential pathogenic consequences of these two variants were evaluated using bioinformatics tools and protein modeling.

2. Materials and Methods

2.1. Patients and genetic analysis

The study included two non-related affected male patients who fulfilled the classically recognized criteria of Lowe syndrome diagnosis including mental retardation, bilateral congenital cataract, congenital hypotonia, and tubulopathy. One child originated from Brazil and the other one from Spain. Neither of them had a family history of the disease. Written informed consent for the genetic analysis was obtained from the patient's parents. The Ethics Committee of Hospital Universitario Nuestra Señora de Candelaria (Santa Cruz de Tenerife, Spain) approved the protocols of this study, which was conducted according to the Declaration of Helsinki.

We performed a mutational analysis of the affected patients and their relatives by direct DNA sequencing. Genomic DNA was extracted from peripheral blood samples using the Gen Elute Blood Genomic DNA kit (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's instructions. Coding exons and intronic flanking regions of *OCRL* were amplified by polymerase chain reaction (PCR) using intronic primers previously described (*9*). PCR products were purified with the NucleoSpin Gel and PCR Clean-up (Macherey-Nagel, Düren, Germany). Purified products were sent out to Macrogen Spain Inc. (Madrid, Spain) for DNA sequencing. Mutations were confirmed by sequencing additional independent amplification products. Variant position was based on the cDNA sequence (Ensembl *OCRL* transcript ID: ENST00000371113.9) using the first coding ATG of exon 1 as initiation codon. We examined several databases, including Human Gene Mutation database (HGMD, *http://www.hgmd.cf.ac.uk/ac/index.php*), ClinVar (*https://www.ncbi.nlm.nih.gov/clinvar/*), 1000 Genomes Project (*http://www.1000genomes.org/*) and gnomAD database (*https://gnomad.broadinstitute.org/*), to verify that the variants detected were not common polymorphism and to confirm that they were novel.

2.2. Bioinformatics analysis

The potential functional effect of amino acid substitutions generated by the variants on the OCRL1 protein was assessed using several bioinformatics tools including SIFT (*http://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html*) (*24*), PolyPhen-2 (*http://genetics.bwh.harvard.edu/pph2/*) (*25*), CADD (*https://cadd.gs.washington.edu/*) (*26*), MutPred2 (*http://mutpred.mutdb.org/*) (*27*), and MutationTaster (*http://www.mutationtaster.org/*) (*28*). We also applied VarSome, an integrated search engine that allows accessing multiple pathogenicity prediction tools and databases (*https://varsome.com/*) (*29*). In this case, variant pathogenicity is reported using an automatic classifier that evaluates the submitted variant according to the American College of Medical Genetics and Genomics (ACMG) guidelines (*30*). Protein stability modifications resulting from missense variants were assessed with web-based programs MUpro (*http://mupro.proteomics.ics.uci.edu/*) (*31*) and INPS (*https://inpsmd.biocomp.unibo.it/inpsSuite*) (*32*) using the OCRL1 protein sequence. These applications provide the calculated free energy change value (DDG). A DDG value below zero indicates that the stability of the protein has decreased, whereas a DDG higher than zero means it has increased. The

protein sequence of human OCRL1 (901 amino acids) was obtained from the NCBI database (*https://www.ncbi.nlm.nih.gov/protein/*; accession number NP_000267.2).

2.3. Protein modeling

The three-dimensional (3-D) structures of wild-type and mutant ASH domains were predicted using the online modeling server SWISS-MODEL (*https://swissmodel.expasy.org/*) with the crystal structure of OCRL1 (amino acid residues 540-678) in complex with Rab8a:GppNHp (3qbt.3.B) as a template (*21*).

3. Results

3.1. Clinical characteristics of patients

Patient 1, a 16-year-old boy from Spain, was the third child of a healthy mother. His father had hypercholesterolemia. The patient's medical history revealed that he was born at term after a normal pregnancy. His birth weight was 3,200 g. Ophthalmological examination at the age of 2 months revealed bilateral congenital cataracts, and he had cataract surgery at three months of life. He showed mild muscular hypotonia and mild psychomotor retardation, but the results of neuroimaging tests were normal. In the first years of life he also presented ocular nystagmus with horizontal wide-angle eye movements, a slight coordination impairment in rapid alternating movements of upper limbs, a distinctive facial expression, and initial rejection of solid foods due to difficulty in chewing that has improved with speech therapy treatment. Lowe syndrome was suspected, and a renal function study was performed that confirmed data of proximal tubulopathy with incomplete Fanconi syndrome (hypercalciuria and tubular proteinuria). Glycosuria, phosphaturia and metabolic acidosis were not observed. His clinical evolution was appropriate, with a normal estimated glomerular filtration rate (103 ml/min/1,73m^2). He maintained a moderate hypercalciuria (calcium/creatinine ratio of 0.3-0.4 mg/mg) until 10 years of age when it was normalized. The urinary elimination of citrate was adequate (citrate/creatinine ratios > 300 mg/g), with some hypocitraturia in periods of higher calciuria. Ultrasound examination showed that he had not developed nephrocalcinosis. His tubular proteinuria is moderate, with protein/creatinine ratios between 1-0.8 mg/mg at the expense of β_2 microglobulin. In the psychometric assessment at age 14, administration of the Wechsler Intelligence Scale for Children-IV, showed that his intelligence quotient was 67. The child is in school with curricular adaptation and psychoeducational support.

Patient 2 was an 11-year-old boy from Brazil whose mother descends from a Spanish family. He was born with 37 weeks gestation. His birth weight and length were 3,120 g and 50 cm, respectively. During pregnancy his mother had hypertension and polyhydramnios. His sister was healthy. Muscular hypotonia and congenital cataracts were diagnosed at the age of 1 month. Clinical tests showed proteinuria by the age of 9 months. He evolved with delayed development (cognitive deficit) and partial loss of vision due to bilateral congenital cataracts that were corrected surgically, glaucoma and nystagmus. The child presents typical facies of Lowe syndrome with front protrusion and deep eyes, and short stature (-2.3 SD). He also has attention deficit disorder and hyperactivity. Further examination revealed metabolic acidosis, hypercalciuria, hyperuricosuria and proteinuria (protein/creatinine ratios between 8.7-4.4 mg/mg), and no nephrocalcinosis. His last estimated glomerular filtration rate is normal (111 ml/min/1,73m^2).

3.2. Identification of novel pathogenic *OCRL* variants and bioinformatics predictions

To confirm the clinical diagnosis of Lowe syndrome, we carried out a mutational analysis of the *OCRL* in the two patients and his relatives. The results revealed two novel hemizygous variants in exon 18 (Figure 1). These results were confirmed by bidirectional sequencing of independent PCR products. The variant identified in patient 1 consisted in the substitution of a T for an A in codon 636, which predicts the change of valine for glutamic acid in the OCRL1 protein (c.1907T>A; p.V636E; g.36496T>A; Chr23:128710321T>A; Ensembl transcript ID: ENST00000371113) (Figure 1A). His mother and one of his sisters were healthy carriers of this mutation, while the father and the other sister showed the normal sequence (Figure 1A). The variant detected in patient 2 involved an A to C change in codon 660 that predicts the substitution of histidine for proline (c.1979A>C; p.H660P; g.36568A>C; Chr23:128710393A>C; Ensembl transcript ID: ENST00000371113) (Figure 1B). The mother of patient 2 was not a carrier of the variant, suggesting this mutation occurred *de novo* (Figure 1B). His father also displayed the normal sequence. Examination of the protein sequence indicates that both variants are located in the ASH domain of OCRL1 (Figure 2). The amino acid residues affected by these two variants are highly conserved among different species (human, mouse, pig, chicken, fish and frog) (Figure 2B), and, therefore, they are probably important for the structure and function of the protein. These *OCRL* variants, c.1907T>A (p.V636E) and c.1979A>C (p.H660P), had not been reported in the literature and were absent from population and clinical databases including gnomAD, ClinVar, 1000 Genomes Project, and Human Gene Mutation Database. Interestingly, the search in gnomAD retrieved variant p.V636M, found in only one allele (frequency in total population 5.46e-6), which has not been associated with disease. The novel variants

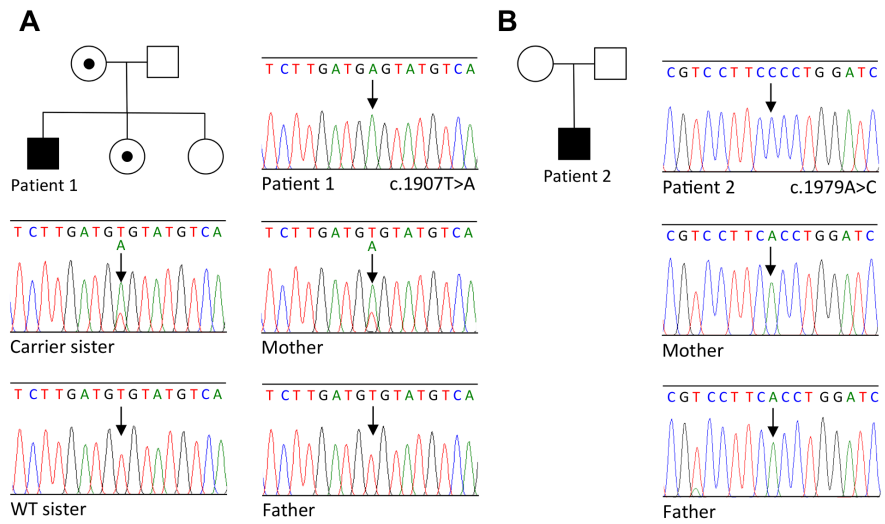

Figure 1. Novel *OCRL* mutations identified in two patients with Lowe syndrome. (A) Partial DNA sequence of *OCRL* exon 18 in patient 1 and his relatives, and family pedigree. Arrows indicate the altered nucleotide position and hemizygous mutation c.1907T>A, p.V636E in the patient. The patient's mother and one of his sisters are carriers of the mutation. The father and the other sister (WT, wild-type) show the normal sequence. **(B)** Partial sequence of exon 18 of *OCRL* in patient 2 and his parents, and family pedigree. Arrows indicate the affected nucleotide position and hemizygous mutation c.1979A>C, p.H660P in the patient. Both parents show the normal sequence suggesting the mutation was originated *de novo*. Circles and squares represent female and male individuals, respectively; black squares represent affected individuals; black circles within open frames denote heterozygous individuals; open circles and squares represent family members with the normal sequence.

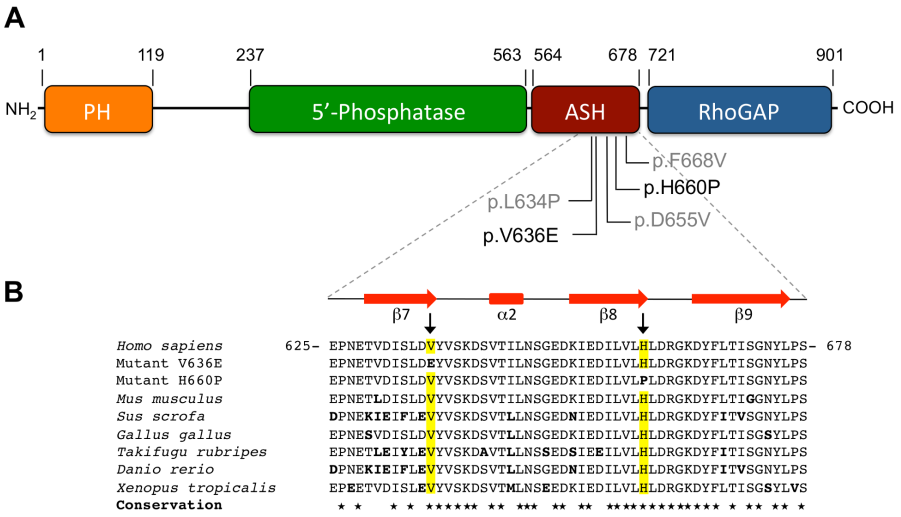

Figure 2. Schematic representation of OCRL1 and location of mutations in the ASH domain. (A) The different protein domains are shown: Pleckstrin homology domain (PH), 5-phosphatase catalytic domain, ASPM, SPD-2, Hydin domain (ASH) and RhoGAP-like domain. The two missense mutations identified in this study, p.V636E and p.H660P, and three previously reported mutations, p.L634P, p.D655V and p.F668V (*3,20,23*) are indicated in black and grey, respectively. **(B)** Multiple sequence alignment analysis of the ASH domain showing evolutionary conservation of valine 636 and histidine 660 (yellow background) among OCRL1 proteins. These amino acid residues are located in β-sheets 7 (β7) and 8 (β8), respectively. Regular and bold letters represent fully conserved and non-conserved residues, respectively.

described here were submitted to ClinVar, National Center for Biotechnology Information (*https://www.ncbi.nlm.nih.gov/clinvar/*) and were included with accession numbers VCV000689472.1 and VCV000689471.1 for c.1907T>A (p.V636E) and c.1979A>C (p.H660P), respectively.

Bioinformatics analysis of p.V636E with five different bioinformatics tools predicted that this variant is pathogenic (Table 1). In addition, the software VarSome classified this substitution as "likely pathogenic" because it met two moderate (PM1 and PM2) and three supporting (PP2, PP3 and PP5) pathogenicity criteria established by the ACMG. All thirteen algorithms included in VarSome inferred the conclusion of pathogenicity. Furthermore, variant p.V636E caused a decrease in OCRL1 protein stability according to programs that predict protein stability of mutated proteins including INPS (DDG in kcal/mol: -2.447) and

Table 1. Bioinformatics predictions of pathogenicity for *OCRL* mutations affecting the ASH domain

Mutation	SIFT (score)	PolyPhen-2 (score)	CADD (score)	MutationTaster (score)	MutPred2 (score)
p.V636E	Affects function (0.00)	Probably damaging (0.999)	Damaging (27.9)	Disease causing (121)	Pathogenic (0.858)
p.H660P	Affects function (0.00)	Probably damaging (0.998)	Damaging (26.6)	Disease causing (77)	Pathogenic (0.915)

Amino acid substitutions with SIFT scores < 0.05 are predicted to be deleterious (scores closer to 0 are more confidently predicted to be deleterious). Variants with HumVar PolyPhen-2 scores in the range 0.85 to 1.0 are more confidently predicted to be damaging. CADD-PHRED scaled CADD score equal or higher than 15 indicates that the variant is damaging. The MutationTaster score is taken from the Grantham Matrix for amino acid substitutions and it ranges from 0.0 to 215. The general score of MutPred2 ranges from 0.0 and 1.0, with a higher score indicating a greater predisposition to be pathogenic.

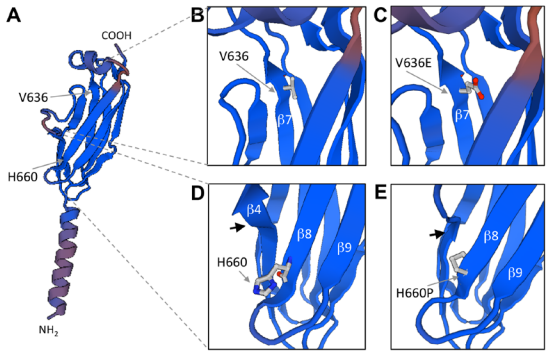

Figure 3. Protein structure modeling of wild-type and mutated ASH domains in ribbon-presentation. The figure shows the ASH domain and α-helix 1 of the 5-phosphatase domain (amino acid residues 564-678). The ASH domain of OCRL1 (residues 564-678) is composed of nine β-strands and a small α-helix. The β-sheets form two layers. Hydrophobic side chains from the two layers occupy the interior of the sandwich. (B and C) Close-ups showing wild-type valine 636 and mutant glutamic acid 636, respectively. (D and E) Close-ups showing wild-type histidine 660 and mutant proline 660, respectively. The black arrow indicates the β-strand 4 (β4) that appears shifted in the mutants ASH domain.

MuPro (DDG in kcal/mol: -1.632). *In silico* analysis of the amino acid change resulting from p.H660P showed that this variant also has a deleterious effect on OCRL1 protein function (Table 1). VarSome analysis classified this variant as likely pathogenic because it met two moderate (PM1 and PM2) and three supporting (PP2, PP3 and PP5) pathogenicity criteria established by the ACMG (*30*). The VarSome pathogenic outcome derived from predictions from eleven out of fourteen scores (versus 3 benign predictions). Furthermore, variant p.H660P caused a decrease in OCRL1 protein stability according to both INPS (DDG in kcal/mol: -1.022) and MuPro (DDG in kcal/mol: -0.924) programs.

3.3. The 3-D structure of mutant ASH domains

To evaluate the structural impact of missense mutations p.V636E and p.H660P, we constructed 3-D models of the wild-type and mutants ASH domains using the SWISS-MODEL software (Figure 3). The protein sequence used contained, in addition to the ASH domain, α-helix 1 of the 5-phosphatase domain (amino acid residues 540-678). Amino acid residue V636 is at the C-terminal end of β-strand 7 that is buried in the core of the ASH domain (Figure 2 and Figure 3). The mutant residue in p.V636E has a larger and less hydrophobic side chain than the wild-type residue, which would cause loss of hydrophobic interactions in the ASH domain core. Additionally, the charge of the mutant residue is negative while the wild-type residue charge is neutral. These differences in amino acid properties could lead to disruptions in proper folding and function. Conversely, residue H660 is positioned close to the C-terminal end of β-strand 8, which is on the surface of the ASH domain (Figure 2 and Figure 3). The mutant residue, proline, is smaller and more hydrophobic than the wild-type residue. The size difference between wild-type and mutant residues makes that the new residue is not in the correct position to create the same hydrogen bond as the original residue did. In addition, the difference in hydrophobicity will affect hydrogen bond formation causing a likely loss of external interactions. Also, the predicted 3-D of the mutant p.H660P ASH domain shows a displacement of β-strand 4 (Figure 3E).

4. Discussion

Patients with Lowe syndrome usually present in childhood with cataracts, progressive growth failure, hypotonia, and proximal tubulopathy, although a wide clinical variability among patients has been described (*1*). The clinical diagnosis can be confirmed by mutational analysis of the *OCRL* gene. More than two hundred *OCRL* variants associated with Lowe syndrome have been identified. Most of the missense variants modify conserved residues in the 5-phosphatase domain, altering its folding, substrate specificity or catalytic activity (*2,14*). There are also several pathogenic missense mutations located in the ASH and RhoGAP domains, some of which abolish interactions with OCRL1 partners and alter the cellular localization of the OCRL1 protein (*19-22*). Fibroblasts derived from Lowe syndrome patients harboring some

of these mutations have reduced 5-phosphatase activity, suggesting that mutations in the ASH and RhoGAP domains somehow disturb OCRL1 activity (*17,33*). These two domains are widely interconnected and form a single folding module; therefore, the destabilization of one of them will affect the stability of the other (*12*). The ASH-RhoGAP module mediates the interactions of OCRL1 with protein partners that facilitate its targeting to different cellular locations such as early endosomes, Golgi complex, lysosomes and primary cilium (*2,19*). The ASH domain has a β-sheet structure similar to the immunoglobulin G domain and contains a Rab-binding site that allows the interaction of OCRL1 with Rab GTPases (*12,21,34*). This interaction is needed for targeting of OCRL1 to the Golgi complex and endosomal membranes. Mutations in the Rab-binding site perturb the interaction between the OCRL1 protein and its partners, and result in membrane targeting defects. In the present study, we analyzed by DNA sequencing the *OCRL* gene of two unrelated patients with the clinical diagnosis of Lowe syndrome; both presented congenital cataracts, developmental delay (mild in one of them), muscular hypotonia and proximal tubulopathy. Two novel missense variants, c.1907T>A (p.V636E) and c.1979A>C (p.H660P) were identified, and both are located in the ASH domain. Variant c.1979A>C (p.H660P) seems to have originated from the patient's DNA or from his mother's germ cells. Genetic studies of mothers of affected males have shown the existence of *de novo* mutation in 30 to 37% of cases (*17,18*).

The ASH domain is comprised of two layers of β-sheets forming a sandwich, and hydrophobic side chains from the two layers occupy the interior space. Amino acid residue valine 636 is located in β-strand 7 of the ASH domain and its replacement for glutamic acid causes loss of hydrophobic interactions in the interior of the ASH domain. Therefore, we suggest that mutation p.V636E causes a folding defect in the ASH domain. Another Lowe syndrome-causing mutation, p.L634P, located very close to valine 636 on the same β-strand 7, seems also to disrupt the correct structure of the ASH domain and the ASH-RhoGAP module, and has been shown to abolish binding to the endocytic protein APPL1 (*20,22*). The histidine residue affected by mutation p.H660P is located on β-strand 8 on the surface of the ASH domain. The substitution of histidine for proline could impair binding of OCRL1 with other proteins, and, therefore, disturb its function. Furthermore, histidine 660 forms a hydrogen bond with the aspartic acid in position 666 on β-strand 9, which is involved in the interaction of the ASH domain with Rab8a (*21*). Therefore, mutation p.H660P could affect this interaction and OCRL1 membrane recruitment. Two known pathogenic mutations p.D655V and p.F668V, located in β-strands 8 and 9, respectively, on the same face of the ASH domain have been shown to abolish binding of OCRL1 to Rab proteins (*21-23*). We should also take into account that some missense mutations located in the ASH-RhoGAP module can destabilize OCRL1 (*17*), and in fact, both mutations describe in our study reduce the protein stability according to bioinformatics predictions.

In conclusion, the identification of two novel missense mutations located in the ASH domain may shed more light on the functional importance of this domain. Our results extend the range of *OCRL* pathogenic mutations in patients with Lowe syndrome. Based on the results obtained with other patient mutations located in the ASH-RhoGAP-like domain of OCRL1, we suggest that p.V636E and p.H660P cause Lowe syndrome by disrupting the interaction between OCRL1 and some of its partners or by destabilizing de protein.

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Conflict of Interest: The authors have no conflict of interest to disclose.

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Case Report

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Genetic variant affecting the myosin light chain 2 related to familial hypertrophic cardiomyopathy

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SUMMARY Familial hypertrophic cardiomyopathy (FHCM) is a genetic disease characterized by left ventricle (LV) or interventricular septum hypertrophy. FHCM is a common heart disease (affecting 1 out of 500 individuals) associated with genetic variants in genes related to the sarcomere, including the *MYL2* (myosin light chain 2) gene that is affected in 1 to 3% of the cases. As described in this report, the genetic mutation p.Gly87Ala, rs 397516399 in the *MYL2* gene is likely pathogenic. Reported here is the case of a 37-year-old Colombian man with asymmetric septal hypertrophic cardiomyopathy and ventricular tachycardia. The man had progressive symptomatology, a family history of FHCM with a dominant inheritance pattern, a mother and 2 brothers with FHCM, and 2 brothers who died suddenly before the age of 35. A molecular panel of 17 genes for hypertrophic cardiomyopathy identified a heterozygous variant, p.Gly87Ala, of the *MYL2* gene. This variant can be found in Ensembl, dbSNP, and ClinVar, where it has conflicting interpretations: it either has an uncertain significance or it is likely pathogenic. This is the first report of a Colombian case of FHCM secondary to a mutation in the *MYL2* gene, highlighting the importance of molecular diagnosis, genetic counseling, and bioinformatic analysis in these patients.

Keywords heart diseases, cardiomyopathy, hypertrophic, familial, hypertrophy, left ventricle

1. Introduction

Familial hypertrophic cardiomyopathy (FHCM) is a hereditary disease characterized by left ventricle (LV) or interventricular septum thickening in the absence of a secondary cause; myofibrillar disarray and interstitial fibrosis may be observed microscopically, and an asymmetric pattern of wall thickening is evident macroscopically, with localized hypertrophy of the interventricular septum being the most common pattern of hypertrophy. The phenotype is heterogenous, even within the same family, and ranges from being asymptomatic to manifesting as dyspnea, arrhythmia, progressive heart failure, and even sudden death (*1*). The frequency of FHCM is approximately 1 in 500 individuals (*2*).

FHCM is a monogenic disease that exhibits an autosomal dominant inheritance pattern, with incomplete penetrance and variable expressivity. The mutations affect genes that code for proteins involved in sarcomere activity: *MYH7* and *MYBPC3* are affected in 50% of cases, though *TNNT2*, *TPM1*, *MYL2*, *MYL3*, *ACTC1*, and *TNNI3* may be affected in some cases. In cases of recessive inheritance, other genetic conditions like Noonan syndrome and LEOPARD syndrome should be considered; in addition, non-genetic disorders such as amyloidosis can mimic FHCM (*3-5*). The *MYL2* (myosin light chain 2) gene is affected in only 1 to 3% of FHCM cases (*6,7*).

The introduction of next-generation sequencing and its integration with bioinformatic tools, which are constantly evolving, has facilitated the application of advances in molecular biology to the identification of genetic diseases in clinical practice. However, decision-making based on molecular tests is still a challenge.

This case report adds to the medical literature by presenting a new case of FHCM secondary to a mutation in the *MYL2* gene, the first such case reported in Colombia, and it highlights the importance of molecular diagnosis in patients with FHCM in order to facilitate genetic counseling.

2. Case Report

A 37-year-old man from Tuluá, Valle del Cauca,

Colombia worked in agriculture and construction, which demanded considerable physical effort. At the age of 25, his symptomatology started, including intense fatigue and mild thoracic pain due to physical activity. Three years later, a routine checkup revealed an abnormal heart murmur. Supplementary electrocardiography revealed hypertrophy of the myocardium but no other abnormalities. Over the next two years, the man's symptoms worsened even with less physical effort. At the age of 32, an echocardiogram revealed asymmetric septal hypertrophic cardiomyopathy (Figure 1). The thickness of the interventricular septum was 19.5 mm in the basal third, 22 mm in the middle third, and 23 mm in the apical third, and ventricular tachycardia was also present. Given the previous diagnosis, alcohol septal ablation was performed. However, ventricular tachycardia and disabling symptoms he persisted, so he required an implantable cardioverter-defibrillator at the age of 35.

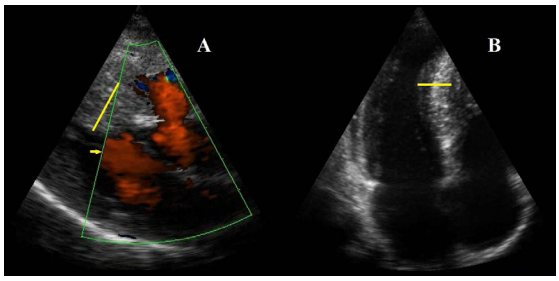

Figure 1. Patient's echocardiogram. (A), In a long-axis view of the heart, asymmetric septal hypertrophy is evident, as identified by the yellow line. Doppler ultrasound suggests a partial obstruction of the left ventricular outflow tract, the yellow arrow indicates the obstruction; **(B)**, Four-chamber cardiac view, the yellow line depicts the thickness of the septum.

During genetic assessment, a family history of hypertrophic cardiomyopathy (HCM) was detected. The patient's mother had HCM and died at the age of 66; two living brothers have MCH (51 and 60 years of age). In addition, two of his brothers under the age of 35 died suddenly, two more brothers died from an unknown cause before the age of 5, and yet another brother is being examined for possible heart disease. Findings indicated a dominant inheritance pattern for HCM in this family (Figure 2).

In light of the clinical diagnosis of FHCM, a molecular panel for HCM including 17 genes (*ACTC1*, *DES*, *FLNC*, *GLA*, *LAMP2*, *MYBPC3*, *MYH7*, *MYL2*, *MYL3*, *BLN*, *PRKAG2*, *PTPN11*, *TNNC1*, *TNNI3*, *TNNT2*, *TPM1*, and *TTR*) was performed. This panel identified a heterozygous variant of the *MYL2* gene, p.Gly87Ala, dbSNP: rs397516399, that is likely pathogenic (Figure 3).

A routine echocardiogram at the age of 36 yielded the following findings: an LV of normal size, mild hypertrophy of the septum, normal systolic function, and diastolic dysfunction with slow LV relaxation. The patient still suffers dyspnea during physical effort and occasionally thoracic pain with palpitations.

A search of the literature in PubMed, Embase, Lilacs, ScienceDirect, Ovid Medline, and the Cochrane Central

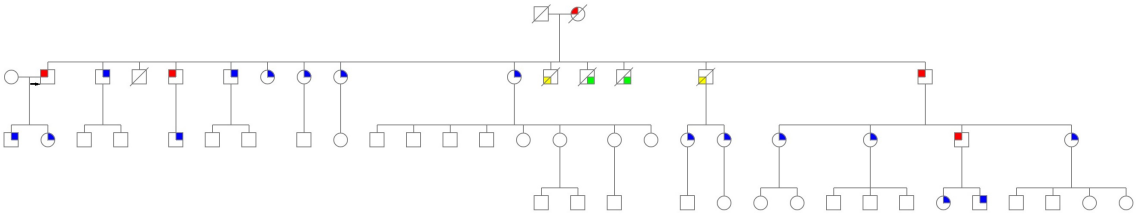

Figure 2. Patient's genogram. Genogram of three generations suggesting an autosomal dominant inheritance pattern of familial hypertrophic cardiomyopathy. **(Black arrow)**, the proband; **(red)**, patient with hypertrophic cardiomyopathy; **(blue)**, genetic testing advised; **(green)**, sudden death before the age of 5; **(yellow),** sudden death.

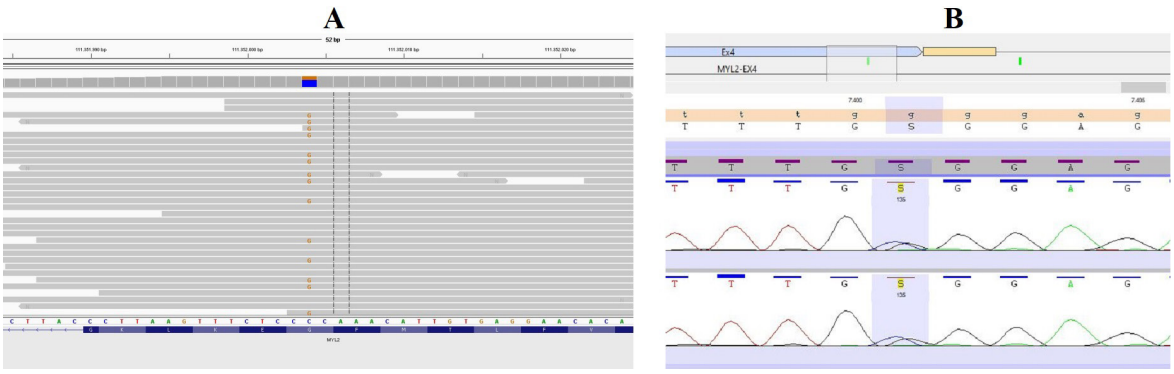

Figure 3. Variation identified in the fourth exon of *MYL2*. (A), Next-generation sequencing alignment of *MYL2*; **(B)**, Sanger sequencing chromatogram focusing on the targeted region including the variation.

Register of Controlled Trials was conducted using combinations of the following keywords: "Colombia" or "Colombian," "hypertrophic cardiomyopathy" or "familial hypertrophic cardiomyopathy," "MYL2" or "MCL2" or "CMH10." The terms were expressed in the respective language of the database, and the thesaurus of each database was used. No language nor year of publication restrictions were imposed. The search returned no results related to a Colombian case of HCM secondary to MYL2 mutations. Thus, the current case is the first reported case of FHCM secondary to a mutation in the *MYL2* gene in Colombia.

3. Discussion

The *MYL2* gene, with a locus at 12q24.11 on chromosome 12 (110,910,819-110,921,443:-1), codes for the ~19 kDa sarcomeric protein MYL2, which is involved in the contraction force of the heart by increasing the stiffness of the myosin lever arm, improving myosin head diffusion with actin. During embryogenesis, *MYL2* facilitates the assembly of cardiac myofibrils, which are important in early contractibility (*8*). Associated phenotypes with mutations in the *MYL2* gene are congenital myopathy with fiber-type disproportion (OMIM# 255310) and FHCM (OMIM# 608758) (*9*).

The frequency of mutations in the *MYL2* gene in FHCM is low, if the frequency of this disease is taken into account (1 in 500 individuals) (*2*). Álvarez *et al.* (2011), reported that among 124 patients with FHCM, only 3 (2.4%) exhibited consequences of mutations in the *MYL2* gene (*6*). Walsh *et al.* (2019) analyzed 4,185 probands with HCM and found that 43 (1.03%) had mutations in the *MYL2* gene (*7*). Lopes *et al.* (2015) identified 6 patients (1.6%) with HCM secondary to *MYL2* mutations out of 383 patients with HCM (*10*). Based on these data, the estimated prevalence of FHCM secondary to mutations in *MYL2* should be between 1 in 20,000 individuals and 1 in 50,000 individuals.

Patients with FHCM are usually asymptomatic during the first two decades of their lives (*3*). This pathology is frequently diagnosed incidentally *via* cardiac auscultation, thorax imaging, or *via* a thorough analysis of family history. The current patient was diagnosed as a result of incidental identification of a heart murmur at the age of 28; when asked, however, he reported experiencing symptoms since the age of 25.

FHCM has a broad symptomatology, from mild to moderate cases with mild dyspnea, thoracic pain, and fatigue upon exertion to more severe cases with angina, palpitations secondary to auricular fibrillation or ventricular tachycardia, syncope, ventricular fibrillation, and even sudden death (*1,3*).

A physical exam may reveal a crescendo-decrescendo murmur at the mitral auscultation site; an electrocardiogram may reveal inversion of the T wave below 0.5 mV, depression of the ST segment above 0.1 mV, or a left bundle branch block (*5,11*). These findings may suggest FHCM, but an echocardiogram is the cornerstone for diagnosing that condition. In adults, a myocardial wall thicker than 15 mm in one or more segments of the LV is a diagnostic criterion for HCM. In first-degree relatives of patients with HCM, the diagnostic criterion for LV thickness decreases to 13 mm (*3,5,12*).

HCM has a heterogeneous etiology. About 70% of cases are explained by genetic causes and the remaining 30% are idiopathic. In patients with HCM, cardiologists and geneticists should design a genogram to search for a family history of cardiomyopathies or sudden deaths of parents or siblings; if evident, a diagnosis of FHCM should considered, and treatment should proceed to molecular studies including the genes that code for proteins involved in cardiac sarcomere performance (*3*).

The current patient meets the clinical criteria for FHCM, and a molecular study identified a missense variant of the *MYL2* gene, NM_000432.3:c.260G>C dbSNP: rs397516399, in which a cytosine nucleotide is replaced by a guanine, substituting glycine for alanine at amino acid 87 of the protein.

This variant is reported in Ensembl, dbSNP and ClinVar, where it has two conflicting interpretations: it either has an unknown significance or is likely pathogenic.

The variant of the *MYL2* gene reported here was also identified in 2015 by Lopes *et al.* in a proband from a cohort of 383 patients with FHCM; 6 of those patients had variants associated with *MYL2* (*10*). It was also reported by Captur *et al.* (2020), which found this variant in 1 of 3 patients with *MYL2* mutations out of 110 patients with HCM (*13*). The current case of FHCM associated with the rs397516399 variant would be a new case involving this association.

The variant NM_000432.3:c.260G>C p.Gly87Ala has not been reported in controls in large population databases (GnomAd and the 1000 Genomes Consortium). It has not been identified in EF-hand domains, which are critical for protein function. A functional impact analysis of different in silico predictors yielded varied results (Table 1).

The variant has only been reported when patients with FHCM are index cases, it has not been found in population controls, and some in silico predictors classify it as deleterious. Given these facts, the current case corroborates the contention that the variant NM_000432.3:c.260G>C p.Gly87Ala is likely pathogenic.

In cases of FHCM with a pathogenic or likely pathogenic genetic mutation, individuals should be tested for the identified mutation and receive genetic counseling in order to predict outcomes for patients (*3*). In the current case, individuals shown in blue in the genogram should be tested (Figure 2).

Table 1. In silico prediction of the functional impact of the variation

Gene	Type	Mutation	Prediction tool	Predicted pathogenicity	Score
MYL2	Missense	p.Gly87Ala rs397516399	Mutation Assessor	NEUTRAL	0.6
			PROVEAN	DELETERIOUS	-4.44
			SIFT	TOLERATED	0.091
			PolyPhen-2	LIKELY HARMFUL	1.0
			SUSPECT	Score 15 (0-100)	15

A considerable number of individuals with HCM die without being diagnosed. Molecular testing and genetic counseling of affected individuals and their relatives allow recognition of the etiology, early interventions in asymptomatic individuals, and decrease sudden deaths of undiagnosed individuals, thus improving the prognosis for asymptomatic carriers.

This report of a case of FMCH secondary to a mutation in the *MYL2* gene adds to the medical literature and Colombian epidemiology. The medical community, and especially cardiologists, is encouraged by the molecular testing and genetic counseling of patients with FMCH. Moreover, the current report corroborates the contention that the variant p.Gly87Ala, rs 397516399 in the *MYL2* gene is likely pathogenic, contributing to the clinical significance of the identification of this genetic variant.

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Case Report

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Primary neuroendocrine carcinoma of the breast: a rare presentation and review of the literature

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SUMMARY Primary neuroendocrine carcinoma of the breast (NEBC) is a very rare occurrence accounting for less than 0.1% of all breast cancers. Typically, the tumor presents with ER- and PgR-positive and HER-2-negative status. Despite its luminal type, NEBC is associated with a more aggressive clinical course and poorer prognosis compared to the other types of invasive breast cancer. Clinical and radiological findings are nonspecific. The most common clinical manifestation is a palpable mass whereas in mammography the tumor most commonly appears as a round or oval mass without spiculated margins. Herein, a very rare case of NEBC is described in an asymptomatic patient who presented with an area of architectural distortion and the presence of microcalcifications that was incidentally detected on a screening mammography. A review of the literature has also been conducted. The diagnosis of NEBC requires a thorough investigation to exclude the possibility of a metastatic neuroendocrine tumor from another site because the two entities require different treatment approaches. Due to the rarity of the disease, the optimal therapeutic approach has not been clearly defined. Surgical resection is the mainstay of treatment. Further research is needed to better understand the molecular characteristics of NEBC and identify novel targeted therapies.

Keywords neuroendocrine, carcinoma, breast

1. Introduction

Neuroendocrine neoplasms are rare heterogeneous tumors originating from neuroendocrine cells throughout the body. They are most commonly seen arising from the gastrointestinal and respiratory tracts (*1*). Although foci of neuroendocrine differentiation can be detected in up to 30% of the cases of invasive ductal carcinomas of the breast (*2*), primary NEBC represents a distinct, and very rare entity accounting for less than 0.1% of all breast cancers (*1,3-6*) and less than 1% of all neuroendocrine tumors (*7*). NEBCs exhibit similar morphological and phenotypic features to their counterparts arising in the gastrointestinal and respiratory tracts (*8,9*).

Their exact incidence is difficult to determine because neuroendocrine markers are not routinely used in breast cancer diagnostics (*3,4*). Park *et al.* (*2*), reported that of 12,945 patients with breast cancer diagnosed over 27-years, only 120 (1%) were found to have NEBC. In an epidemiologic based study from the SEER database it was found that among 381,644 cases of stage I-IV breast carcinoma diagnosed within six-years, only 142 (< 0.1%) were NEBC (*6*). In 2003 WHO defined as neuroendocrine breast carcinoma a tumor of epithelial origin with positive staining of one or more neuroendocrine markers in at least 50% of the tumor cells (*10*). However, in the revised 2012 WHO classification it was acknowledged that the 50% cut-off for diagnosis was arbitrary and therefore no specific threshold of tumor cell expression is currently required for a diagnosis of NEBC. Therefore, breast carcinomas with neuroendocrine features were classified into three categories: neuroendocrine tumor well-differentiated, resembling carcinoid tumors originating at other sites, poorly differentiated neuroendocrine carcinoma, or small cell carcinoma which is morphologically identical to small lung carcinoma, and invasive breast carcinoma with neuroendocrine differentiation (*11*).

NEBCs usually run a more aggressive clinical course and tend to have a higher propensity for local and distant recurrence when compared to other types of invasive breast carcinoma (*2,12*). NEBCs do not have specific clinical and radiological features. Besides, their optimal treatment has not been clearly defined. We present a case of NEBC with both rare clinical and imaging features along with a review of the literature.

2. Case Report

A 50-year-old Caucasian woman presented for further evaluation of a mammographic finding that was incidentally detected on a screening mammogram. She denied any symptoms. Her medical history was unremarkable and she had no history of breast or ovarian cancer.

Physical examination revealed an area of asymmetry rather than a discrete mass in the upper inner quadrant of the right breast. There was no palpable axillary and supraclavicular lymphadenopathy. The mammogram showed an area of architectural distortion along with the presence of confluent microcalcifications in the upper inner quadrant of the right breast (Figure 1). Ultrasound revealed two adjacent hypoechoic masses with irregular margins measuring 2.8 cm without prominent acoustic enhancement (Figure 2).

The patient underwent a core needle biopsy under ultrasonographic guidance, which revealed a breast adenocarcinoma with neuroendocrine differentiation. Based on these findings the possibility of an NEBC was considered. Following the core biopsy the patient underwent a thorough staging investigation including hematologic and biochemical evaluation, computed tomography of the chest and abdomen, and bone scintigraphy. All of the above were unremarkable. She then underwent a modified radical mastectomy because the two sentinel lymph nodes were found to harbor metastatic disease in the intraoperative frozen section.

Histological evaluation of the mastectomy specimen showed primary neuroendocrine breast carcinoma of grade II according to the Bloom-Richardson grading system (Figure 3). On gross appearance the tumor measured 3 × 2.7 × 2.5 cm and was solid whitish and elastic. On immunohistochemical evaluation the tumor cells stained strongly positive for estrogen receptors, focally positive for progesterone receptors whereas the expression for Her2 was negative (Figure 4). All tumor cells were strongly positive for synaptophysin and negative for chromogranin A and CD56. Ki-67 proliferation index was 15-20%. Ductal carcinoma in situ (DCIS) was also detected associated with comedo necroses and microcalcifications. Metastatic disease was detected in 2 of the 14 removed axillary lymph nodes. The patient received adjuvant chemotherapy followed by radiotherapy and is currently under hormonal therapy. She is well without any evidence of recurrence 44 months after surgery.

3. Discussion

Primary neuroendocrine tumors of the breast were originally reported in 1977 by Cubilla and Woodruff (*13*),

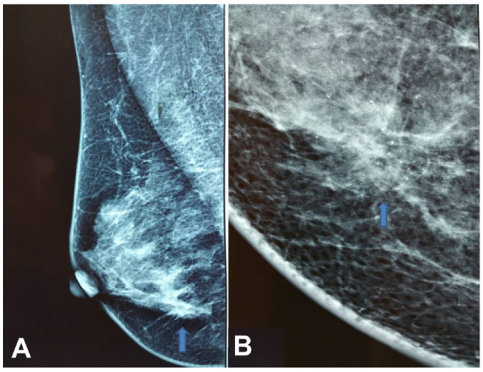

Figure 1. (A) Right mediolateral oblique mammogram showing an area of architectural distortion (arrow); **(B)** Magnification view showing in detail the area of architectural distortion along with confluent microcalcifications (arrow).

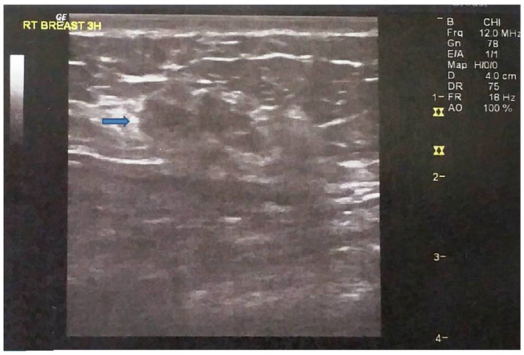

Figure 2. Ultrasonography showing two adjacent hypoechoic nodules with irregular margins (arrow).

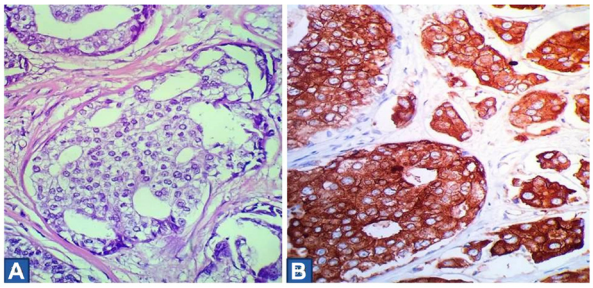

Figure 3. (A) High power photomicrograph, displaying medium-sized, mildly pleomorphic tumor cells, with clear cytoplasm, medium-sized round to oval nuclei with granular chromatin and inconspicuous nucleoli. Mitoses are relatively common. (Hematoxylin & Eosin ×400); **(B)** Tumor cells expressing strong cytoplasmic positivity for synaptophysin (Syn ×400).

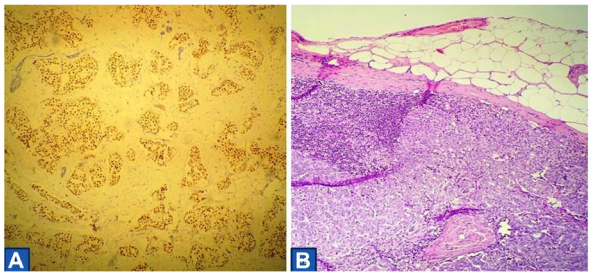

Figure 4. (A) Almost all tumor cells expressing strong nuclear positivity for estrogen receptors (ER ×100); **(B)** Sentinel lymph node with metastasis, which was visible during gross description (Hematoxylin & Eosin ×100).

who described eight patients with a painless breast mass that histologically resembled carcinoids of other sites containing argyrophilic granules.

The exact histogenesis of NEBC is unclear. One theory suggests that the tumor arises from endocrine differentiation of preexisting endocrine cells in the breast. The second theory suggests that the tumor arises from divergent differentiation of neoplastic stem cells into epithelial and endocrine cell lines during early carcinogenesis (*4,7*).

Histologically NEBC is characterized by alveolar structures of solid sheets of cells with a tendency to produce peripheral palisading (*10*). The tumor is more likely to be of luminal subtype, positive for ER and PR expression, and negative for Her2 expression (*1,3,9,12,14-16*). Wei *et al.* (*12*), reported 92% positivity for ER and 69% positivity for PR, in patients with NEBC whereas in patients with other types of breast cancer the relevant rates were 72% and 57% respectively. Synaptophysin and chromogranin A are currently the most specific immunohistochemical markers for the evaluation of NEBC whereas Neurone-specific enolase and CD56 are less sensitive and specific (*4,16*).

The clinical presentation of patients with NEBC is similar to that in other types of invasive breast cancer (*4,10,16*). The tumor most commonly occurs in postmenopausal women in their sixth and seventh decades of life (*3-6,9,14,17,18*) who have reported being significantly older than patients with conventional invasive breast cancer (*6*). Premenopausal patients have been reported in up to 15% of the cases (*12*). Extremely rare cases have been reported affecting male patients (*2*). The most common clinical manifestation is a painless palpable retro areolar lump (*19*). Nipple retraction, skin alterations, and bloody nipple discharge may be present (*19*). Nipple discharge has been reported in 54.5% of the cases (*5*), and should therefore alert the clinician to the possibility of an underlying NEBC.

Locally advanced NEBCs have been reported in 4.7-9.3% of the cases (*3,8,12*), whereas axillary metastases are detected in 43-47% of the cases at presentation (*2,20*). Lee *et al.* (*8*), reported a case of neuroendocrine breast carcinoma manifesting as inflammatory breast cancer.

The median tumor size at presentation is 3.1 cm (*2*), and has been reported to be significantly larger than that of other types of breast cancer (*6*). Multifocality and multicentricity have been reported in 6.9% and 9.2% of the cases respectively (*2*). Patients with NEBC present at a higher clinical stage and higher histologic grade compared to patients with conventional breast cancer (*6*). Well-differentiated and poorly defined NEBCs have been reported in 45% and 40% of the cases respectively (*21*).

The radiological findings of NEBCs are nonspecific (*15,16,21*). On a mammogram, the tumor most commonly appears as a high-density round or oval mass with circumscribed margins (*5,10*). Spiculated margins and the presence of microcalcifications have been reported in 18% and 26.4% of the cases respectively (*2*). The mammographic appearance, however, may mimick benign entities such as fibroadenomas or intramammary lymph nodes (*5*).

On ultrasonography, NEBC appears as a hypoechoic, irregular mass with indistinct margins and increased vascularity (*4,5*). Posterior enhancement is infrequently reported. Jeon *et al.* (*5*) reported the absence of acoustic enhancement in 82% of the cases. MRI findings include an irregular mass with heterogeneous rapid enhancement and washed out pattern (*2,8,16*). In 43% of the cases with multiple lesions reported by Park *et al.* (*2*), multicentricity or multifocality was detected only with MRI.

The diagnosis of NEBC is established by core needle biopsy (*15,16*), although a precise diagnosis may not be possible in up to 40% of the cases (*5*). Fine needle aspiration may not be adequate for the diagnosis (*16*), because the cytology findings, of NEBC, may overlap with those of invasive ductal carcinoma and intraductal papilloma (*19*).

The differential diagnosis of NEBC includes metastatic neuroendocrine tumors to the breast, Merkel cell carcinoma, melanoma, and lymphoma (*16*). Differentiating between primary and metastatic breast neuroendocrine tumors is essential since the two entities require different therapeutic approaches (*4,14,20,21*). The presence of an intraductal component with similar cytologic features is suggestive of a primary breast tumor (*10,15,16*) since it has been reported in 68% of the cases (*2*). The most specific immunohistochemical markers indicative of a primary breast tumor are GATA3, mammaglobin, and GCDFP15 which are stained negative in metastatic tumors (*4*). The absence of an intraductal component, negativity for ER, PR, and absence of axillary metastases are suggestive of a metastatic tumor (*16*). Metastases to the breast account for less than 1% of breast tumors whereas metastatic neuroendocrine tumors account for 1-2% of the metastases to the breast (*4*). Thorough imaging investigation of the patient with CT scans and PET CT is mandatory to exclude any other primary site. A detailed clinical history and complete physical examination are essential in the assessment of the patient with NEBC.

Perry *et al.* (*22*), reported 18 metastatic neuroendocrine tumors to the breast of whom 62% were of gastrointestinal tract origin, 28% were from the lungs and 10% were of intermediate origin. Interestingly, 44% of these cases were initially misdiagnosed as primary breast carcinomas. All the metastatic tumors stained positive for synaptophysin and chromogranin and 83% were stained positive for NSE or CD56 (*22*).

Metastases from NEBC may occur many years after the initial treatment and thus a long-term follow-up is mandatory. Most common metastatic sites include the liver, bones, lungs, soft tissues, pleura, brain, mediastinal

lymph nodes, adrenal glands, ovaries, and pancreas (*9,12,16*).

There is no standard treatment protocol for NEBC and the therapeutic approach is similar to that for other types of breast cancer (*5*).

Surgery is the mainstay treatment in patients with NEBC (*8,16*). The type of surgery depends on the tumor location and clinical stage and can be either lumpectomy or mastectomy with sentinel node biopsy, or modified radical mastectomy in cases with metastatic sentinel nodes. Lack of surgical treatment, along with higher tumor stage, larger tumor, and negative ER, PR status has been associated with shorter overall survival in NEBC patients compared to that of patients with invasive breast carcinoma (*6*). There are limited data on oncoplastic conservation and immediate reconstruction in patients with NEBC. As the tumor may develop a pagetoid pattern of spread, the assessment of surgical margins may be difficult especially in the intraoperative frozen section (*20*).

Chemotherapy can be used either in the adjuvant setting in patients with a high risk of relapse or as a neoadjuvant treatment in patients with locally advanced disease not amenable to surgery (*16,18*). It can also be used for downstaging large tumors to allow for breast conservation treatment.

However, the optimal chemotherapy regimen has not been clearly defined and the current consensus is to treat NEBC with the chemotherapeutic regimens that are used in the treatment of conventional breast cancer and pulmonary small-cell carcinoma (*1,18,20*). Several regimens including anthracyclines and or taxanes that are used for other types of breast cancer and a combination of platinum and etoposide are commonly administered (*16,19*). Suhani *et al.* (*15*) reported good results in four patients with NEBC who were treated with Cyclophosphamide, Adriamycin, and 5-Fluorouracil (CAF) based adjuvant chemotherapy, irradiation of chest wall, and hormonal therapy. Interestingly, in some series the patients who received chemotherapy appeared to have both shorter overall survival and disease-free survival than those who did not receive chemotherapy. This difference, however did not reach statistical difference likely due to the small number of patients, and the fact that chemotherapy is used depending on the clinical stage and tumor histological characteristics (*12,18*). The poor response to chemotherapy in patients with NEBC may be attributed either to the chemoresistance commonly seen in neuroendocrine tumors in other sites or to the lack of an optimal chemotherapeutic regimen (*4*).

Hormonal therapy should be given in cases with positive hormonal status (*2,15*). Patients who received endocrine therapy have been associated with longer overall survival and distant recurrence-free survival (*12*).

There are conflicting reports for prognosis in patients with NEBC (*21*). Most authors report significantly worse outcomes for overall survival, local recurrence-free survival, and distant recurrence-free survival in patients with NEBC compared to the matched group of patients with invasive ductal cancer (*4,6,9,12*). On the contrary, Jeon *et al.* (*5*) reported that all 11 patients of their study showed favorable prognosis and were free of locoregional disease 21-76 months after treatment. A 15% and 34% risk for local and distant recurrence has been respectively reported at five years (*12*).

The 5-year overall survival has been reported from 70-80% (*17,21*). In a literature review by Lu *et al.* (*18*), including 86 primary NEBCs the overall survival at 48 months was 83.5%. A more favorable prognosis has been reported for tumors detected at an early stage (*5*). Large tumors, high tumor stage, negative hormonal status, regional metastases, and ki-67 > 14% have been associated with worse overall survival and disease-free survival (*12,16*). In the multivariate analysis of the population-based study from the SEER database, it was found that older age and positive lymph node status were independent prognostic factors for overall survival in patients with NEBC tumors, whereas positive lymph nodes, negative PR status and lack of surgical treatment were independent prognostic factors for disease-specific survival (*6*). In the same study it was shown that radiation therapy did not prolong survival (*6*). Patients with early NEBC without axillary dissection are associated with better overall survival compared to advanced-stage patients treated with mastectomy and axillary dissection (*18*). The presence of mucinous differentiation has been reported as a favorable prognostic factor (*10*).

The published data on molecular characteristics of NEBCs is scarce. Molecular analysis of 47 NEBCs showed that these tumors are part of the spectrum of luminal carcinomas. An equal distribution between A and B subtypes was observed. In addition, only three (7%) of the cases were found harboring a PIK3CA mutation and 7% were harboring TP53 mutations (*9*). Despite its luminal phenotype NEBC is associated with an aggressive clinical course and poor prognosis (*23*).

In an effort for the development of novel targeted therapies in patients with NEBC, Vranic *et al.* (*23*), identified several potential targets for novel therapies including farletuzumab and mirvetuximab soravtansine (FOLR1), sacituzumab govitecan (TROP-2) and HDAC inhibitors (H3K36Me3). Novel therapeutic approaches should further be explored (*6,12*).

After conducting a literature review, we were able to find twenty-five retrospective reviews and case series (*1-3,5-7,14,15,18,19,24-38*) and thirty-one case reports (*20,21,39-67*) of neuroendocrine breast carcinoma published since 2000. The clinicopathological characteristics are summarized in Table 1 and Table 2.

Similar to the majority of the published cases, our patient had an ER and PR positive and Her2 negative tumor. Regarding the clinical presentation findings, in the vast majority of the published cases the patients presented with a palpable breast mass. On the contrary,

Table 1. Summary of clinicopathological findings of primary neuroendocrine breast carcinoma published in retrospective reviews and case series since 2000

Author/Year (*Ref.*), Study design	Patients/sex	Age	Size (mm)	Pathological, Immunohistochemical Findings	Treatment	Follow-up (months)	Outcome
OZDIRIK/2020 (*19*), CS	5 F	67 (49-73)	12 (9-53)	ER+: 60%, PR +: 80%, HER2 - : 60% ER +, PR -, HER2 - : 100% Syn+: 100%, CgA+ : 80% N+: 20%, N/A: 40%	BCS+ALND: 20% BCS+SNLB: 40% No Surgery: 40% Chemo : 40% RT: 40%, ET:20%	45.4 (11-130)	Alive: 60% Died :40%
CANBAK/2020 (*14*), RR	11 F	68 (49-86)	N/A	ER+, PR+: 90% N/A:10% HER2 - :100% Syn+:, CgA+: 100% N: N/A	BCS: 64% MT: 36%	N/A	Alive: 90% Died :10%
HEJJANE/2020 (*25*), CS	2 F	71 48	22 40	ER+, PR+, HER2- N- ER+, PR+, Syn+, CgA+ HER2-, N+	MRM + ALND ET BCS+ ALND Chemo, ET	21 28	Alive Alive with recurrence at 12 months
ZHANG/2020 (*24*), CS	2 F	46 34	65 30, 20	ER-, PR-, HER2 – Syn+, CgA-, N:- ER+, PR+ ,HER2 + Syn+, CgA+ N-	MRM + ALND Chemo N/A	N/A N/A	N/A N/A
LI/2017 (*26*), RR	119 F 7 M	53.2 (N/A)	T1:23% T2:31% T3:19% T4:6.3% N/A:20.6%	ER+: 81%, PR+: 72.2%, HER2+: 15.1%, N: +15.1%, N/A: 46%	MT: 79.4% BCS: 14.3% Other: 5.5% ALND: 82.5% Chemo: 43.7% RT: 17.5% ET: 63.5%	4-144	Alive without tumor: 80.2% Alive with tumor:7.1% Died of disease: 5.6% Died of other causes:0.8% Lost to follow-up: 6.3%
ROININEN/2017 (*3*), RR	43F	66	25.3 (18.9-31.7%)	ER+: 97.7%, PR+: 58.1%, HER2+: 4.7%, Syn+: 100%, CgA+: 69.8% N+: 44.2%	MT+ALND: 44.2% BCS+ ALND: 25.6% Other type: 14% Chemo: 30.3% RT: 74.4% ET:76.7%	35.4 (23.5-47.2)	Local recurrence: 7.6%

CS: case series, RR: retrospective review, LR: literature review, F: female, M: male, ER: estrogen receptors, PR: progesterone receptors, Syn: synaptophysin, CgA: chromogranin A, MT: mastectomy, BCS: breast conserving surgery, SLNB: sentinel lymph node biopsy, ALND: axillary lymph node dissection, N: nodes, Chemo: chemotherapy, RT: radiation therapy, ET: endocrine therapy, N/A: not available.

Table 1. Summary of clinicopathological findings of primary neuroendocrine breast carcinoma published in retrospective reviews and case series since 2000 (continued)

Author/Year (*Ref.*), Study design	Patients/sex	Age	Size (mm)	Pathological, Immunohistochemical Findings	Treatment	Follow-up (months)	Outcome
YANG/2017 (*1*), RR	19 F	59.2 (17-82)		ER+: 93.3%, PR+: 73.3%, HER2+: 4.7%, HER2-: 93% Syn+: 77.8% CgA+: 42.1% N+: 22.2%	MT +ALND: 89% MT: 5.3% MT+SLNB: 5.3% Chemo: 61% RT: 16.6% ET:44%, N/A: 44%	59.2 (15.5-114) N/A:32%	Alive: 52.6% Died: 10.5% N/A: 36.8%
COLLADO-MESA/2017 (*7*), CS	2 F	58	15, 8	ER+, PR+, HER2- Syn+, CgA+, PN-	BCS+SLNB (-)	N/A	N/A
		62	17	ER+, PR+, HER2- Syn+, CgA+, N-	BCS+SLNB (-)	N/A	N/A
LOCURTO/2016 (*27*), CS	5 F	59.4 (50-75)	29 (2.3-4.5)	ER+: 100%, PR+: 80% HER2+: 100% Syn+: 80%, CgA+: 60% N+: 60%	MRM -ALND: 20% BCS- ALND :60% BCS+SLNB: 20% (-) Chemo: 60%, RT: 100%, ET:100%	N/A	Alive: 80% Died: 20%
LU/2014 (*18*), LR	85 F 1 M	53.9 (25-83)	27.5 (0-180)	ER+:59%, PR+: 66% HER2+: 27% Syn+: 80%, CgA+: 60%	MRM : 50% BCS: 50% Chemo: 41.7%, RT: 28.3%, ET:13.3%	38.1 (3-99)	Alive with no evidence of recurrence 79%. OS: 94%, 86%, 73% in I, II, III stages respectively
PARK/2014 (*2*), RR	84 F 3 M	62.9 (28-89)	31 (6-110)	ER+:98.9%, PR+: 77% HER2+: 2.4%	N/A	N/A	N/A
SUHANI/2014 (*15*), RR	4 F	58 (50-65)	51 (4-6.5)	ER+:100%, PR+: 80% HER2- : 100% Syn+: 50%, CgA+: 75% N+: 75%	MRM: 100% Chemo: 100% RT: 100% ET: 100%	27.7 (9-48)	Alive:100%

CS: case series, RR: retrospective review, LR: literature review, F: female, M: male, ER: estrogen receptors, PR: progesterone receptors, Syn: synaptophysin, CgA: chromogranin A, MT: mastectomy, BCS: breast conserving surgery, SLNB: sentinel lymph node biopsy, ALND: axillary lymph node dissection, N: nodes, Chemo: chemotherapy, RT: radiation therapy, ET: endocrine therapy, N/A: not available.

Table 1. Summary of clinicopathological findings of primary neuroendocrine breast carcinoma published in retrospective reviews and case series since 2000 (continued)

Author/Year (*Ref.*), Study design	Patients/sex	Age	Size (mm)	Pathological, Immunohistochemical Findings	Treatment	Follow-up (months)	Outcome
WANG/2014 (*6*), RR, SEER	139 F 3 M	64 (26-99)	31.9	ER+:54.2%, PR+: 37.3% HER2 : N/A N+: 28.2%	Surgery: 76.8% RT:35.9% Chemo: N/A ET: N/A	N/A	Median survival:26 months (12-48)
JEON/2014 (*5*), RR	11 F	54.7 (29-79)	18 (0.5-4)	ER+:100%, PR+: 100% HER2 -: 55%, N/A in 5 cases N+: 28.2%	BCS+SLNB: 55% BCS: 9% MRM+SLNB:27% MRM:9% Chemo: N/A, RT: N/A, ET: N/A	38.6 (21-76)	Alive free of disease: 100%
ZHU/2013 (*28*), RR	22 F	52.5 (29-77)	22 (0.5-4)	ER+:91%, PR+: 95% HER2 -: 75%,	MRM:100% Chemo: 64% ET: 91% RT: 0%	64.5 (4-89)	Alive free of disease: 95% Alive with recurrence: 5%
ROVERA/2013 (*29*), RR	61 F	70 (42-87)	2.05 (0.6-6)	ER+: 90%, PR+: 75% HER2- :98% N+: 21.3%	BCS:49% MRM:48% Chemo:5% RT: 48% ET: 72%	88 (4-242)	Local or systemic recurrence: 14.8% after a median of 53.7 months
ZHANG/2012 (*31*), RR	107 F	65 (25-95)	8-50	ER+: 94.3%, PR+: 85.05% HER 2- :97.2% N+:24.3%	N/A	27 (3-134)	Overall survival: 85.1% Local recurrence: 3.7% Distant recurrence: 5%
WU/2012 (*32*), RR	13 F	53 (36-78)	25.5 (10-40)	ER+, PR+: 100% HER2+: 100% Syn+, CgA+: 54% N+: 7.7%	N/A	67.5 (41-89)	Alive free of disease: 85% Died: 7.5% Lost: 7.5%
MENENDEZ/2012 (*33*), CS	4 F	44	20	ER:+, PR:-, HER2: -, N:+ CgA+, N+	BCS+ALND, Chemo,RT	48	Alive disease free
		68	36	ER, RP, HER2:N/A, CgA+, Syn:N/A, N+	BCS+ALND, Chemo, RT, ET	24	Alive disease free
		58	10	ER:+, PR:-, N: -, HER2:-	BCS+SLNB, Chemo, RT	8	Alive with liver metastasis
		69	14	ER:+, PR:+, HER2:-, Syn:+, CgA: +, N: -	BCS+SLNB, Adjuvant treatment: N/A	2	Alive disease free

CS: case series, RR: retrospective review, LR: literature review, F: female, M: male, ER: estrogen receptors, PR: progesterone receptors, Syn: synaptophysin, CgA: chromogranin A, MT: mastectomy, BCS: breast conserving surgery, SLNB: sentinel lymph node biopsy, ALND: axillary lymph node dissection, N: nodes, Chemo: chemotherapy, RT: radiation therapy, ET: endocrine therapy, N/A: not available.

Table 1. Summary of clinicopathological findings of primary neuroendocrine breast carcinoma published in retrospective reviews and case series since 2000 (continued)

Author/Year (*Ref.*), Study design	Patients/sex	Age	Size (mm)	Pathological, Immunohistochemical Findings	Treatment	Follow-up (months)	Outcome
BRASK/2013 (*30*), RR	13 F	67.8 (42-89)	15.1 (8-30)	ER+: 100%, PR: N/A, HER2-: 100%, Syn+: 92%, CgA+:30% N+: 23%	BCS: 77% MRM: 23% Adjuvant treatment: N/A	N/A	N/A
KAWASAKI/2012 (*34*), RR	24 F	47.8 (28-74)	4.9 (1-25)	ER+, PR+, HER2-: 100%, Syn:+, CgA: +, N: -	BCS: 63% MRM: 37% Adjuvant treatment: N/A	83.7 (64-101)	Alive free of disease:100%
GHANEM/2011 (*35*), RR	7 F	56 (50-82)	40 (14-130)	ER+, PR+: 85% HER2-: 100%, Syn+, CgA+: 100%, N +: 71%	BCS+ALND: 29% MRM +ALND: 71% Chemo:66%, RT: 86% ET: 71%	28 (0-38)	Alive free of disease: 86% Alive with recurrence: 14%
ADEGBOLA/2005 (*36*), CS	3F	46	10	ER:-, PR:-, HER2: - Syn:+, CgA: +, PN: -	BCS, Chemo, RT	48	Alive free of disease
		60	17	ER:-, PR:-, HER2: - Syn:+, CgA: +, PN: -	BCS, Chemo, RT	20	Died
		61	17	ER:-, PR:-, HER2: - Syn: -, CgA: +, N: +	BCS, Chemo, RT	6	Alive with disease
ZEKIOGLU/2003 (*38*), RR	12F	66.5 (43-79)	23.5 (8-70)	Syn+, CgA+: 66%,	BCS+ALND: 50% MT+ALND:50%	24-54	Alive free of disease
SHIN/2000 (*37*), RR	9F	54.1 (43-70)	26 (13-50)	ER+, PR+: 92% HER2-: N/A, Syn+:42%, CgA+: 92%, N+: 50%	BCS:67% MT: 33% ALND: 89% Chemo:78%, RT:44% ET:22%	3-35	Metastases:22% All alive

CS: case series, RR: retrospective review, LR: literature review, F: female, M: male, ER: estrogen receptors, PR: progesterone receptors, Syn: synaptophysin, CgA: chromogranin A, MT: mastectomy, BCS: breast conserving surgery, SLNB: sentinel lymph node biopsy, ALND: axillary lymph node dissection, N: nodes, Chemo: chemotherapy, RT: radiation therapy, ET: endocrine therapy, N/A: not available.

Table 2. Summary of clinicopathological findings of case reports of primary neuroendocrine breast carcinoma published since 2000

Author/Year/ (*Ref.*)	Age	Size (mm)	Clinical Findings	Imaging Findings Mammogram/US	Pathological Immunohistochemical Findings	Treatment	Follow-up	Outcome
SALEMIS/2020 (present case)	50	30	None	Architectural distortion, Calcifications Hypoechoic mass. Irregular margins	ER+, PR+, HER2- Syn+, CgA-, N+	MT+ALND Chemo, RT, ET	44	AWD
VALENTE/2020 (*39*)	69	15	Palpable mass, nipple, areolar erosions	Nodule with calcifications Nodule with irregular margins and posterior shadowing	ER-, PR-, HER2- CgA+, N+ Concomitant invasive lobular carcinoma	BCS+ALND Chemo, RT, ET	96	AWRD
PULAT/2019 (*40*)	73	40	Pain, swelling, skin changes	Macrolobular mass Irregular, hypoechoic mass	ER+, PR+, HER2- N-	MT+ALND ET	48	AWD
TREMELLING/2017 (*41*)	65	50	Palpable mass	Irregular mass with increased vascularity	ER-, PR-, HER2-, Syn+, CgA+ N+	Chemo, RT	3	AWD
SOE/2017 (*42*)	57	40	Palpable mass	Oval, lobulated dense with mass calcifications Hypoechoic lobulated mass with marked posterior enhancement	ER+,PR+, Syn+,CgA+	BCS+Chemo	18	AWRD
BERGSTROM/2017 (*43*)	53	80	Palpable mass	Irregular mass	ER+, PR-, HER2- Syn+, CgA+	Chemo	N/A	N/A
VATS/2017 (*44*)	32	60	Palpable mass	BIRADS-5	ER+, PR+, HER2+ Syn+, CgA+, N+	Chemo, RT	12	AWRD
MEKIAROVA/2016 (*45*)	42	32	Palpable mass	Circumscribed mass with calcifications Hypoechoic mass with enhancement	ER-, PR-, HER2-, Syn+, CgA+	BCS+SLNB	36	AWD
MARINOVA/2016 (*46*)	42	35	None	Distinctive mass with microcalcifications	ER+, PR+, HER2-, N+	BT+ALND Chemo, RT, ET	12	AWD
YOSHIMURA/2015 (*47*)	34	N/A	Palpable mass	Microlobulated, hyperdense mass	ER+, PR+, HER2-, N+	MT+ALND. Refused adjuvant treatment	48	AWD
WRONSKI/2015 (*48*)	45	22	None	N/A	ER+, PR+, HER2- Syn+, CgA+ , N+	BCS+ALND	N/A	N/A

CS: case series, RR: retrospective review, LR: literature review, ER: estrogen receptors, PR: progesterone receptors, Syn: synaptophysin, CgA: chromogranin A, MT: mastectomy, BCS: breast conserving surgery, SLNB: sentinel lymph node biopsy, ALND: axillary lymph node dissection, N: nodes, Chemo: chemotherapy, RT: radiation therapy, ET: endocrine therapy, AWD: alive without disease, AWRD: alive with recurrent disease, N/A: not available

Table 2. Summary of clinicopathological findings of case reports of primary neuroendocrine breast carcinoma published since 2000 (continued)

Author/Year/ (*Ref.*)	Age	Size (mm)	Clinical Findings	Imaging Findings Mammogram/US	Pathological Immunohistochemical Findings	Treatment	Follow-up	Outcome
TATO-VARELA/2015 (*21*)	62	20	Palpable mass	Increased trabeculation	ER+, PR+, HER2- Syn+, CgA+, N+	MT+ALND Chemo, RT	N/A	AWD
WEI/2014 (*49*)	43	83	Palpable mass	Irregular mass Lobulated lump Hypoechoic mass	ER+, PR-, HER2+ Syn+, N+	MT+ALND Chemo	N/A	N/A
PANJVANI/2015 (*50*)	70	25	Breast swelling	Hypoechoic mass with increased vascularity	Syn+	MT+ALND	N/A	N/A
LEE/2015 (*51*)	48	24	Breast enlargement, eythema	Diffuse skin thickening Hypoechoic irregular mass	ER+, PR+, HER2- Syn+, N+	Chemo, RT, ET	N/A	AWRD
JANOSKY/2015 (*52*)	34	43	Palpable mass	Irregular mass with calcifications	ER-, PR-, HER2- CgA+, Syn+	Chemo, MT, RT	N/A	AWRD
ALVA/2015 (*53*)	53	50	Palpable mass	Irregular lump	ER-, PR-, HER2- CgA+, Syn+, N-	MT, Chemo	N/A	AWD
VALENTIM/2014 (*54*)	75	19	None	Ovoid, well defined mass Hypoechoic irregular mass without posterior enhancement	CgA+, Syn+	BCS	N/A	N/A
PAGANO/2014 (*55*)	51	35	Palpable lump	Irregular mass	ER+, PR+, HER2- CgA+, Syn+, N+	MT+ALND Chemo, ET	126	AWD
BOZKURT/2014 (*56*)	75	31	Palpable mass	Spiculated Mass Hypoechoic mass with regular borders	ER+, PR+, HER2- CgA+, Syn+,	MT,Chemo, RT	N/A	AWD
LINGAPPA/2014 (*57*)	80	100	Palpable mass	Well-defined mass	CgA+, N+	MT+ALND Chemo	N/A	N/A
TAJIMA/2013 (*58*)	78	20	None	N/A	ER+, PR+, HER2- CgA+, Syn+, N+	BCS+ALND, ET	N/A	N/A
MURTHY/2013 (*59*)	34	25	Palpable mass	Ill defined-mass	ER+, PR+, HER2- CgA+, Syn+, N-	MT+ALND Chemo, TR,ET	6	AWD

CS: case series, RR: retrospective review, LR: literature review, ER: estrogen receptors, PR: progesterone receptors, Syn: synaptophysin, CgA: chromogranin A, MT: mastectomy, BCS: breast conserving surgery, SLNB: sentinel lymph node biopsy, ALND: axillary lymph node dissection, N: nodes, Chemo: chemotherapy, RT: radiation therapy, ET: endocrine therapy, AWD: alive without disease, AWRD: alive with recurrent disease, N/A: not available

Table 2. Summary of clinicopathological findings of case reports of primary neuroendocrine breast carcinoma published since 2000 (continued)

Author/Year/ (*Ref.*)	Age	Size (mm)	Clinical Findings	Imaging Findings Mammogram/US	Pathological Immunohistochemical Findings	Treatment	Follow-up	Outcome
HANNA/2013 (*60*)	60	20	Axillary mass	None Enlarged axillary node	ER+, PR+, HER2-	BCS+ALND Chemo	N/A	N/A
ANGARITA/2013 (*20*)	51	32	Breast lump	Spiculated mass with calcifications	ER+, PR-, HER2- CgA+, Syn+,	Chemo, RT, MT+ALND, ET	13	AWRD
WATROWSKI/2012 (*61*)	56	17	Palpable mass	Hypoechoic mass with angular margins and posterior shadowing	ER+, PR-, HER2-, Syn+ N-	BCS, Chemo, RT, ET	15	AWD
SANGUINETTI/2012 (*62*)	63	65	Bulky mass, skin changes	Mass with suspicious characteristics	ER+, PR+, HER2-, Syn+ CgA+, N+	Chemo, MT+ALND, ET	6	AWRD
PSOMA/2012 (*63*)	46		Palpable mass	Asymetric Hyperdense Microlobulated Area Hypoechoic irregular mass with posterior enhancement	CgA+, Syn+ Paget disease.	MT+ALND, RT	6	AWD
NAWAWI/2012 (*64*)	22	80	Palpable mass	Hypoechoic mass	N/A	Chemo	N/A	Died
GRACA/2012 (*65*)	83	24	Palpable lump	Nodule with Benign characteristics. Heterogeneous benign nodule	ER+, PR+, Syn+	BCS+SLNB ET	N/A	AWD
ZHANG/2011 (*66*)	29	85 left 20 right	Bilateral Nipple discharge	Bilateral Suspicious lesions	ER+, PR+, HER2-, Syn+ N-	Bilateral BCS+ Axillary sampling, Chemo	N/A	N/A
GHANEM/2011 (*67*)	64	80	Palpable mass	Suspicious characteristics	ER+, PR+, HER2-, Syn+ N+	Chemo, MT+ALND, ET	6	AWRD

CS: case series, RR: retrospective review, LR: literature review, ER: estrogen receptors, PR: progesterone receptors, Syn: synaptophysin, CgA: chromogranin A, MT: mastectomy, BCS: breast conserving surgery, SLNB: sentinel lymph node biopsy, ALND: axillary lymph node dissection, N: nodes, Chemo: chemotherapy, RT: radiation therapy, ET: endocrine therapy, AWD: alive without disease, AWRD: alive with recurrent disease, N/A: not available

our patient was completely asymptomatic and the tumor was incidentally detected on a screening mammogram. The asymptomatic presentation of neuroendocrine breast carcinoma is an exceedingly rare manifestation.

Besides, the mammographic appearance of architectural distortion, as observed in our case, is very rare as well. The presence of architectural distortion and calcifications, as seen in our case, is an extremely uncommon mammographic manifestation and has been reported in only 2.3% of the cases (*2*).

In conclusion, NEBC is a very rare breast malignancy with unclear histogenesis, which is associated with a more aggressive clinical course compared to other types of invasive breast cancer. Due to the rarity of the tumor the optimal treatment has not been clearly defined and is currently treated similarly to conventional breast cancer. Surgery is the mainstay of treatment. The distinction of primary from metastatic neuroendocrine breast tumors is crucial as these two entities require different therapeutic approaches. Further research is needed to understand the molecular profile of the tumor and identify novel targeted therapies.

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Case Report

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Cortical deafness as a sequela to meningitis: a single case study

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SUMMARY The study reports a case that was responding well to sounds and suddenly stopped hearing following fever. She contracted bacterial meningitis at the age of 5 months and had sought an audiological opinion at the age of 7 years. On evaluation, the objective test results showed normal peripheral hearing, but behaviorally she did not respond to any sounds presented during pure-tone audiometry (PTA). Thus, she was evaluated for higher auditory function using late latency response (LLR) and the responses were absent bilaterally. This confirmed cortical deafness post meningitis. Meningitis can thus also cause cortical deafness in addition to peripheral hearing loss.

Keywords meningitis, pure-tone audiometry, late latency response

1. Introduction

Cortical deafness is a condition where a patient presents with normal peripheral hearing but may be unable to hear the sounds due to a lesion in the auditory cortex. The literature primarily shows evidence of cortical deafness only through limited case studies (*1-4*). In addition, the available data is not considered due to lack of consensus in the nomenclature (*5*) and lack of a consistent protocol in the assessment of cortical deafness (*6*). Thus, understanding of this disorder is partial.

Some of the causes for cortical deafness reported in the literature reveal head injury/trauma, cerebro vascular accident (CVA), infections (*3,7,8*) and seldom meningitis (*9*). The literature on meningitis focuses primarily from the view of a peripheral lesion, which became more evident with the advent of cochlear implants. In addition, ossification is a primary consequence of meningitis. It starts as early as 21 days after infection and is completed within a few months usually less than six months (*10*). Ossification in meningitis has been deeply studied to evaluate the benefits from cochlear implants (*11*).

2. Case Report

The case presented in this study is a 7-year-old girl who was first seen for a hearing screening at a village camp. She presented with not hearing nor speaking age appropriately and was referred for a detailed audiological evaluation. Her history and medical records revealed that she was admitted for evaluation of a high-grade fever at the age of 5 months, was diagnosed as acute bacterial meningitis, and was treated accordingly. A computed tomography (CT) scan finding was normal and no other imaging reports were available. A neurosonogram was recommended but a follow up was not done. The parents reported that until the age of 5 months the child used to respond to sounds and suddenly stopped responding, following the onset of fever.

At the age of 7 years when she reported at our institute for the purpose of receiving a handicap certificate, she did not follow verbal instructions but could understand gestural commands. She was conditioned to respond to pure-tone audiometry (PTA) by making her raise her hand on the presentation of a vibrotactile stimulation using a bone vibrator and then a PTA and speech audiometry was performed using a GSI-61 audiometer. An immittance test was carried out using GSI Tympstar Pro. Tympanometry using a 226Hz probe tone was carried out and reflexometry was obtained bilaterally to ipsilateral and contralateral stimulation. Distortion product otoacoustic emissions (DPOAEs) were recorded bilaterally using a ILO V6 instrument with a frequency range of 1,000Hz to 8,000Hz. Based on basic audiological findings, the test battery was further extended to include auditory brainstem response (ABR) tracked using clicks, 500Hz and 4,000Hz tone burst stimuli. Late latency response (LLR) was also performed using a 1000Hz tone burst stimulus at an intensity of 70dBnHL. Both the auditory evoked potential testing was done using Biologic Navigator Pro Version 7.2. Detailed speech and language evaluation was carried out. The child was referred for an imaging test; however, they could not follow-up due to financial constraint and travel inconvenience and thus is a limitation.

The child was very well conditioned to vibrotactile

stimulation but was completely unresponsive to pure-tone audiometry. The PTA revealed no response to air conduction stimulation even at maximum limits from 250Hz to 8,000Hz in both ears (Figure 1). Similarly, there was no response to bone conduction stimulation of the right ear from 250Hz to 4,000Hz. She could understand gestural commands and it could be understood that she was not able to hear any sounds. The middle ear analysis was done using tympanometry, which revealed normal middle ear functioning with bilateral 'A' type tympanograms (Figure 2). A peculiar finding was noted in reflexometry. Both ears showed absent acoustic reflexes on ipsilateral recording, but acoustic reflexes were present in the right ear on contralateral presentation of the stimulus and absent in the left ear. The consistency of this result was confirmed by repeating three trials. This made us extend our test battery. DPAOEs revealed normal emissions with SNR (signal to noise ratio) more than 6dBSPL from 1.4KHz-8KHz with good amplitude bilaterally as shown in Figure 3. Subsequently, bilateral peak I, III &V of ABR (Figure 4) could be recorded until 30dBnHL in response to clicks and peak V till 30dBnHL in response to 4,000Hz tone burst stimuli. Peak V was present till 40dBnHL and absent at 30dBnHL in response to 500Hz tone burst stimuli, bilaterally. This clearly suggested that the child had normal peripheral hearing and thus brought us to the next level of testing.

The possible reason for her unresponsiveness to sounds could lie in the central auditory system. Since she had no response to PTA, no other behavioral test would help us look into the central auditory system. This led us to the selection of LLR, which revealed that there was absence of all the components of LLR *i.e.*

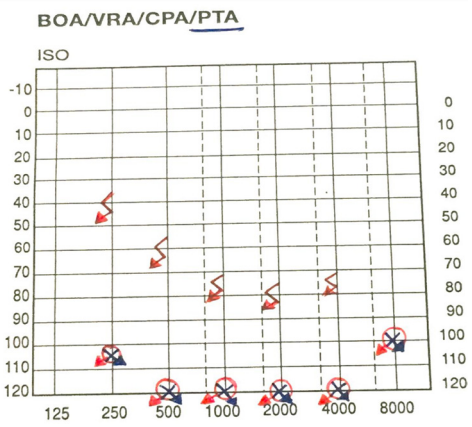
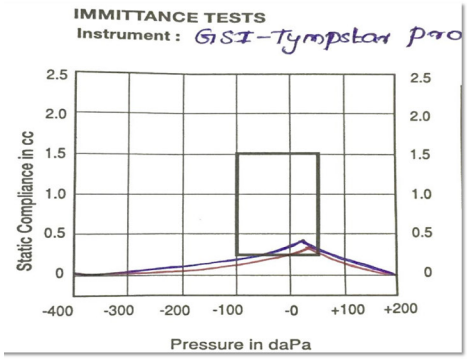


Figure 1. Pure tone audiometry (PTA) showing bilateral profound hearing loss.



Test ear	500Hz	1KHz	2KHz	4KHz	BBN
Right contra	115	115	120	NR	105
Right ipsi	← NR →				
Left contra	NR	NR	NR	NR	105
Left ipsi	← NR →				

Figure 2: Immittance showing bilateral 'A' type of tympanogram with contralateral acoustic reflexes present at higher intensities.

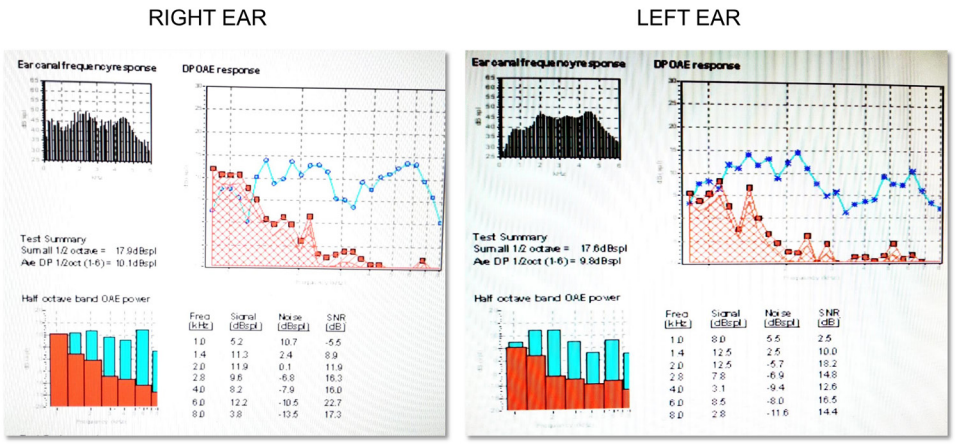


Figure 3. DPgram showing bilateral presence of distortion product otoacoustic emissions (DPOAEs).

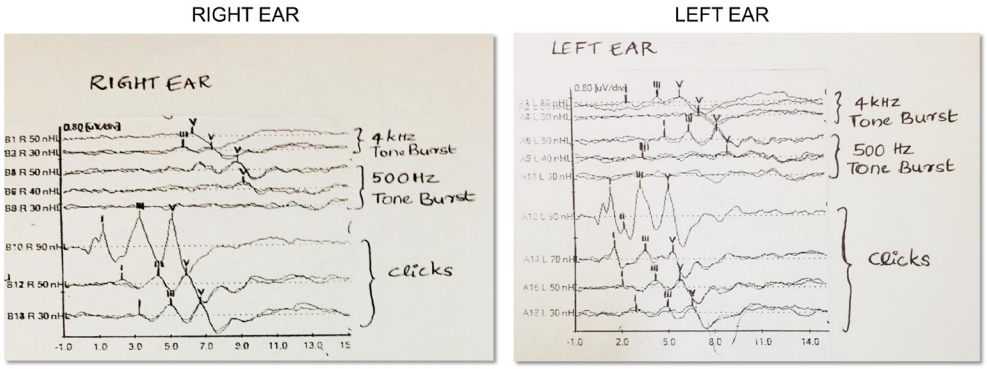


Figure 4. Auditory brainstem response (ABR) showing bilateral presence of waves I, III & V in response to clicks, 500Hz & 4KHz toneburst stimuli.

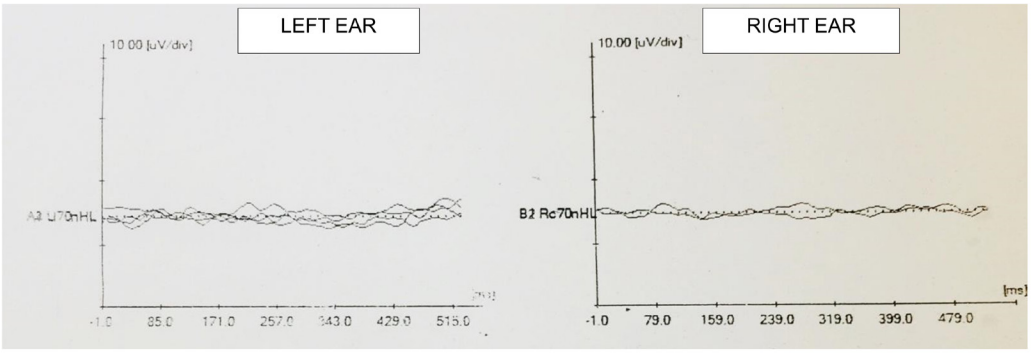


Figure 5. Late latency response (LLR) showing bilateral absence of peaks.

P1, N1, P2 & N2 at 70dBnHL. Two recordings ensured the replicability of each wave and both recordings showed no replicable peaks throughout the time window bilaterally. Figure 5 shows absent LLR bilaterally on two recordings of each ear. Detailed speech & language evaluation reveals delay in receptive and expressive language skills (receptive and expressive language age of 0-0.5 months respectively, based on Assessment of Language Development test). Reading and writing could not be assessed, as the child was not sent to school. The child was recommended for magnetic resonance imaging (MRI) scan but did not follow up.

3. Discussion

It is known that cortical deafness is an extremely rare condition (*4*) reported in the literature with single case studies. Among them, meningitis as a cause for cortical deafness is even rarer. With the advent of cochlear implantation, meningitis has caught the attention of several researchers and clinicians as it presents with peripheral hearing loss and cochlear ossification (*11*) thereby needing immediate management. In certain cases, meningitis also presents with normal peripheral hearing but cortical deafness has not been reported so far in the recent literature. Hood *et al.* (*3*) in their discussion have quoted a case of cortical deafness due to meningitis (*9*). The symptoms of the child presented in this study were very much similar to those of pre-lingual deafness with totally no response to pure-tone audiometry or to any speech stimulus presented at the maximum levels of the audiometer. Most often, the studies on cortical deafness reveal complete deafness on pure-tone audiometry results, although cases with residual hearing or even normal hearing cannot be ruled out (*4*). There can also be instances of inconsistent or variable responses to sound (*12*).

The bilateral absence of acoustic reflexes to ipsilateral stimulation and the presence of reflexes in only one ear to contralateral stimulation at very high intensities can be explained with a possible lesion in the reflex pathway. However, this is contrary to the general findings of cortical lesion where acoustic reflexes are present at normal levels (*3*). In addition, the ABR is also normal in both the ears with good morphology and lower thresholds suggesting normal brainstem function. Among the objective tests performed on patients with cortical deafness ABR has found to be normal in many cases (*4,12*) as they have normal peripheral hearing. However, the possibility of a lesion in the reflex pathway could not be explained. The presence of normal peripheral mechanism has been further highlighted in this case with the presence of normal outer hair cell function as evident from DPOAEs. Authors (*4,13*) also report normal otoacoustic emissions (OAEs) in a cortical lesion. The only objective test that explained the absence of response

to pure-tone audiometry was the absence of cortical response, LLR. The diagnosis of cortical deafness is based predominantly on the objective measures (*3*) with middle latency response (MLR) and LLR being sensitive. In this study, however the MLR was not done on this case. The upper brainstem and subcortical structures could have been explored using MLR (*14*) and possibly a correlation with reflexometry would have given a better understanding of the condition.

This child would need to rely on sign language or an alternative augmentative communication to meet her communication demands. Early intervention in children with cortical deafness would have improved the quality of life to some extent. In cases of cortical deafness, post-stroke patients are recommended for follow up for the possibility of long-term recovery (*4*). In India, the awareness towards, the importance of follow up is lacking. This case had never sought an audiological evaluation until a free hearing screening was set up. The study also highlights the outreach to the rural population.

A test battery in the diagnostic protocol of meningitis must include a combination of subjective and objective tests. Even if the ABR exhibits normal results, the incorporation of LLR is a mandate. Since the focus of tests in a patient with meningitis has always remained on identifying ossification of the cochlea, many cases of cortical deafness would have been overlooked. The study also highlights the importance of contralateral acoustic reflexes which would give an insight into the involvement of the brainstem not picked up by the ABR and suggests how sometimes physiological tests might be more informative than electrophysiologcal tests. The availability of imaging would have been ideal for correlating the present audiological results but is not present because the client failed to follow up and this remains the drawback of this paper.

A combination of both audiological and imaging tests are recommended in order to investigate cortical deafness (*4*). However, cortical deafness can be identified accurately by means of an audiological test battery, which turns out to be economical especially for patients from a rural background.

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Case Report

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Primary leiomyoma of the liver in an immunocompetent patient

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SUMMARY Primary leiomyoma of the liver (PLL) is a rare benign tumor occurring in immunosuppressed people. From 1926 less than fifty cases are reported in the scientific literature and about half are in immunocompetent patients. Etiology of this kind of lesion is not yet well known. We report a case of primary hepatic leiomyoma in a 60-year-old immunocompetent woman. The patient presented with lipothymia with unexpected vomiting. She underwent an ultrasound (US), and a computed tomography (CT) scan that revealed the presence of a single, solid lesion about 9 cm located between the S5 and S8 segment of the liver. It showed a well-defined, heterogeneous hypodensity with internal and peripheral enhancement and various central hypoattenuating areas and no wash-out in the portal and the late phases. Because of her symptoms and the risk of malignancy, the patient underwent a surgical liver resection. Histological diagnosis was primary leiomyoma of the liver. The patient had an uneventful recovery and was discharged after 7 days. At 30 months follow-up there were no symptoms and no evidence of disease. Leiomyoma of the liver is a rare benign neoplasm of which clinical symptoms are nonspecific and the exact radiological diagnosis still remains a challenge for radiologists. Etiology is still unclear and usually PLL represents an incidental diagnosis. Surgery plays a primary role not only in the treatment algorithm, but also in the diagnostic workout.

Keywords liver resection, liver leiomyoma, liver neoplasm, rare liver diseases

1. Introduction

Primary leiomyoma of the liver (PLL) is a rare benign tumor occurring in immunosuppressed patients and arising from the muscularis of the gut or the media of the blood vessels, usually in the urogenital and gastrointestinal tracts (*1,2*). However it can originate from any organ or tissue such as the biliary tract and large vessels of the liver (*3*). Its occurrence in immunocompetent patients is extremely rare. Less than fifty cases are reported in literature; twenty-three of them in immunocompetent patients (*4,5*). This is the 24th described. Etiology is still unclear and usually PLL represents an incidental diagnosis. Surgery plays a primary role not only in the treatment algorithm, but also in the diagnostic workout. We report a case of a resected hepatic mass with an unexpected diagnosis of PLL in an immunocompetent patient.

2. Case Report

A 60-year-old woman with abdominal pain and nausea was referred to our institute because of an incidental finding of liver neoplasm detected by US examination performed in the Emergency Department of another hospital for lipothymia with unexpected vomiting. An hypoechoic and heterogenic liver mass about 9 cm diameter between V-VIII segments of the liver was revealed (Figure 1). The patient had a history of hypertension and type 2 diabetes mellitus. Neither liver disease nor immunosuppression were reported. Upon admission, liver function and routine blood exams were within normal limits and viral marker tests like hepatitis B virus (HBV), hepatitis C virus (HCV), human immunodeficiency virus (HIV), Epstein-Barr virus (EBV) were negative. Tumor markers showed normal values: alphafetoprotein (AFP) 1.35 ng/mL, carcinoembryonic antigen (CEA) 1.21 ng/mL, carbohydrate antigen 19-9 (CA199) 17 U/mL, and cancer antigen 125 (CA125) 24 U/mL. Further diagnostic triphasic CT confirmed a single solid lesion of about 9 cm (Figure 2) without characteristics of malignancy or clear signs of benign liver neoplasms. The patient underwent a percutaneous US-guided biopsy and diagnosis was unspecified

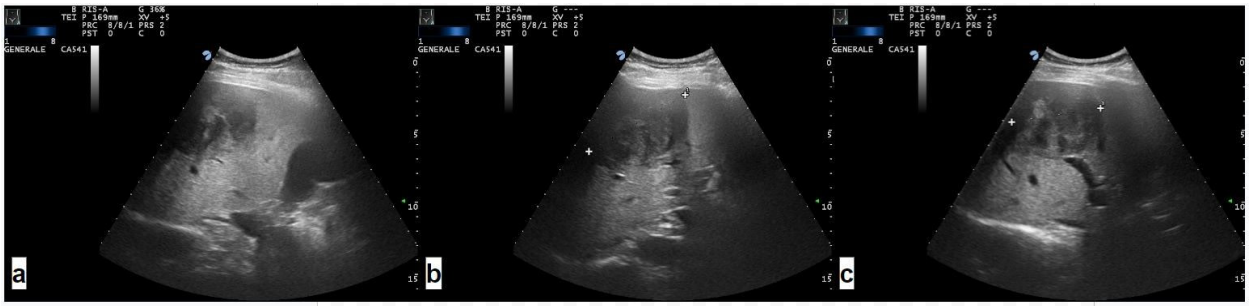

Figure 1. Ultrasound images: different slices of the hepatic leiomyoma.

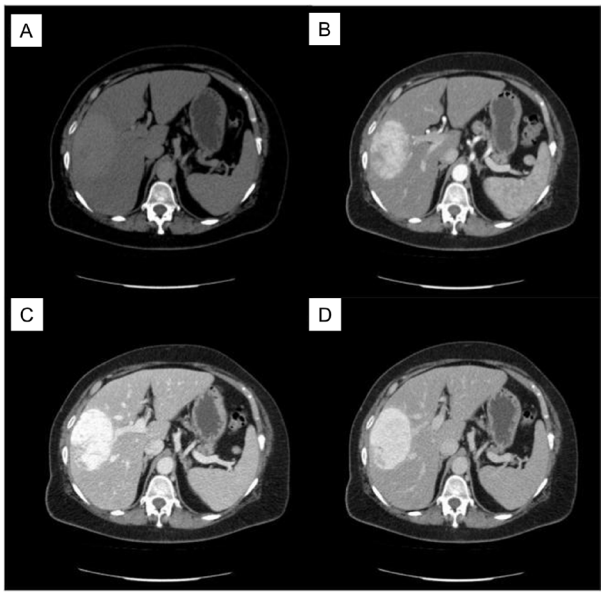

Figure 2. CT Scan. (A), basal phase; (B), arterial phase; (C), venous phase; (D), portal phase.

myopericytoma. Because of her symptoms and the risk of malignancy, the patient was scheduled for liver resection. Anatomical bisegmentectomy of V-VIII liver segments by kellyclasia technique was performed (Figure 3). After an uneventful recovery, the patient was discharged on postoperative day 7. At histological examination the liver specimen showed spindle cells with eosinophilic cytoplasm forming a pattern of interlacing bundles, separated by sclero-hyaline tissue with no mitoses or atypia. Immunohistochemical examination revealed staining positivity for Smooth-Muscle-Actin, Vimentin and Desmin, and negativity for CD31 and CD34 (Figure 4). Diagnosis was PLL. At 30 months follow-up there is no evidence of disease.

3. Discussion

Leiomyoma is relatively common, tends to originate from the muscularis of the gut or the media of the blood vessels, and usually develops in the urogenital and gastrointestinal tracts (*1-3*). However it can originate from any organ or tissue such as the biliary tract and large vessels of the liver (*6,7*). PLL is rare and has

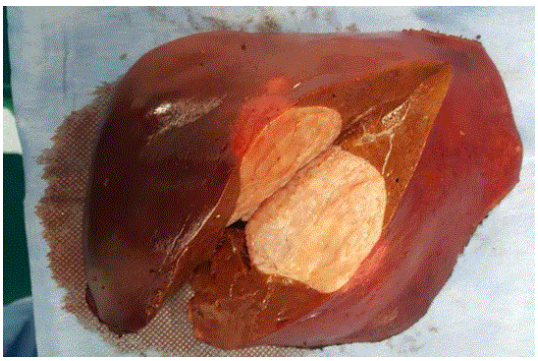

Figure 3. Specimen. Hepatic parenchyma within the center a whitish encapsulated liver mass.

its own particular clinical and biological features (*1*). Hepatic leiomyomas can be multiple or solitary lesions and can grow to a large size sometimes exceeding 20 cm in diameter with a low tendency for necrosis and hemorrhage (*6*). On the cut surface they appear yellowish to whitish in color and may be fibrous in texture (Figure 3). Some authors described in their papers that PLL may contain cystic parts (*8-10*).

The first case was described in 1926 in a 42-year-old woman (*11*). Ninety years after the first case of primary leiomyoma of the liver was reported, to the best knowledge of this author, 44 cases of primary leiomyoma of the liver have been reported in the literature. The age of presentation is very variable. The mean age of diagnosis is 41 years (range 3-87). Primary leiomyomas of the liver have been reported to have female sex predilection. Familial predispositions have not been reported.

To diagnose primary hepatic leiomyoma Hawkins *et al*. proposed two criteria. First, the tumour must be composed of leiomyocytes. Second, the presence of leiomyoma in other intraabdominal sites, especially in urogenital tissue, must be excluded (*12*). Both benign and malignant smooth-cell liver neoplasms are often found in immunosuppressed (child and adult) patients, like EBV-infected, HIV-infected and organ transplant recipients. However, they also occur in immunocompetent patients, as in our case (*13-16*). There is strong evidence for the role that some viruses exercise in the pathogenesis of certain human

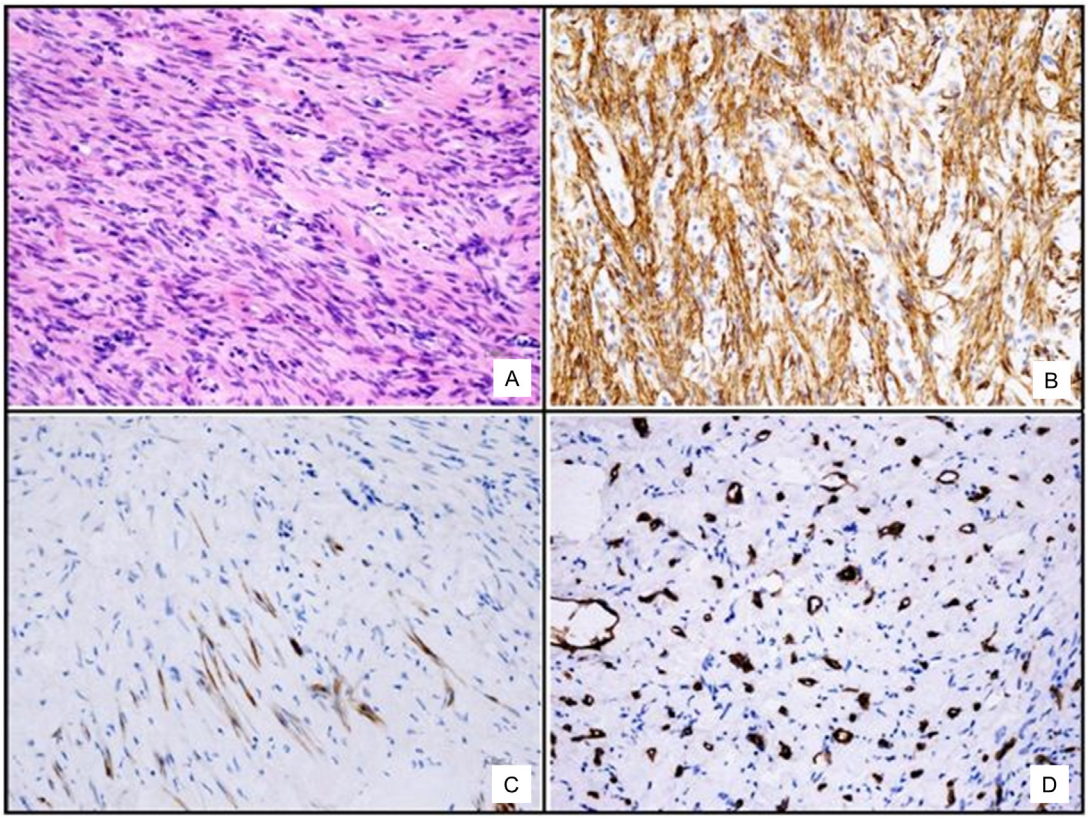

Figure 4. (A), Haematoxylin-Eosin staining. The hepatic tumor was composed of bundles of spindle-shaped cells with elongated nuclei and eosinophilic cytoplasm. No mitotic activity was seen; (B), Smooth Muscle Actin staining of tumor cells, showing diffuse positivity of spindle cells; (C), Desmin staining of tumor cells showing focal positivity; (D), CD34 staining of the tumor, showing positivity in blood vessels and negativity of tumor cells.

neoplasms. Patients with an immune deficiency condition are at risk for virus-associated malignancies (*17*). Kaposi Sarcoma and smooth-muscle tumors might arise from a common stem cell under the influence of some unknown factor produced during HIV infection. This relationship was demonstrated in 1990 in cultured cells (*18,19*). Epstein-Barr virus shows a near consistent association with smooth muscle tumors of the liver. This association has been reported several times; so much that some authors have proposed to term these lesions EBV-associated smooth muscle tumors (EBV-SMT) (*7, 20-21*). The marked expression of EBV early antigen (EBER) in the nuclei of numerous smooth muscle cell tumours has been well documented since 1993. EBER in the nuclei was first detected in neoplastic smooth-muscle cells in three cases of post-transplant spindle cell tumor (*7,22*). Then some researchers detected EBER using in situ hybridization/ISH, which is the gold standard for detection and localization of latent EBV in tissues in both adults and children (along with quantitative polymerase chain reaction). Nonetheless, EBV infection is not necessary nor sufficient for the development of primary liver leiomyoma (*14,16,23*).

Usually, in immunosuppressed patients, these lesions are detected during routine follow-up for the underlying disease, so tumors tend to be found at a smaller size when compared with tumors found in patients without immunosuppressive disorders. Instead, in immunocompetent patients, because symptoms are induced by compression of the surroundings organs, they are generally discovered when they are greater than 10 cm in diameter (*24*). Microscopically PLL generally shows a proliferation of spindle cells with eosinophilic cytoplasm forming a pattern of interlacing bundles, often separated by sclero-hyaline tissue. No mitoses are observed. Some authors suggested the limit of 10 mitoses per HPF as one of the criteria to discern leiomyomas from leiomyosarcomas. Atypia, if present, is slight. Immunohystochemical examination reveals positive staining for SMA and Vimentin, less often for Desmin, and negative for CD31, CD34, S-100 and HMB-45. The proliferation activity, based on Ki-67, was lower than 5% in all the papers we reviewed. The most common symptoms, when present, are abdominal, epigastric, or right upper quadrant pain, abdominal mass, abdominal discomfort, dyspepsia, vomiting and liver dysfunction, listed in order of decreasing frequency. Blood liver function tests and tumor markers are generally negative.

The exact radiological diagnosis of liver neoplasms (and rare tumors overall), which have often variable imaging behaviors and still unknown patterns, is still a challenge for radiologists. By US, hepatic leiomyomas have been repeatedly described as well defined hypoechoic lesions with variable degrees of

heterogeneity (*2,25,26*). Some authors reported an echo-poor halo surrounding the lesion (*27*). The CT findings that have been described are similar to those of other smooth muscle tumors in the body (*28*). By CT scan, most authors reported hypodense lesions with brisk enhancement in arterial phase, occasionally mainly peripheral, and a persistent enhancement in portal and delayed phase (*27-29*).

By angiography, marginal, diffuse or irregular hypervascularization were variously reported. Nevertheless, in all cases reported the authors concluded that the angiography study was nondiagnostic, but could perhaps play a role in a therapeutic process, as in the rare case of acute bleeding.

On MRI, these lesions were hypointense in Tl-weighted images, partially hyperintense in T2-weighted sequences, and showed marked gadolinium enhancement in both early and equilibrium phases. Gadobenatedimeglumine is a liver-specific, gadolinium-based MR contrast agent with a vascular-interstitial distribution in the first minutes after bolus injection, followed by delayed hepatobiliary excretion. In a study published in 2008 (*30*), the most common imaging pattern and the imaging protocol that the authors used is very well described. On pre-contrast T2- and T1-weighted images, the lesion showed isointensity and marked hypointensity respectively to the surrounding liver. As with the previous CT examination, the lesion showed intense enhancement on T1-weighted VIBE images acquired during the arterial phase, followed by persistent and homogeneous enhancement during the hepatic venous and equilibrium phases. During the delayed hepatobiliary phase, the lesion showed lack of contrast retention, and thus, was identified as a well-defined, hypointense focus against the highly enhanced background liver (*30*). Despite that, there are primary leiomyomas of the liver characterized by hypointensity in the T2-weighted MRI image that may be related to its dense fusocellular nature, composed by intramuscular actin, myosin and collagen, that decrease extracellular fluid compared with surrounding tissue (*26*).

The feasibility of an accurate preoperative diagnosis by fine needle aspiration (FNA) versus biopsy is still a matter of debate. Some authors reported the inadequacy of FNA for preoperative diagnosis of PLL because of the difficulty of getting an adequate sample (*31*) and favoured biopsy (*26*), defined more appropriate in cases of smooth muscle neoplasm samples (*13,28,32*). In our case, like most reported, the preoperative imaging tests and the biopsy were inconclusive (*4*).

When preoperative tests do not present a clear diagnosis, surgery has a primary role not only in the treatment algorithm, but also in the diagnostic process. As for other benign lesions of the liver, surgery should be performed in all symptomatic PLL, in those with dimensions > 10 cm with a higher risk to develop malignant changes, and for those in which radiological diagnosis can not be reached. Surgery should be performed in institutes and units that with surgical skills for these kind of procedures. Laparoscopic resections have been described by some authors (*24,33*). Mininvasive liver surgery, including robotic surgery, for benign lesions is feasible and safe for well-selected patients, and may play a role in patients with immuno-suppressive conditions in which abdominal incisions carry a higher risk of wound complications (*24*).

In conclusion, leiomyoma of the liver is a rare neoplasm which can also grow in immunocompetent patients. Clinical symptoms are nonspecific and the exact radiological diagnosis of these liver neoplasms is still a challenge for radiologists. Due to that, we think that surgery may play a key role in the diagnostic process and in the treatment algorithm.

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Case Report

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A case of coronavirus disease 2019 in acquired immunodeficiency syndrome patient: a case report and review of the literature

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SUMMARY Coronavirus disease 2019 (COVID-19) is a respiratory illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus that was identified in December 2019. The impact of COVID-19 virus on Acquired Immunodeficiency syndrome (AIDS) patients has been reported with variable outcome. We reported a patient that was immunosuppressed by AIDS disease and chemotherapy for cancer who contracted SARS-CoV-2 infection and had a mild disease. We did literature review for the cases published that had human immunodeficiency virus (HIV) infection and COVID-19 disease and analyzed the characteristics and outcomes of the reported cases. Our review yielded three case reports and four case series for patients with HIV infection and COVID-19 disease. The majority of patients had mild disease, and some had critical disease or death. Those who had severe disease usually had other comorbidities. The findings from the case reports and case series indicate that the risk of death or severe disease from COVID-19 in HIV positive patients was lower than observed in the general population, which may indicate a possible protective effect of uncontrolled HIV in preventing the complications associated with the massive inflammatory response.

Keywords COVID-19, coronavirus, HIV, AIDS, chemotherapy, SARS-CoV-2, pneumonia

1. Introduction

Beginning in late December 2019, numerous cases were emerging from Wuhan, China, of a new type of severe pneumonia of unknown etiology. The etiologic pathogen has since been identified as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus has since spread rapidly to many countries throughout the world (*1*). This is the seventh coronavirus identified so far and differs from the other coronaviruses that cause the common cold and mild pneumonia (229E, OC43, NL63, and HKU1) (*2*). In the United States, 1.2 million people are living with human immunodeficiency virus (HIV). Of note, in March 2020, the Centers for Disease Control and Prevention (CDC) identified people living with HIV (PLWH),cancer patients and those receiving chemotherapy as high risk for severe illness from the new coronavirus disease known as coronavirus disease 2019 (COVID-19) compared to the general population (*3*).

Here, we report a case of COVID-19 infection in an immunocompromised HIV patient on chemotherapy that resulted in a mild disease with full recovery.

2. Case Report

A 37-year-old man with a history of AIDS and Kaposi's sarcoma presented to the infusion clinic to receive his second dose of doxorubicin. On arrival, he complained of high-grade fever for two days associated with sore throat, mild cough, occasional headaches, chills, and night sweats. A review of systems was negative for shortness of breath, chest pain, diarrhea, skin changes, or loss of smell or taste. He denied any sick contacts.

He was diagnosed with AIDS two years ago, was nonadherent with antiretroviral therapy (ART). He had recent hospitalization for severe *pneumocystis* pneumonia from which he recovered. He was diagnosed with Kaposi sarcoma two months ago and was started on doxorubicin. Since the diagnosis of Kaposi's sarcoma, he was adherent with his ART. He also history of treated chronic hepatitis C, syphilis, anxiety, and

depression.

His medications include bictegravir-emtrictabine-tenofovir alafenamide, atovaquone, prochlorperazine, ondansetron, and tramadol as needed. He is allergic to trimethoprim/sulfamethoxazole and intolerant to Dapsone. He is a never smoker, denies alcohol use, had remote history of methamphetamine and marijuana use, but has been sober for 3 years. Family history was unremarkable. He used to work as a bus driver in the past, currently unemployed. He denied any recent travel outside Nebraska state or recent exposure to COVID-19 or sick patients.

On examination, his temperature was 38.2°C, heart rate of 118 beats per minute, blood pressure was 136/72 mmHg, respiratory rate of 20 breath per minute, and his oxygen saturation was 99% on room air. The patient had normal respiratory effort, lungs were clear to auscultation. There was a healed incision in right groin, pruritic rash in bilateral inguinal areas. He had shallow perianal ulcers with minimal bleeding. The rest of his examination was normal.

Laboratory testing at the time of presentation was notable for leukocytosis, and mildly elevated procalcitonin (Table 1 and Table 2). A recent HIV viral load of 517 copies/milliliter with a cluster of differentiation 4 (CD4) cell count of 67 and both respiratory pathogen screen and group-A streptococcus screening have been negative, and the rest of laboratory workup are listed in Table 1. The chest radiograph at the time for admission showed no signs of cardio/pulmonary disease (Figure 1), and computed tomography for abdomen and pelvis showed no focus of

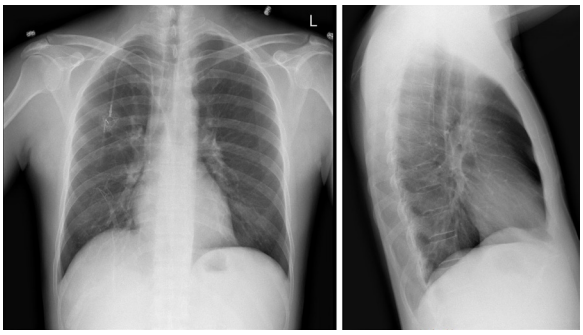

Figure 1. Chest X-ray on admission. Postero-anterior chest x-ray showing normal lung fields with no reported abnormality.

Table 1. Laboratory investigation on admission and on discharge

Component	Reference range in adults	On admission	On discharge
White blood cells (k/ul)	4.0-12.0	15.1	10.8
Red blood cells (m/ul)	4.30-5.90	4.14	3.68
Hemoglobin (gm/dl)	13.5-17.5	12.9	11.7
Platelets (k/ul)	140 000-440 000	88	125
Absolute neutrophil count (k/ul)	1.5-8.0	10.3	
Absolute lymphocytes count (k/ul)	1.0-4.5	1.1	
Creatinine (mg/dl)	0.60-1.30	0.84	0.62
Sodium (mmol/L)	135-145	135	140
Potassium (mmol/L)	3.7-5.1	3.5	3.9
Albumin (gm/dl)	3.5-5.0	3.0	2.9
Aspartate aminotransferase (u/l)	10-40	27	24
HIV viral load (Copy/mL)	Negative	517	
T-cell count differential			
CD4/T4 cells (%)	40.0-60.0%	4.2	
CD4 T cell abs. (cells/ul)	436-2,168	67	
CD8/T8 cells (%)	15.0-43.0	47.3	
CD8 T cell abs. (cells/ul)	164-1,456	757	
CD19 cells (%)	5.0-22.0	19.0	
CD19 abs. (cells/ul)	56-745	290	
CD56 cells (%)	3.0-21.0	14.1	
Absolute CD56 (cells/ul)	33-711	215	
CD4/CD8 ratio	0.9-3.4	0.1	
Infectious tests			
Blood culture	Negative	No growth at 5 days	
Respiratory pathogen panel	Negative	No respiratory pathogens detected by multiplex PCR	

Table 2. Inflammatory markers related to COVID-19

Component	Reference range in adults	Hospital day 1	Hospital day 2	Hospital day 4
Ferritin (ng/mL)	22-388	452	430	416
C-reactive protein (mg/L)	≤ 9.00	41.90	35.80	25.50
D-dimer quantitative (mg/L)	< 0.25	0.79		0.90
Fibrinogen (mg/dl)	200-400	437		
Procalcitonin (ng/mL)	≤ 0.05	0.55		
COVID-19 qualitative	Not detected	detected		

infection. Blood cultures were drawn, and COVID-19 testing was sent (Table 1 and Table 2).

Given his recent methicillin-resistant *Staphylococcus aureus* (MRSA) abscess infection, a current cutaneous candidiasis infection, and overall septic picture, he was started on empiric therapy with vancomycin, cefepime, metronidazole, fluconazole, and topical Miconazole. On day 3 of admission, COVID-19 testing was positive, rest of the work up was negative so the antibiotics were stopped. During his entire hospital stay, he did not require any supplemental oxygen therapy, and all his symptoms of fever, sore throat, headache, and mild cough completely resolved. Anticoagulation was deferred due to his thrombocytopenia and recent episode of rectal bleeding. He was discharged home on day 4 of hospitalization in stable condition with instructions for self-quarantine for 14-days. The patient remained asymptomatic and healthy at post discharge follow up visit.

3. Discussion

Multiple risk factors have been linked to worse outcomes in COVID-19 infection including, age (> 60 years), hypertension, diabetes, cardiovascular disease, lung disease, and chronic kidney disease (*4*). Immunosuppressed patients are at a higher risk of being infected with COVID-19. Multiple studies and case reports showed the role of massive immune response and excessive release of inflammatory cytokines - which the CD4 T-cells play a significant role - in the damage that occurs in the lung tissues (*5-7*). However, the question of whether being immunosuppressed is a risk factor for more severe disease or not is still under investigation.

We conducted a systematic review of the literature for studies published to date in PubMed, Scopus, Web of Science, and Cochrane Central databases. The following search terms were used: "acute respiratory syndrome coronavirus 2 (SARS-CoV-2)", "COVID-19" and "Human Immunodeficiency Virus". Our search was limited to individuals 18 years and older. Our search revealed a total of 3 case reports and 4 case series.

Zhu *et al.* (2020) was among the first to report a case of SARS-CoV-2 and HIV co-infection in a patient from Wuhan. The patient was diagnosed with COVID-19 pneumonia and was found to be HIV positive during hospitalization (*8*). Despite his hospital course complicated by severe pneumonia requiring treatment with steroids, he recovered completely from the illness. Our findings were similar to Louisa *et al.* (2020), who reported a SARS-CoV-2 infection from a patient with previously diagnosed HIV infection, on antiretroviral therapy. The patient developed a mild illness and recovered completely without any specific therapy for COVID-19 (*9*). Also, Wu *et al.* (2020) reported a patient with HIV on antiretroviral therapy (tenofovir disoproxil fumarate, lamivudine, and efavirenz), stage-4 diffuse large B-cell lymphoma and previously treated pulmonary tuberculosis, who was diagnosed with COVID-19 after presenting to the hospital with fever and symptoms of viral respiratory tract infection that progressed to pneumonia then he recovered (*10*).

Furthermore, four prior case series were found, the first case series by Blanco *et al.* (2020), describes five HIV positive patients who were generally less than 50 years old. Two patients were virologically suppressed with protease inhibitor (darunavir-boosted cobicistat) based antiretroviral therapy, while the other two were suppressed with integrase inhibitor (dolutegravir) based antiretroviral therapy. Nevertheless, the fifth patient had elevated viral load, low CD4 count, and was antiretroviral therapy naïve. Mortality was low amongst these patients, with four cured of COVID-19 and one remaining in ICU at the time of publication of the study (*11*). The second case series by Haerter *et al.* (2020) was that 33 people living with HIV patients were included. All patients were on antiretroviral therapy at the time of diagnosis of COVID-19, 60% of patients included had comorbidities, including hypertension, COPD, diabetes mellitus, cardiovascular disease, and renal impairment. 76% of patients had mild disease, 6% had severe disease, while the remaining were critical cases (*12*).

The third case series by Gervasoni *et al.* (2020) reported 28 HIV patients with COVID-19. Out of these 28 patients, 13 required hospitalization, and 6 had severe disease. The majority (96%) of patients in this cohort recovered with good outcomes, while the rest (4%) died (*13*). The fourth case series by Aydin *et al.* (2020) reported four cases of patients diagnosed with COVID-19 pneumonia who had co-existent previously diagnosed HIV infection. Most of the patients were maintained on antiretroviral therapy, except for one noncompliant patient. All patients without comorbidities (three patients out of the four) recovered; and the fourth patient who died had co-comorbidities; diabetes mellitus, essential hypertension, and chronic obstructive pulmonary disease (*14*).

Our patient was immunosuppressed, as evidenced by his low CD4, high viral load, and being on chemotherapy. However, he did not develop any complications such as pneumonia, acute kidney injury, stroke, or coagulopathy. We hypothesize that the fact his immunocompromised state with low CD4 count resulted in a lessened immune response and fewer disease complications, in addition to the possible potential protective effect of antiretroviral therapy (bictegravir, emtricitabine & tenofovir alafenamide). Our assumption was supported by the prior studies which showed similar outcomes of patients with high viral load and low CD4 T-cell count, and in organ transplant patients who are on immunosuppressive therapy that contracted COVID-19 and had mild symptoms (*15*). The possible protective

effect of antiretroviral therapy was based on the current ongoing trials that are being done to evaluate the role of tenofovir and emtricitabine in protection against COVID-19 infection (*16*). However, our review of the literature failed to support it.

4. Conclusion

Findings from the above case series indicate that the risk of death, severe disease, or admission to ICU from COVID-19 in HIV positive patients was lower than observed in the general population. This might suggest a possible protective effect of poorly controlled HIV in avoiding the cytokine storm induced COVID-19 complications despite being more susceptible to infection. However, as current knowledge about COVID-19 is still evolving, more studies are needed to validate this observation.

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Case Report

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Intraventricular pilocytic astrocytoma in an adult patient

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SUMMARY Pilocytic astrocytomas are tumors of the central nervous system mostly during the first two decades of life. Although they are mostly common in the midline structures of children, pilocytic astrocytoma within the ventricular system of an adult is extremely rare. We report a case of a 38-year old woman with obstructive hydrocephalus secondary to a brain tumor within the third ventricle. On histological examination, the tumor exhibited biphasic growth pattern comprising compacted cellular areas with Rosenthal fibers and loose textured microcystic areas with eosinophilic granular bodies. Mitosis or necrosis was not present. Immunohistochemical studies demonstrated glial fibrillary acid protein (GFAP), Olig2, and ATRX positivity as well as NeuN and EMA negativity. Ki67 labeling index was less than 1%. Molecular studies revealed that there are no isocitrate dehydrogenase (*IDH*) gene mutation and *H3F3A* mutation. This clinical presentation along with the histologic and molecular findings is consistent with a pilocytic astrocytoma arising in the third ventricle of this adult brain, which indicates that pilocytic astrocytoma can present as an intraventricular tumor in an adult patient and should be routinely included in the differential diagnosis of intraventricular brain neoplasm.

Keywords pilocytic astrocytoma, ventricle, central nervous system, adult

1. Introduction

Pilocytic astrocytomas are tumors of the central nervous system (CNS) most commonly occurring during the first two decades of life with a peak incidence between 8 and 13 years of age. Although these tumors are typically located in the midline structures of CNS, like posterior fossa, cerebellum, thalamus, hypothalamus, etc., they rarely arise within a cerebral ventricle of an adult patient (*1*). In contrast to other low grade astrocytomas, pilocytic astrocytomas are associated with absence of isocitrate dehydrogenase (*IDH*) gene mutation. In some of cases, but not all cases, *KIAA 1549/BRAF* gene fusion is present. Literature review indicates that only a single prior case of intraventricular pilocytic astrocytoma has been histologically and molecularly identified in an adult patient (*2*). We report another histologically and molecularly confirmed case here to further support that pilocytic astrocytoma can occur intraventricularly in an adult patient.

2. Case Report

2.1. Clinical history

The patient was a 38-year-old woman with no significant medical history who reported a new brain mass and obstructive hydrocephalus recently diagnosed in another hospital. She presented to our institution for further evaluation and treatment. She described five months of intermittent diplopia, tremors, and clumsiness. She was previously treated with levetiracetam and acetazolamide. At our institution, computed tomography (CT) confirmed moderate hydrocephalus with dilation of the lateral and third ventricles, without dilation of the fourth ventricle, as well as transependymal resorption of CSF indicating acute hydrocephalus. Magnetic resonance imaging (MRI) revealed a 12 mm rounded mass along the floor of the third ventricle with mild to moderate heterogeneous contrast-enhancement, obstructing the cerebral aqueduct (Figure 1). Subsequently, she underwent endoscopic ventriculostomy, in which a third ventricular tumor was identified and excised. The resulting specimen was sent to our neuropathology laboratory for evaluation.

2.2. Pathology

The surgical specimen comprised multiple small fragments of soft, pink-tan tissue. Cytological preparation for intra-operative consultation exhibited bipolar piloid cells with long, hair-like processes and elongated, moderately pleomorphic nuclei as well as smaller cells with short, cobweb-like processes

and round to oval nuclei. Rosenthal fibers and eosinophilic granular bodies were present. Mitosis was not identified. Histological examination of the paraffin-embedded tissue revealed a biphasic growth pattern composed of compacted cellular areas with Rosenthal fibers and loose textured microcystic areas with eosinophilic granular bodies. Mitosis, vascular endothelial hyperplasia or necrosis was not observed (Figure 2). Immunohistochemical studies demonstrated that the neoplastic cells expressed glial fibrillary acid protein (GFAP), Olig2, and ATRX, while IDH1 R132H, NeuN, and epithelial membrane antigen (EMA) were not present in the neoplastic cells. Ki67 labeling index was less than 1% (Figure 3). Next-generation sequencing study showed that there were no *IDH* mutation, *BRAF* alterations including *KIAA1549/BRAF* fusion, *H3F3A* mutation, and any other reported glioma-related genomic abnormalities (data not shown).

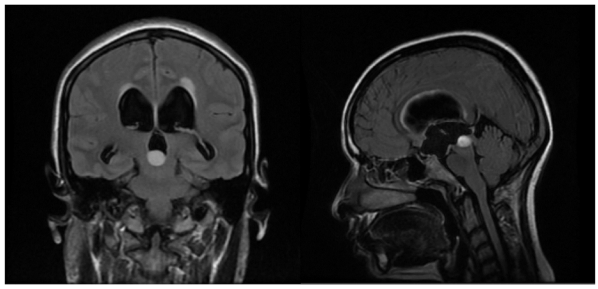

Figure 1. Magnetic Resonance Imaging, T2-weighted, showed a mild to moderate heterogeneous contrast-enhanced mass in the third ventricle.

3. Discussion

An intraventricular tumor is far more likely to arise from choroid plexus, ependyma, and subependymal tissue. Other less common intraventricular tumors include meningioma, lymphoma, and metastasis. Third ventricle tumors are rare and only comprise 0.6-0.9% of all brain tumors among all age groups (*3,4*). Within the category of third ventricle tumors, the most common includes colloid cysts, choroid plexus papillomas, and ependymomas. Occasionally, a pineal tumor can be located within the third ventricle. Although intraventricular pilocytic astrocytomas have been reported radiologically with or without limited histological examination (*5,6*), only one histologically and molecularly confirmed pilocytic astrocytoma within the lateral ventricle has been recently documented in the literature (*2*). In our case, neuroimaging demonstrated an obvious obstructing third ventricle mass which histologically exhibited classic pilocytic astrocytoma features, including biphasic growth pattern comprising compacted cellular areas and loose textured microcystic areas with Rosenthal fibers and eosinophilic granular bodies. Immunohistochemical studies demonstrated that the tumor cells are positive for glial fibrillary acid protein (GFAP), ATRX, and Olig2 with Ki67 labeling index of less than 1%, indicating astrocytic nature of low-grade glial neoplasm. Molecular studies revealed that there is no *IDH* gene mutation. The above supports the diagnosis of pilocytic astrocytoma. This case further supports that pilocytic astrocytoma can occur in the cerebral ventricular system including the third ventricle and

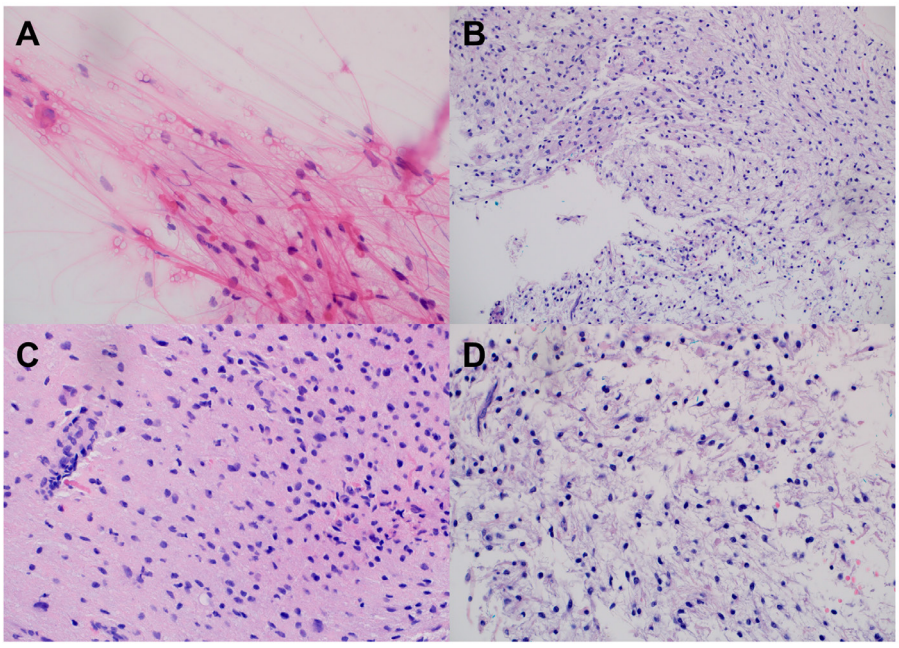

Figure 2. Histological examination observed two cell populations, bipolar piloid cells with long, hire-like processes and smaller cells with short, cobweb-like processes as well as a biphasic growth pattern composed of compacted cellular areas with Rosenthal fibers and loose textured microcystic areas with eosinophilic granular bodies. (A), smear preparation, H&E, 400×; **(B)**, tissue section, H&E, 200×; **(C)** and **(D)** tissue section, H&E, 400×.

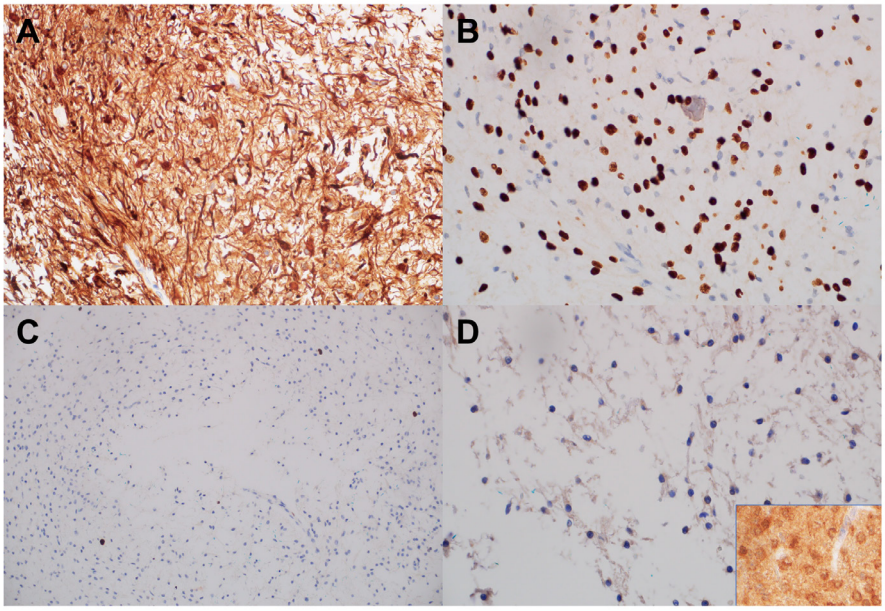

Figure 3. Immunohistochemical studies demonstrated that the neoplastic cells expressed GFAP and Olig2 with low Ki67 labeling index, but not IDH1 R132H mutant protein. (A), GFAP immunostaining, 400×; **(B)**, Olig2 immunostaining, 400×; **(C)**, Ki67 immunostaining, 200×; **(D)**, IDH1 R132H immunostaining, 400×, insert: positive control.

should be in the differential diagnosis of intraventricular tumors.

Pilocytic astrocytoma accounts for 1.3% of all central nervous system tumors. It is the most common glioma in the pediatric population during the first two decades of life with median age of 12 years old, which declines dramatically from 14 years old to 15-19 years old. Approximately one third of gliomas in 0-14 years old patients are pilocytic astrocytoma (*1,7*). In adults, pilocytic astrocytoma is much less common. It usually occurs a decade earlier than diffuse astrocytoma and is rarely present in patients older than 50 years. It is worth noting that in contrast to another previously reported intrventricular pilocytic astrocytoma (*2*), this case does not harbor *KIAA 1549/BRAF* gene fusion. However, *KIAA 1549/BRAF* gene fusion is known to present much less frequently in adult patients with pilocytic astrocytoma, which suggests that the pathogenesis of intraventricular pilocytic astrocytoma may not necessarily be different from other extraventricular pilocytic astrocytomas (*8*).

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Conflict of Interest: The authors have no conflict of interest to disclose.

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Letter

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Primary cutaneous follicle center lymphoma of the breast: Management of an exceedingly rare malignancy

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SUMMARY Primary cutaneous follicle center lymphoma (PCFCL) is defined as a low-grade B-cell non-Hodgkin's lymphoma, which primarily occurs and remains confined to the skin, without evidence of extracutaneous or systemic involvement at the time of diagnosis. PCFCL affecting the breast skin is an exceedingly rare entity with only two cases reported in the English literature. We present a case of PCFCL affecting the periareolar breast skin and review the relevant literature. Our patient was a 64-year-old female who presented with an erythematous plaque in the periareolar region of the left breast. The diagnosis of PCFCL was confirmed by a biopsy performed with a seven-month delay, as the tumor had been initially misdiagnosed as a benign lesion. The patient was successfully treated with local radiation therapy. PCFCL is an indolent lymphoma associated with an excellent prognosis. For localized lesions, skin-directed therapies mainly consisting of radiation therapy or complete surgical excision are curative therapeutic approaches, while systemic chemotherapy should be reserved for patients with extensive disease. This case highlights the need to consider PCFCL as an important differential diagnosis in patients presenting with non-resolving erythematous breast skin lesions. A timely biopsy should be obtained to avoid delays in the initiation of appropriate treatment.

Keywords PCFCL, cutaneous, lymphoma, follicle, center, breast

Follicular lymphoma is the third most common lymphoma affecting the breast, accounting for 14-19% of all breast lymphomas and represents, in most cases, a manifestation of disseminated disease (*1*). Primary cutaneous follicle center lymphoma (PCFCL) involving the breast skin is an exceedingly rare clinical entity with only two cases reported in the English literature (*2,3*).

A 64-year-old woman presented with a 7-month history of a non-tender slowly enlarging erythematous plaque, in the areolar region of her left breast. She denied any symptoms, whereas her past medical history was unremarkable, and she had no family history of breast cancer. She had been prescribed local treatment by another institution without any clinical response.

Physical examination revealed a non-tender erythematous plaque, measuring approximately 4 cm, in the upper outer areolar region of the left breast (Figure 1). The mammogram was unremarkable, but the ultrasonogram showed a focal thickening of the outer aspect of the left areolar region. A biopsy was performed and the histological and immunohistochemical findings were suggestive of primary cutaneous follicle center lymphoma (Figure 2).

Following the biopsy, a thorough staging investigation was performed to rule out extracutaneous disease, including hematological and biochemical parameters, and computed tomography scans of the abdomen, chest, and pelvis. All of the above were unremarkable. The patient treated with superficial radiation therapy with a total dose of 3600 cGy delivered in 200 cGy doses for a total of 18 fractions. She exhibited a complete clinical response and is well without any evidence of tumor recurrence 27 months after treatment.

PCFCL is an indolent lymphoma of unknown histogenesis originating from the germinal-center B cells without evidence of extracutaneous disease at the

time of diagnosis. It most commonly occurs in patients in their fifth to seventh decades of life at a median age of 50 years, with a male to female ratio of 1.5:1 (*4,5*).

The most common clinical presentation of PCFCL is a solitary or less commonly multiple firm erythematous or violaceous papules, plaques, or tumors of variable size and a smooth surface (*6*). The lesions tend to enlarge slowly and may reach several centimeters in size. The most commonly affected areas are the head and neck, and the trunk (*3-5,7*). Multiple lesions are reported in 60% of the patients, 30-40% of which tend to occur in a localized area (*8*). Histologically, PCFCL is characterized by dense dermal infiltration of large centrocytes derived from germinal center B cells in a follicular, diffused, or mixed growth pattern (*8*). The neoplastic infiltrate spares the epidermis from which is separated by a grenz zone (*7,8*).

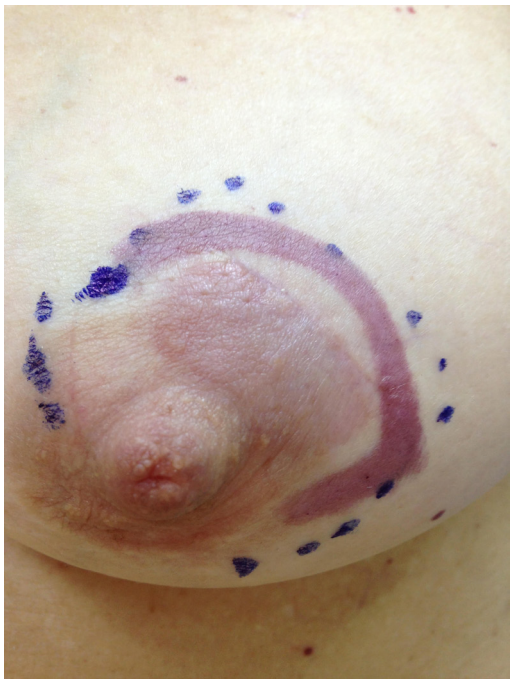

Figure 1. Photo showing the erythematous lesion of the left areolar region during radiation treatment planning.

On immunohistochemical analysis, the neoplastic lymphocytes express B-cell markers such as CD19, CD20, CD22, CD79A, and PAX5 and at least one follicle center marker which is BCL-6 and less commonly CD10 (*8,9*).

The differentiation of PCFCL from systemic follicular lymphoma (SCFL) is of paramount importance (*1*) since the two entities require different treatments (*7*).

The diagnosis of PCFCL is confirmed by an excisional on punch biopsy and subsequent thorough pathologic and immunohistochemical analysis. After the diagnosis of PCFCL is confirmed, a thorough staging investigation is mandatory to rule out extracutaneous involvement.

The treatment options for PCFCL depend on the extent of the disease and include local or systemic treatment. For solitary lesions, radiation therapy and complete surgical excision constitute curative therapeutic approaches (*6,8*). Surgical resection is considered for small well-demarcated solitary lesions (*6*).

Complete remission of PCFCL after radiation therapy is reported in up to 100% of the cases (*5*), whereas recent publications suggest that PCFCL can be successfully treated, with significantly lower doses of radiotherapy (*8*).

The prognosis of patients with PCFCL is excellent

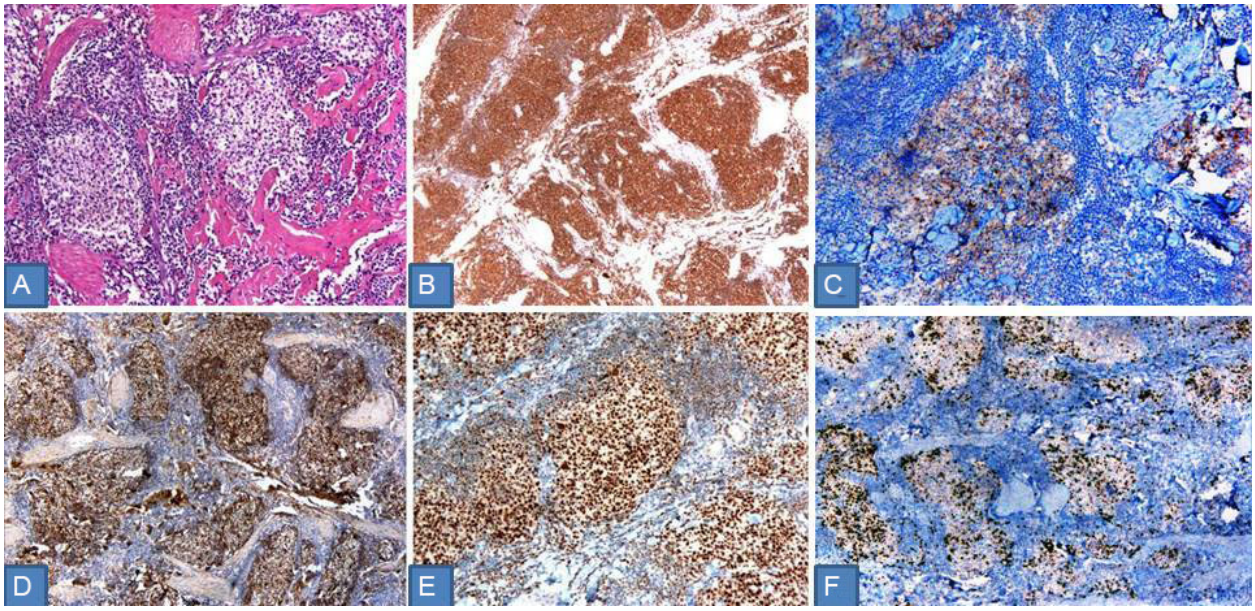

Figure 2. Histopathological and immunohistochemical findings of PCFCL. (A), Dermal lymphoid infiltrate with diffuse and nodular growth pattern (H&E ×20); **(B)**, Tumor cells showing strong and diffuse positive staining for CD20 (CD20 ×20); **(C)**, Negativity or focal weak positive staining for CD10 (CD10 ×40); **(D)**, Tumor cells stained positive for CD23 (CD ×20); **(E)**, Tumor cells stained positive for BCL-6 (BCL ×40); **(F)**, Reduced expression of ki-67 proliferation index in the nodules (Ki67 ×20).

even in cases with multifocal or recurrent disease, with a 5-year disease specific survival over 95% (*4,5,7,8*). Approximately 30% of the patients may exhibit a relapse (*5*). The extracutaneous spread most commonly involves the regional nodes and the bone marrow (*8*).

PCFCL may become locally aggressive if left untreated, whereas transformation to diffuse large B cell lymphoma has been suggested (*10*).

Although PCFCL involving the breast skin is exceedingly rare, a biopsy is indicated in any periareolar skin changes that do not resolve with topical treatment. Temporary resolution of the skin changes with or without topical treatment may occur, resulting in a delayed biopsy. A repeated biopsy should be considered in selected cases with discordance between clinical and pathological findings (*9*). In our patient, the biopsy was performed with a seven-month delay, as the tumor had been initially misdiagnosed as a benign lesion at another institution.

In conclusion, we present an exceedingly rare case of PCFCL affecting the periareolar breast skin. PCFCL is a clinical entity associated with an indolent clinical course and an excellent prognosis. A timely biopsy of any breast erythematous skin change that does not respond to local therapy should always be considered to avoid delays in initiating appropriate treatment.

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