Clinical, Pathological, and Electron Microscopic Findings in Two Thai Children with Pompe Disease

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Abstract

The authors report on a Thai boy who first presented at age 7 months and an unrelated Thai girl in her neonatal period with hypotonia, cardiomegaly and hepatomegaly. Their chest roentgenograms showed markedly enlarged hearts, EKGs showed abnormally shortened PR intervals with gigantic QRS complexes, and electron microscopic studies of their skin samples showed glycogen accumulations surrounded by membranes. The boy died at age 22 months and the girl at age 9 months due mainly to cardiorespiratory failure. Autopsy of the girl showed marked accumulation of glycogen in the liver, heart and numerous additional tissues including her brain. The clinical, pathological, and electron microscopic findings of these two children are consistent with the diagnosis of Pompe disease.

Pompe disease is an autosomal recessive disorder of glycogen metabolism resulting from deficiencies in activity of the lysosomal acid α-glucosidase. Definite diagnosis of the disease can be made from a biochemical test or a mutation analysis. To the authors' knowledge, no service laboratories in Thailand offer the tests. Because Thai children have occasionally been reported to be affected by Pompe disease, an attempt to establish a definite diagnostic test for Pompe disease in Thailand should be encouraged. With a definite diagnosis, the proper genetic counseling and prenatal diagnosis could be offered to the families.

Key word : Glycogen Storage Disease, Pompe Disease, Electron Microscopy

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Pompe disease or glycogen storage disease type II is an autosomal recessive disorder of glycogen metabolism resulting from deficiencies in the activity of the lysosomal hydrolase acid α-glucosidase in all tissues of affected individuals[1]. The clinical manifestation of Pompe disease includes a range of phenotypes, all of which involve varying degrees of myopathy. The most severe type is infantile-onset disease, with hypotonia, cardiomegaly, hepatomegaly, and death due to cardiorespiratory failure, usually before the age of 2 years[2]. The deficiency of the enzyme results in accumulation of glycogen of normal structure within lysosomes in numerous tissues, most marked in cardiac muscle, skeletal muscle, and hepatic tissues[3]. Electron microscopy reveals a specific vacuoles tightly packed with glycogen particles surrounded by a single membrane[4].

The authors report two Thai children with clinical, pathologic, and electron microscopic findings characteristic of Pompe disease. With the diagnosis, proper genetic counseling and prenatal diagnosis could be offered to the families.

MATERIAL AND METHOD

Patient 1

The patient, a boy, was born at term to a 24-year-old G1P0 Thai mother and a 30-year-old nonconsanguineous Thai father. The pregnancy and labor were uncomplicated. Birth weight was 3,100 g. He had pneumonia at age 7 months. During hospitalization, hypotonia and cardiomegaly with congestive heart failure were found. Diuretics, digitalis, and enalapril were given. He was rehospitalized 4 more times for pneumonia or congestive heart failure at ages 10, 19, 20 and 22 months. He held his head up at age 3 months, rolled over at 5 months, but was not able to sit at age 10 months. At age 22 months, he measured 75 cm (-3 SD), weighed 8.0 kg (-3 SD), and had a head circumference of 46.5 cm (-2 SD). Cardiac examination revealed a systolic murmur grade 3/6 on his left upper sternal border. His liver was palpated 5 cm below the right costal margin but the spleen was not palpable.

Electrocardiogram (EKG) showed a short PR interval, large QRS voltage, signs of left atrial dilatation and biventricular hypertrophy (Fig. 1). Roentgenograms of his chest showed marked cardiomegaly. The echocardiogram showed severe ventricular hypertrophy. Electron microscopy on a skin biopsy at age 7 months showed vacuoles filled with glycogen (Fig. 2). He died of cardiopulmonary failure with septicemia at the age of 22 months. Hemoculture was positive for *Morganella morganii*.

Patient 2

The patient, a girl, was born at full-term to a 32-year-old G1P0 Thai mother and a 37-year-old nonconsanguineous Thai father. The pregnancy was complicated by maternal gestational diabetes mellitus. The patient was born by Cesarean section with forcep extraction due to fetal distress. Birth weight was 3,600 g. APGAR scores were 6 and 8 at 1 and 5 minutes, respectively. After birth, she had dyspnea requiring hospitalization for 4 weeks. Hypotonia and cardiomegaly with congestive heart failure were found. She was hospitalized three times at ages 3, 4, and 6 months for pneumonia. At age 6 months, she could hold her head up but could not roll over. Her weight was 5.3 kg (-2.5 SD). She had respiratory distress, bilateral rhonchi on both lungs, systolic ejection murmur grade 2/6 on left sternal border, and hepatomegaly.

Her liver enzymes were elevated with alanine aminotransferase (ALT, SGPT) 89 U/L (normal: 5-45) and aspartate aminotransferase (AST, SGOT) 185 U/L (normal: 15-55). EKG showed a short PR interval, massive QRS voltage, and signs of biventricular hypertrophy. Chest roentgenograms showed striking cardiomegaly. The echocardiogram showed severe ventricular hypertrophy with low left ventricular systolic function and mild tricuspid and mitral valve regurgitation. Mitochondrial DNA analysis at position 3,243, 8,344, and 8,993 was negative. She died of cardiopulmonary failure at the age of 9 months.

At the postmortem examination, the main pathology was observed in the heart, liver, and the brain. The heart was enlarged, with the weight of 195 grams (normal: 41 ± 5). The left and right ventricular walls were thickened, and respectively measured 1.8 cm and 1.0 cm. There was also marked eccentric thickening of the interventricular septum. The liver weighed 250 g (normal: 288 ± 67), showing yellow brown cut surfaces. The brain weighed 720 g (normal: 810 ± 82). Coronal sections revealed diffusely increased firmness with gray discoloration of the white matter of both the cerebral hemispheres. The gray structures were relatively intact.

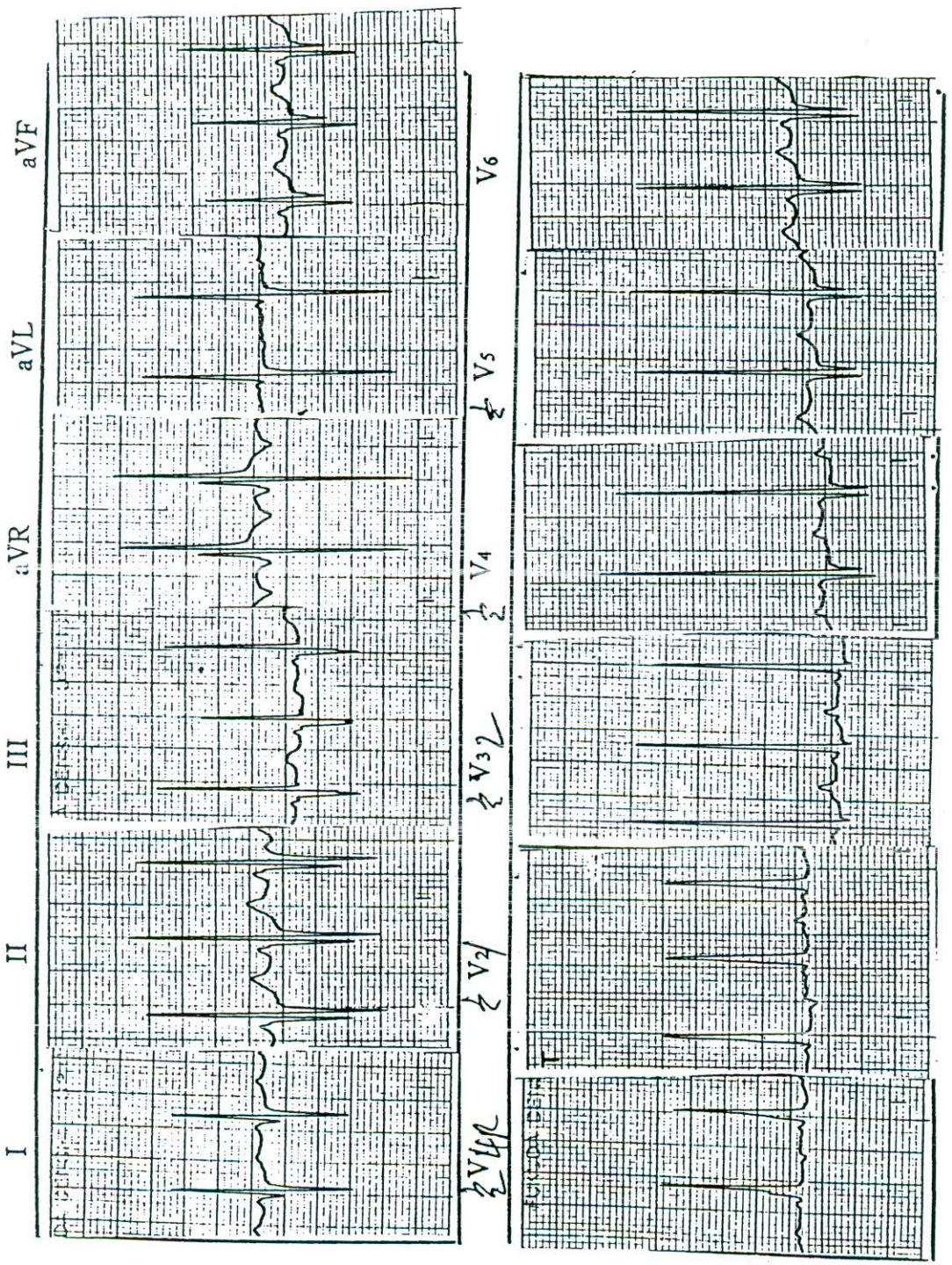

Fig. 1. The electrocardiogram of Patient 1 shows a short PR interval with large QRS complexes.

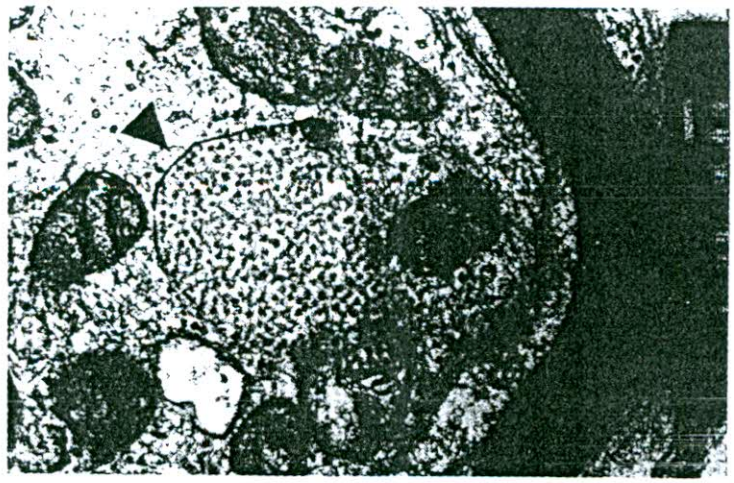

Fig. 2. Electron microscopic findings of patient 1. Note an accumulation of glycogen particles surrounded by a single membrane (arrow head).

On routine stain, virtually all cardiac muscle fibers contained cytoplasmic clear vacuoles (Fig. 3A). All of the hepatocytes were expanded with multiple small round vacuoles in the cytoplasm (Fig. 3B). Many of the neurons in the dentate nuclei of the cerebellum (Fig. 3C) and in the basal ganglia were distended with fine cytoplasmic vacuoles whereas the cortical neurons were well-preserved. Astrocytes in the white matter possessed enlarged cytoplasm, with foamy appearance (Fig. 4A). Periodic acid Schiff (PAS) staining method demonstrated PAS-positive diastase-sensitive glycogen material in the above abnormal cells, most prominent in the white matter astrocytes (Fig. 4B and 4C). Antemortem electron microscopy was performed on a skin biopsy, which demonstrated vacuoles filled with glycogen.

DISCUSSION

The authors report two Thai patients with findings characteristic of Pompe disease. One was a boy and the other a girl. Pompe disease is transmitted as an autosomal recessive trait[5]; therefore, affected individuals can be of either sex. Both of our patients had their first symptoms in their infancy; Patient 1 at age 7 months and Patient 2 in her neonatal period. Age of onset of individuals with Pompe disease varies. The most severe phenotype is the classic infantile-onset disease which presents within the first few months of life[6], even in the neonatal period[7]. The two presented patients manifested hypotonia, cardiomegaly, and congestive heart failure. Roentgenograms showed markedly enlarged hearts. EKG showed an abnormally shortened PR interval with gigantic QRS complexes. The diagnoses of Pompe disease were first suspected because of the floppy baby appearance, the cardiomegaly on chest X-rays and the findings of EKGs, which are all typical features of Pompe disease[8-10]. Patient 1 died at age 22 months and patient 2 at age 9 months due to cardiorespiratory failure, consistent with the rapidly progressive course with death usually before 2 years of age in patients with Pompe disease[2,11].

Hepatic enzymes of patient 2 were slightly elevated similar to those found in patients with Pompe disease[12]. Electron microscopic features of skin samples from both patients showed glycogen accumulations surrounded by membranes, specific for Pompe disease[4,13-15]. The intracellular vacuoles full of glycogen found in electron microscopy can be used as a rapid, safe, and reliable method for prenatal diagnosis[16]. Autopsy was performed on Patient 2. She was found to have marked accumulation of glycogen in liver, heart and numerous additional tissues including her brain. All these autopsied findings are consistent with the diagnosis of Pompe disease[3,17].

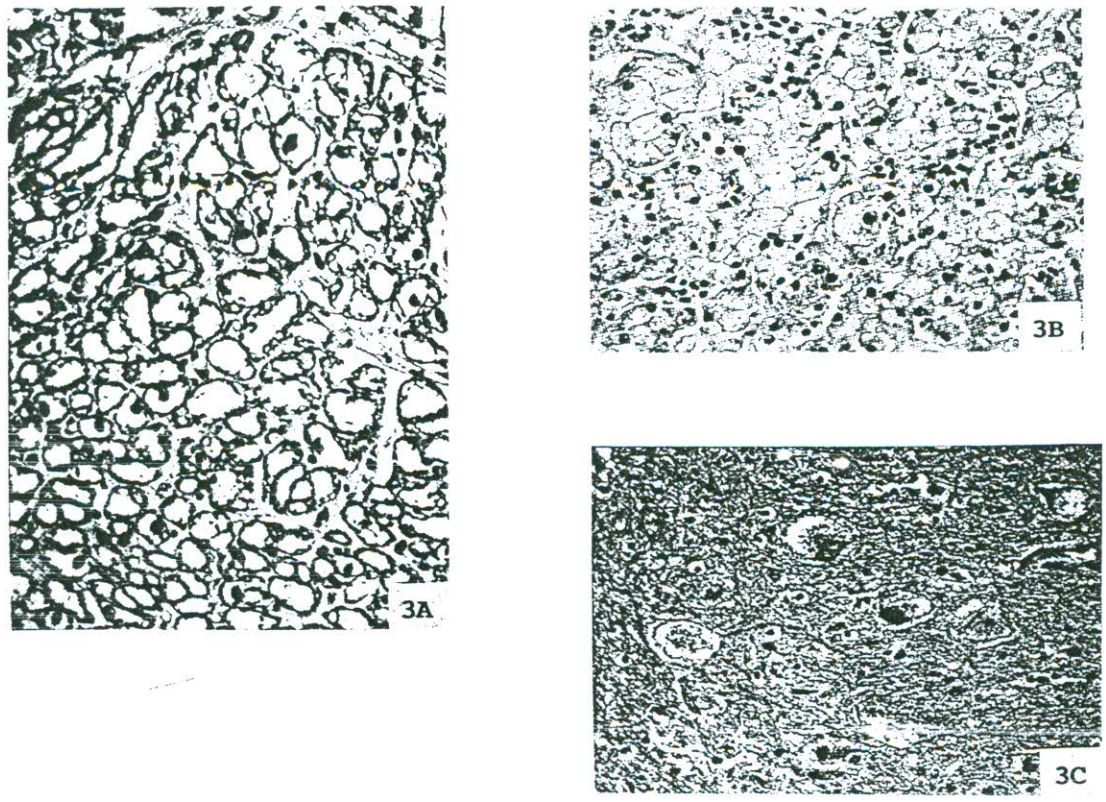

Fig. 3. Pathology of Patient 2. All of the cardiac muscle cells contain intracytoplasmic round to oval clear space (A). The liver cells are enlarged with multiple cytoplasmic vacuoles (B). Neurons in the dentate nucleus of the cerebellum are distended with cytoplasmic foamy substance, displacing the nucleus into the periphery (C), (A-C, H&E).

Even though clinical, pathological, and electron microscopic features are specific for Pompe disease, they are not pathognomonic. Cardiac abnormalities, skeletal involvement and the intravacuolar accumulation of glycogen are also found in Danon syndrome, which has normal acid α-glucosidase. Danon syndrome is inherited as an X-linked trait caused by primary deficiency of a lysosomal membrane protein, LAMP-2[18]. The diagnosis of infantile-onset Pompe disease can be confirmed by virtual absence of acid α-glucosidase in muscle biopsies or cultured fibroblasts[19-25]. Purified lymphocytes also exhibit the enzyme defects but misdiagnosis may occur with imperfectly fractionated peripheral blood lymphocytes. Assay of unfractionated leukocytes is not reliable[26-30]. Another method, which can be used to definitely diagnose patients with Pompe disease, is to perform mutation analysis. Both the cDNA and structural gene for human acid α-glucosidase have been isolated and characterized. The cDNA has 2,859 nucleotides of coding sequence predicting 952 amino acids. The structural gene contains 20 exons in approximately 20 kb of genomic DNA and has been localized to chromosome 17q25. Mutations associated with Pompe disease are various including missense mutations, nonsense mutations, deletions, and insertions[31-35]. Unfortunately, no diagnostic laboratories offering either the biochemical or molecular tests as a service for definite diagnosis of Pompe disease are available in Thailand.

A few Thai patients with Pompe disease have been reported[36-39]. The authors diagnosed two additional children during a two-year period in a single hospital suggesting that children with Pompe disease could occasionally be encountered in

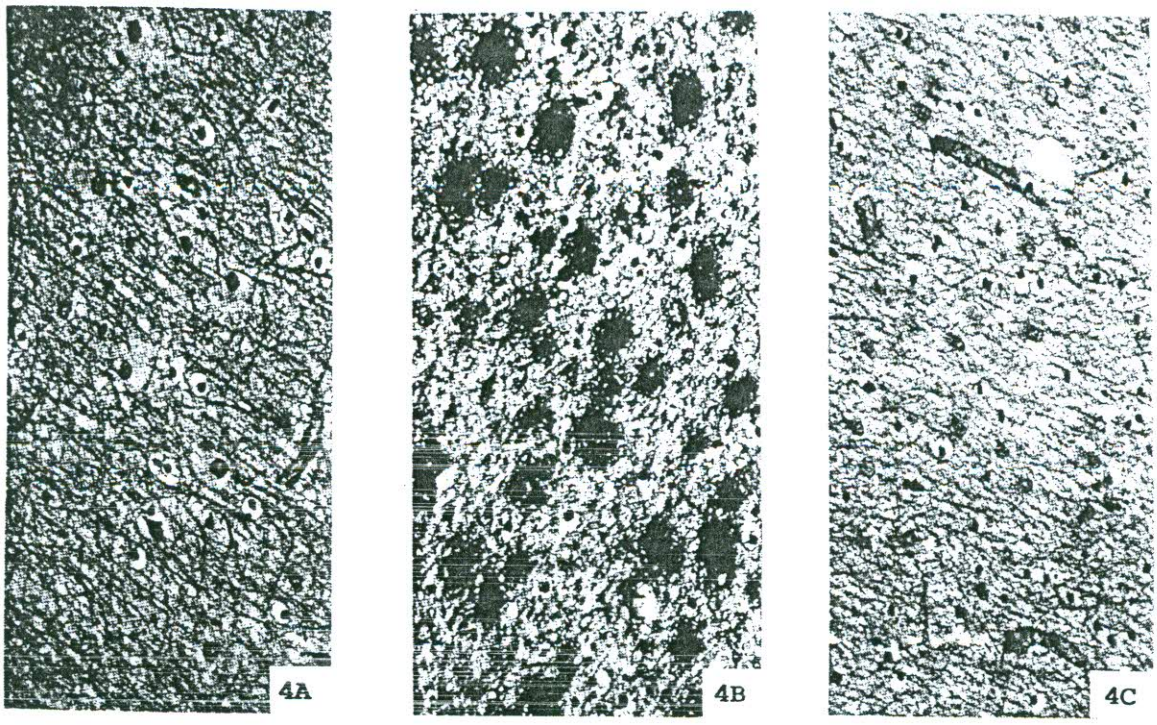

Fig. 4. Pathology of Patient 2. Astrocytes in the cerebral white matter are enlarged with foamy cytoplasm (A), which is PAS (periodic acid Schiff)-positive (B) diastase-liable (C), characteristic of glycogen (A, H&E; B, PAS; C, PAS with diastase pretreatment).

Thailand. The disease should be in the differential diagnosis for patients presenting with cardiomegaly and skeletal myopathy.

Some of the previously reported cases were definitely diagnosed by biochemical studies[36,38]. However, none of the studies were performed in Thailand. Attempts to establish the biochemical or molecular studies to definitely diagnose Pompe disease in Thailand should be encouraged. With the definite diagnosis, proper genetic counseling and prenatal diagnosis could be offered to the families.

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ลักษณะทางคลินิก พยาธิสภาพและกล้องจุลทรรศน์อิเลคตรอนของเด็กไทยซึ่งป่วยด้วยโรคปอมเปสองราย

วรศักดิ์ โชติเลอศักดิ์, พ.บ., ชนพ ช่วงโชติ, พ.บ.**, ไพโรจน์ โชติวิทยธารากร, พ.บ.*, วิเชียร เชาวน์ศรีกุล, พ.บ.**, วรรณี วัฒนเสริมกิจ, ค.บ.**, คุ้างค์ มณีศรี, วท.ม.**, เพ็ญพรรณ์ นวลบุญมา, วท.บ.**, ชูศักดิ์ วิรัชชัย, พ.บ.**, พงษ์พีระ สุวรรณกูล, พ.บ.***

รายงานผู้ป่วยเด็กชายไทย 1 ราย เริ่มมีกล้ามเนื้ออ่อนแรง หัวใจโต และตับโต ตั้งแต่อายุ 7 เดือน และเด็กหญิงไทย 1 ราย มีอาการเช่นเดียวกันตั้งแต่ช่วงทารกแรกเกิด การตรวจทางรังสีของผู้ป่วยทั้งสองรายพบหัวใจโต, คลื่นไฟฟ้าหัวใจมีช่วง PR สั้น, QRS complexes ใหญ่ และการตรวจทางกล้องจุลทรรศน์อิเลคตรอนของชิ้นผิวหนังพบไกลโคเจนสะสมอยู่ในอวัยวะเซลล์ ผู้ป่วยเสียชีวิตด้วยระบบหัวใจและหายใจล้มเหลวขณะอายุ 22 เดือนและ 9 เดือนตามลำดับ การชันสูตรศพของผู้ป่วยหญิงพบมีไกลโคเจนสะสมอยู่ในตับ กล้ามเนื้อหัวใจ และเนื้อเยื่ออื่น ๆ รวมทั้งสมองลักษณะทางคลินิก, พยาธิสภาพ, และกล้องจุลทรรศน์อิเลคตรอนของผู้ป่วยทั้ง 2 รายเข้าได้กับโรค Pompe

โรค Pompe เป็นโรคที่มีการถ่ายทอดทางพันธุกรรมแบบยีนด้อย เกิดจากความผิดปกติของ acid α–glucosidase ในไลโซโซม ซึ่งเป็นส่วนหนึ่งของกระบวนการเมแทบอลิซึมของไกลโคเจน การวินิจฉัยที่แน่ชัดทำได้โดยการตรวจระดับการทำงานของเอ็นไซม์หรือการตรวจหาการกลายพันธุ์ อย่างไรก็ตามยังไม่มีห้องปฏิบัติการในประเทศไทยที่ให้บริการการตรวจนี้ เนื่องจากมีรายงานผู้ป่วยโรค Pompe ในประเทศไทยอยู่ประปราย จึงควรมีการสนับสนุนให้มีการตรวจทางห้องปฏิบัติการดังกล่าวเพื่อให้สามารถให้การวินิจฉัยที่แน่ชัดแก่ผู้ป่วยได้ ทั้งนี้เพื่อประโยชน์ในการให้คำปรึกษาแนะนำทางพันธุศาสตร์และการวินิจฉัยก่อนคลอด

คำสำคัญ : โรคสะสมไกลโคเจน, โรคปอมเป, จุลทรรศน์อิเลคตรอน

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