Original article

Whole Exome Sequencing (WES) for *ALMS1* Mutation in a Thai Boy with Alström syndrome-A First Report at Phramongkutklao Hospital

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Abstract:

Alström syndrome (ALMS) is a rare autosomal recessive multi-system disorder with a phenotypic variability and is characterized by cone-rod dystrophy, sensorineural hearing loss, obesity, insulin resistance/type 2 diabetes mellitus (T2DM), cardiomyopathy and progressive pulmonary, hepatic and renal dysfunction. ALMS is caused by mutations in the Alström syndrome protein 1 (ALMS1) gene. Herein, we reported a 15-year-old Thai boy with ALMS presenting with childhood onset retinal degeneration, obesity with T2DM, hypertriglyceridemia and non-alcoholic steatohepatitis, sensorineural hearing loss, and dilated cardiomyopathy (DCM). Whole exome sequencing (WES) of the patient's genomic DNA identified two compound heterozygous mutations, namely, one frameshift mutation; c.6166_6167dup or p.Leu2057PhefsTer17, in exon 8, and one nonsense mutation; c.10822C>T or p.Arg3608Ter, in exon 16 of ALMS1 gene. Both mutations were predicted to cause either absence or truncation of ALMS1 proteins. This report highlighted a clinical utility of WES as a powerful tool for diagnosis of genetic heterogeneity disorders and rare diseases including ALMS.

Keywords: ● *Alström syndrome* ● *Whole exome sequencing* ● *ALMS1 gene*

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นิพนธ์ต้นฉบับ

การตรวจการกลายพันธุ์ของยีน *ALMS1* ด้วยวิธี Whole exome sequencing ในผู้ป่วยเด็กชายกลุ่มอาการ Alström รายแรกในโรงพยาบาลพระมงกุฎเกล้า

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บทคัดย่อ

กลุ่มอาการ Alström เป็นกลุ่มอาการที่ส่งผลต่อร่างกายในหลายระบบและมีลักษณะอาการที่หลากหลาย พบได้น้อยมาก และมีการถ่ายทอดแบบยีนด้อยบนโครโมโซมร่างกาย อาการแสดงที่สำคัญประกอบด้วย cone-rod dystrophy การสูญเสียการได้ยิน ภาวะอ้วน ภาวะดื้อต่ออินซูลินหรือเบาหวานชนิดที่ 2 ภาวะกล้ามเนื้อหัวใจทำงานผิดปกติ รวมถึงการทำงานของปอด ตับ และไตที่ผิดปกติ กลุ่มอาการ Alström เกิดจากการกลายพันธุ์ของยีน ALMS1 ในที่นี้รายงานผู้ป่วยเด็กชายไทยอายุ 15 ปีที่มีอาการแสดงเข้าได้กับกลุ่มอาการ Alström ได้แก่ ภาวะจอประสาทตาเสื่อมในวัยเด็ก ภาวะอ้วนและเบาหวานชนิดที่ 2 ภาวะไตรกลีเซอไรด์ในเลือดสูง ภาวะไขมันพอกตับที่ไม่ได้เกิดจากแอลกอฮอล์ การสูญเสียการได้ยิน และภาวะหัวใจโต การตรวจ whole exome sequencing (WES) จากดีเอ็นเอของผู้ป่วยรายนี้พบการกลายพันธุ์บนยีน ALMS1 2 ตำแหน่ง ได้แก่ การกลายพันธุ์แบบ frameshift c.6166_6167dup หรือ p.Leu2057PhefsTer17 ใน exon 8 และการกลายพันธุ์แบบ nonsense c.10822C>T หรือ p.Arg3608Ter ใน exon 16 ซึ่งการกลายพันธุ์ทั้งสองตำแหน่งนี้อาจทำให้เกิดการขาดหายหรือการตัดส่วนของโปรตีน ALMS1 รายงานนี้แสดงให้เห็นถึงประโยชน์ของการใช้ WES ในทางคลินิกเพื่อเป็นเครื่องมือสำคัญที่ใช้ในการวินิจฉัยโรคที่เกิดจากความผิดปกติทางพันธุกรรมที่หลากหลาย และโรคหายาก รวมถึงกลุ่มอาการ Alström

คำสำคัญ: ● *กลุ่มอาการ Alström* ● *Whole exome sequencing* ● *ยีน ALMS1*

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Introduction

Alström syndrome (ALMS; OMIM 203800) is a rare autosomal recessive genetic disorder with an estimated prevalence of less than 1:1,000,000 and was first reported in 1959[1-2]. ALMS is a progressive multisystem disease characterized by cone-rod retinal dystrophy, sensorineural hearing loss, truncal obesity, hyperinsulinemia and insulin resistance/type 2 diabetes mellitus (T2DM), hypertriglyceridemia, cardiomyopathy, and progressive pulmonary, hepatic and renal dysfunction[1].

ALMS is caused by mutations in the Alström syndrome protein 1 (*ALMS1*; OMIM 606844) gene which is located on chromosome 2p13. The *ALMS1* comprises of 23 exons and encodes for a 4,169 amino acids ALMS1 protein[3,4]. ALMS1 is widely expressed and localized to the centrosomes and basal bodies of ciliated cells of tissue including the central nervous system, photoreceptor, cardiopulmonary, endocrine, and genitourinary system. Although exact biological functions of ALMS1 have not been fully delineated, current evidences suggested that ALMS1 protein possibly involved in maintaining ciliary functions, cell cycle regulation, intracellular trafficking and adipocyte differentiation[3,5].

The aim of our study was to report a first 15-year-old Thai boy presented with classical ALMS phenotypes at Phramongkutklao Hospital. Two compound heterozygous mutations of the *ALMS1* were identified by whole exome sequencing (WES).

Patients and methods

Patient

A 15-year-old Thai boy was sent to a genetic clinic due to bilateral progressive vision loss. He was the third child of both healthy and non-consanguineous parents. The pedigree of the patient's family is shown in Figure 1. Photophobia and decreased vision started since 2 years of age which subsequently progressed to nearly blindness at the age of 9 years. Fundoscopic examination revealed optic disc atrophy and retinal hyperpigmentation. Leber congenital amaurosis was diagnosed by ophthalmologist. Type 2 diabetes mellitus (T2DM) and hypertriglyceridemia have been diagnosed since 13 years of age. Laboratory investigations revealed fasting plasma glucose 161 mg/dL, HbA1C 9.5%, serum C-peptide 3.7 ng/mL (normal 0.9-7.1), serum insulin 2941.9 pmol/L (normal 17.8-173), negative for both anti-GAD and pancreatic islet cell antibody. Lipid profiles showed serum cholesterol 157 mg/dL, triglyceride 251 mg/dL, HDL 31 mg/dL and LDL 91 mg/dL.

Physical examinations at the time of genetic consultation revealed an obese boy. His weight was 67 kg (> 97th percentile) and his body mass index (BMI) was 26.5 kg/m^2. Dysmorphic facial features including round face, frontal balding with sparse hair, prominent supraorbital ridge, deep set eyes, thickened ala nasi and ear helices, and bilateral nystagmus was detected. Dermatologic examinations revealed greasy skin and acanthosis nigrican.

Further laboratory investigations revealed AST 48 U/L, ALT 73 U/L, BUN 13 mg/dL and Cr 0.7 mg/dL. Urinalysis showed protein 2+, glucose 2+, urine Cr 134.2 mg/dL, urine micro-albumin 992 mg/L (normal 0-29 mg/L), and urine micro-albumin/creatinine ratio 739.2 (normal 0-20.33). Abdominal ultrasound revealed

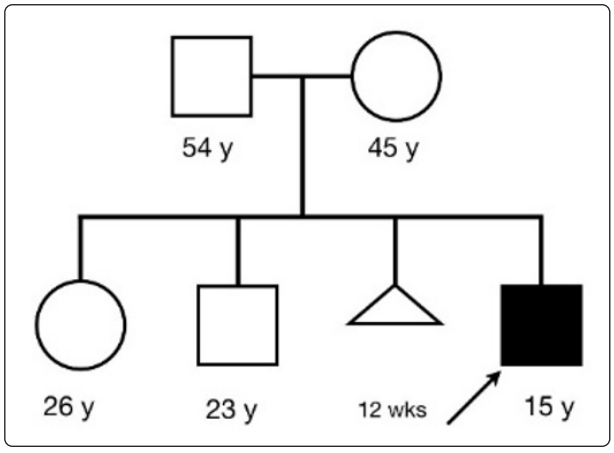
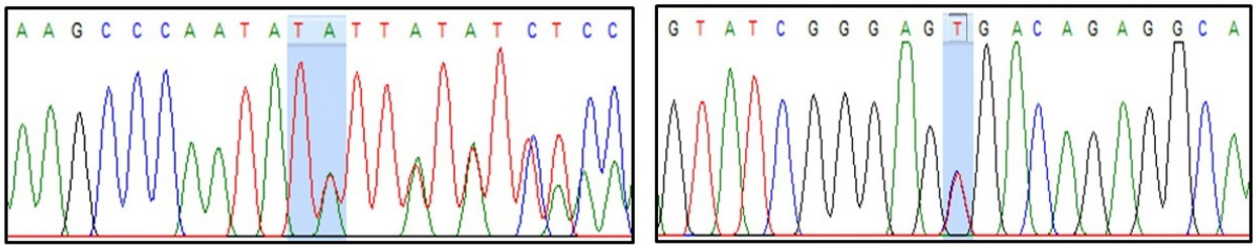


Figure 1 Pedigree of the patient's family



A) Heterozygous c.6166_6167dup mutation **B)** Heterozygous c.10822C>T mutation

Figure 2. Electropherogram revealed a heterozygous c.6166_6167dup mutation in exon 8 (**A**) and a heterozygous c.10822C>T mutation in exon 16 (**B**) of *ALMS1* in the patient DNA.

severe fatty liver and bilateral hyperechogenicity of renal medullas. These findings suggested non-alcoholic fatty liver disease (NAFLD) and progressive kidney disease. Auditory brainstem response (ABR) demonstrated bilateral sensorineural hearing loss. Echocardiogram revealed mild left ventricular dilatation and mildly impaired diastolic function. Normal LV systolic function was detected with the ejection fraction (EF) of 60 percent. Chromosome studies revealed normal male karyotype (46,XY).

Molecular Analysis

This study was approved by the Institutional Review Board of the Royal Thai Army (IRBRTA1483/2563). After informed consent was obtained, genomic DNA was extracted from peripheral blood leukocytes according to manufacturer's protocol. Whole exome sequencing (WES) was performed by Macrogen Inc (Seoul, South Korea). DNA was captured on SureSelect Human All Exon V7 (Agilent Technologies, Santa Clara, CA, USA) and then sequenced on Novaseq 6,000 platform (Illumina, San Diego, CA, USA). Sequence reads were aligned to UCSC hg19 using Burrows-Wheeler Alignment Tool (BWA v0.7.12; http://bio-bwa.sourceforge.net/). Single nucleotide variants (SNVs) and small insertions and deletions (InDels) were identified using Genome Analysis Toolkit (GATK v3.4.0; https://gatk.broadinstitute.org/hc/en-us). The sequence variants were annotated with SnpEff (v4.1g; https://pcingola.github.io/SnpEff/) and novel variants were filtered against 1000Genome, dbSNP, ClinVar and ESP6500 (https://evs.gs.washington.edu/EVS/). Existing SNVs or known pathogenic mutations were subsequently filtered out using the Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/index.php) and Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/). The mutations were confirmed by PCR and Sanger sequencing.

Results

Two heterozygous pathogenic mutations, namely, one frameshift mutation: c.6166_6167dup (p.Leu2057PhefsTer17) in exon 8, and one nonsense mutation: c.10822C>T (p.Arg3608Ter) in exon 16, were identified in the *ALMS1* by both WES and subsequently verified by Sanger sequencing (Figure 2). The reference of genome version was UCSC hg19 and the reference sequences were NM_015120.4 and NP_055935.4 for *ALMS1* cDNA and ALMS1 amino-acid position, respectively. Both mutations were predicted to create premature termination codons resulting in truncated or disrupted ALMS1 protein.

Discussion

Alström syndrome (ALMS) is a rare multi-system genetic disorder characterized by early-onset cone-rod dystrophy, obesity with insulin resistance/type 2 diabetes mellitus (T2DM), and progressive sensorineural hearing loss. Other organ systems including liver, kidney, neurological, cardiac, and pulmonary can also be involved in ALMS[1]. Diagnosis of ALMS is usually based on the cardinal clinical features which can be confirmed by

ALMS1 mutation analysis. In this report, we described a 15-year-old Thai boy who has been presented with progressive visual loss since the age of 2 years. Obesity, T2DM, and hypertriglyceridemia have been detected since 13 years of age. At 15-year-old, a time of first genetic consultation, bilateral sensorineural hearing loss, non-alcoholic fatty liver disease (NAFLD), progressive renal disease, and cardiomyopathy were also detected. ALMS was suspected in our patient since all clinical features were compatible with ALMS diagnostic criteria[6]. One major (vision problems) and three minor clinical criteria (sensorineural hearing loss, cardiomyopathy, and obesity with complications such as insulin resistance/ T2DM and hypertriglyceridemia) were detected in our patient. Although these clinical features do not meet the diagnostic criteria for a 15-year-old child, molecular analysis of the *ALMS1* is the next step of investigation to confirm the diagnosis of ALMS in our patient.

To date, more than 300 *ALMS1* pathogenic variants have been previously reported in the literatures[1,7-8]. Almost all mutations are either nonsense or frameshift mutations which usually associated with premature termination codons resulting in absence or truncation of ALMS1 protein. The majority of the *ALMS1* mutations are located in exon 8, 10, and 16 suggesting the mutational hotspots in these regions. According to the large size and highly heterogeneous mutation spectrum of *ALMS1*, whole exome sequencing (WES) was used as a molecular tool to confirm the diagnosis of ALMS in our patient. Two pathogenic mutations are one frameshift mutation; c.6166_6167dup (p.Leu2057PhefsTer17), in exon 8, and one nonsense mutation; c.10822C>T (p.Arg3608Ter), in exon 16 were identified in the *ALMS1*. Both mutations have been previously reported[7-9]. The rare c.6166_6167dup mutation has been exclusively reported in Asian populations with as estimated allelic frequency of 0.000008 (1/118864) in the Exome Aggregation Consortium (ExAC) database.

Our study is the second report of *ALMS1* mutation in Thai populations. In 2017, WES was used to identified the genetic causes of Leber congenital amaurosis (LCA) in eight Thai patients[10]. Two *ALMS1* mutations, including c.3896C>A (p.Ser1299Ter) and c.8041G>T (p.Glu2681Ter), were identified in two unrelated patients and both mutations were localized in exon 8 and 10, respectively. Ethnicity is one of strong contributors to the distribution of mutations. In East Asian descents, most of the mutations were clustered within exon 8, 10, and 16[8,9]. These exons were also detected to be the mutational hot spots in Thai populations. One of the possible explanation is the largest size of exon 8 which encompasses approximately half of the *ALMS1* coding regions and harbors almost half of the variants. Although, only a few variants have been reported more than once in the East Asian populations, both mutations identified in our study were recurrent and have been previously reported only in either East Asian and Chinese populations[7-9]. This possibly suggested the common ancestor between Chinese and Thai populations.

Although, there is no specific treatment for ALMS at the present, early diagnosis and intervention can alleviate the progression of the disease and improve the survival and quality of life of the patients and their families. Thus, molecular diagnosis should be performed as early as possible for diagnostic confirmation, leading to early intervention and surveillance in order to improve the prognosis and long-term survival in ALMS patients. Appropriate genetic counselling should be given to the patients and their families.

Conclusion

In our study, whole exome sequencing (WES) was used to identify compound heterozygous nonsense and frameshift mutations of the *ALMS1* in a 15-year-old boy

presented with clinical features of ALMS. This report highlighted the clinical utility of WES as an alternative powerful molecular tool for diagnosis of genetic heterogeneity disorders and rare diseases including ALMS.

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