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Short Communication

Diagnosis of Arboleda-Tham syndrome by whole genome sequencing in an Asian boy with severe developmental delay

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ABSTRACT

Diagnosis of a 9-month-old boy brought to our genetics clinic with chief complaints of developmental delay (DD), failure to thrive, microcephaly, trunk hypotonia and hypertonia of the extremities. Multiple congenital defects but no significant syndromes or diseases were impressed. The chromosomal analysis and array comparative genomic hybridization (aCGH) revealed no significant pathogenic changes. Whole Genome Sequencing (WGS) identified a p.Glu1139fs *de novo* mutation of the *KAT6A* gene. The patient's phenotype was consistent clinically with Arboleda-Tham syndrome (ARTHS). Reviewing the literature showed that this is the first patient in Taiwan detected by WGS and that it involves a novel mutation. Comparing the highly variable clinical presentations of this syndrome with our patient, this boy's features and severe developmental defects seem to be due to a late-truncating mutation at the carboxyl end of the KAT6A protein. Our study demonstrates the power of WGS to confirm a diagnosis within 4 weeks for this rare condition.

1. Introduction

Intellectual disability (ID) or development delay (DD) is a common complaint at pediatric and genetics out-patient clinics. In DSM-5, global developmental delay (GDD) is a diagnosis whereby children younger than 5 years of age have a significant delay in achieving developmental milestone in two or more domains of development [1]. As diagnostic tools have developed, including karyotyping, gene microarray analysis, screening for inborn errors of metabolism, and imaging studies, a large number of patients who previously would be diagnosed as unexplained GDD/ID can now be given a specific diagnosis. An emerging technology is next-generation genome sequencing. Whole exon sequencing (WES) provides an additional yield of definitive diagnoses of between 30% and 40%, while WGS adds about 15% to WES [2]. In this study, we present a 9-month and 2-week-old boy with DD, hypotonia, microcephaly, failure to thrive, and multiple dysmorphic features. There was no definite diagnosis after we conducted a chromosome study and aCGH. Ultimately, the boy was given a confirmed diagnosis of ARTHS after WGS was carried out. This emphasizes the importance of WES/WGS when diagnosing unexplained GDD/ID.

2. Materials and methods

We collected the boy's medical records, family history and clinical presentations completely. Then a blood sample from the boy was obtained and DNA was extracted and analyzed by cytogenetic analysis and aCGH. DNA extracted from the boy's and his parents' leukocytes was subjected to shotgun library preparation using the KAPA HyperPrep Kits, and short-read sequencing was conducted on an Illumina NovaSeq6000 instrument. The variant calling was performed using the software CLC Genomic Workbench 12.0 (Qiagen). Before mapping, we trimmed the low quality bases ($Q < 30$) and then aligned to human reference genome (GRCh37/hg19). The mapping parameters were set to default except the mapping length for the read and the mapping

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similarity was set to 0.9. We only allowed read mapped to reference genome once. To identify the disease-causing variants, variants were filtered by comparison with common variant database (dbSNP version 150 and Taiwan BioBank). The Human Phenotype Ontology database was used to identify candidate genes according to patient's phenotype including global developmental delay (HP:0001263), failure to thrive (HP:0001508 and HP:0001531) and hypertonia (HP:0001276). The variants located in the candidate genes were further filtered by its functions involved in the CDS region, 5'-UTR, 3'-UTR or splicing site. The variants located in the CDS region were performed protein structure changes analysis by using SIFT [3] and PROVEAN [4] software. To further filter the variants, we filtered out the variants that has been reported in the ClinVar database and type is benign or likely benign. We also used patient's parents WGS data to perform trio-based analysis. As a next step, we collected blood samples from the parents in order to perform trio analysis with Sanger sequencing. All collected information has been de-identified in the study. The corresponding authors had full access to all of the data and had final responsibility for the decision to submit for publication. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The study protocol was approved by the Institutional Review Board of TVGH (TVGH-2018-09-006A), and performed after parental informed consent form was obtained with a full explanation of the study objectives and procedures.

3. Results

The medical records of the patient were reviewed. He has two older brothers without any abnormalities and there was no congenital or inherited disease associated with the family history. He was born as a full-term baby *via* caesarean section; he was symmetrical, but small for his gestational age (SGA). His birth body weight (2.352 kg, −2.37 SD), body height (41 cm, −4.68 SD) and head circumference (31 cm, −2.75 SD) were below the third percentile for his gestational age. He was found

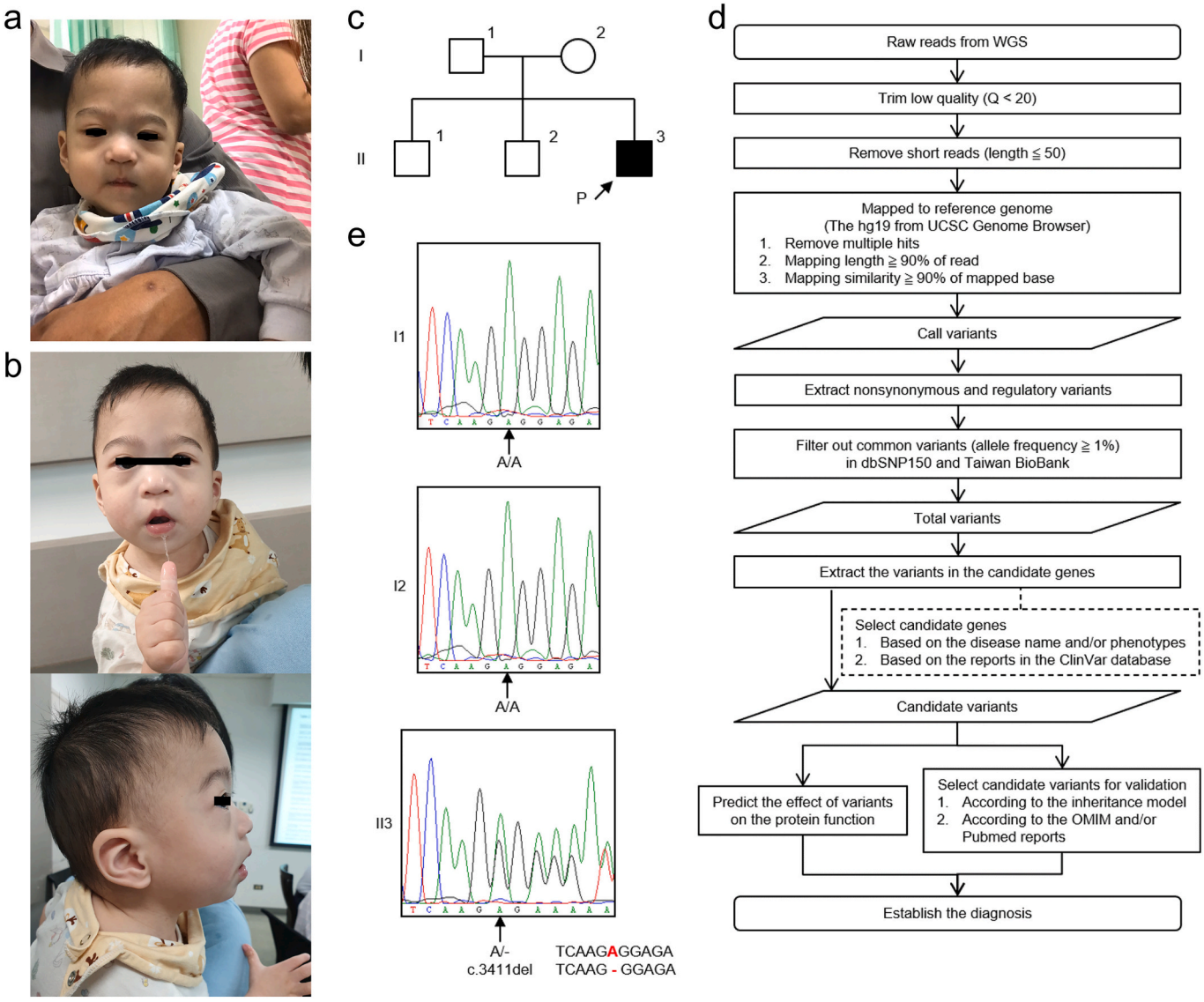

Fig. 1. The photos of the patient and analysis of the genetic data.
a. Front photograph at his age of 9 months and 2 weeks.
b. Front and ride side photographs at his age of 1 year and 1 month.
c. Pedigree of the patient.
d. Whole genome sequencing data analysis workflow for rare diseases.
e. Sanger sequencing of the patient and his parents.

at birth to have a right undescended testis. The results of newborn screening testing and hearing screening tests were normal. Patent foramen ovale (PFO) and peripheral pulmonary stenosis were reported in a cardioechogram at 2 months old. A reducible left inguinal hernia was diagnosed at 5 months and 3 weeks, and he underwent bilateral herniorrhaphy and orchiopexy of right side at 8 months and a week. Furthermore, closure of the anterior fontanelle and DD were also detected at 8 months old. Brain magnetic resonance imaging (MRI) showed no structural abnormalities. A lumbar spine MRI reported that the conus medullaris ended at around the L3 level, which raised a suspicion of a mild tethered spinal cord. At this point the boy was referred to Taipei Veterans General Hospital (TVGH) and early intervention was started immediately.

At TVGH, he was noted to be microcephalic and that there was a failure to thrive. At 9 months and 2 weeks old, he had a head circumference of 40 cm (−4.00 SD), a body weight of 5.8 kg (−5.45 SD) and a body height of 65.5 cm (−3.00 SD). He was unable to sit without support, could not imitate speech sounds, failed to reach for objects, and failed to stare at his hands. He was also noted to have hypotonia and dysmorphic features (Fig. 1a,b), including ocular hypertelorism, a flat nasal bridge, epicanthic folds, low-set posteriorly rotated ears, a bulbous nose tip, and a thin and tented upper lip. A chromosome study and aCGH were conducted and no abnormalities were found. WGS was carried out and this revealed three significant *de novo* mutations affecting the *KAT6A* gene, the *ATXN3* gene and the *NEFH* gene (Supplement Table 1). All of them are classified as deleterious with SIFT predictions. The second one was a point deletion in the *ATXN3* gene, NM_004993.6:c.873del (located at ch14: 92537397), NP_004984.2:p.(Lys291Asnfs40). Mutations in ATXN3 were known to cause spinocerebellar ataxia type 3. Patients with this disease may progressively present with ataxia, spasticity, dystonia, speech difficulty, swallowing difficulty, and peripheral neuropathy [5]. And, they typically present in the third decades of life. More importantly, the mutation was reported to be benign in LVOD3 database. The third was a base change in the *NEFH* gene, NM_021076.4:c.1933G>A (located at ch22: 29885562), NP_066554.2:p.Glu645Lys, which is known to be associated with axonal Charcot-Marie-Tooth disease, type 2CC [6]. This disease typically affects teenagers, and causes peripheral neuropathy in the lower extremities. After comparing the manifestations of these three diseases with his phenotype, it was concluded that the *KAT6A* mutation is most highly likely to be the causative mutation, and the mutation corresponds to ARTHS.

Sanger sequencing of the *KAT6A* gene from the patient and his parents confirmed a heterozygous *de novo* mutation, NM_006766.5:c.3411del (located at ch8: 41792327), NP_006757.2:p.Glu1139SerfsTer41 (Supplement Table 1; Fig. 1c,d,e). Importantly, this mutation is the first of its kind to be reported. It is a novel mutation in the *KAT6A* gene. The deletion of the nucleotide putatively results in a frame shift within the encoded protein and the new reading frame encounters a stop codon at the 41st amino acid. A diagnosis of ARTHS was made. Upon follow-up assessment when the child was 1 year and 1 months old, his head circumference was 41 cm (− 4.15SD), his body length was 69 cm (−3.24SD), and his body weight was 6.8 kg (− 3.38SD). Severe DD was still noted. He could roll over and reach out for objects, but still could not sit without support. No improvement in language development and personal-social development was found. An ophthalmological evaluation revealed severe myopia. The patient remains under early intervention and is regularly followed-up at our hospital.

4. Discussion

To our best knowledge, this is the first Taiwanese ARTHS patient detected by WGS and he has a novel mutation. The *KAT6A* gene consists of 17 exons and locates at chromosome 8p11. The gene encodes the protein lysine acetyltransferase 6 A (KAT6A, also known as MOZ and MYST3), which belongs to the MYST family [7]. The KAT6A forms a complex with other proteins and then this complex is able to modify the lysine of histone H3 and then to take part in epigenetic modification [8,9].

ARTHS was firstly described by Arboleda et al. [10] and Tham et al. [11] in 2015. Missense, splicing, and protein-truncating mutation have all been identified in this disease. There are three hotspot pathogenic variants at amino acid positions 1019, 1024, and 1129, which are within the last two exons [12]. In a previous review, the mutations located in the last two exons (16th and 17th exons) were considered to be late-truncating mutations, while those located in the 1st to 15th exons were considered to be early-truncating mutations [12]. The clinical presentations seem to be more severe and frequent in a late-truncating mutation. ID and speech delay were observed in all the patients, but both are more severe in late-truncating variants. Most patients have neonatal hypotonia, feeding difficulties, gastroesophageal reflux (GER), and constipation. About half of the patients were found to have simple forms of congenital heart disease (CHD). Strabismus, ptosis, and visual defects are also common. Common facial features include a broad nasal tip and a thin upper lip. Other reported symptoms are SGA, seizure, frequent infection, behavior problems, and sleep disturbance.

In our study, a *de novo* and heterozygous truncating mutation affecting the *KAT6A* gene, NM_006766.5:c.3411del, NP_006757.2:p.(Glu1139SerfsTer41), and related to ARTHS, is reported. This mutation has not been reported in previous studies. The diagnosis was made based on the high similarity of the phenotypes between the patient and other patients having a mutation in this gene. The mutation locates in the 17th exon of the *KAT6A* gene and therefore the mutation is a late-truncating mutation. Severe DD, hypotonia, and microcephaly were observed. The findings are similar to previous studies, which have shown that the presentations are more severe in late-truncating mutations.

In a plenty of genetic disorders, it is extremely difficult to reach a diagnosis only by recognizing patients' phenotypes. In addition, many diseases or syndromes share similar phenotypes between patients of different ethnicities. We report here the first patient in Taiwan with ARTHS. There was no abnormality found by chromosome study and aCGH. WGS was found in this patient to be an effective diagnostic tool for the differential diagnosis of patients with DD and associated syndromic features. A physician might consider ARTHS if there is a patient with similar presentations and in such circumstances arranging a WES/WGS promptly is recommended. Such an approach is especially important if the presenting phenotypes are not in good agreement with a well-known syndrome or disease.

There are some limitations in our study. There is much information yielded by WGS, which make physician difficult to identify the causative mutation and the association between the genotype and the phenotype of patients. However, we still eager to present a patient diagnosed as ARTHS with a novel mutation in the KAT6A gene in manner of WGS. As the genetic databases become more complete, the use of WGS and/or WES in diagnosis such patients with syndromic DD will be more powerful.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgmr.2020.100686.

CRediT authorship contribution statement

Yung-Feng Lin: Investigation, Writing - original draft, Data curation. **Tzu-Ching Lin:** Investigation, Writing - original draft, Formal analysis. **Ralph Kirby:** Writing - review & editing, Formal analysis. **Hui-Ying Weng:** Data curation, Formal analysis. **Yen-Ming Liu:** Methodology, Visualization. **Dau-Ming Niu:** Methodology, Formal analysis, Resources. **Shih-Feng Tsai:** Project administration, Supervision. **Chia-Feng Yang:** Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing - review & editing.

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Research data sharing statement

In order to protect patient's personal privacy, research data would remain confidential and would not be shared.

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