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Case Report

Identification of a c.544C>T mutation in WDR34 as a deleterious recessive allele of short rib-polydactyly syndrome

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ABSTRACT

Objective: Single-nucleotide polymorphism (SNP) microarrays and whole-exome sequencing (WES) are tools to precisely diagnose rare autosomal recessive (AR) diseases. In this study, SNP chip and WES were used to identify a mutated location in WDR34 in a baby born to consanguineous parents.
Case report: The baby, born at 36 gestational weeks had a small thoracic cage, symmetric short proximal bones, and polydactyly. Radiography showed short ribs with reduced lung volume and pulmonary opacities, compatible with asphyxiating thoracic dystrophy or short rib-polydactyly syndrome (SRPS). At 4 months of age, she died of pulmonary hypoplasia and sepsis. SNP microarray and evaluation tool confirmed WDR34 as the candidate gene. WES detected an AR mutation at c.554C > T [p.Arg182Trp] in WDR34.
Conclusion: This study was the first to identify c.544C > T [p.Arg182Trp] mutation in WDR34 in a patient with SRPS. According to the database, the homozygous mutation of c.544C > T in WDR34 was deleterious and the prevalence of heterozygous mutation was relatively higher in Asian population. More studies of this mutation in patients with SRPS are required.

Introduction

The diagnosis of some rare Mendelian disorders based on only clinical characteristics and specific radiographic findings is difficult because multiple diseases share common symptoms and signs. However, tools such as single-nucleotide polymorphism (SNP) microarrays and whole-exome sequencing (WES) can facilitate the diagnosis and identify the causative genes of heritable diseases [1]. Current commercially available genomic microarrays contain 0.4–2.7 million oligonucleotide probes for SNP in the human genome. SNP genotyping has been applied to detect regions of homozygosity (ROHs). ROHs are the copies that individuals inherit from their parents and increase the risk of autosomal recessive (AR) diseases, especially in consanguinity [2]. After identifying the ROHs, an online SNP array evaluation tool can be used to further identify AR disease-related candidate genes based on key clinical features and establish a precise diagnosis [3]. Recently, WES has been the most powerful platform to identify rare disease-causing genes and inherited mutations. More than 300 disease-causing genes have been identified through WES [4,5].

In this study, we used SNP probes on the Affymetrix SNP 6.0 chip and WES to identify an AR disorder and detect the mutation in a disease-related gene in a baby born to consanguineous parents.

Case presentation

This study was approved by the Institutional Review Board of the Chang Gung Memorial Hospital (IRB No. 99-0229B). Written

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informed consents were obtained from the legal guardian of the patients.

Clinical patient

A female baby was born to consanguineous parents at 36 gestational weeks. Her mother, a 15-year-old primigravida did not undergo antenatal examination, and her pregnancy was diagnosed at 34 weeks of gestation when she complained of abdominal distension. Prenatal ultrasound showed a fetus with short long bones, curved femurs, and a small ventricular septal defect. The baby's parents were generally healthy, except for psychiatric disorders. The mother previously had a diagnosis of major depressive disorder with a poor compliance to fluoxetine. She denied smoking, illicit drug use, nor alcohol abuse before and during gestation. The baby's father was her younger brother who was 13 years old and had attention deficit hyperactivity disorder. They had no family history of congenital abnormalities.

At birth, the baby weighed 2330 g (>97 percentile) and measured 42 cm (<3 percentile) in height with Apgar scores of 7 (1 min) and 8 (5 min). The head, thoracic, and abdominal circumferences were 33 cm (15–50 percentile), 25 cm (<3 percentile), and 27 cm (<3 percentile), respectively. Intubation was performed 30 min after birth because of progressive respiratory distress. Physical examination revealed a prominent forehead, small thoracic cage, symmetric short proximal bones, and postaxial polydactyly of the left foot (Fig. 1A). Radiography showed small scapulas, curved clavicles, short ribs with pulmonary opacities, reduced volume of both lung fields, and trident-shaped acetabular roofs (Fig. 1B). The findings were compatible with those of asphyxiating thoracic dystrophy (ATD) or short rib-polydactyly syndrome (SRPS). The peripheral blood karyotype was 46 XX. During hospitalization, she received pressure-controlled ventilation and inhaled nitric oxide for persistent pulmonary hypertension. An inotropic agent and broad-spectrum antibiotics were administered for severe sepsis. Phenobarbital was used due to frequent bronchospasm episodes and serial postnatal brain sonography did not show a structural abnormality except for progressive ventriculomegaly at 3 months of age. She died of respiratory failure as a complication of sepsis and pulmonary hypoplasia at 4 months of age. The clinical presentations are summarized in Table 1.

Affymetrix microarrays analysis and online SNP evaluation tool

Genomic DNA was extracted according to manufacturer's instructions (Gentra Puregene Blood Kit, Qiagen, Valencia, CA). An Affymetrix SNP 6.0 chip containing 0.9 million SNP probes was used to identify possible AR disorders. The SNP microarray confirmed the consanguinity status and identified 134 ROHs based on inheritance in the patient and subsequently inputted the data into the online genomic oligoarray and the SNP array evaluation tool, version 2.0, developed by the Center for Computational Science at University of Miami and the Section of Genetics in the Department of Pediatrics at Oklahoma University Health Sciences Center [3]. The evaluation tool searched for candidate genes associated with AR diseases that map to these regions based on key clinical features. The search criteria included Online Mendelian Inheritance in Man (OMIM) genes and the description of “short ribs AND polydactyly” as specific clinical features. Four candidate genes associated with AR diseases, namely lamin B receptor (LBR), intraflagellar transport (IFT)172, CD96, and WD repeat domain 34 (WDR34) were filtered from the output based on literature review on OMIM and PubMed.

Among the four genes, LBR is associated with Greenberg dysplasia [6] and CD96 is associated with C syndrome, characterized by trigonocephaly, unusual facies, psychomotor retardation, redundant skin, abnormalities of joints and limbs, and visceral anomalies [7]. However, these findings were not compatible with those of our case. IFT172 and WDR34 have been reported as mutation genes related to SRPS [8–10]. However, the clinical presentations of the IFT172 mutation including nephronophthisis result in end-stage renal disease, retinal degeneration, and liver fibrosis were not observed in the baby. Thus, we suspected WDR34 to be the mutated gene associated with SRPS in our patient. To

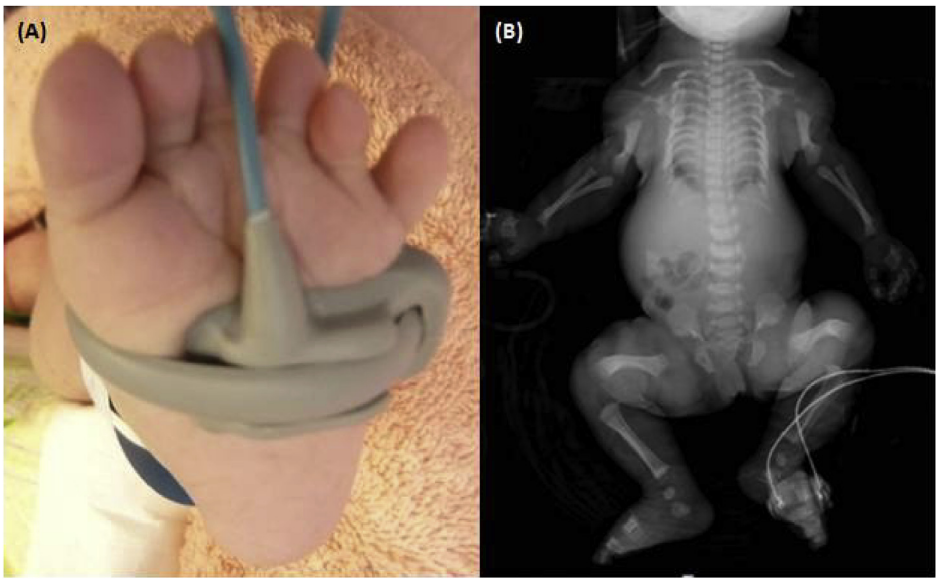


Fig. 1. Clinical presentation and radiological characteristics of the patient (A) Polydactyly of the left foot (B) Small thoracic cage, short proximal bones, small scapulas, curved clavicles, short ribs with pulmonary opacities, reduced volume of both lung fields, and a trident-shaped acetabular roof.

Table 1
Clinical scenario of the baby born to consanguineous parents.

Day after birth	Clinical presentations and impressions
Day 0	(i) Respiratory distress with pulmonary hypertension (ii) Ventricular septal defect 4.2 mm and patent ductus arteriosus 2.5 m
Day 21	Bronchospasm and focal epileptiform activity at right temporal area on EEG
Day 38	Septic shock, intravenous catheter related
Day 60	Sepsis, ventilation associated pneumonia related
Day 80	Persisted pulmonary hypertension
Day 92	Hydrocephalus with increased intracranial pressure
Day 102	Central venous line infection
Day 103–139	Seizure episodes
Day 143	Gastrointestinal bleeding, stress ulcer and thrombocytopenia related
Day 163	Expired due to sepsis and pulmonary hypoplasia

further identify the definite mutation in WDR34, WES was performed.

Whole exome sequencing (WES) analysis

The trio samples from maternal, paternal, and the patients' peripheral blood were prepared using the Ion AmpliSeq Exome Enrichment Kit Library (Thermo Fisher Scientific Inc.) and they were sequenced on the Ion Proton sequencer. The sequencing data were analyzed using the Ion Torrent Mapping Alignment Program, v4.2-18, and Ion Torrent Variant Caller, v4.2-18, which are plug-ins for the Ion Torrent Software Suite, v.4.2 (Thermo Fisher Scientific). The called variants were then annotated using the Variant Effect Predictor (VEP), v74 and gene sets associated with diseases were extracted from the MalaCards database.

Through Ion Torrent exome sequencing, we identified 645 homozygous AR variants and 313 heterozygous de novo variants. Only one AR mutation in WDR34 (c.554C > T [p.Arg182Trp]) was considered deleterious and associated with ATD according to the MalaCards human disease database (Figs. 2 and 3). According to the prediction of SIFT and PolyPhen-2, the mutation c.554C > T [p.Arg182Trp] in WDR34 was probably damaging.

Confirmation of the WDR34 mutation through sanger sequencing

Fig. 4 illustrates the genomic structure of WDR34. WDR34 included nine exons, and the mutation c.544C > T [p.Arg182Trp] was the initial amino acid in the fourth exon. The mutated nucleotide was located at 128636919 (GRCh38)/131399198 (GRCh37). Sanger sequencings with primer sequences as follows:

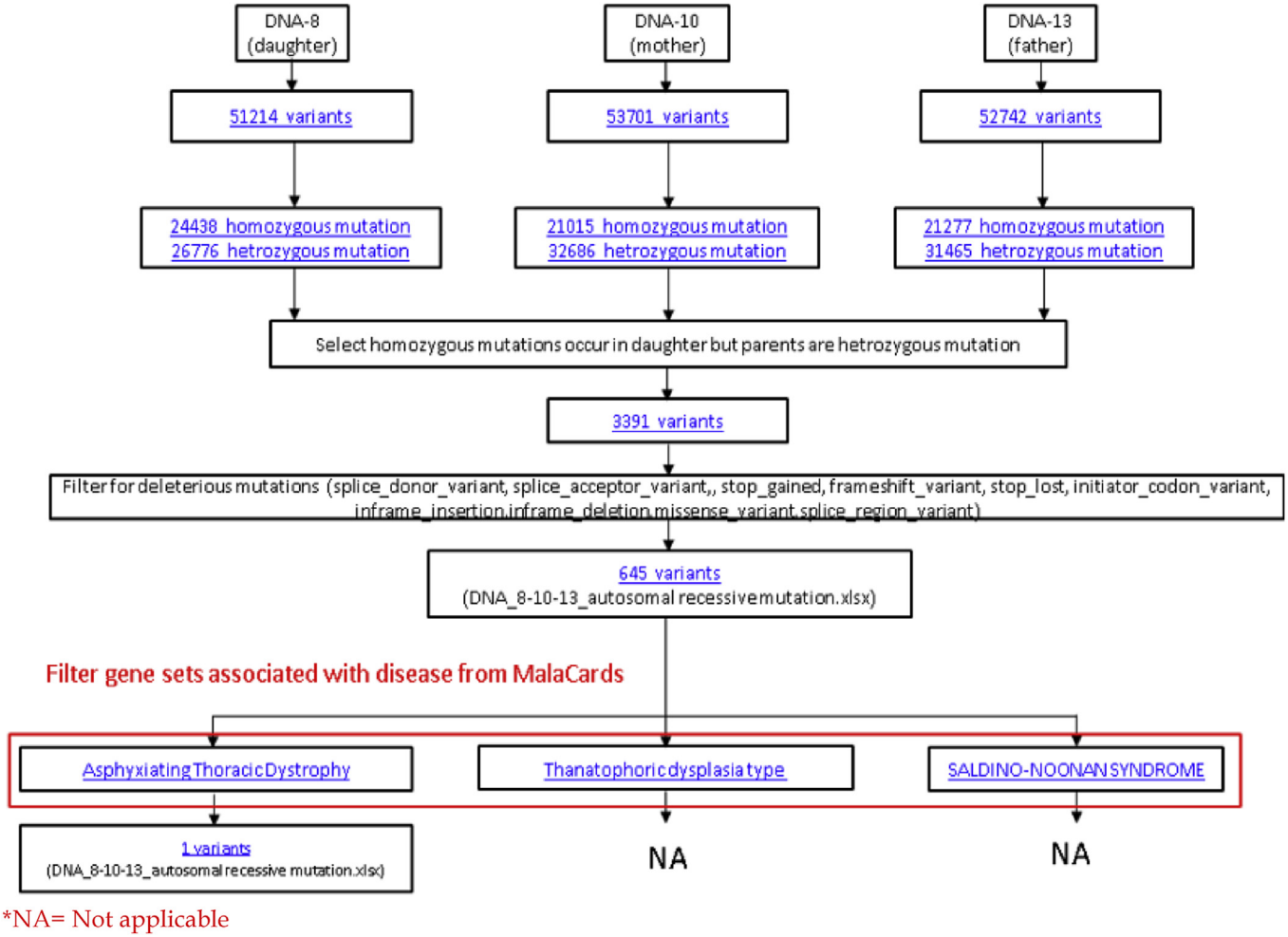


Fig. 2. Detection of autosomal recessive mutations associated with the disease through whole-exome sequencing.

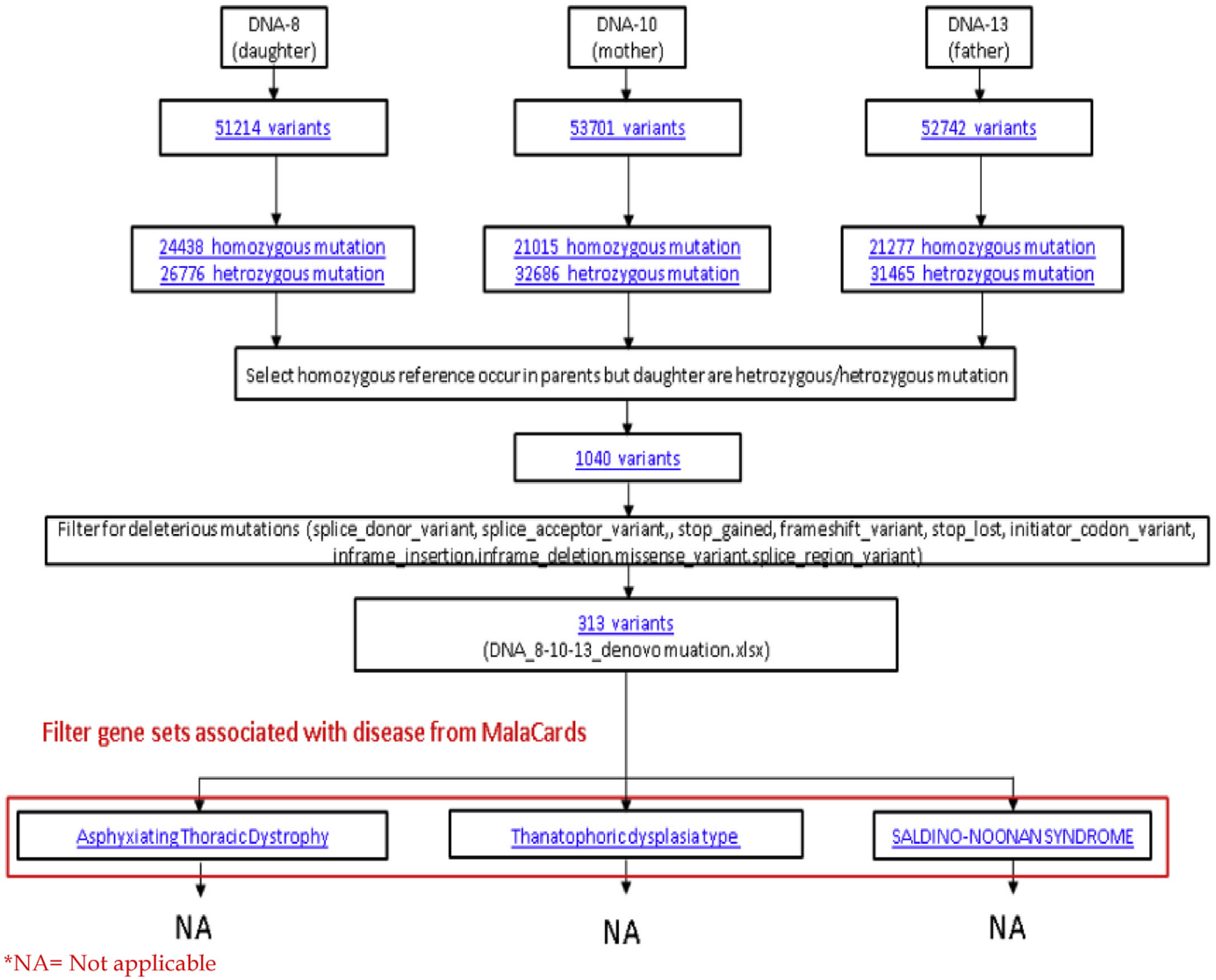


Fig. 3. Detection of de novo mutations associated with the disease through whole-exome sequencing.

5′-gtctgcatgtgaccagcatc-3′ (sense) and 5′-ggatggccaagctcttagaa-3′ (antisense) were performed on the samples obtained from family members and showed wild type C/C of the grandfather and heterozygous C/T of the grandmother and parents but deleterious homozygous T/T of the patient. The pedigree and the results of Sanger sequencing are presented in Fig. 5A and B, respectively.

Fig. 4. Location of the mutation in the WDR34 genomic structure.

Genetic database search

The whole process of detecting a definite mutation in the patient with SRPS is summarized in Fig. 6. We searched the WDR34 mutation in the databases of the Clinical Variation of National Center for Biotechnology Information (NCBI), Taiwan View (Taiwanese Biobank), and the Exome Aggregation Consortium (ExAC) [11]. In the NCBI, there was no report of the mutated c.544C > T, but nine other variations of the nucleotide in WDR34 were related to SRPSs, which indicated a strong association of WDR34 with SRPS. No deleterious homozygous mutation in WDR34 c.544C > T was found in the Taiwan View or ExAC; however, its heterozygous prevalence was 0.000504 in the Taiwan View. Two cases of heterozygous variant (rs555817074) were reported in the East Asian population registered in the ExAC, resulting in a frequency of 0.00001909 in the total registered population and of 0.0002546 in the East Asian population.

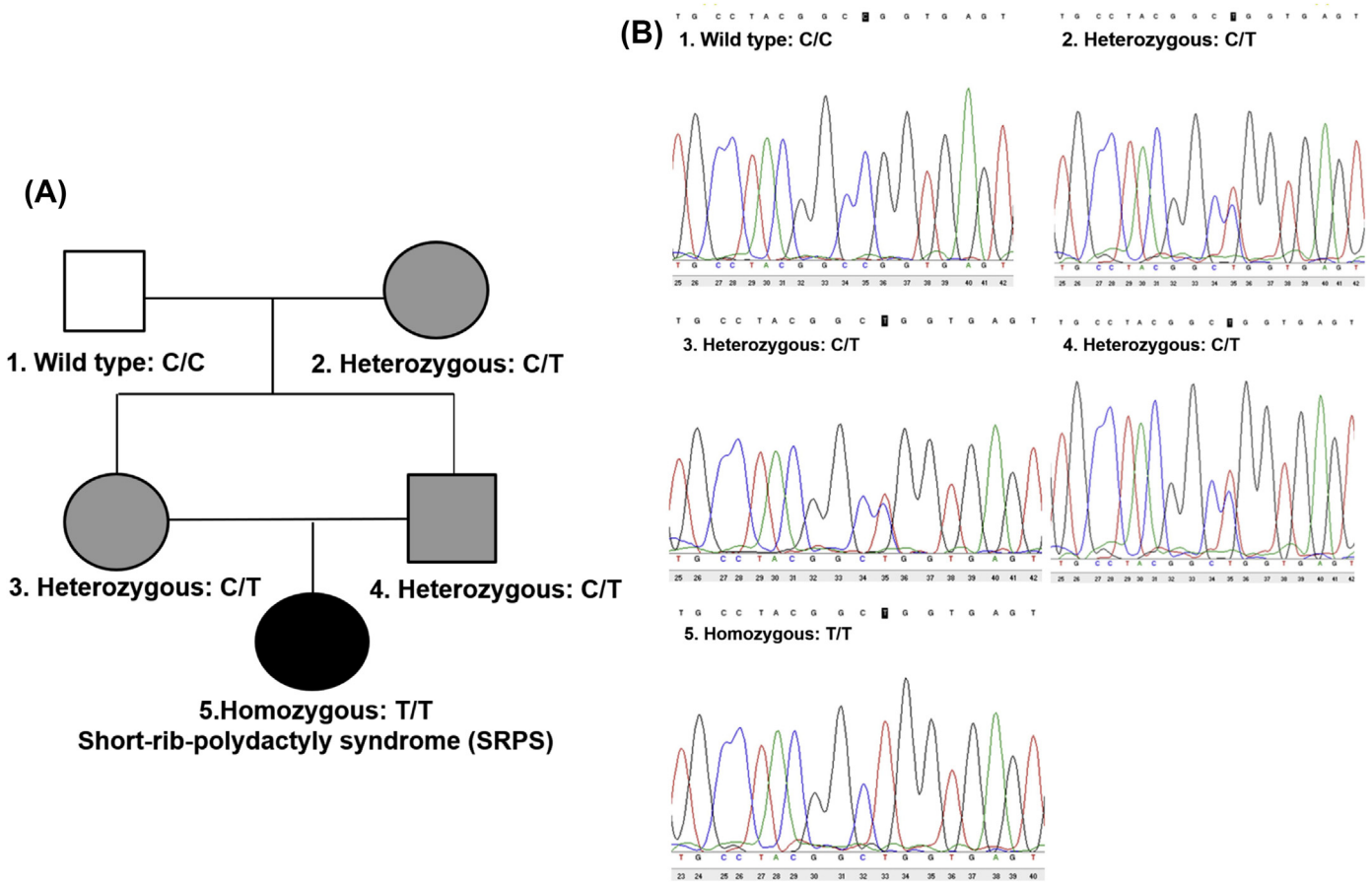


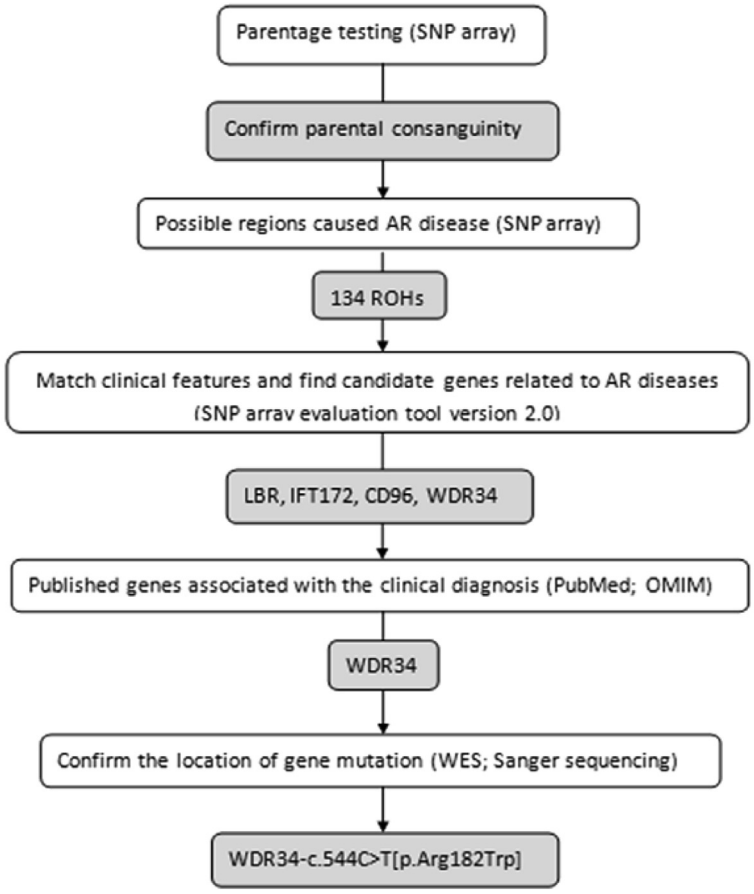
Fig. 5. The pedigree of the patient's family (A) and Sanger sequencing: 1 and 2 are grandparents, 3 and 4 are parents, and 5 is the patient with SRPS (B).

Discussion

This study was the first to report that the WDR34 c.544C > T variant is a mutation associated with SRPS. SRPS are a group of lethal skeletal dysplasias classified as Saldino–Noonan (SRPS type I), Majewski (SRPS type II), Verma–Naumoff (SRPS type III), and Veemer–Langer syndromes (SRPS type IV). All SRPSs are inherited with an AR pattern and share the overlapping clinical and radiological features [12]. The clinical diagnosis of SRPS depends on the phenotypes and specific radiographic findings of short long bones, a narrow thorax, short ribs, a trident-shaped acetabular roof of the pelvis, and polydactyly, which were also noted in patients with ATD. ATD is defined as a nonlethal skeletal dysplasia, but its lethality increases with retinal degeneration, respiratory insufficiency, renal cysts, and liver abnormalities [8]. SRPS and ATD share not only clinical presentations but also related gene mutations. Genes including IFT80, DYNC2H1, WDR34, WDR60, IFT172, TTC21B, NEK1, IFT43, IFT122, WDR19, and WDR35 have been identified for SRPS [9,13,14]. By contrast, IFT80, IFT172, DYNC2H1, and WDR34 have been determined for ATD [8–10,15]. Both SRPS and ATD are AR osteochondrodysplasias and mutated genes cause skeletal ciliopathies. WDR34 encoding a WD-repeat protein with five WD40 domains is required for cytoplasmic dynein-2 function. Mutations in WDR34 affect the retrograde IFT and are related to ciliogenesis and shorter primary cilia in fibroblasts [9,10]. Defects in the components of the IFT and dynein-2 complexes or regions of the basal body affected the normal formation, maintenance, and function of the primary cilium [15]. In addition, WDR34 inhibits the NF-κB pathway, which is involved in the pathogenesis of human skeletal ciliopathies [16].

SNP microarrays and WES work in different ways to identify disease-related genes in this case. With the assistance of a web-based tool [4], SNP microarrays efficiently determined the hereditary pattern and candidate genes. The online evaluation tool [4] helped in establishing a diagnosis by integrating the detected ROHs and key clinical features. WES ultimately identified nucleotide mutations and amino acid changes in disease-associated genes. McInerney-Leo et al. performed whole-exome massive parallel sequencing in 11 patients with SRPS and 993 unaffected individuals to determine the genetic cause of SRPS and identified six genes (IFT172, DNC2H1, TTC21B, WDR60, WDR34, and NEK1) in 10 patients, with sensitivity of 90.9% and specificity of >99% [13]. Compared with SNP data, the interpretation of WES data is more complicated and time consuming; however, WES is the ideal platform to identify AR or de novo mutations in babies born to consanguineous parents.

In conclusion, this study was the first to identify the c.544C > T [p.Arg182Trp] mutation in WDR34 in a patient with SRPS. Our subsequent bioinformatics studies of this genetic variation also provided its clinical interpretation and the mutation frequency in selected populations. According to the database, the homozygous mutation of c.544C > T in WDR34 was considered disease causing and the prevalence of heterozygous mutation was relatively higher in Asian population. More studies of this mutation in patients with SRPS are required to ascertain its role in this disease in Asians.



* SNP = single-nucleotide polymorphism; AR = autosomal recessive; ROHs = regions of homozygosity; WES = whole exome sequencing

Fig. 6. Flowchart illustrating the identification process of the location of gene mutations in this patient.

Conflicts of interest

All authors report no conflict of interest.

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